FoodSmartphone

Smartphone analyzers for on-site testing of food quality and safety

H2020-MSCA-ITN-2016

Joint Training Manual

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

Revision history

Version	Date	Modified by	Comments
V0.1	12 November 2019	Ms Ciara Sarsfield (QUB)	First Draft of D7.4 for Consortium Approval
V0.2	4 December 2020	Mrs Joanna Scott (QUB)	Amendments to report due to EU privacy laws as report to be made public
V0.3	10 December 2020	Michel Nielen (WR)	Minor revisions on D7.4 title page and document control page based on new GA contract 07/09/2020
V0.4	14 December 2020	Michel Nielen (WR)	Cover pages revised for use on public website www.foodsmartphone.eu

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Preface

A paradigm shift in food quality and safety testing is required in order to free resources for an intensified combat against fraud in the food chain. As an enabling technology solution to the problem, the FoodSmartphone project proposes the development of smartphone-based (bio)analytical sensing and diagnostic tools, for simplified on-site pre-screening of quality and safety parameters and wireless data transfer to servers of relevant stakeholders. The consortium has been built upon highly complementary disciplines: (bio)analytical chemists, biologists, physicists, micro/nano-engineers, mathematicians, organic- and food chemists work together on the joint supra-disciplinary goal. The scientific training in novel smartphone-based sensing technologies plus the complementary skills training provided, may have a major impact on future EU monitoring practices and, moreover, pave the road for future Citizen Science. More information can be found at www.FoodSmartphone.eu.

The FoodSmartphone project is a European Training Network and offered the early-stage researchers an extensive programme of both mandatory and optional network-wide training events and intersectoral secondments. This Joint Training Manual provides a compilation of the presentations given at the four mandatory network-wide training events:

- N1 was the summer school on Smartphone-based Assay Development & Open Science hosted by Wageningen Food Safety Research (formerly known as RIKILT) in Wageningen (NL),
- N2 was the summer school on Food Applications, QA/QC and Validation hosted by the University of Chemical Technology in Prague (CZ),
- N3 was the summer school on Software Design and FoodSmartphone Exploitation hosted by Queen's University Belfast (UK),
- N4 was the final FoodSmartphone network conference, called Workshop on SmartTech for Food, that was hosted by CSIC in Barcelona (E).

The Graduate School

VLAG

1st Summer School on

Smartphone-based Food Analysis

26-30 June 2017
Wageningen, The Netherlands

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CONTENTS

Smartphone-based Food Analysis

26-30 June 2017

Course: Smartphone-based Food Analysis (SFA)

Programme of the 1st edition, 2017

Week 26: 26-30 June, 2017
H2020 Marie-Curie project FoodSmartphone in co-operation with Graduate School VLAG
Course Director: Prof. Michel W.F. Nielen
Course organizer: Mrs. Chantal Doeswijk (Graduate School VLAG)
Co-organizer: Mr. Wim Beek (H2020 FoodSmartphone project)
Course venue: RIKILT, Room 0016/0017, Building #123 (Vitae) Wageningen Campus (Akkermaalsbos 2)

Monday, 26 June 2017				
10:30	Registration with coffee / tea			
11:15-11:30	Welcome, short introduction to the programme		**Prof. Michel Nielen** (RIKILT, WU)	
11:30-12:30	Setting the scene 1: EU monitoring practices		**Dr. Leen van Ginkel** (RIKILT)	
12:30-13:30	*Lunch (Orion building #103))*			
13:30-13:45	*Group photo outside Orion*			
13:45-14:45	Setting the scene 2: the RASFF		**Dr. Hans Marvin** (RIKILT)	
14:45-15:45	Validation and benchmarking of screening assays		**Dr. Bjorn Berendsen** (RIKILT)	
15:45-16:15	*Coffee, tea & refreshments*			
16:15-17:15	Foodsmartphone concepts		**Prof. Michel Nielen** (RIKILT, WU)	
17:15-18:00	Introduction to hot paper studies		**Prof. Michel Nielen** (RIKILT, WU)	
19:00-21:00	*Course Dinner **Colors World Food***		**Markt 15, 6701 CX Wageningen**	
Tuesday, 27 June 2017				
08:30-09:00	*Coffee & tea*			
09:00-10:00	Biorecognition		**Dr. Monique Bremer** (RIKILT)	
10:00-11:00	Ligand binding assays		**Dr. Monique Bremer** (RIKILT)	
11:00-11:30	*Coffee & tea*			
11:30-12:30	How to speed-up binding assays		**Dr. Monique Bremer** (RIKILT)	
12:30-13:45	*Lunch (Orion building # 103)*			
13:45-15:45	Hands-on labwork 1-15, Forum (P703, building #102)	Comm. workshop (16-30, RIKILT building)	**ing. Jeroen Peters** (RIKILT)	**Marcella Bos & Hanneke van Marle** (Bos Matchworks BV)
15:45-16:15	*Coffee, tea & refreshments*			
16:15-17:15	Hands-on labwork (continued)	Comm. workshop (continued)	**ing. Jeroen Peters** (RIKILT)	**Marcella Bos & Hanneke van Marle** (Bos Matchworks BV)
17:15-18:00	Happy hour debates (RIKILT building)		**Prof. Michel Nielen** (RIKILT, WU)	
Wednesday, 28 June 2017				
08:30-09:00	*Coffee & tea*			
09:00-10:00	Surface Chemistry		**Prof. Han Zuilhof** (Organic Chemistry, WU)	
10:00-11:00	Membranes and microsieves		**Prof. Cees van Rijn** (Aquamarijn microfiltration)	
11:00-11:30	*Coffee & tea*			
11:30-12:30	Electrochemical detection		**Dr. Louis de Smet** (Organic Chemistry, WU)	
12:30-13:45	*Lunch (Orion building)*			
13:45-14:45	Hot paper debates		**Prof. Michel Nielen** (RIKILT, WU)	
14:45-15:45	Optical detection		**Prof. Michel Nielen** (RIKILT, WU)	
15:45-16:15	*Coffee, tea & refreshments*			
16:15-17:15	Microfluidics		**Prof. Daniel Filippini** (Linköping University, SE)	
17:15-18:00	Happy hour presentations 1-3		**Prof. Michel Nielen** (RIKILT, WU)	
18:15-21:30	ESRs business meeting (ESRs only)		**Dr. Arjen Gerssen** (RIKILT)	

Thursday, 29 June 2017				
08:30-09:00	*Coffee & tea*			
09:00-10:00	CAD, 3D printing		**Prof. Daniel Filippini** (Linköping University, SE)	
10:00-11:00	Image data handling		**Dr. Jeroen Jansen** (Radboud University)	
11:00-11:30	*Coffee & tea*			
11:30-12:30	Commercial smartphone assays		**Mr. R. Niemeijer** (R-Biopharm AG)	
12:30-13:45	*Lunch (Orion building)*			
13:45-15:45	Hands-on labwork (15-28, Forum (P703, building #102)	Comm. workshop (1-14, RIKILT building)	**ing. Jeroen Peters** (RIKILT)	**Marcella Bos & Hanneke van Marle** (Bos Matchworks BV)
15:45-16:15	*Coffee, tea & refreshments*			
16:15-17:15	Hands-on labwork (continued)	Comm. workshop (continued)	**ing. Jeroen Peters** (RIKILT)	**Marcella Bos & Hanneke van Marle** (Bos Matchworks BV)
17:15-18:00	Happy hour presentations 4-7		**Prof. Michel Nielen** (RIKILT, WU)	
Friday, 30 June 2017				
08:30-09:00	*Coffee & tea*			
09:00-10:00	Smartphone-based NIR scanners		**Dr. Yannick Weesepoel** (RIKILT)	
10:00-11:00	Mobile Microscopy, Sensing and Diagnostics through Computational Photonics		**Prof. Aydogan Ozcan** (UCLA, Los Angeles, USA)	
11:00-11:30	*Coffee & tea, meet the expert*			
11.30-11.45	Course certificates			
11:45-12:45	*Farewell Lunch (Orion building)*			
12:45-16:15	ESRs e-newsletter (ESRs only)		**Mr. Wim Beek** (RIKILT)	

Smartphone-based Food Analysis (SFA)

Setting the scene: EU monitoring practices

Leen van Ginkel

26 June 2017

FoodSmartphone.eu

European Union Reference Laborato

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Why do we want to analyse our food?

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Why we want to analyse our food?

- Food composition: how healthy is our food?
- Food quality: is the quality up to the standards?
- Food authenticity: is the food what it claims to be (origin, biological, sustainably produced)
- Food safety: is it safe to consume this food?

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Food safety issues

Residues of treatment

Environmental contaminants

Natural toxins

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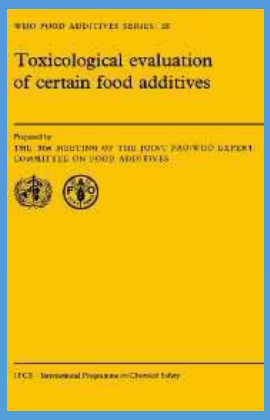
How big is the problem?

Risk = hazard X exposure of the consumer



Monitoring

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The lègal basis for monitoring

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The legal basis for monitoring

- **Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products,**

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However, for now two questions

- How and when did it start?
- What are the current monitoring practices?

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Use of growth promoting hormones in meat production.

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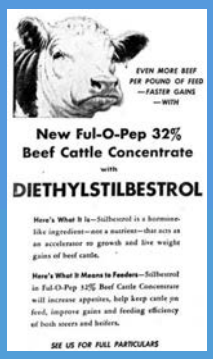
Illegal use of growth promoting hormones

- Use in EU countries in which the use was already banned
- Use of non-registered products



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DES and food

DES was a mainstay of the livestock industry, too. It was used in animal feed to fatten cattle, lamb, and chicken. A large percentage of the hamburgers, veal, poultry, and steaks on our dinner plates in the 1950s, '60s, and '70s, were DES-fed livestock.

Geknoei met slachtvee duurt

Food and Agriculture Organization of the United Nations

English Español Français العربية 中文 Русский

AGRIS SEARCH Find resources...

Is the calf with hormones toxic? [meat quality, DES (diethylstilboestrol), use of anabolizers] [1981]

ROTTERDAM, 12 SEPT. Wie met vlees slechte dingen wil uithalen, kan ongestraft zijn gang gaan. Opsporing oratoria loopt achter op de Die weten bijvoorbeeld precies waar ze die lichaamsdelen, die bij de slacht toch en op de dag af nauwkeurig hoe lang ze uiken om geen sporen achter te laten, van is terug te vinden.


Hoe gevaarlijk is DES?

Reportage

Hormonen: gemakkelijk te krijgen moeilijk aan te tonen

April 7th, 1981

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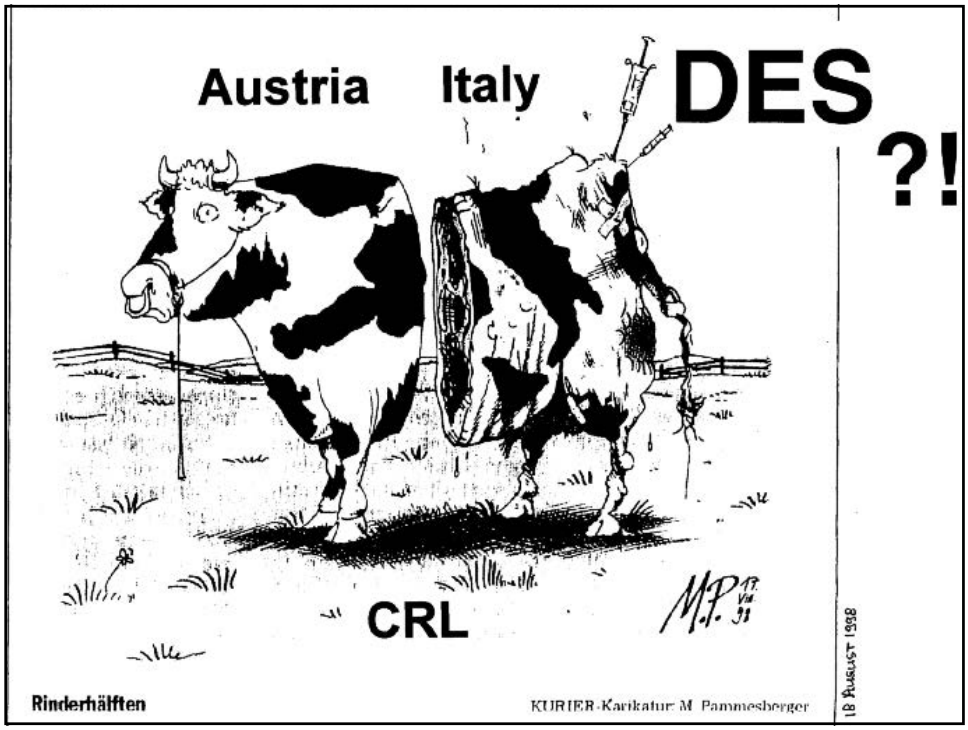

Summarized, the situation 70's and early 80's was

- "Toxic" compounds were used in food production
- Poor legislation, little harmonization between countries
- Little official control
- Limited possibilities for laboratories

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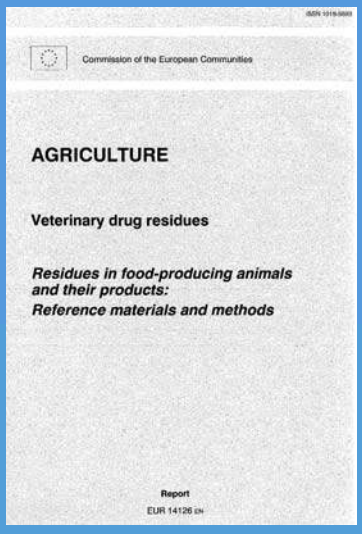
Control strategy for hormone abuse

COUNCIL DIRECTIVE
of 16 July 1985
supplementing Directive 81/602/EEC concerning the prohibition of certain substances having a hormonal action and of any substances having a thyrostatic action
(85/358/EEC)

Pending decisions to this effect, the Member States shall, in the event of dispute, recognize the findings obtained by radio-immunoassay (RIA) and by thin layer chromatography or by gas chromatography.

1. Where the analysis referred to in Article 5 confirms the presence of prohibited substances or of residues either exceeding the maximum natural physiological levels for the authorized substances or proving that authorized substances have been used abusively, the competent authorities shall be informed immediately of :


Commission of the European Communities

AGRICULTURE

Veterinary drug residues

Residues in food-producing animals and their products:
Reference materials and methods

Report
EUR 14126 EN

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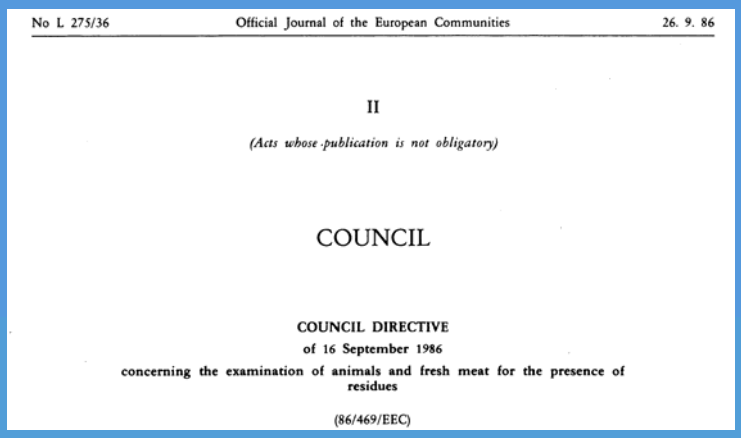
Start of official European legislation



No L 275/36 Official Journal of the European Communities 26. 9. 86

II

(Acts whose publication is not obligatory)

COUNCIL

COUNCIL DIRECTIVE
of 16 September 1986
concerning the examination of animals and fresh meat for the presence of residues
(86/469/EEC)

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Development of the EU monitoring program

- 1985
- 1986
- 1988
- 1989
- 1991

- First Directive on methods
- First Residue Directive
- Routine Field labs designed
  Start Annual National Plan
- Criteria for confirmative methods
  CRL powers and mandates
- Four CRLs designated (RIVM (later RIKILT, BVL, AFSSA, Roma)

Residues in meat only

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However, in spite of the new legislation

- In the 90's several major food safety incidents in Europe
- The most dramatic were the Belgium dioxin incident in 1999 and the UK BBE crisis in 1997-2001

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BBC NEWS MAY 31, 1999
BELGIAN CHICKENS
BBC NEWS JUNE 2, 1999
'Faster, cheaper dioxin tests needed
CAER SCARE
BBC NEWS JUNE 3, 1999
NEW FEARS OVER BELGIAN FOOD

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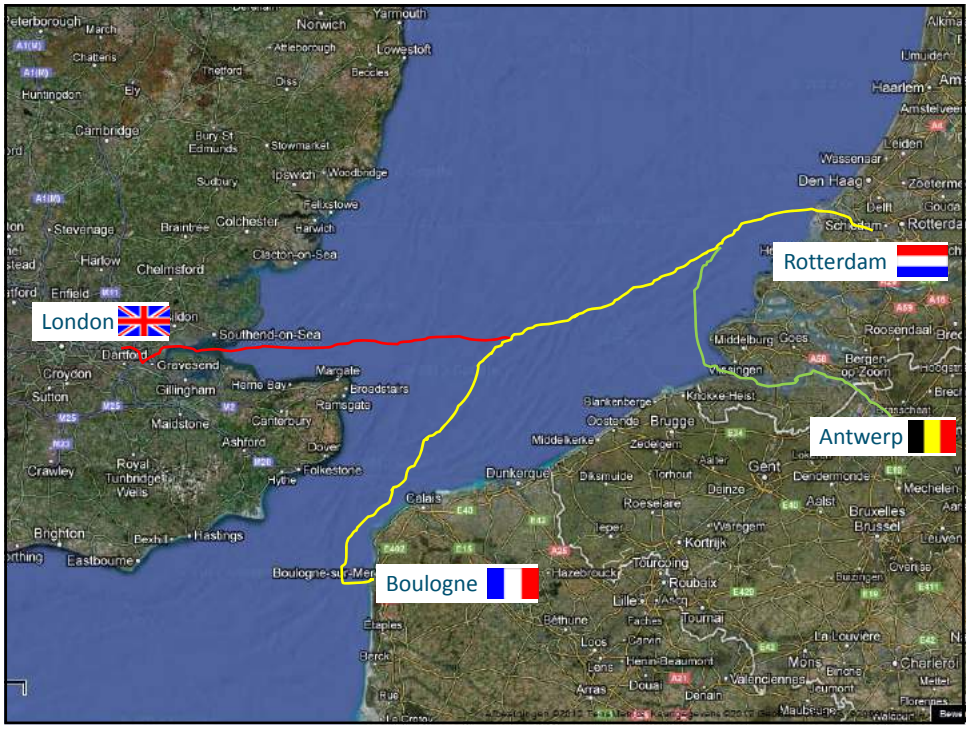

Consequently the EU issued in 2002 the General Food Law (2002/178)

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General Food Law: principles

- Operators in feed/food business have the primary responsibility for food safety
- Competent authorities monitor, verify and enforce at all stages of production, processing and distribution
- EC evaluates the work of the national authorities through audits and inspections

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General Food Law

- Precautionary principle: better safe then sorry
- Obligation for the producer to report any incident to the government within 24 hours
  → all alerts are reported to the EU Rapid Alert System Food & Feed (RASFF)
- Obligation to withdraw products from the market when there is any doubt on the safety

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General Food Law

- Establishment European Food Safety Authority


efsa
European Food Safety Authority

The EFSA Journal (2007) 510, 1-62

OPINION OF THE SCIENTIFIC PANEL ON CONTAMINANTS IN THE FOOD CHAIN ON A REQUEST FROM THE EUROPEAN COMMISSION RELATED TO HORMONE RESIDUES IN BOVINE MEAT AND MEAT PRODUCTS

Question N° EFSA-Q-2005-048

Risk = hazard X exposure of the consumer

- Organization of a trusted system for the official control by the EU countries

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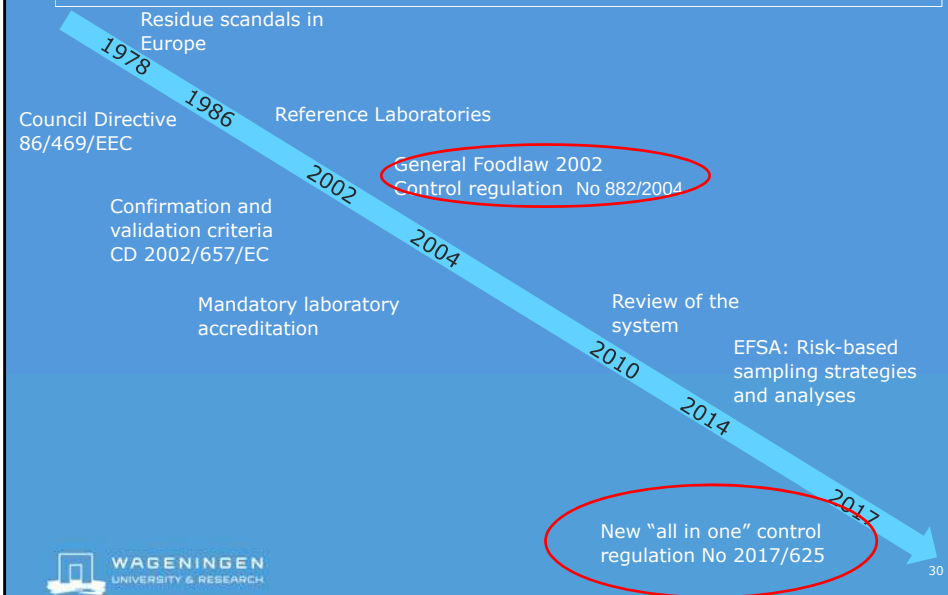
From 1980's till ....



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Control Regulation No 882/2004

It is all about: Official control in the EU:

Regulation (EC) No 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

- Analyses of official samples ask for
  - Analyses in accordance with internationally approved procedures.
- Are supported by
  - A Network of laboratories.

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EU food/feed safety monitoring system is based on a network of laboratories

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EU food safety monitoring system
The vertical network

EU-Reference Laboratories (EURLs)

National Reference Laboratories (NRLs)

Official Laboratories (OLs)

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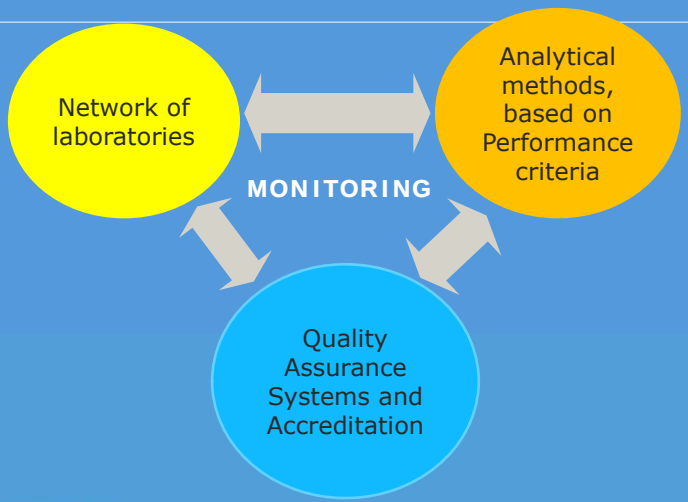
Cornerstones for Residue Control under the General Foodlaw



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EU Monitoring at different levels

National Residue Plans

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National Residue Plans

- Annual programmes within each EU Member State, based on production data (e.g. number of animals) of the previous years and the percentage on non-compliant results (violations)
  - National production
  - Routine, based "solid" techniques and methodology
  - Publicly known content
  - Static
  - Minimum program

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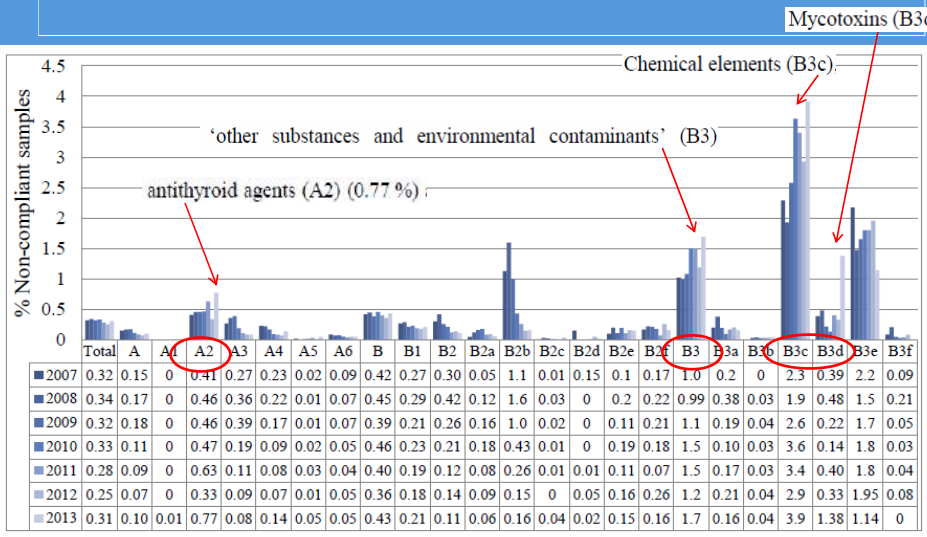
Non-compliant results



	Total	A	A1	A2	A3	A4	A5	A6	B	B1	B2	B2a	B2b	B2c	B2d	B2e	B2f	B3	B3a	B3b	B3c	B3d	B3e	B3f
2007	0.32	0.15	0	0.41	0.27	0.23	0.02	0.09	0.42	0.27	0.30	0.05	1.1	0.01	0.15	0.1	0.17	1.0	0.2	0	2.3	0.39	2.2	0.09
2008	0.34	0.17	0	0.46	0.36	0.22	0.01	0.07	0.45	0.29	0.42	0.12	1.6	0.03	0	0.2	0.22	0.99	0.38	0.03	1.9	0.48	1.5	0.21
2009	0.32	0.18	0	0.46	0.39	0.17	0.01	0.07	0.39	0.21	0.26	0.16	1.0	0.02	0	0.11	0.21	1.1	0.19	0.04	2.6	0.22	1.7	0.05
2010	0.33	0.11	0	0.47	0.19	0.09	0.02	0.05	0.46	0.23	0.21	0.18	0.43	0.01	0	0.19	0.18	1.5	0.10	0.03	3.6	0.14	1.8	0.03
2011	0.28	0.09	0	0.63	0.11	0.08	0.03	0.04	0.40	0.19	0.12	0.08	0.26	0.01	0.01	0.11	0.07	1.5	0.17	0.03	3.4	0.40	1.8	0.04
2012	0.25	0.07	0	0.33	0.09	0.07	0.01	0.05	0.36	0.18	0.14	0.09	0.15	0	0.05	0.16	0.26	1.2	0.21	0.04	2.9	0.33	1.95	0.08
2013	0.31	0.10	0.01	0.77	0.08	0.14	0.05	0.05	0.43	0.21	0.11	0.06	0.16	0.04	0.02	0.15	0.16	1.7	0.16	0.04	3.9	1.38	1.14	0

Figure 5: Percentage of non-compliant samples reported in relation to the total number of targeted samples analysed for the respective group in 2007, 2008, 2009, 2010, 2011, 2012 and 2013 (substance groups are detailed in Appendix E)

EU Monitoring at different levels

Surveillance studies

National Residue Plans

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Surveillance studies

- Additional analyses on potentially new risks; meaning new compounds, other commodities. (e.g. new medicines, designer drugs (growth promotors).
  - National activity (optional reporting to the EC)
  - Higher "surprise" factor
  - Results provide input for "next years" National residue Plan (more "risk based" approach)
  - Also in imported products

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Ractopamine in imported beef from USA en South America

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Surveillance studies

- Testing new analytical approaches based - combining fast and broad initial screening with instrumental targeted methods.

Broad screening:
Activity based – on site - untargeted

Confirmation:
Accurate quantification and identification(MS)

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EU Monitoring at different levels

Method innovation and research

Surveillance studies

National Residue Plans

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Method innovation and research

- Why
  - Technology push: things that were not possible before become within reach:
    - More sensitive methods,
    - On-site measurements
  - New problems ask for new approaches
  - New clients become important, not only official inspection services, but also consumer(s)organisations.

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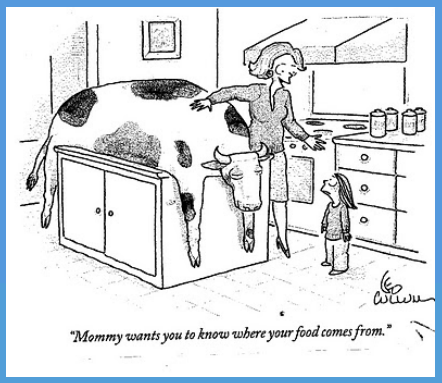
New food related concerns

- New banned substances
- Antibiotic resistance
- New toxins
- Food fraud issues



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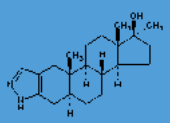
New banned substances



Stanozolol

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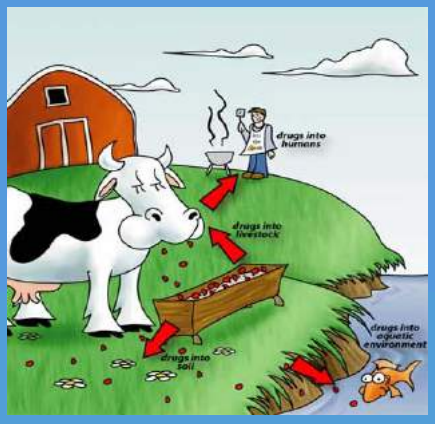
Antibiotic resistance

From monitoring te the level of allowable Maximum Residue Limits

Due to an increased concern about resistance

Monitoring of the total exposure of feed and food for antibiotics



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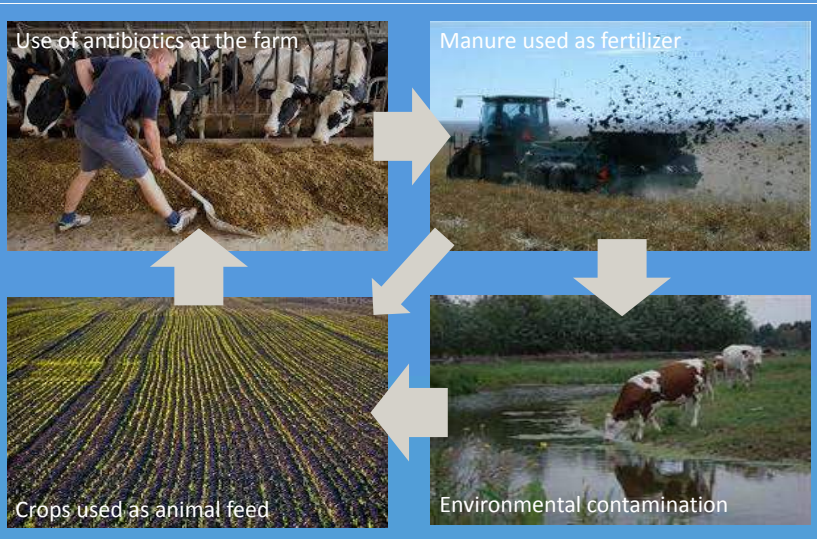
Antibiotics - Dissemination



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New methods for antibiotics

- Not only in consumable (MRL) matrices but also
  - Organs and hair or feathers.
  - Excreta (environment)
  - Soil
- Fast and on-site measurements at farms (verification of treatment records)

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On-site screening for antibiotics

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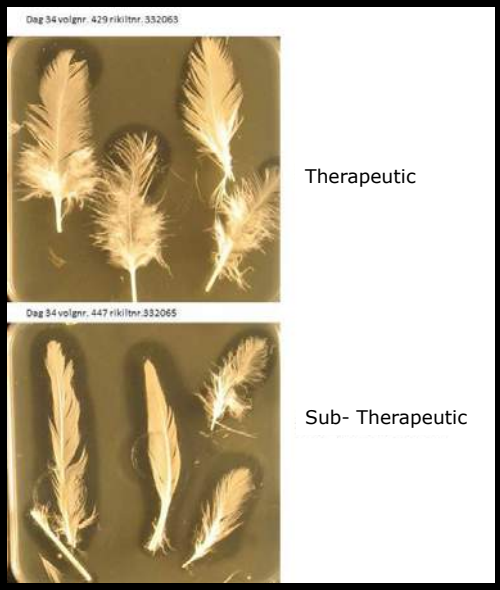


First detection of TTX in the Netherlands

nrc.nl

Tropisch gif dringt door tot de Oosterschelde

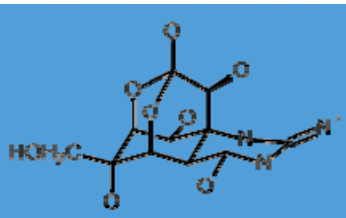
Schelpdieren met het gif tetrodotoxine zijn gevaarlijk voor mensen. Ineens zit het gif in de Oosterschelde en bedreigt het er de mosselen.

Hester van Santen
28 juni 2016

NOS Nieuws Sport Uitzendingen

Gif in deel mosselen Oosterschelde

TTX=tetrodotoxin found in dutch mussels (25-200 µg/kg)



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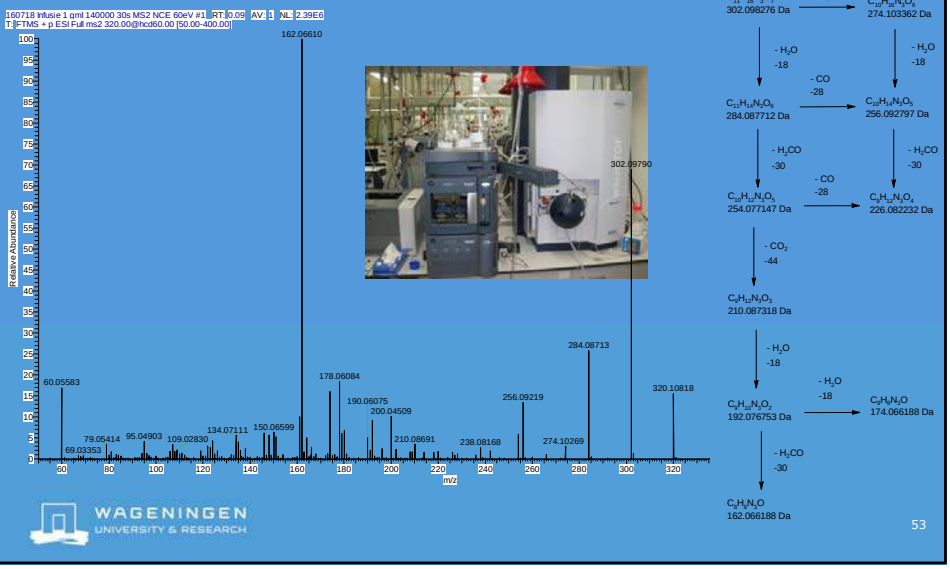
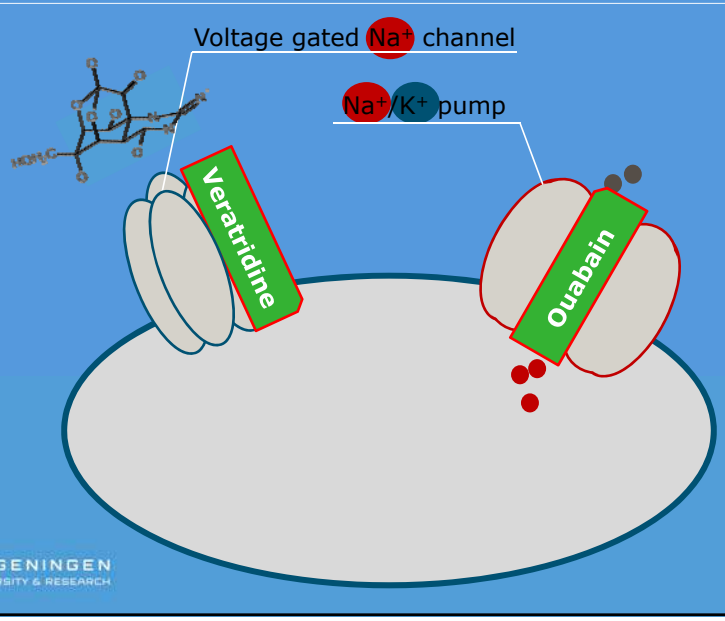
Use of *in vitro* bioassay and Mass Spectrometry

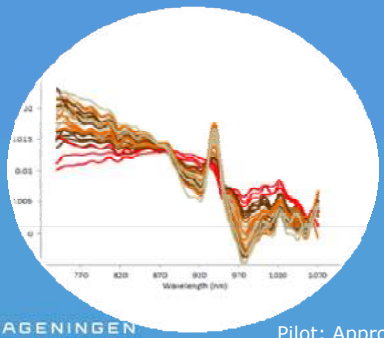
Structural elucidation (FWHM res 140.000)

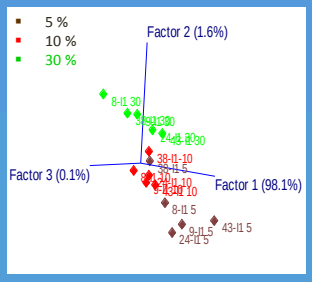


Activity based screening



Powders: Adulteration of ground nutmeg





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Pilot: Approximately 30% adulteration detectable

Conclusion and outlook

- Current monitoring programs for residues and contaminants are based on incidents and crises that occurred in the previous century and have resulted in a high level of consumer protection.
- Future developments will have to deal with the relationship that exists between food security and food safety at a global level.
- Fast and simple on-site techniques will have to supplement advanced in-lab techniques in order to fulfil future demands of consumers and (local) producers for a first quick assessment.
- Smartphone-based analyses potentially can make an important contribution.

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Questions

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Monitoring: Rapid Alert for Food and Feed (RASFF)

Hans Marvin [1]

As part of the General Food Law (178/2002/EC), the Rapid Alert System for Food and Feed (RASFF) was established. RASFF provides a knowledge and the technological platform for the EU-28 national food safety authorities, the European Commission, European Food Safety Authority, Norway, Liechtenstein, Iceland and Switzerland. The objective of RASFF is to provide food and feed control authorities with an effective tool to exchange information about measures taken responding to serious risks detected in relation to food or feed. Four types of notifications are reported in RASFF: 1) alerts (serious health risk, rapid action is required), 2) information (a risk identified, no rapid action needed by other members), 3) border rejection (consignments tested and rejected at the external borders of the EU), 4) news (any other relevant information). The European Commission is responsible for managing the system including among others a check on completeness, legislative requirement, scope and classification. Furthermore, the European Commission is responsible for the communication with non-EU countries when a product is a subject to a notification and the product has been exported or has been imported from that country.
All notifications are publically available and can be found in the RASFF portal:
(https://webgate.ec.europa.eu/rasff-window/portal/?event=SearchForm&cleanSearch=1).

Till date (May 2017), more than 47,000 notifications can be found in the RASFF portal. This data source provides a wealth of data that has been used in many scientific studies.

Suggestions for further reading:

[1] The Rapid Alert System for Food and Feed, 2015 annual report. European Commission (https://ec.europa.eu/food/sites/food/files/safety/docs/rasff_annual_report_2015.pdf)

[2] Kleter, G.A., Prandini, A., Filippi, L., and Marvin, H.J.P.(2009) Identification of potentially emerging food safety issues by analysis of reports published by the EU Rapid Alert System for Food and Feed (RASFF) during a four-year period. Food Chem. Toxicol. 47, 932-950. DOI:10.1016/j.fct.2007.12.022

[3] Petróczi, A., Taylor, G., Nepusz, T., Naughton D.P (2010) Gate keepers of EU food safety: Four states lead on notification patterns and effectiveness. Food and Chem. Toxicol.48, 1957–1964. Doi.org/10.1016/j.fct.2010.04.043

[1] E-mail; hans.marvin@wur.nl; Wageningen University & Research, RIKILT, Bu Toxicology Novel Foods and Agrochains, Wageningen, The Netherlands

Monitoring: Rapid Alert for Food and Feed (RASFF)

Hans Marvin (RIKILT)

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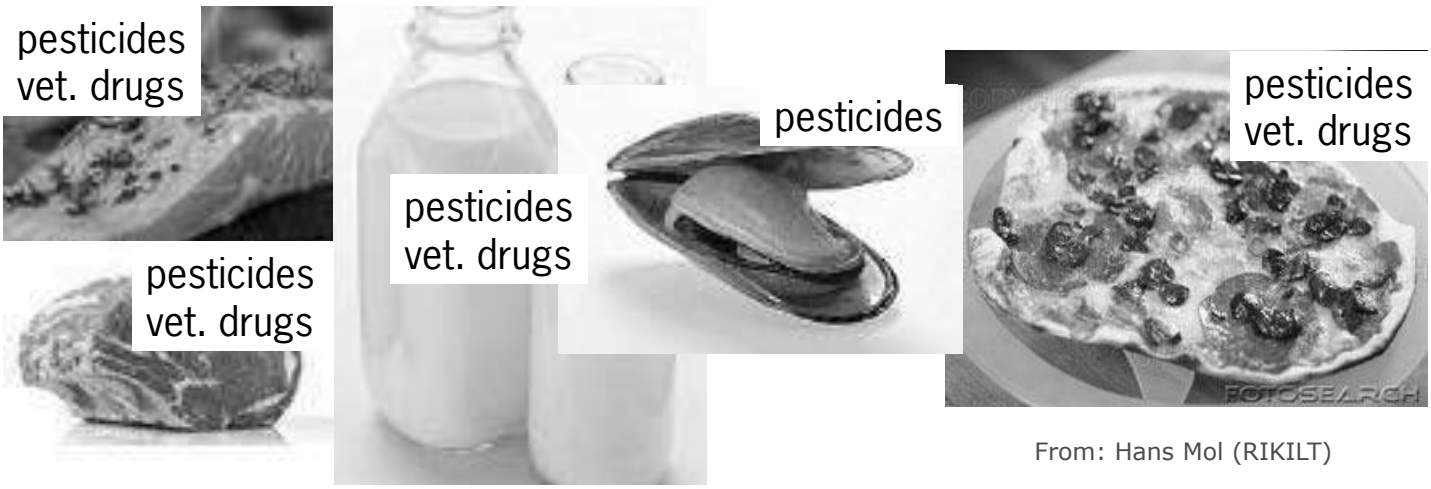
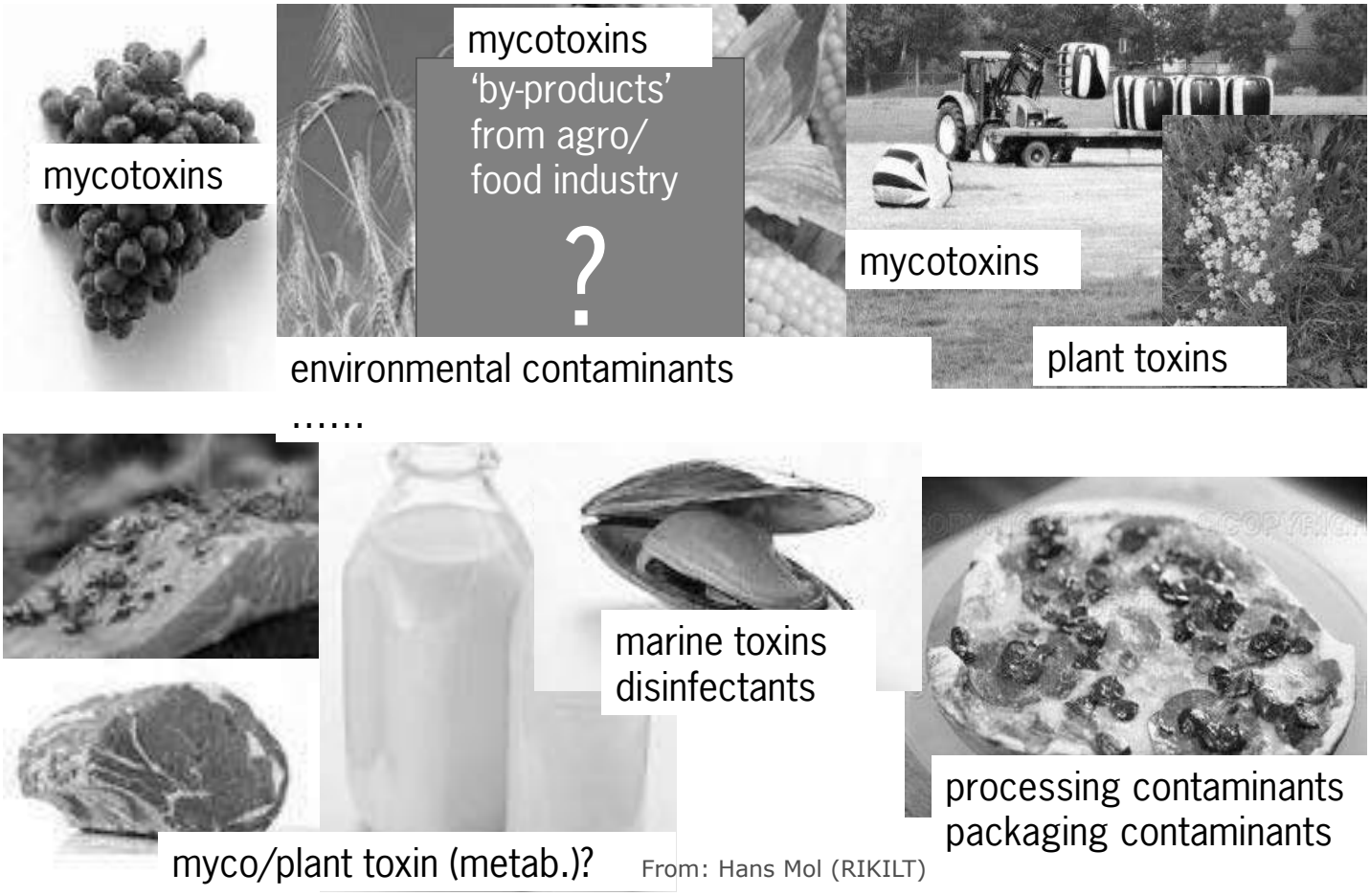
Foodsmartphone PhD course, Wageningen 26-06-2017

Food may be contaminated with residues…



From: Hans Mol (RIKILT)

and contaminants…



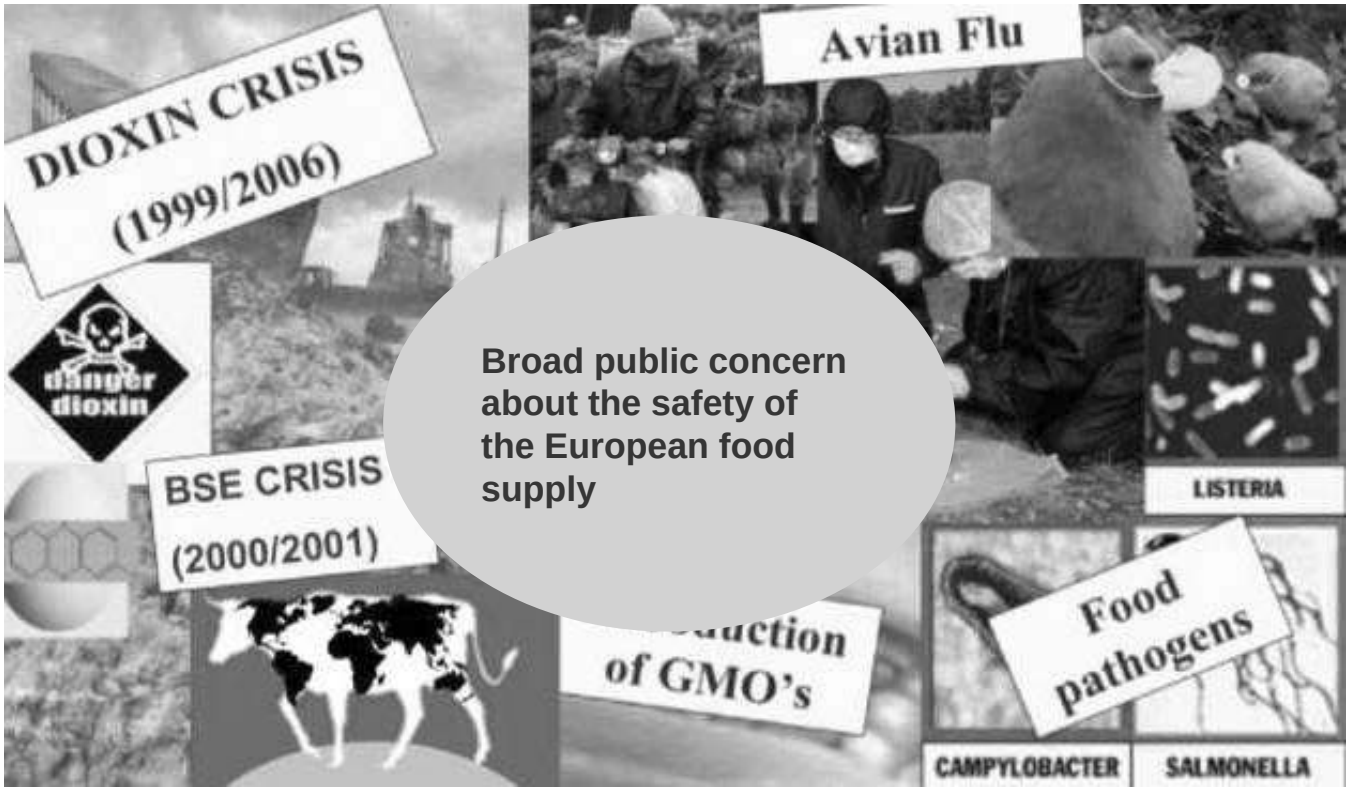
From: Hans Mol (RIKILT)

Potential large number of hazards……

<u>Residues</u>	
Pesticides	> 1000
Veterinary drugs	> 300
<u>Environmental contaminants</u>	> 1000
dioxins, PCB, PAH, flame retardants, perfluorinated compounds, biocides, anti-fouling, endocrine disruptors, heavy metals	
<u>Natural contaminants</u>	
Mycotoxins	> 500
Fytotoxins	> 500
Fycotoxins	> 100
<u>Processing contaminants & aldulterants</u>	>> 100
acryl amide, heterocyclic amines, furan, 3-MCPD, decontamination agents, solvent residues, packaging contaminants (badge, ESBO, SEM, phthalates, Sn) marker substances (spoilage, irradiation), dyes, melamine	

From: Hans Mol (RIKILT)

Food Safety incidents



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Low public trust in:

- Food safety
- The way food crises were handled
- The regulatory system in Europe

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European Commission reacted

- EU White Paper on Food Safety

Strategic priorities:

- Establishment of the European Food Safety Authority (EFSA) in 2002
- "Farm to Fork" approach in EU legislation
- Defining responsibilities for food safety

- General Food Law (Regulation 178/2002)

- Rapid Alert System for Food and Feed (RASFF)

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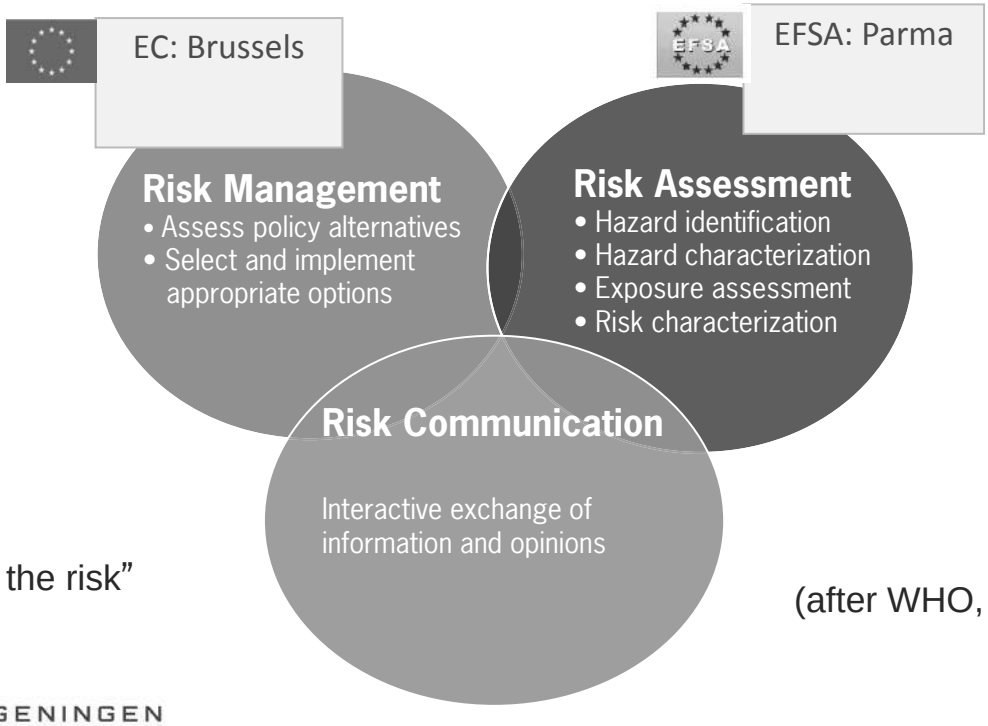
Risk Analysis Framework for food safety in EU



"To minimize the risk"

(after WHO, 1998)

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RASFF: Rapid Alert System for Food and Feed

- Centralized system
  - Required by General Food Law 178/2002/EC
  - Managed by European Commission
  - Members: EU-28 national food safety authorities, Commission, EFSA, Norway, Liechtenstein, Iceland and Switzerland

- Objective: to provide food and feed control authorities with an effective tool to exchange information about measures taken responding to serious risks detected in relation to food or feed.

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Types of notifications in RASFF

- **Alerts** (serious health risk, rapid action is required)

- **Information** (a risk identified, no rapid action needed by other members)

- **Border rejection** (consignments tested and rejected at the external borders of the EU)

- **News** (any other relevant information)

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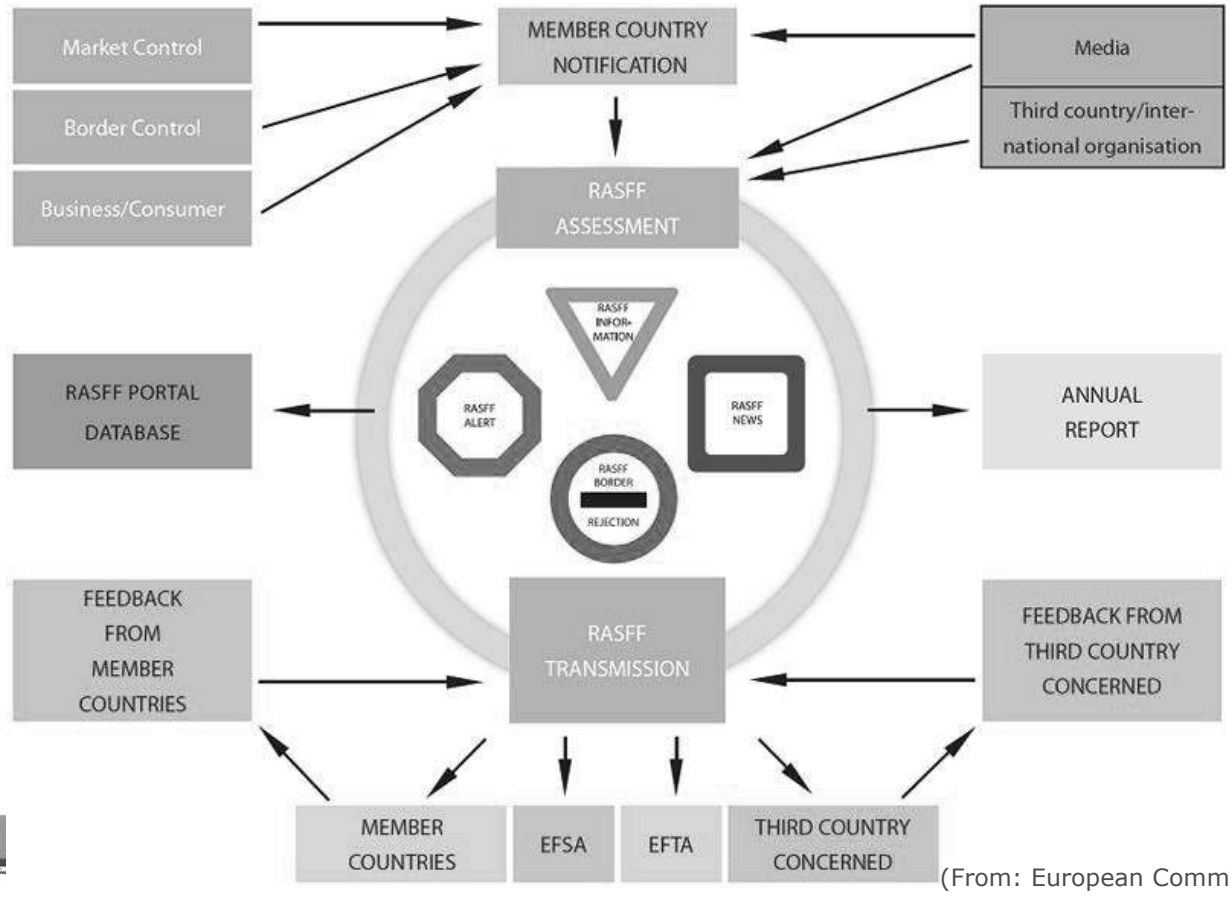
Role of European Commission

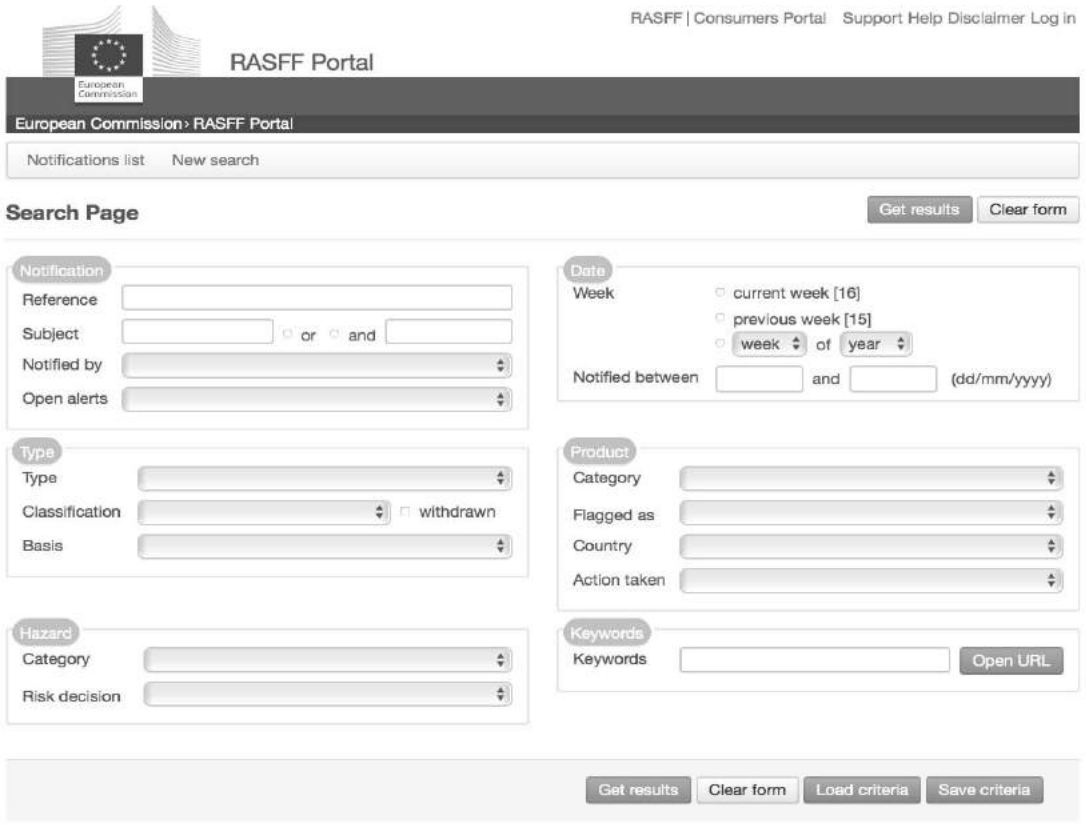
- Managing of the RASFF system
- Providing knowledge and the technological platform of RASFF
- Performs checks on each notification, prior to making them available to all members of the network.
- The following is checked: i) completeness check, ii) legislative requirements, iii) verification if the notification falls within the scope of the RASFF, iv) translation into English, v) classification of the notification, vi) members of the network flagged for action, vii) recurrences of similar problems relating to the same professional operator and/or hazard/country of origin.

RASFF how does it work?

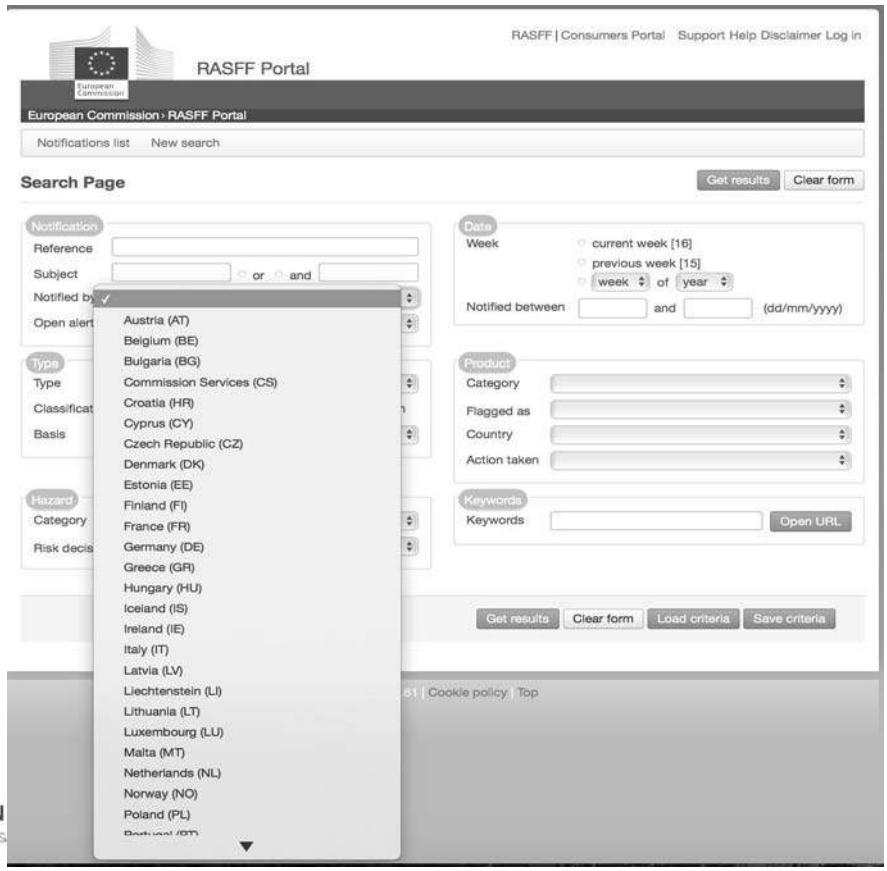


(From: European Commission)

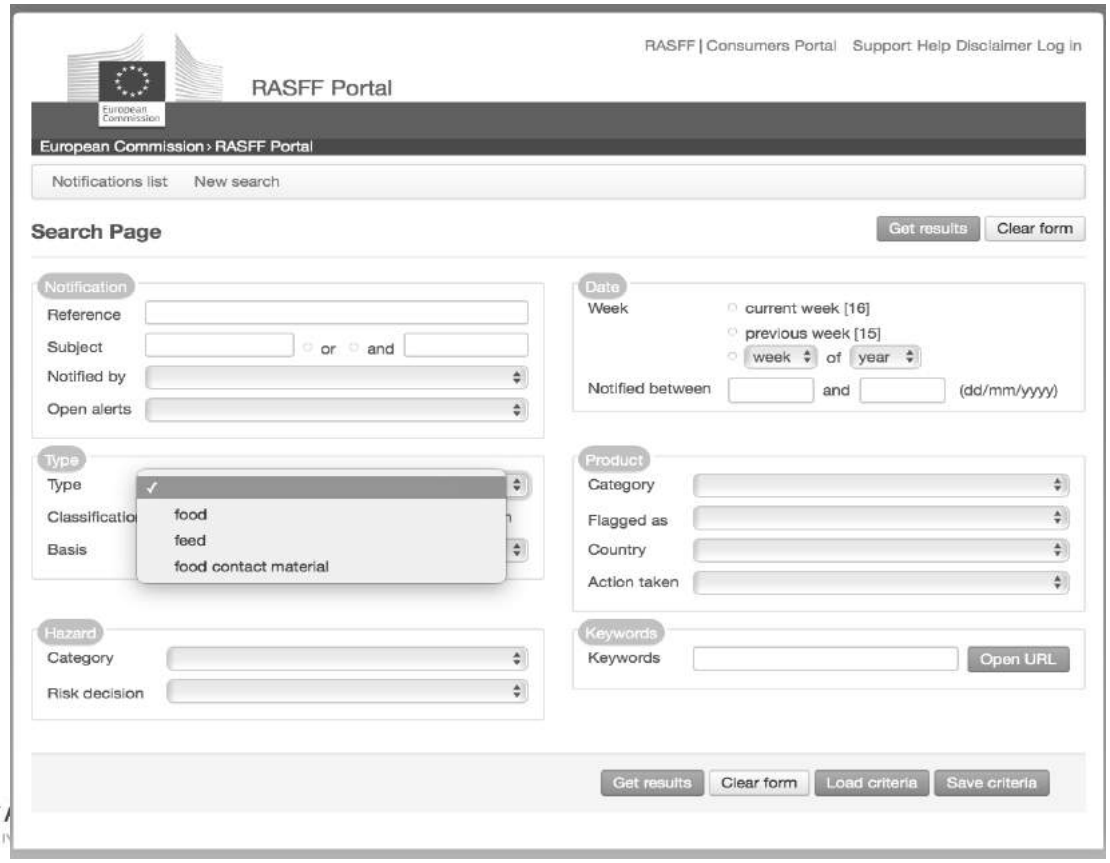
RASFF Portal



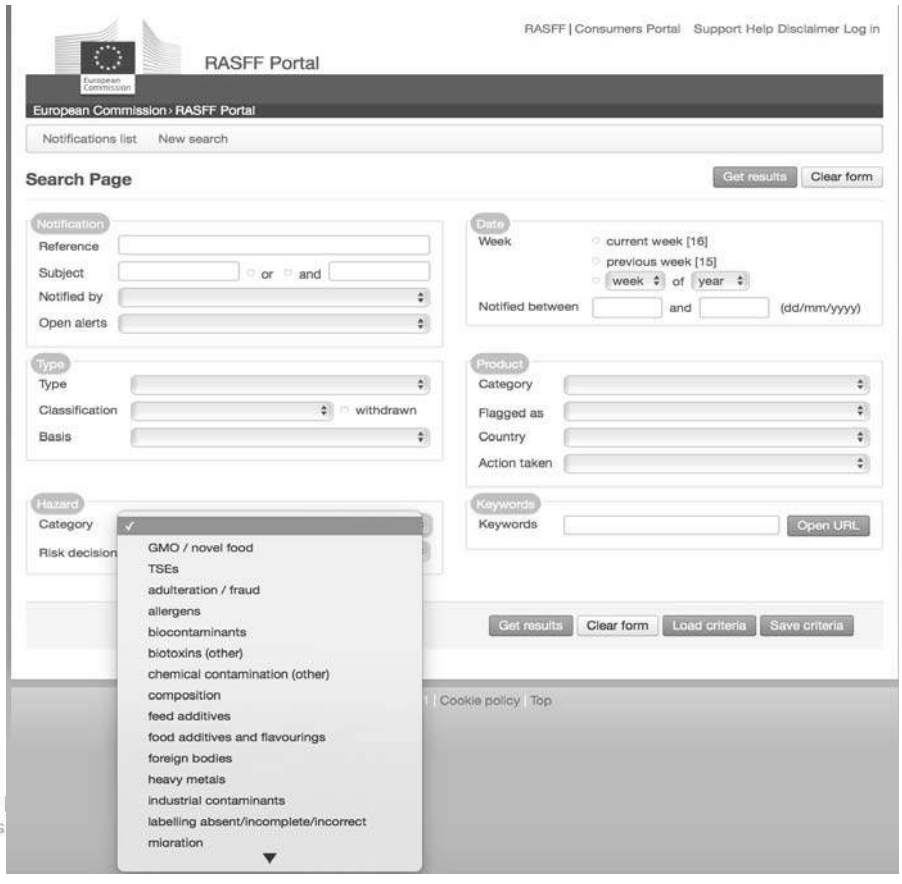
RASFF portal



RASFF portal



RASFF portal



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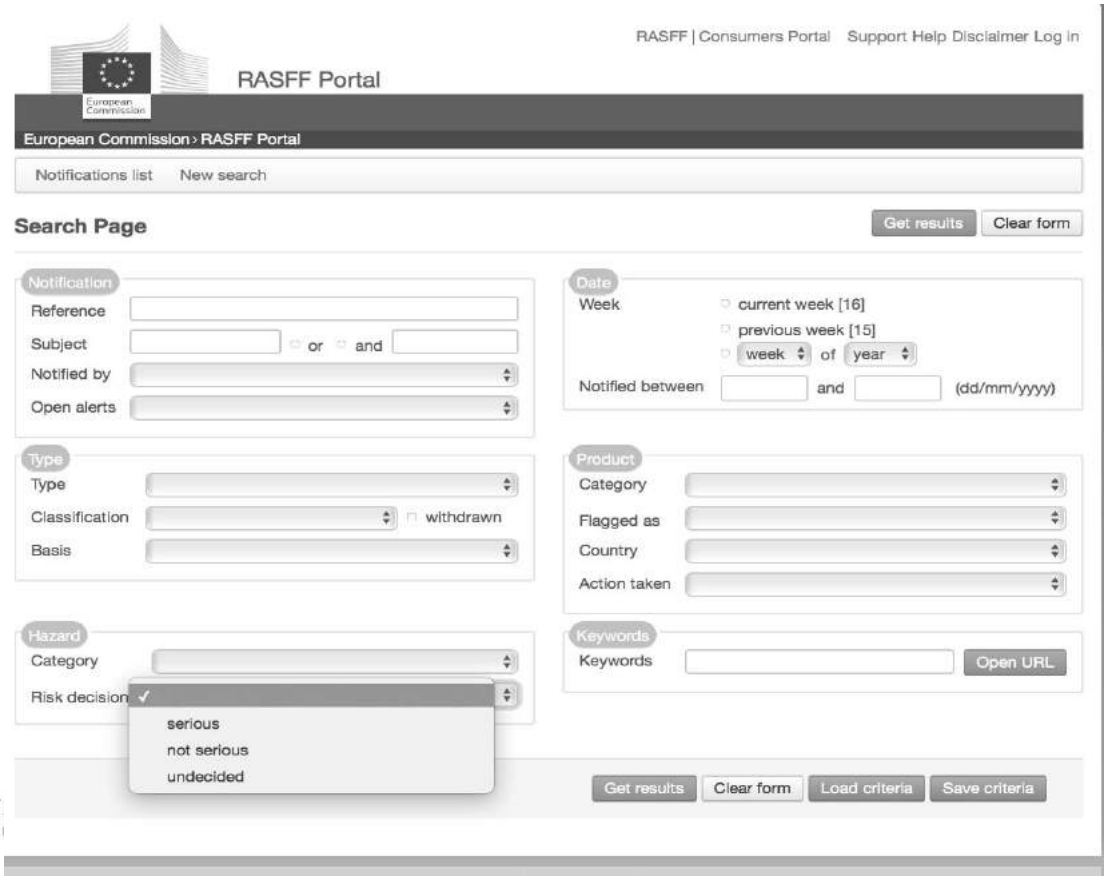

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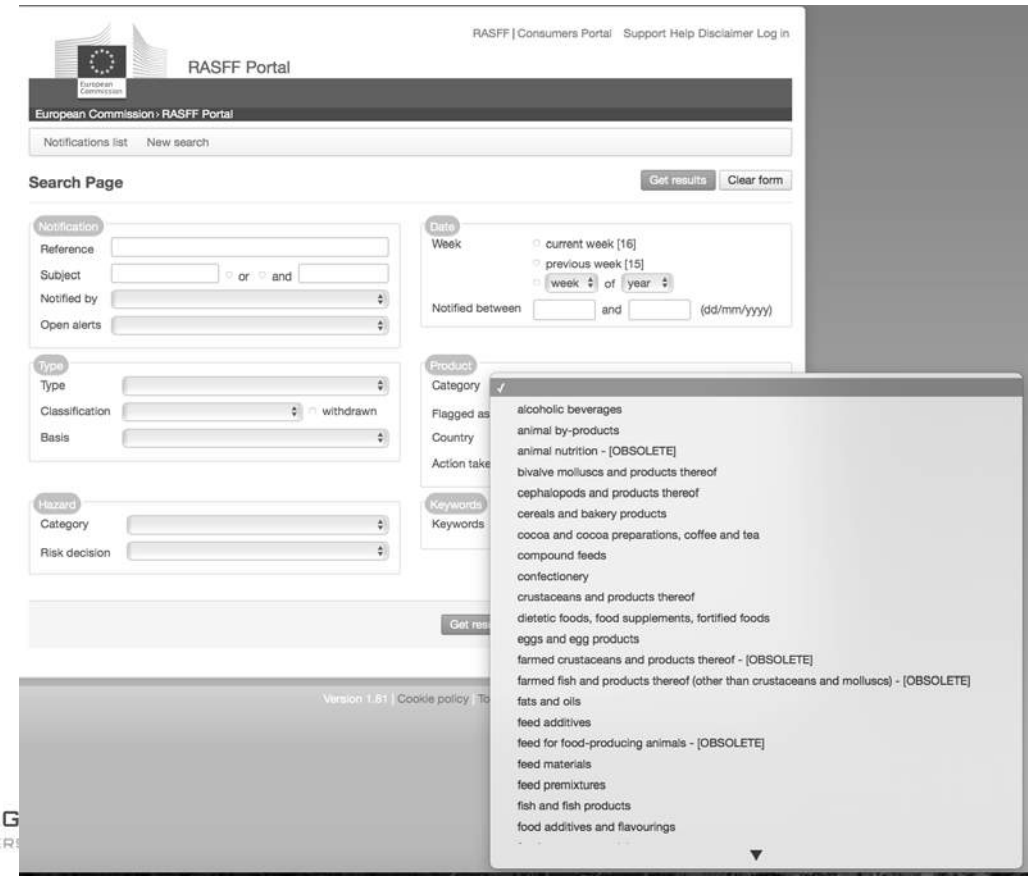
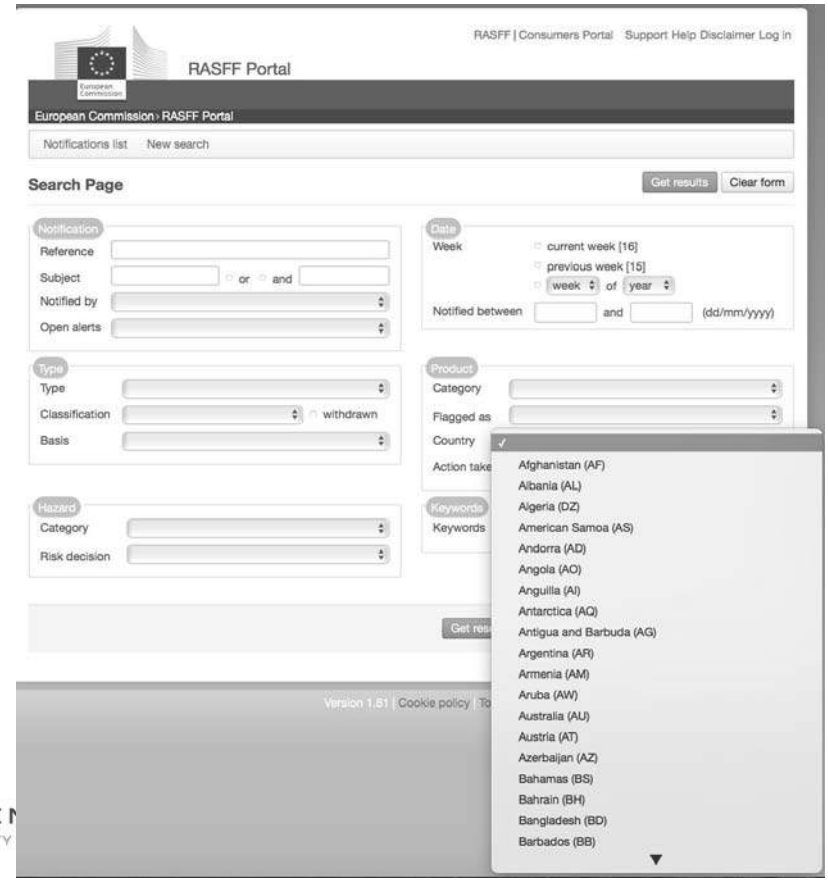
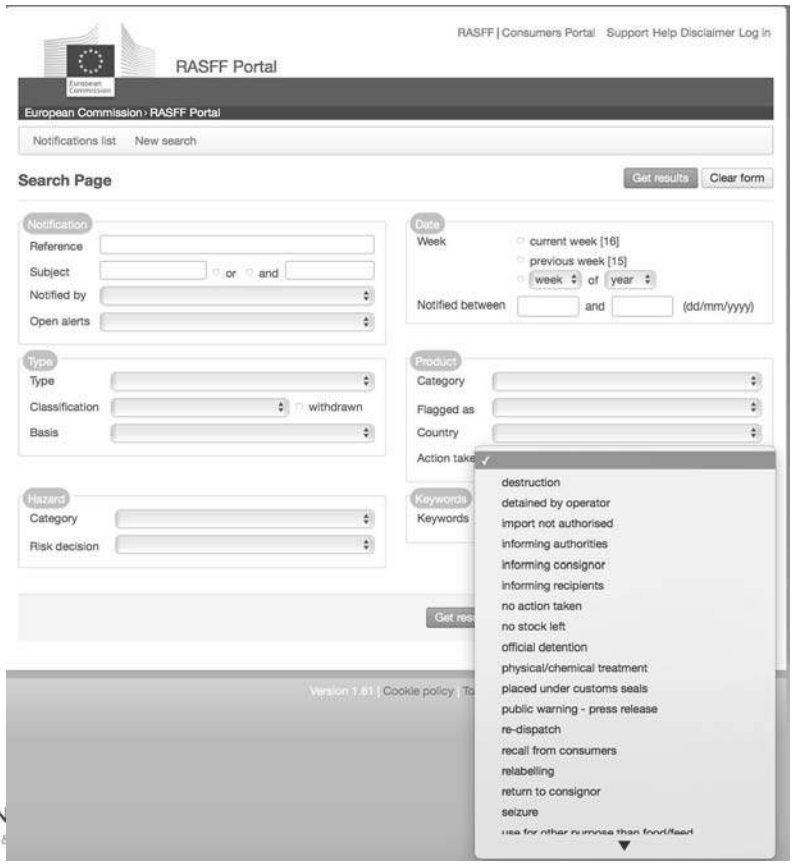


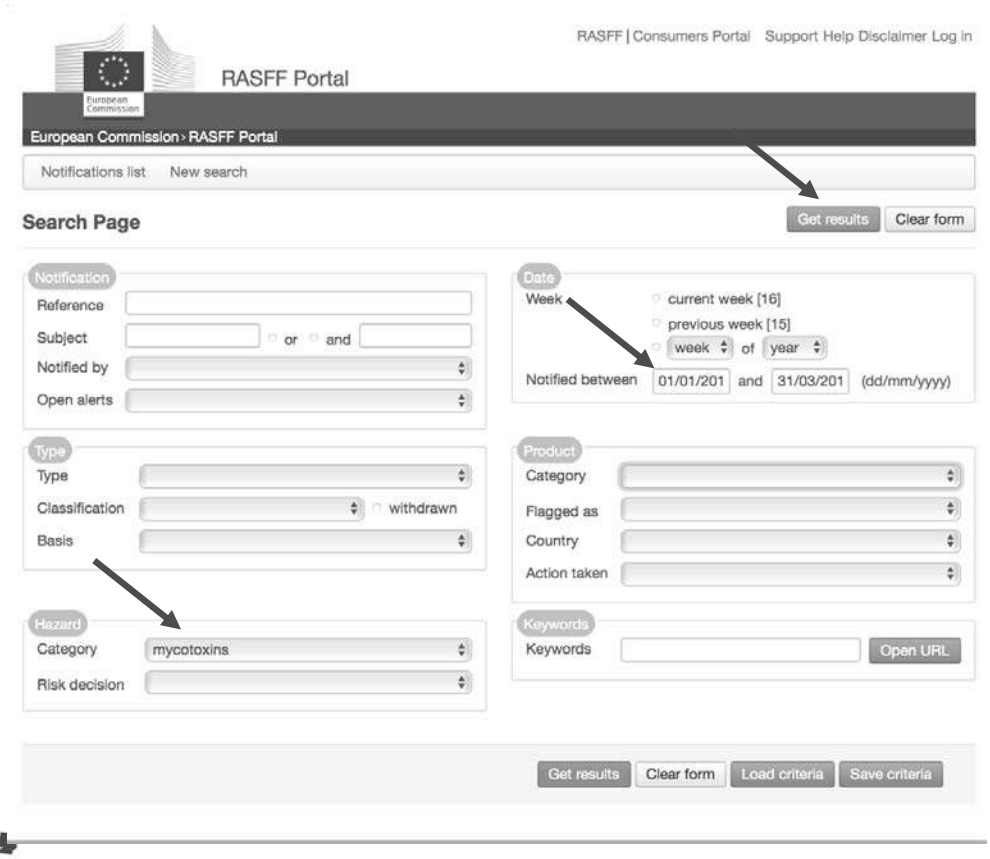
RASFF portal



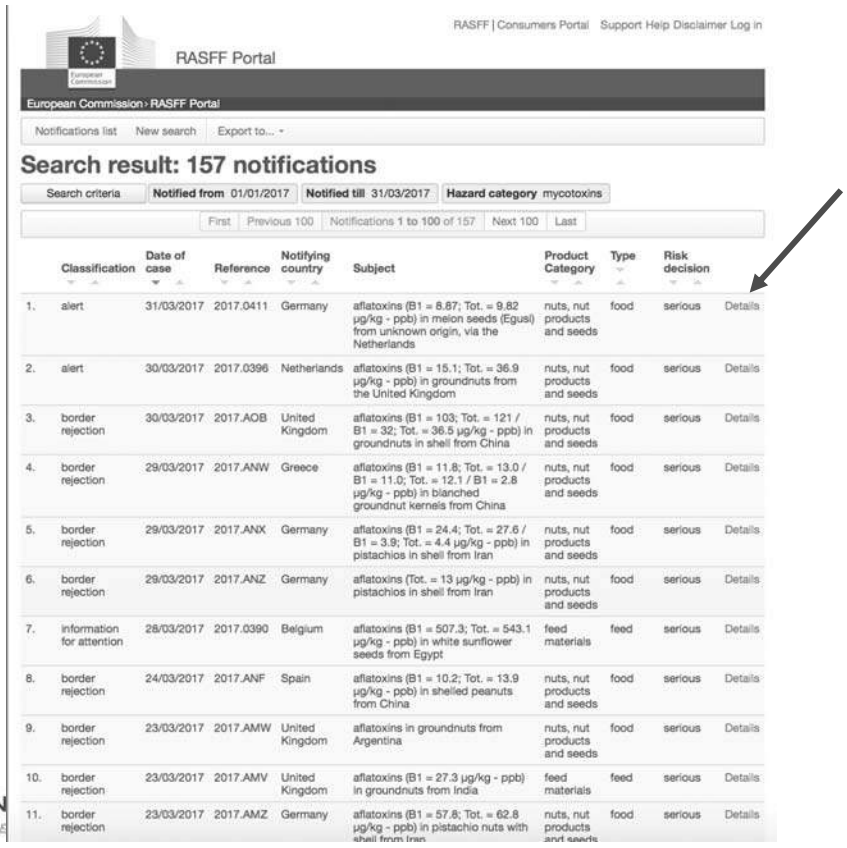
RASFF portal



RASFF portal

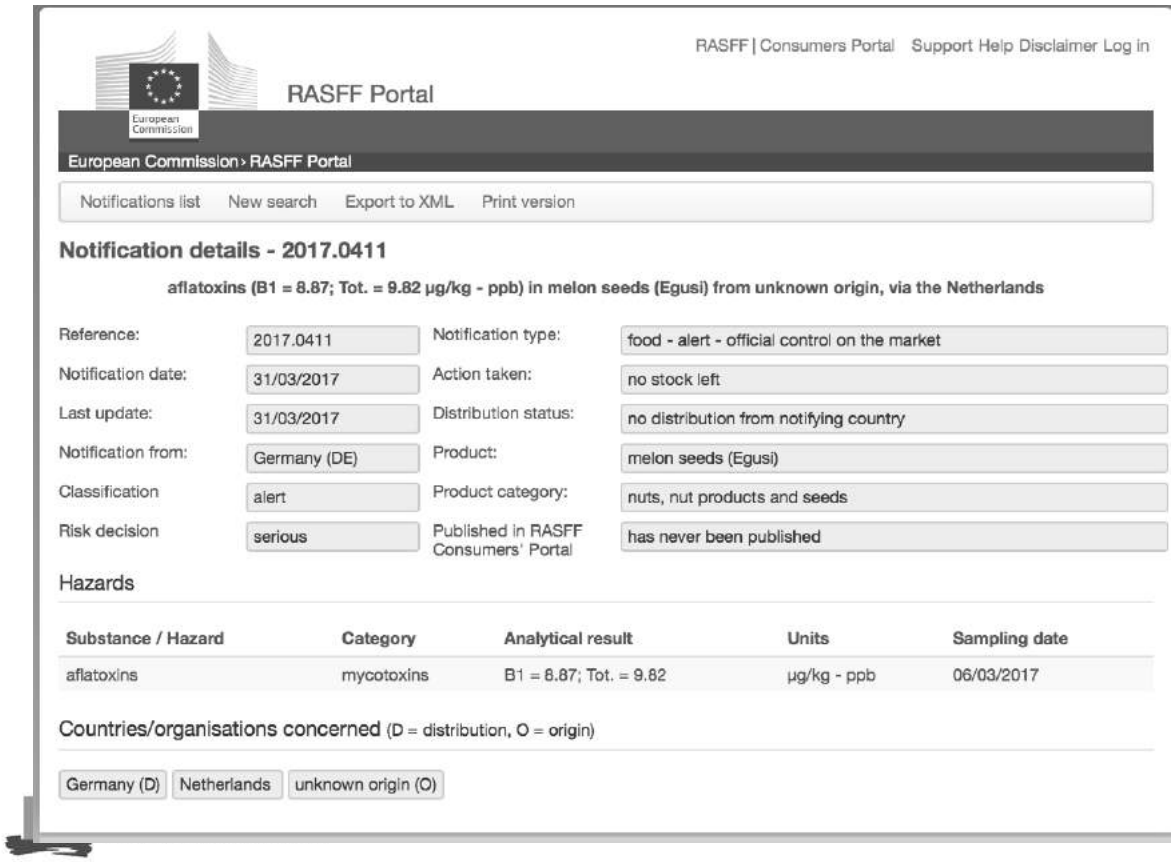


RASFF portal



	Classification	Date of case	Reference	Notifying country	Subject	Product Category	Type	Risk decision	
1.	alert	31/03/2017	2017.0411	Germany	aflatoxins (B1 = 8.87; Tot. = 9.82 µg/kg - ppb) in melon seeds (Egusi) from unknown origin, via the Netherlands	nuts, nut products and seeds	food	serious	Details
2.	alert	30/03/2017	2017.0396	Netherlands	aflatoxins (B1 = 15.1; Tot. = 36.9 µg/kg - ppb) in groundnuts from the United Kingdom	nuts, nut products and seeds	food	serious	Details
3.	border rejection	30/03/2017	2017.AOB	United Kingdom	aflatoxins (B1 = 103; Tot. = 121 / B1 = 32; Tot. = 36.5 µg/kg - ppb) in groundnuts in shell from China	nuts, nut products and seeds	food	serious	Details
4.	border rejection	29/03/2017	2017.ANW	Greece	aflatoxins (B1 = 11.8; Tot. = 13.0 / B1 = 11.0; Tot. = 12.1 / B1 = 2.8 µg/kg - ppb) in blanched groundnut kernels from China	nuts, nut products and seeds	food	serious	Details
5.	border rejection	29/03/2017	2017.ANX	Germany	aflatoxins (B1 = 24.4; Tot. = 27.6 / B1 = 3.9; Tot. = 4.4 µg/kg - ppb) in pistachios in shell from Iran	nuts, nut products and seeds	food	serious	Details
6.	border rejection	29/03/2017	2017.ANZ	Germany	aflatoxins (Tot. = 13 µg/kg - ppb) in pistachios in shell from Iran	nuts, nut products and seeds	food	serious	Details
7.	information for attention	28/03/2017	2017.0390	Belgium	aflatoxins (B1 = 507.3; Tot. = 543.1 µg/kg - ppb) in white sunflower seeds from Egypt	feed materials	feed	serious	Details
8.	border rejection	24/03/2017	2017.ANF	Spain	aflatoxins (B1 = 10.2; Tot. = 13.9 µg/kg - ppb) in shelled peanuts from China	nuts, nut products and seeds	food	serious	Details
9.	border rejection	23/03/2017	2017.AMW	United Kingdom	aflatoxins in groundnuts from Argentina	nuts, nut products and seeds	food	serious	Details
10.	border rejection	23/03/2017	2017.AMV	United Kingdom	aflatoxins (B1 = 27.3 µg/kg - ppb) in groundnuts from India	feed materials	feed	serious	Details
11.	border rejection	23/03/2017	2017.AMZ	Germany	aflatoxins (B1 = 57.8; Tot. = 62.8 µg/kg - ppb) in pistachio nuts with shell from Iran	nuts, nut products and seeds	food	serious	Details

RASFF portal



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RASFF Portal

European Commission › RASFF Portal

Notifications list New search Export to XML Print version

Notification details - 2017.0411

aflatoxins (B1 = 8.87; Tot. = 9.82 µg/kg - ppb) in melon seeds (Egusi) from unknown origin, via the Netherlands

Reference:	2017.0411	Notification type:	food - alert - official control on the market
Notification date:	31/03/2017	Action taken:	no stock left
Last update:	31/03/2017	Distribution status:	no distribution from notifying country
Notification from:	Germany (DE)	Product:	melon seeds (Egusi)
Classification	alert	Product category:	nuts, nut products and seeds
Risk decision	serious	Published in RASFF Consumers' Portal	has never been published

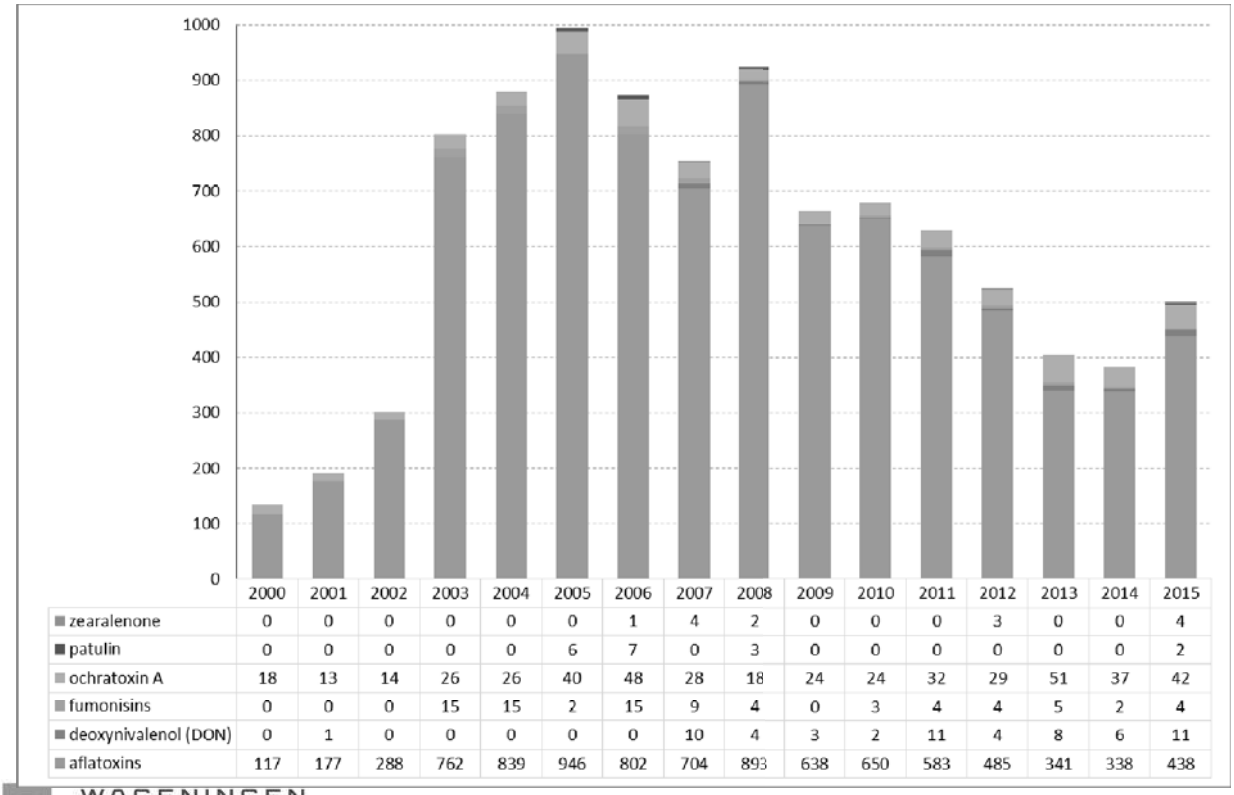
Hazards

Substance / Hazard	Category	Analytical result	Units	Sampling date
aflatoxins	mycotoxins	B1 = 8.87; Tot. = 9.82	µg/kg - ppb	06/03/2017

Countries/organisations concerned (D = distribution, O = origin)

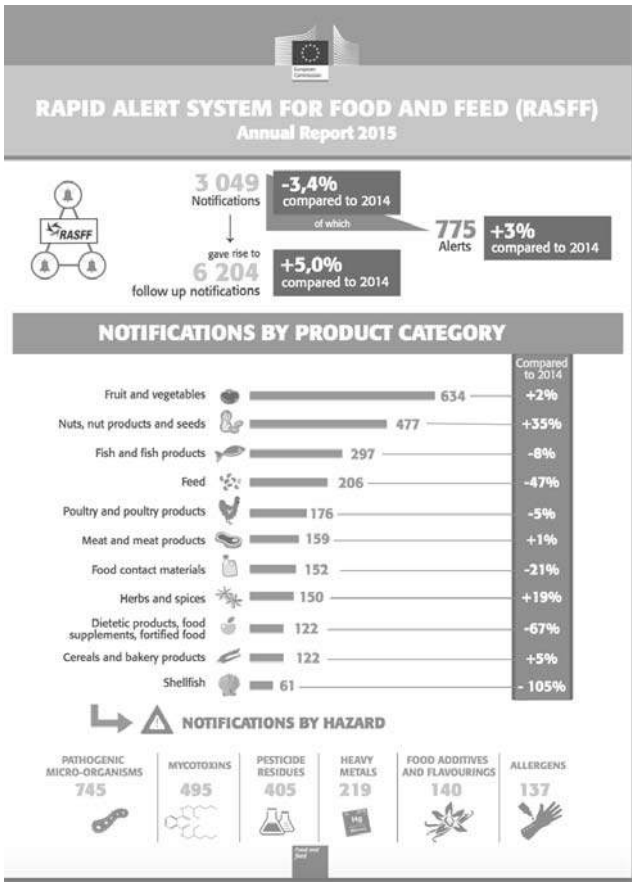
Germany (D) Netherlands unknown origin (O)

Reports on mycotoxins in RASFF



	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
zearalenone	0	0	0	0	0	0	1	4	2	0	0	0	3	0	0	4
patulin	0	0	0	0	0	6	7	0	3	0	0	0	0	0	0	2
ochratoxin A	18	13	14	26	26	40	48	28	18	24	24	32	29	51	37	42
fumonisins	0	0	0	15	15	2	15	9	4	0	3	4	4	5	2	4
deoxynivalenol (DON)	0	1	0	0	0	0	0	10	4	3	2	11	4	8	6	11
aflatoxins	117	177	288	762	839	946	802	704	893	638	650	583	485	341	338	438

EC publish annually



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Many scientific studies (2 examples)

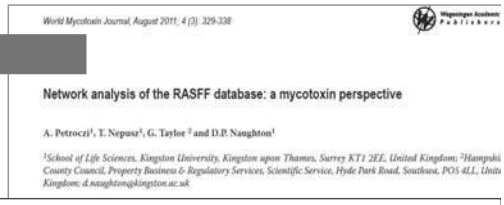

Food and Chemical Toxicology
Volume 47, Issue 5, May 2009, Pages 932–950
ELSEVIER

Identification of potentially emerging food safety issues by analysis of reports published
Rapid Alert System for Food
year period

G.A. Kleter, A. Prandini, L. Filippi, H.J.P
Show more

http://doi.org/10.1016/j.fct.2007.12.022

Abstract
The SAFE FOODS project undertakes to
identification of emerging food safety haz
notifications filed through RASFF, the Eur
Food and Feed, to identify emerging trend
alert notifications published in the four-ye
assigned to categories of products and ha


World Mycotoxin Journal, August 2011; 4 (3): 329-338

Network analysis of the RASFF database: a mycotoxin perspective

A. Petroczi[1], T. Nepusz[1], G. Taylor [2] and D.P. Naughton[1]

[1]School of Life Sciences, Kingston University, Kingston upon Thames, Surrey KT1 2EE, United Kingdom; [2]Hampshi
County Council, Property Business & Regulatory Services, Scientific Service, Hyde Park Road, Southsea, PO5 4LL, Unit
Kingdom; d.naughton@kingston.ac.uk


Food Control 79 (2017) 143–149
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journal homepage: www.elsevier.com/locate/foodcont
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Analysis of foreign bodies present in European food using data from Rapid Alert System for Food and Feed (RASFF)

Ilija Djekic [a, *], Danijela Jankovic [a], Andreja Rajkovic [a, b]

[a] Department of Food Safety and Quality Management, University of Belgrade – Faculty of Agriculture, Belgrade, Serbia
[b] Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Belgium

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Foreign bodies

ABSTRACT

This paper contains a comprehensive review of different types of foreign matter reported in Rapid Alert System for Food and Feed (RASFF) during the period 1998–2015. It provides information on 1446 incidents of foreign matter contamination discussed and mined in terms of types of foreign bodies, food products involved and geographic distribution within indicated European regions.

Regional distribution shows that the scattering of number of notifications is rather similar between regions, with the most notifications coming from Eastern Europe. The top three foreign bodies are pests, glass and metals. Main food categories in which foreign bodies occur are fruits and vegetables, nuts, nut products, confectionery and bakery products.

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Kleter *et al*: RASFF trend analysis

- **Research study:**
  - Can data be used for trend analysis
    - Early recognition of trends possible?
  - Links between hazards and other parameters?
    - Certain regions of origin?
    - Certain products ?
  - Four-years data, 11,403 records (next slides)
    - Alert 29%, information 71%

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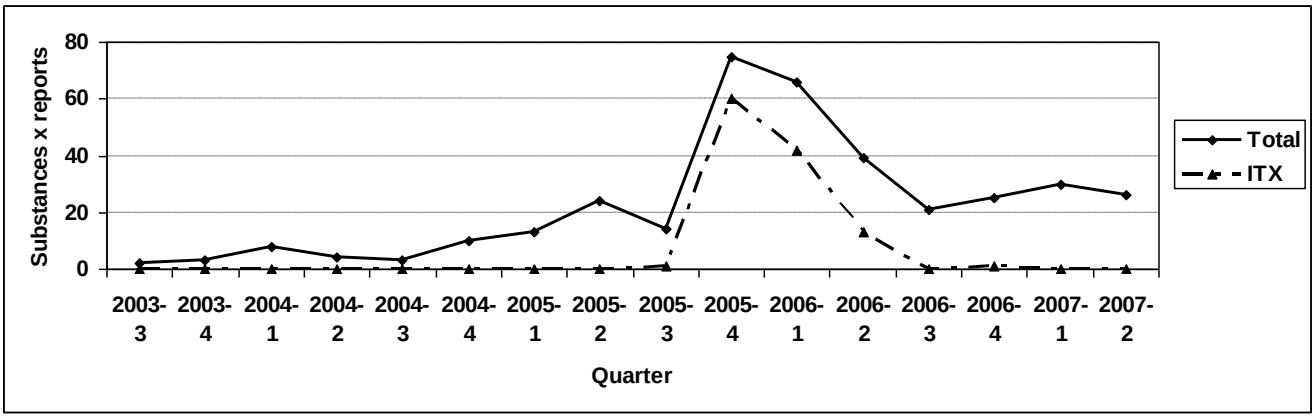
Kleter *et al.:* RASFF trend analysis



- Increasing trend and temporary high in reports on food contact substances
  - Cutlery and other kitchenware, particularly from China
  - ITX reports (packaging of juices etc., EU)

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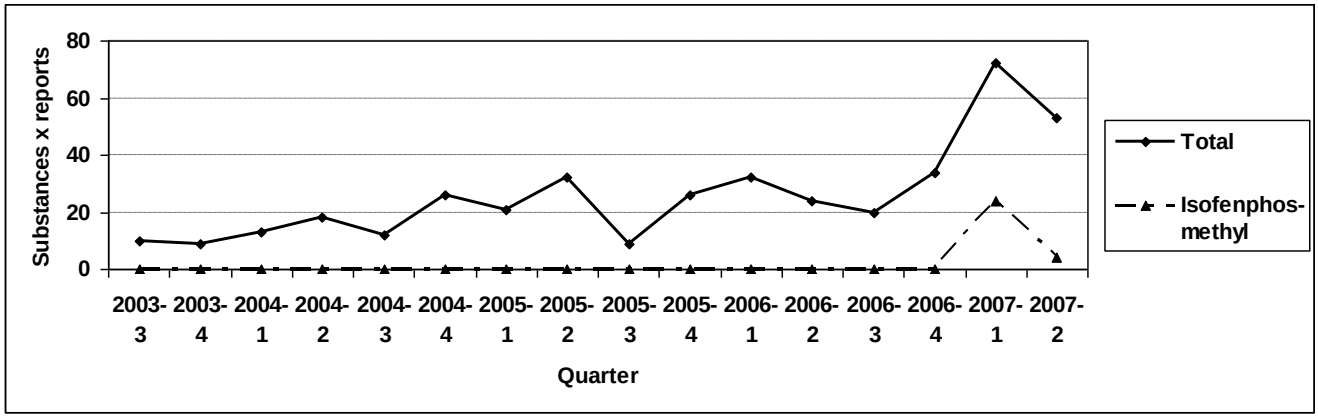
Kleter *et al.*: RASFF trend analysis



- Temporary high in occurrence of reports on pesticide residues
  - Mainly unauthorized OP-pesticide isophenfos-methyl on bell peppers from Spain

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Many scientific studies (2 examples)

Food and Chemical Toxicology

Volume 47, Issue 5, May 2009, Pages 932–950

Identification of potentially emerging food safety issues by analysis of reports published
Rapid Alert System for Food
year period

G.A. Kleter, A. Prandini, L. Filippi, H.J.P

Show more

http://doi.org/10.1016/j.fct.2007.12.022

Abstract

The SAFE FOODS project undertakes to identification of emerging food safety haz notifications filed through RASFF, the Eur Food and Feed, to identify emerging trend alert notifications published in the four-ye assigned to categories of products and ha

database: a mycotoxin perspective

D.P. Naughton

Food Control 79 (2017) 143–149

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Analysis of foreign bodies present in European food using data from Rapid Alert System for Food and Feed (RASFF)

Ilija Djekic [a, *], Danijela Jankovic [a], Andreja Rajkovic [a, b]

[a] Department of Food Safety and Quality Management, University of Belgrade – Faculty of Agriculture, Belgrade, Serbia
[b] Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Belgium

ARTICLE INFO

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ABSTRACT

This paper contains a comprehensive review of different types of foreign matter reported in Rapid Alert System for Food and Feed (RASFF) during the period 1998–2015. It provides information on 1446 incidents of foreign matter contamination discussed and mined in terms of types of foreign bodies, food products involved and geographic distribution within indicated European regions.

Regional distribution shows that the scattering of number of notifications is rather similar between regions, with the most notifications coming from Eastern Europe. The top three foreign bodies are pests, glass and metals. Main food categories in which foreign bodies occur are fruits and vegetables, nuts, nut products, confectionery and bakery products.

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From Djekic *et al.* (2017)

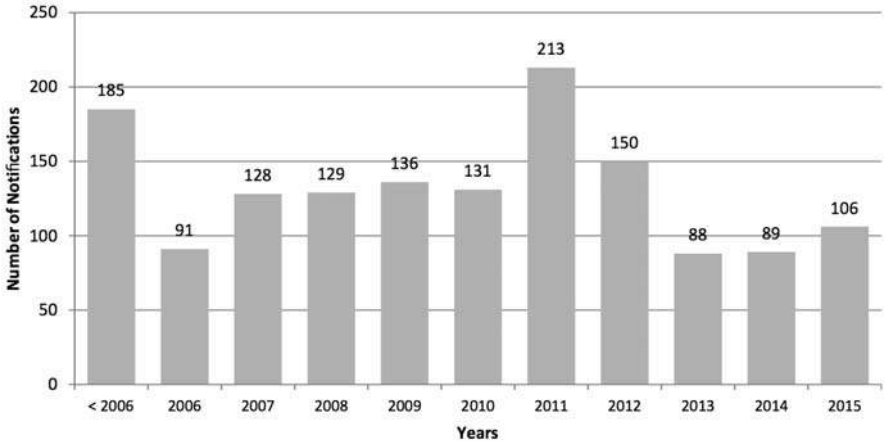

Fig. 1. Foreign body notifications in the RASFF database (1998–2015).

Conclusion: Most notifications coming from Eastern Europe. The top three foreign bodies are pests, glass and metals. Main food categories in which foreign bodies occur are fruits and vegetables, nuts, nut products, confectionery and bakery products.

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Reporting countries (2000-2009)

Food and Chemical Toxicology 48 (2010) 1957–1964

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Gate keepers of EU food safety: Four states lead on notification patterns and effectiveness

A. Petróczi [a,*], G. Taylor [b], T. Nepusz [a], D.P. Naughton [a]

[a] School of Life Sciences, Kingston University, London KT1 2EE, UK
[b] Hampshire County Council, Property Business & Regulatory Services Scientific Service Hyde Park Road, Southsea PO5 4LL, UK

ARTICLE INFO

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Received 22 January 2010
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Market control
Trend analysis
Detector

ABSTRACT

The EU RASFF database has been used to provide information on trend
focus of previous reports has been on either the health hazards arising
in foodstuff or producers of these faulty products. To complement these
to examine the food notifications, recorded via the RASFF between 20
point of view and to compare and contrast detecting activities of those p
safety and security in the EU. Data were scrutinized using network anal
in the EU context and detailed descriptive statistics to generate an in-
that 60% of the notifications were made by Italy, Germany, the UK an
shared among 26 other countries and Commission Services. A distinct
between these key countries with the Netherlands showing vigilance
based on its population, suggesting that countries with major ports
own country as well as guarding the EU's food market. The ethnic co
detection patterns.

© 2010

Gate keepers: Italy (20%), Germany (18%), UK (11%), Spain (10%), Netherlands (7%)

13 countries with <1%

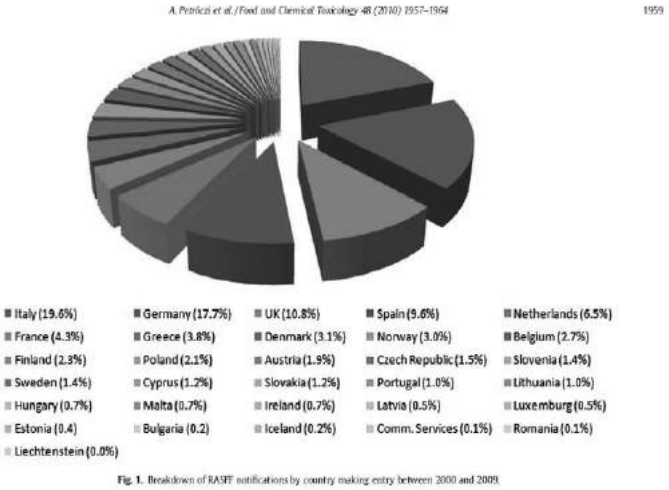

Fig. 1. Breakdown of RASFF notifications by country making entry between 2000 and 2009.

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New approach to study the data in RASFF

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Prediction of food fraud type using data from Rapid Alert System for Food and Feed (RASFF) and Bayesian network modelling

Yamine Bouzembrak, Hans J.P. Marvin*

ARTICLE INFO

ABSTRACT

Because food fraud can harm human health and erode consumer trust, it is imperative that it is detected at an early stage. Therefore the aim of this study was to predict the expected food fraud type for imported products for which the product category and country of origin are known in order to target enforcement activities. For this purpose we used a Bayesian Network (BN) model that was developed based on adulteration/fraud notifications as reported in the Rapid Alert System for Food and Feed (RASFF) in the period 2000–2013. In this period 749 food fraud notifications were reported and were categorised in 6 different fraud types (i) improper, fraudulent, missing or absent health certificates, (ii) illegal importation, (iii) tampering, (iv) improper, expired, fraudulent or missing common entry documents or import declarations, (v) expiration date, (vi) mislabelling. The data were then used to develop a BN model. The constructed BN model was validated using 88 food fraud notifications reported in RASFF in 2014. The proposed model predicted 80% of food fraud types correctly when food fraud type, country and food category had been reported previously in RASFF. The model predicted 52% of all 88 food fraud types

Bayesian Network model for food fraud

⇒ Statistical relationship between all parameters

⇒ High prediction performance (>90%)

Bayesian Network model (all parameters in RASFF; period 2000-2014; to be published)

Strong links between :

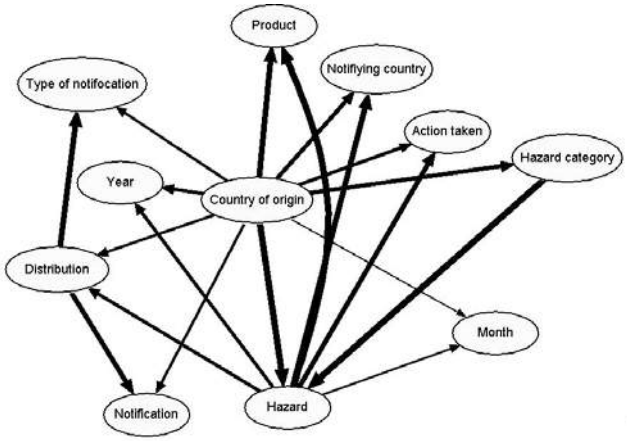
⇒ Hazard and product (expected)

⇒ Distribution and type of notification (expected)

⇒ Hazard category and hazard (expected)

⇒ Notifying country and hazard

⇒ Country of origin and hazard



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Conclusions

- RASFF is a useful platform to exchange information on measures taken by authorities in relation to risks detected food or feed
- The RASFF database contains a huge amount of data (> 47,000 records) which is useful for trend analysis.
- There are big differences between countries in regard to the frequency of reporting in RASFF
- Bayesian Network modelling is a useful tool to understand the relationships between the parameters reported in RASFF and are useful in prediction models

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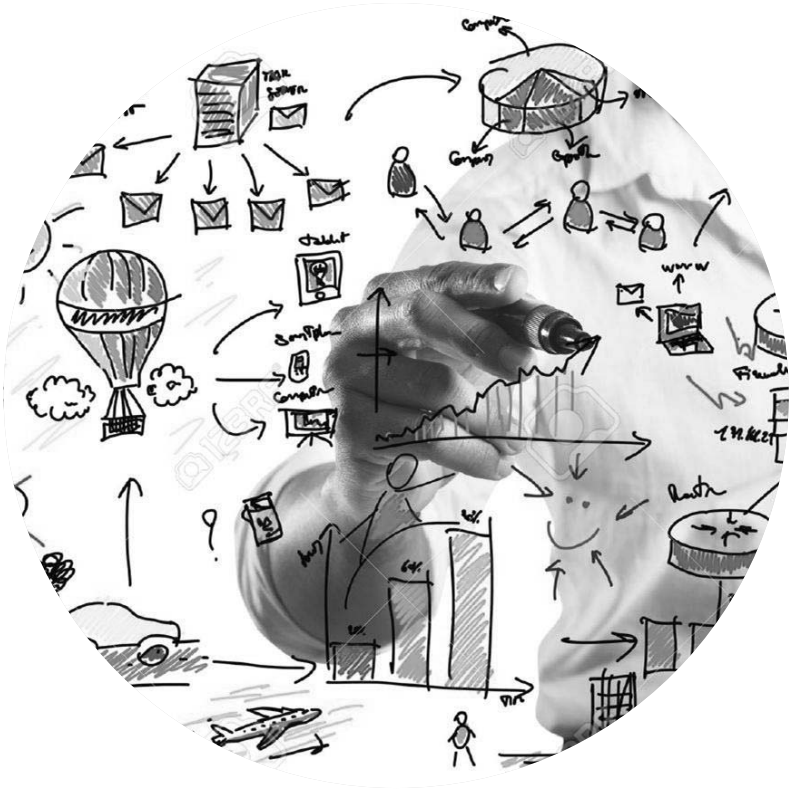
End

Questions?



1st Summer School on Smartphone-based Food Analysis
Wageningen, The Netherlands, 26-30 June 2017

Validation and benchmarking of a screening assays

Bjorn Berendsen [1]

Developing a new method can be a lot of work. You are finally finished, you've written the operating procedure and you can't wait to analyse your first sample. Stop right there! Is the method indeed as good as you think and ready to be used by just anyone? What is the performance of your new method? What levels is it able to reproducibly detect? Is it selective or does it respond to other chemicals as well and does it sometimes result in false positive results? Do you know the answers to these questions, then you are dismissed. If not, read on.

A validation is undeniably an important step before a new method can be applied in practice. During a validation the method performance is determined including detection limits, trueness, precision and selectivity. If a validation is successful (if carried out according to the right standards), this is the ultimate proof that your test results are trustworthy. In other words: only then you are sure what your method is capable of (and what not).

The validation of a screening assay is, compared to a validation of a quantitative confirmatory assay, quite simple. Only a few parameters are of relevance as stated by commission decision 2002/657/EC and related guidelines and therefore the workload is limited. European Reference Laboratory guidelines give clear instructions on the process. A number of blank samples and a number of spiked samples should be analysed under within-laboratory reproducibility conditions. The exact number (at least 20 blanks and 20 spiked samples) depends on the performance of the method. If the spiked population is clearly distinct from the blank population, it is concluded that CCß (the level from which, with 95% probability, a sample will result in a positive/suspect finding) is equal or below the spiked level (also called the target screening concentration).

After validation, can be applied in practice and, if needed, be accreditated. Even though the method is fully validated, it should be monitored in time (benchmarking) to guarantee continued acceptable performance. Benchmarking can be done based on first, second or third line quality control samples, of which the latter regards proficiency testing. Quality control data should not only be assessed, but also trends should studied, e.g. by using a control chart. Finally, regularly (depending on the frequency of use) methods and validation data should be revised to keep data on method performance up to date.

Detailed information about the procedures and advised further reading can be found here:

[1] CD 2002/657/EC in different languages: http://publications.europa.eu/en/publication-detail/-/publication/ed928116-a955-4a84-b10a-cf7a82bad858/language-en

[2] EURL guidelines on validation of a screening assay 20/1/2010, Guidelines for the validation of screening methods for residues of veterinary medicines: https://ec.europa.eu/food/sites/food/files/safety/docs/cs_vet-med-residues_guideline_validation_screening_en.pdf

[3] Stolker AA, Application of EU guidelines for the validation of screening methods for veterinary drugs. Drug Test Anal 2012, Suppl. 1:28-33.

[1] E-mail; bjorn.berendsen@wur.nl; Wageningen University & Research, RIKILT, Bu Veterinary Drugs, Wageningen, The Netherlands

Validation and benchmarking of a screening assays

June 26th, Bjorn Berendsen

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Process

- Idea / proposal
- Development
- Validation
- Benchmarking (ongoing quality control)

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Validation of a screening assay

Validation of a screening assay

- Why?
  - Determine the method's performance
  - Demonstrate the method's performance
  - Challenge the method
  - To be able to work under accreditation
  - Stronger position in case of a dispute
  - To assure correct results

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Validation of a screening assay

- Outline:
  - Parameters
  - Guidelines
  - Validation process
  - Data evaluation

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Validation parameters

Classification of Performance characteristics				
Performance characteristic	Qualitative method		Quantitative method	
	screening	confirmatory	screening	confirmatory
Trueness / Recovery				
Repeatability				
Within- Laboratory reproducibility				
Decision limit (CCα)				
Detection capability (CCβ)				
Specificity				
Ruggedness				
Stability				
Linearity				

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Commission decision 2002/657/EC

Parameters

- Precision
  - Repeatability

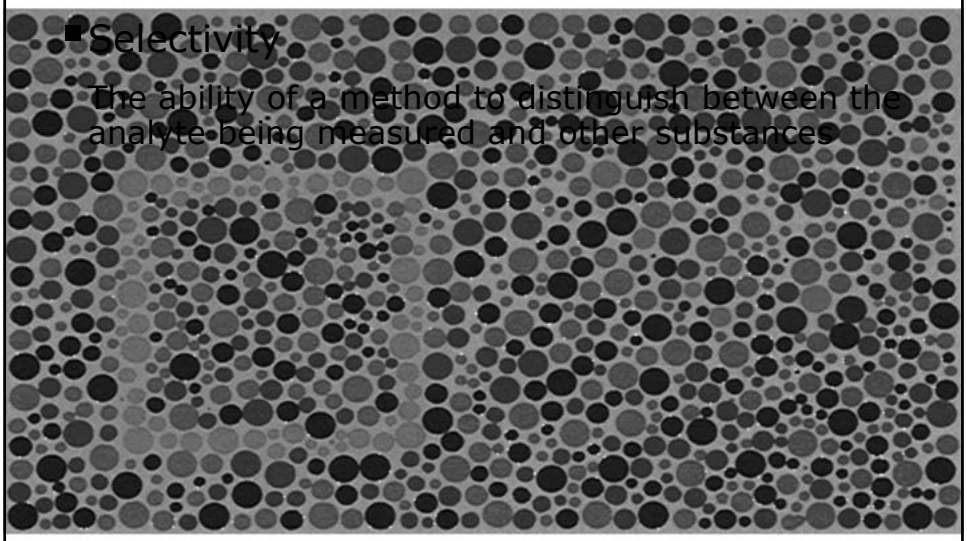
    Closeness of agreement between independent test results obtained under the same conditions
  - Within-lab reproducibility

    Closeness of agreement between independent test results obtained under different conditions

Parameters

- Selectivity

  The ability of a method to distinguish between the analyte being measured and other substances



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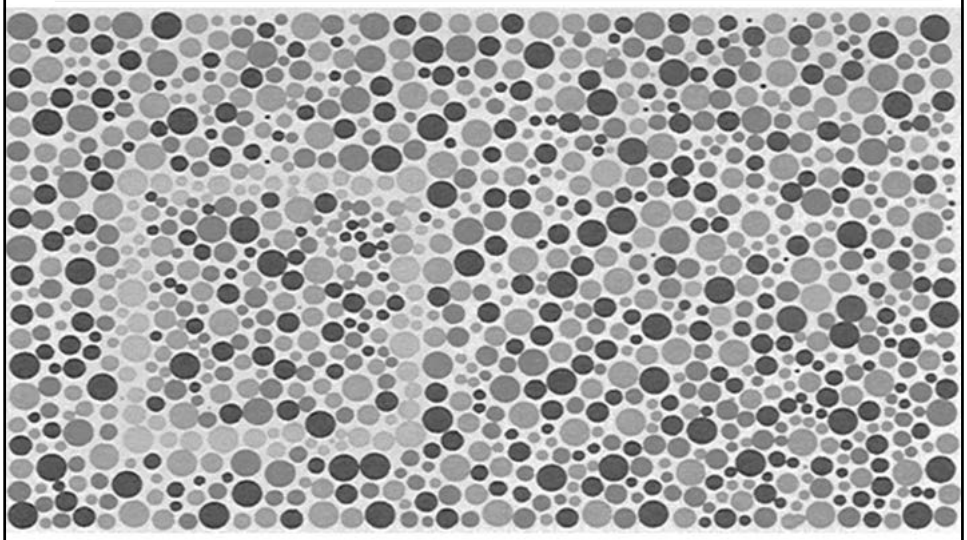
Parameters



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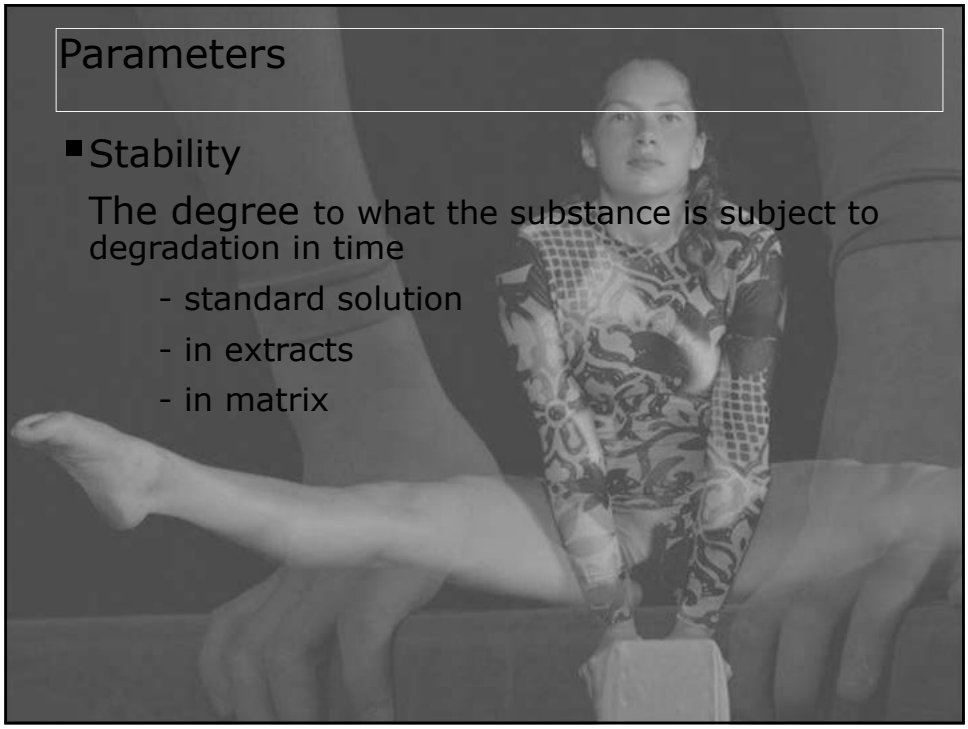
Parameters

- Ruggedness

  The susceptibility of a method to minor changes in experimental conditions

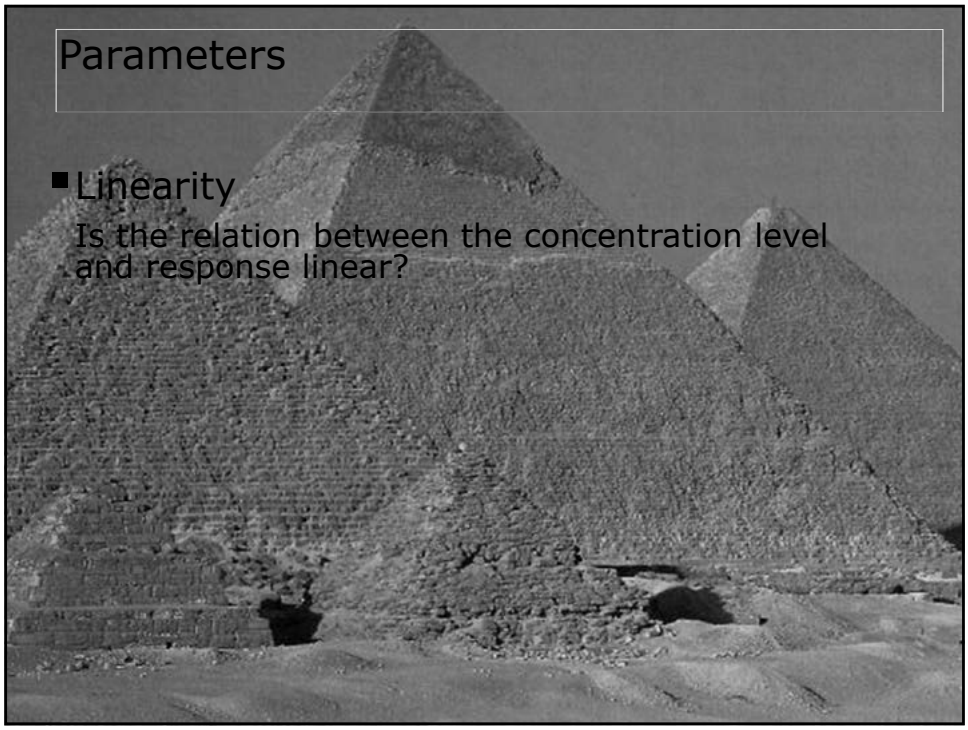


Parameters

- Stability

  The degree to what the substance is subject to degradation in time

  - standard solution
  - in extracts
  - in matrix



Parameters

- Linearity

  Is the relation between the concentration level and response linear?

Validation parameters

Classification of Performance characteristics				
Performance characteristic	Qualitative method		Quantitative method	
	screening	confirmatory	screening	confirmatory
Trueness / Recovery				
Repeatability				
Within- Laboratory reproducibility				
Decision limit (CCα)				
Detection capability (CCβ)				
Specificity				
Ruggedness				
Stability				
Linearity				

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Commission decision 2002/657/EC

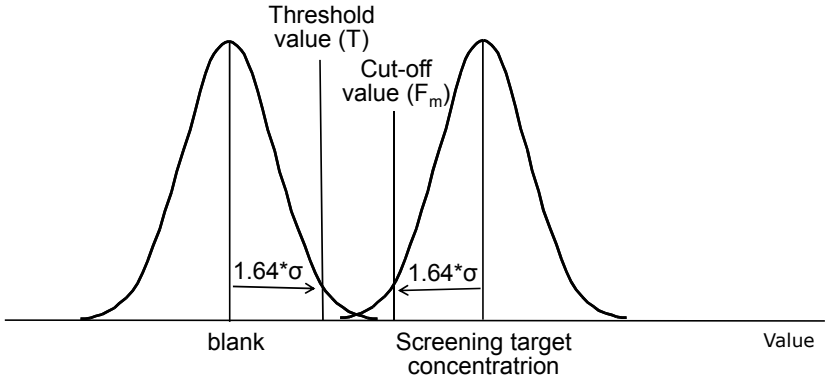
Validation guidelines

- EURL guidelines
  - False negatives vs false positives

Count the number of sheep

Validation guidelines

- Guidelines from the EURL



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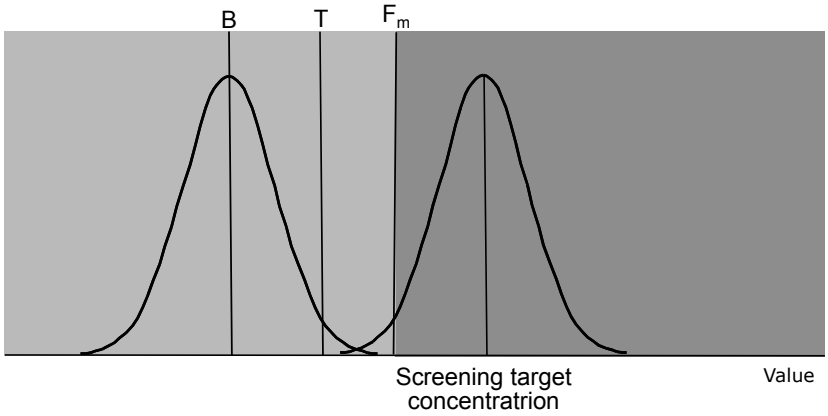
Validation guidelines

$T < F_m$

False negative rate = 5%

False positive rate < 5%

- Guidelines from the EURL



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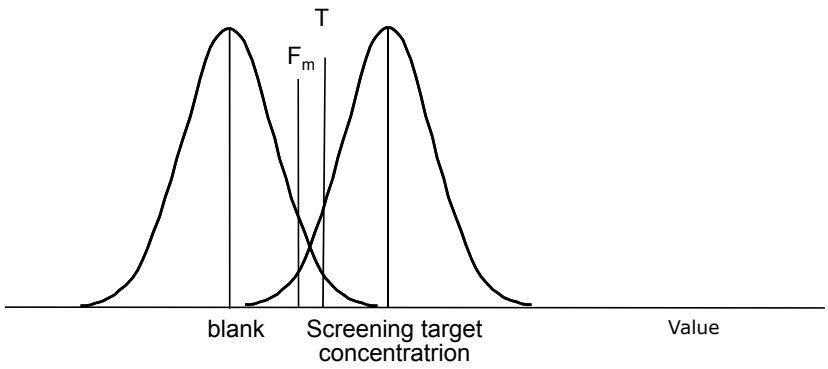
Validation guidelines

- Guidelines from the EURL



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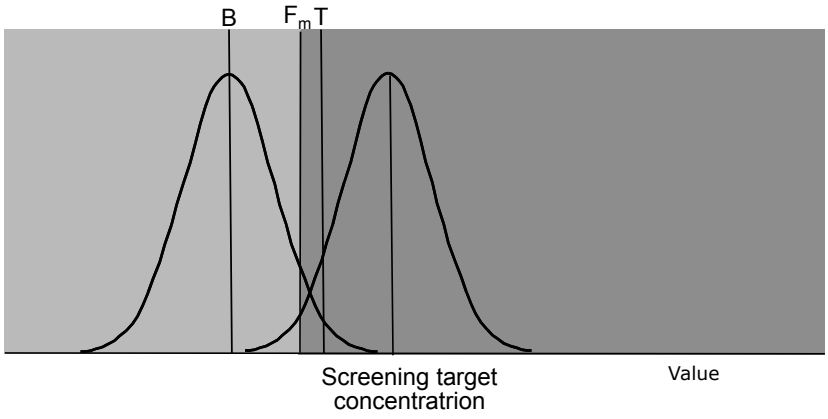
Validation guidelines

$F_m < T$

False negative rate = 5%

False positive rate > 5%

- Guidelines from the EURL



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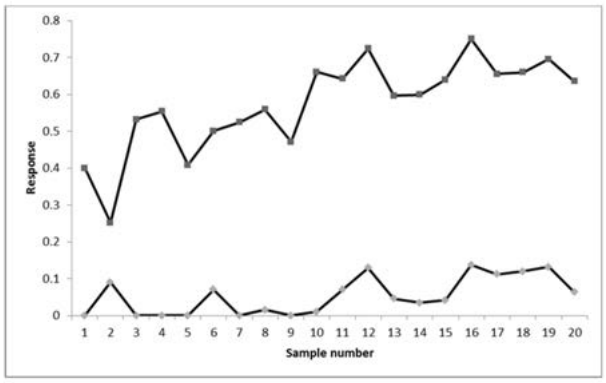
Validation guidelines



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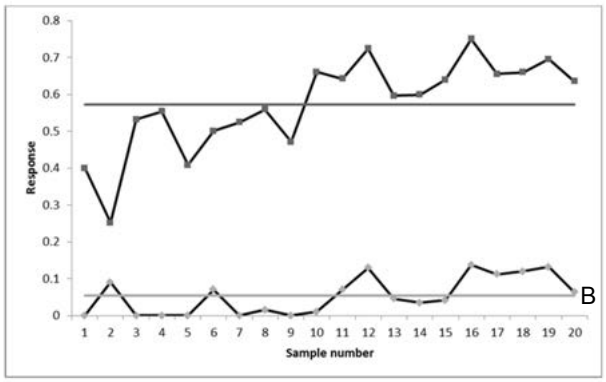
Validation guidelines



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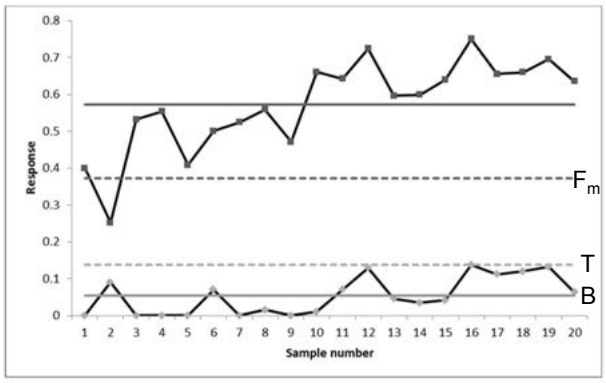
Validation guidelines



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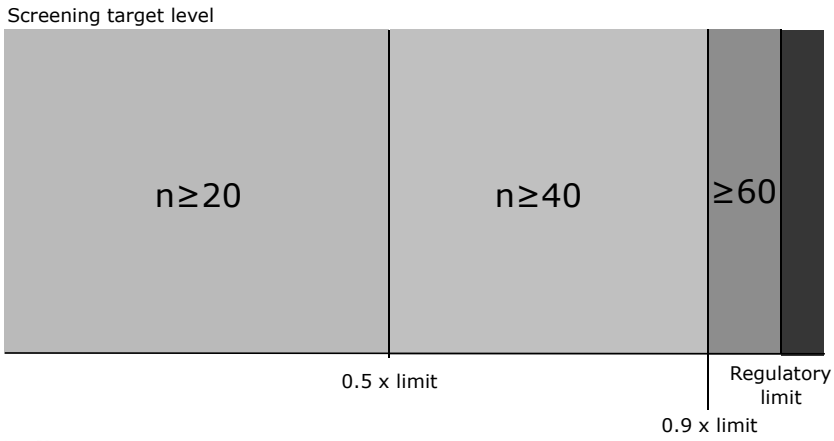
Validation guidelines

- What is the minimum number of samples?



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Validation of a screening assay

- All samples origin from a different batch
- n * spiked at screening target concentration
- n * blank
- Analyse on different days
- Preferably by different technicians

- Iterative process in which screening target concentration is selected until CCß is established.
- You do want to make an informed decision!

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Data evaluation

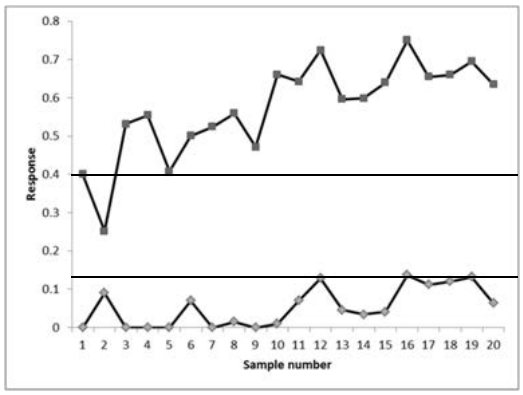
- 2 approaches
  - Amplitude
  - Statistical

RIKILT WAGENINGEN UR

Data evaluation

- Amplitude

Sample no.	Blank	Spike 0.5
1	0	0.4
2	0.09	0.252
3	0	0.532
4	0	0.554
5	0	0.408
6	0.07	0.501
7	0	0.524
8	0.015	0.559
9	0	0.471
10	0.01	0.661
11	0.07	0.642
12	0.129	0.724
13	0.046	0.596
14	0.034	0.599
15	0.041	0.64
16	0.137	0.75
17	0.112	0.655
18	0.12	0.66
19	0.132	0.695
20	0.063	0.635



Threshold = 0.132

Cut-off level = 0.4

$T<F_m$, CCß≤0.5

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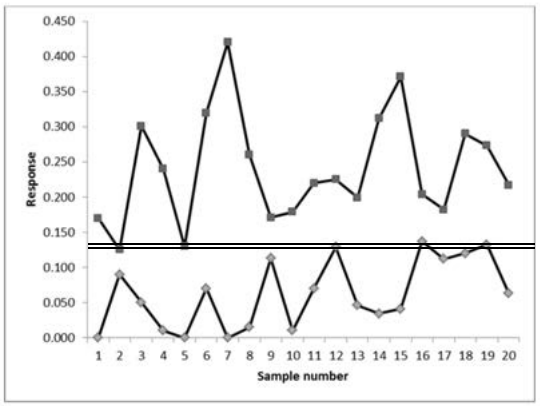
Data evaluation

- Amplitude

Sample no.	Blank	Spike 0.25
1	0.000	0.170
2	0.090	0.125
3	0.050	0.301
4	0.010	0.240
5	0.000	0.130
6	0.070	0.320
7	0.000	0.420
8	0.015	0.260
9	0.113	0.171
10	0.010	0.179
11	0.070	0.220
12	0.129	0.225
13	0.046	0.199
14	0.034	0.312
15	0.041	0.371
16	0.137	0.204
17	0.112	0.182
18	0.120	0.290
19	0.132	0.273
20	0.063	0.217



Threshold = 0.132

Cut-off level = 0.130

$T>F_m$, CCß>0.25

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Data evaluation

- Statistical

Sample no.	Blank	Spike 0.5
1	0	0.4
2	0.09	0.252
3	0	0.532
4	0	0.554
5	0	0.408
6	0.07	0.501
7	0	0.524
8	0.015	0.559
9	0	0.471
10	0.01	0.661
11	0.07	0.642
12	0.129	0.724
13	0.046	0.596
14	0.034	0.599
15	0.041	0.64
16	0.137	0.75
17	0.112	0.655
18	0.12	0.66
19	0.132	0.695
20	0.063	0.635
average	0.053	0.573
std	0.051	0.123
1.64xstd	0.084	0.201
avg+1.64xstd	0.137	
avg-1.64xstd		0.372

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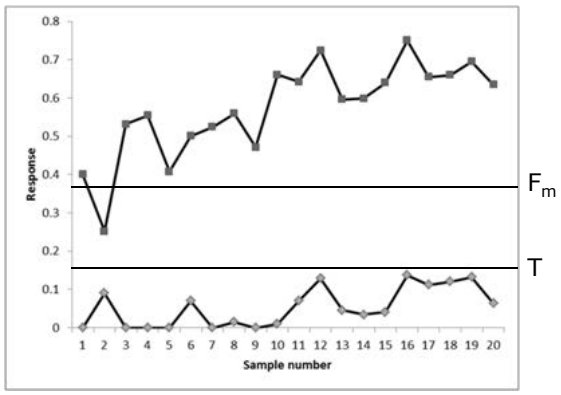

Threshold = 0.137

Cut-off level = 0.372

T< F_m, CCß≤0.5

Data evaluation

- Statistical

Sample no.	Blank	Spike 0.25
1	0.000	0.170
2	0.090	0.125
3	0.050	0.301
4	0.010	0.240
5	0.000	0.130
6	0.070	0.320
7	0.000	0.420
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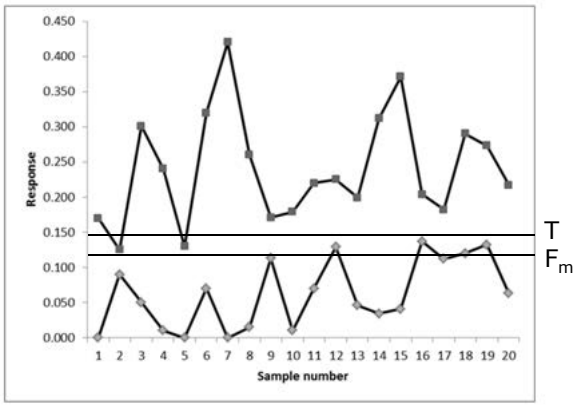
15	0.041	0.371
16	0.137	0.204
17	0.112	0.182
18	0.120	0.290
19	0.132	0.273
20	0.063	0.217
average	0.062	0.240
std	0.049	0.078
1.64xstd	0.080	0.128
avg+1.64xstd	0.142	
avg-1.64xstd		0.113

RIKILT WAGENINGEN UR



Threshold = 0.14

Cut-off level = 0.11

T>F_m, CCß>0.25

Validation of a screening assay

- Iterative process in which screening target concentration is selected until CCß is established.
- You do want to make an informed decision!

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Validation of a screening assay

- If your result is binary (yes/no):

Search for screening target concentration in which:

- ≥19 of 20 samples spiked at the screening target concentration give result = yes
- Number of false positives is acceptable.

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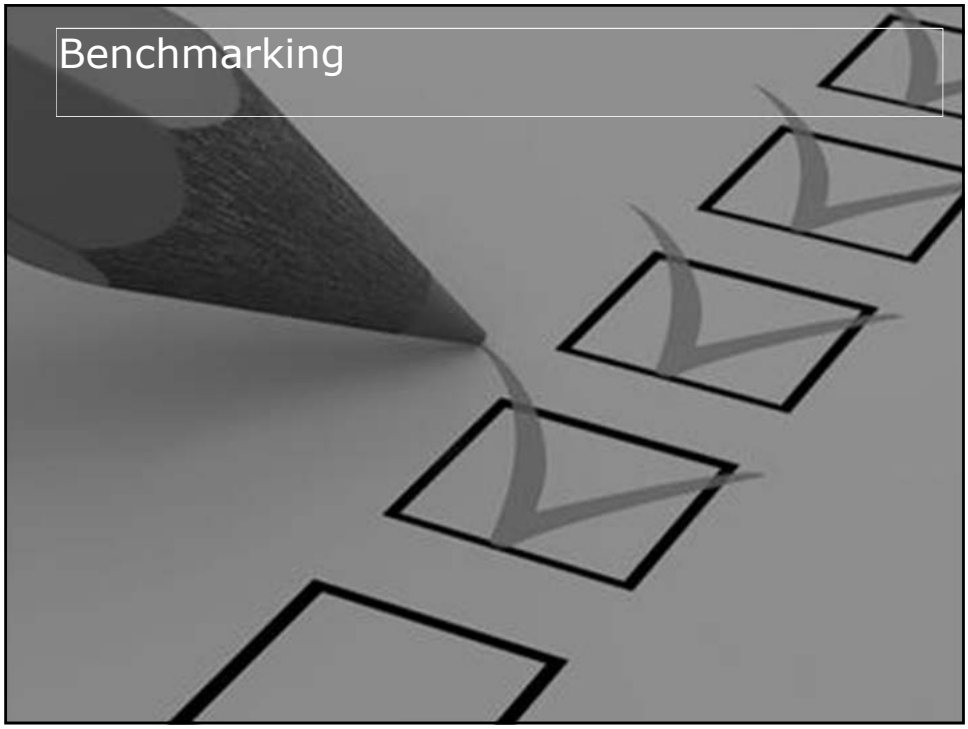

Benchmarking a screening assay

- Outline
  - People involved
  - Quality control samples
    - 1st
    - 2nd
    - 3rd
  - Evaluation and revision of validation data

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People involved in QC

- Lab technicians

- Responsible scientists

- Colleagues from other laboratories

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1st line Quality control samples

- Known to lab technician
  - Certified reference material
  - In-house reference material
  - Spiked sample

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1st line Quality control samples

- In case CRM is available:

  - Analyse CRM
  - Check the validity of the series
  - Register the result

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1st line Quality control samples

- In case no CRM is available:

  - select a blank sample (other than used for calibration)

  - spike at target level
  - Analyse all samples + QC

  - Check the validity of the series
  - Register the result

RIKILT WAGENINGEN UR

2nd line Quality control samples

- Unknown to lab technician
  - Certified reference material selected by responsible scientist
  - In-house reference material selected by responsible scientist
  - Spiked sample prepared by responsible scientist

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2nd line Quality control samples

- In case no CRM is available

- select a blank sample (other than used for calibration)

- spike the sample at a level unknown to the lab technician.

- register the spiked level in confidential journal

  - analyse all samples + QC
  - Report the result of the QC

- Check the validity

- Registration of the result

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3rd line Quality control samples

- Unknown to anyone in the laboratory
  - Proficiency test

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3rd line Quality control samples

- In case no CRM is available

- Prepare, test and distribute the samples

- Analyse the PT sample

- Report the PT sample

- Calculate z-score (=related to trueness)

- Evaluate and register results

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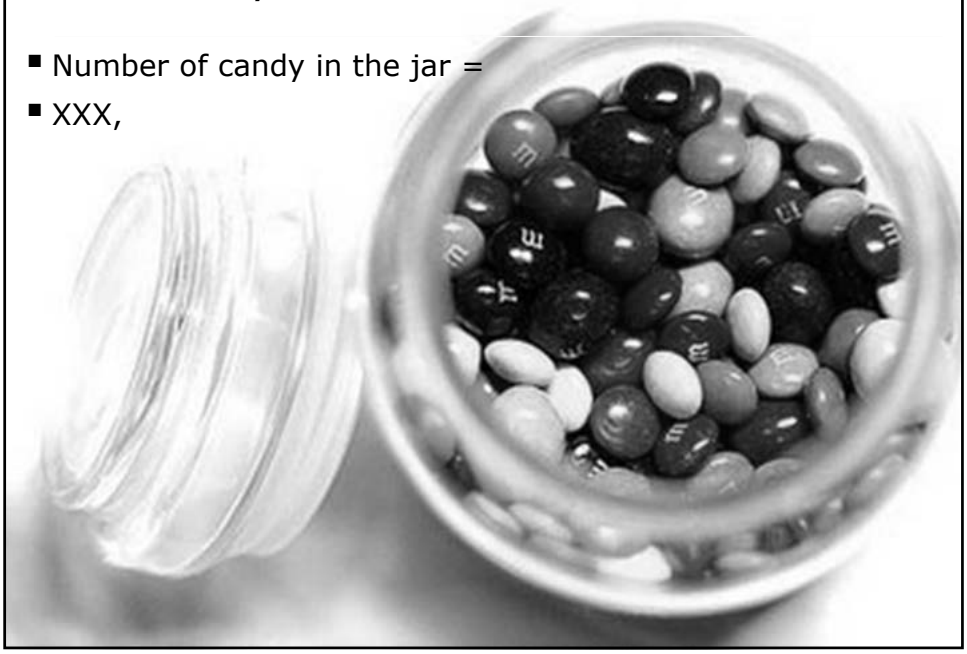
Proficiency test

- Number of candy in the jar =
- XXX,



Proficiency test

- The result of a PT is a z-score
- If -2 < z < 2: satisfactory
- If not: deviating result
    - Investigate and report cause

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Frequency of quality control samples

- 3rd line
  - On regular basis (at least every 3 – 4 years)
- 2nd line
  - Annually if no PT is available
- 1st line
  - Every series

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Monitoring assay trends

- Trends should be monitored

- Possible characteristics for monitoring trends
  - CCß (especially for screening method)
  - Trueness (in case quantitative measure is obtained)
  - ...

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Monitoring trends

- Trends can be monitored based on
  - 1st line control
  - 2nd line control if frequency allows
  - 3rd line control if frequency allows
- Control chart for quantitative parameters (ISO 8258, ISO 7870)

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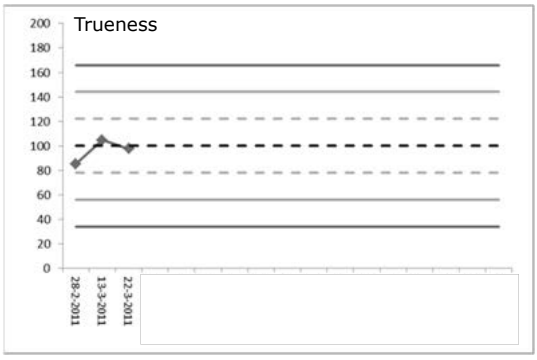
Quality control chart

- Control chart for quantitative parameter
  - 3 data points from your validation
  - Set 1s, 2s and 3s limits

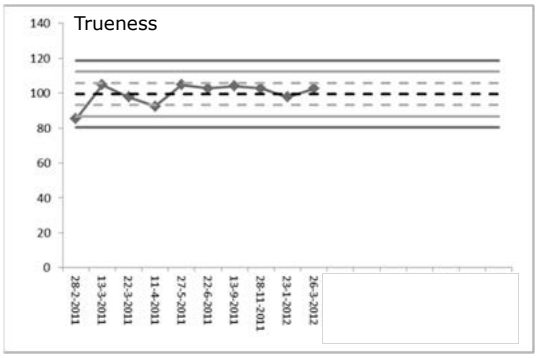
28-2-2011	85.3
13-3-2011	104.8
22-3-2011	97.7



RIKILT WAGENINGEN UR

Quality control chart

- Control chart for quantitative parameter
  - 7 additional routine series
  - Recalculate 1s, 2s and 3s limits



28-2-2011	85.3
13-3-2011	104.8
22-3-2011	97.7
11-4-2011	92.4
27-5-2011	104.8
22-6-2011	102.8
13-9-2011	104.1
28-11-2011	102.8
23-1-2012	97.7
26-3-2012	102.6
average=	99.5
stdev=	6.38801

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Quality control chart

- Out of control if:
  - 1 x outside 3s
  - 4 consecutive outside 1s
  - 11 consecutive points on one side of the average

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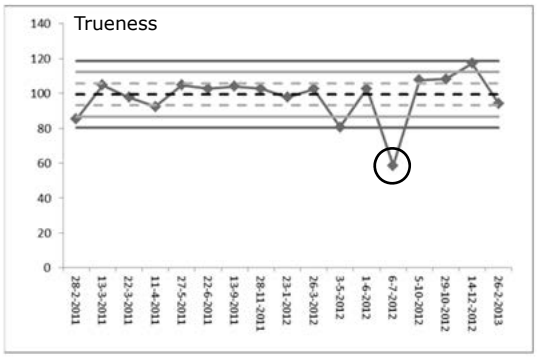
Quality control chart

- Control chart for quantitative parameter
  - additional routine series



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27-5-2011	104.8
22-6-2011	102.8
13-9-2011	104.1
28-11-2011	102.8
23-1-2012	97.7
26-3-2012	102.6
3-5-2012	80.7
1-6-2012	102.7
6-7-2012	58.6
5-10-2012	107.6
29-10-2012	108.3
14-12-2012	117.3
26-2-2013	94.2

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Revising validation characteristics

- Depending on the frequency of use
- At least every 3 years

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Revising validation characteristics

- Check and revise Standard Operating Procedure
  - Are all procedures up to date?
- Check and revise validation characteristics
  - Are all validation parameters up to date?
    - CCß (screening assays)
    - Trueness
    - Within-lab reproducibility
    - Ccα

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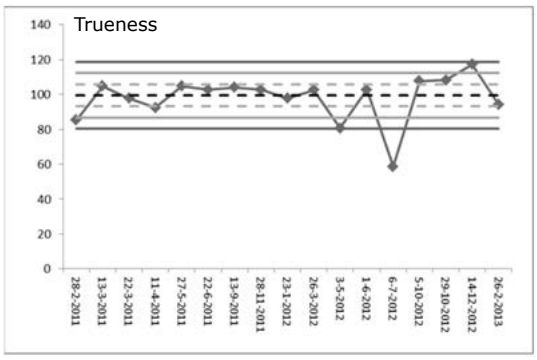
Revising validation characteristics



28-2-2011	85.3
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22-6-2011	102.8
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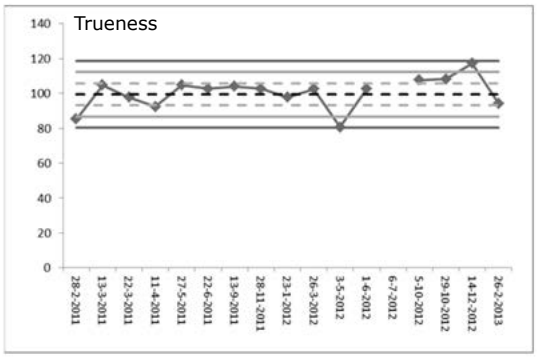
6-7-2012	58.6
5-10-2012	107.6
29-10-2012	108.3
14-12-2012	117.3
26-2-2013	94.2

RIKILT WAGENINGEN UR

Revising validation characteristics



28-2-2011	85.3
13-3-2011	104.8
22-3-2011	97.7
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22-6-2011	102.8
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28-11-2011	102.8
23-1-2012	97.7
26-3-2012	102.6
3-5-2012	80.7
1-6-2012	102.7
6-7-2012	
5-10-2012	107.6
29-10-2012	108.3
14-12-2012	117.3
26-2-2013	94.2
average=	100.4
st dev=	8.97
CV%=	8.9%

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Validation and benchmarking

- Validation is mandatory to determine/demonstrate method performance
- Think before starting the validation
- After validation, an assay is benchmarked continuously
- Benchmarking can be a lot of work but should be incorporated in the laboratory quality system

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Thank you

bjorn.berendsen@wur.nl

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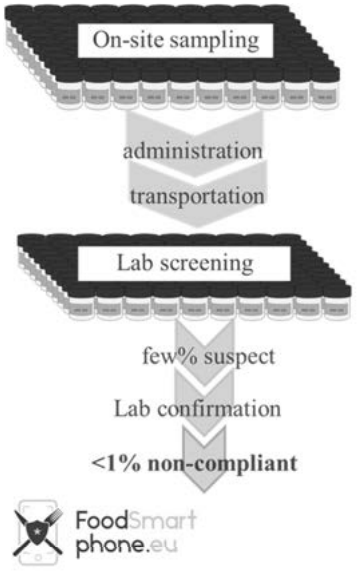
Smartphone-based food analysis: status, concepts and building blocks

Michel Nielen, FoodSmartphone coordinator
RIKILT, Wageningen Research, NL

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

The monitoring issue



Food Fraud Network Activity Report 2015

Imagine.....

- Food Q&S control being more focused and more cost-effective
- Simplified food Q&S screening available on-site
- Everywhere: in the field, at the farm, food industry, retail, border inspection, even at home
- So intuitive, easy and low-cost that even you and me can do it, *i.e.*, citizen science ready

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Why smartphone-based diagnostics

- Wide-spread, battery, computer, graphics, image detector, USB, time, location, wireless data communication, nice Apps, Internet of Things.....
- 3 billion smartphone users in 2020

The Telegraph

World's cheapest smartphone, costing under £3, begins shipping next week

http://www.telegraph.co.uk/technology/2016/07/01/worlds-cheapest-smartphone-costing-under-3-begins-shipping-next/

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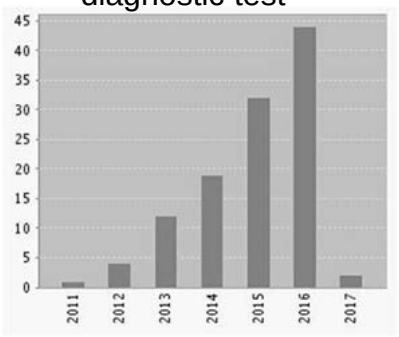
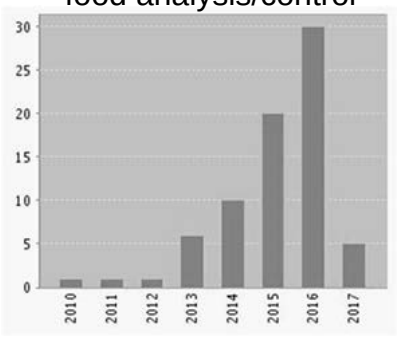
Smartphone or cellphone papers

>5800 scientific papers about smartphone/cellphone

‘diagnostic test’



‘food analysis/control’



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... but also many prototype products without papers!

Initial stakeholder analysis

Dairy farmer, n=1 (NL):

- Testing for somatic cells in milk
- Would like to test for antibiotics because:
  - High penalties when detected by dairy industry
  - Clearance times are different per cow: dispose of too much milk (to be on the safe side) is not a good thing
- Smartphone-based device should be easy to use, cheap (<€100-150), fast (< 1 min), simplified output.

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Initial stakeholder analysis

QA affairs officer dairy industry, n=1 (NL):

- Truck drivers test raw milk loads for antibiotics at the farm using a strip test (only €2 per test, thanks to huge contract)
- Milk trucks also sampled at the industry for lab-based testing
- Smartphone would be beneficial: wireless data upload!
  - Low cost, at least as accurate as a strip test, non-expert use

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Initial stakeholder analysis

Retail managers, n=2 (NL):

- No emphasis on “substances in food”, only on shelf-life and storage conditions.
- Have no time “to bother about food contaminants”.

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Initial stakeholder analysis

Consumers, n=100, mainly students@WU (NL):

- They typically change smartphone models every 2 years
- 16% has installed food-related Apps
- >60% want to spend less than 5 min total analysis time, 25% would accept 5-10 min time
- >60% is willing to pay >€20 for the testing device
- They do worry about food contaminants:

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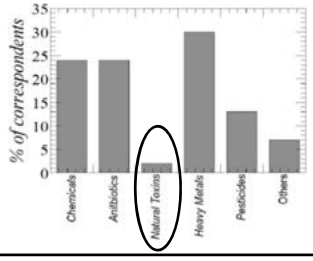
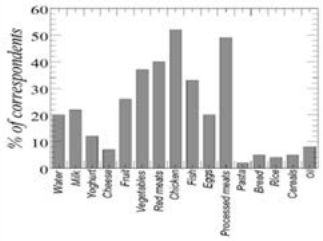




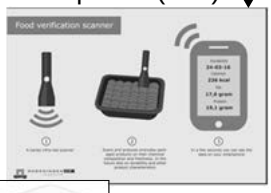
Different on-site device options

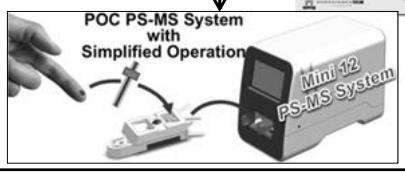
What are you measuring, (only) your target?

False positive and/or false negative test results?

- So how to assure selectivity/specificity???

1. Rely on a chemometric model plus a generic spectral profile (NIR)
2. Rely on a high(er) resolution spectrum (MS)
3. Rely on biorecognition (this course)





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Commercial singleplex biorecognition smartphone assay

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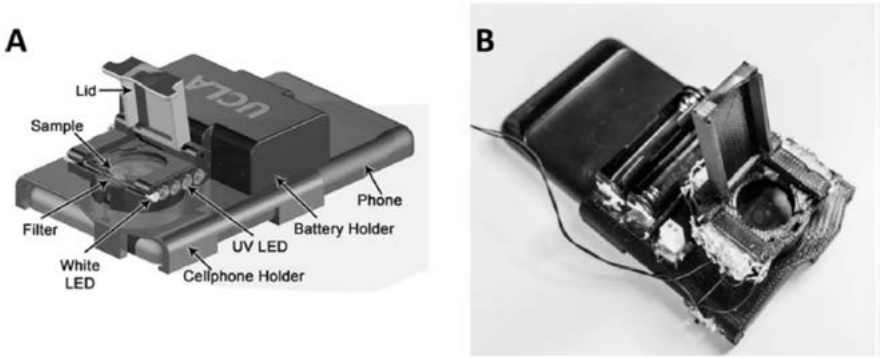
State-of-the-art multiplex biorecognition smartphone assay



Ludwig *et al*., *PLOS ONE*, 10(8): e0134360 (2015)

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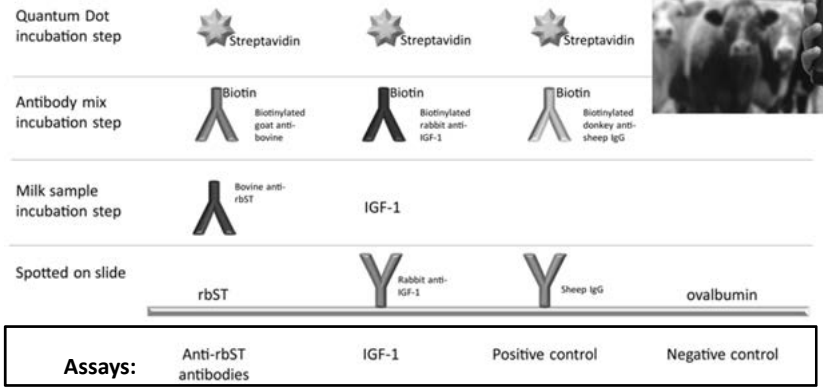
Assay for protein biomarkers in milk



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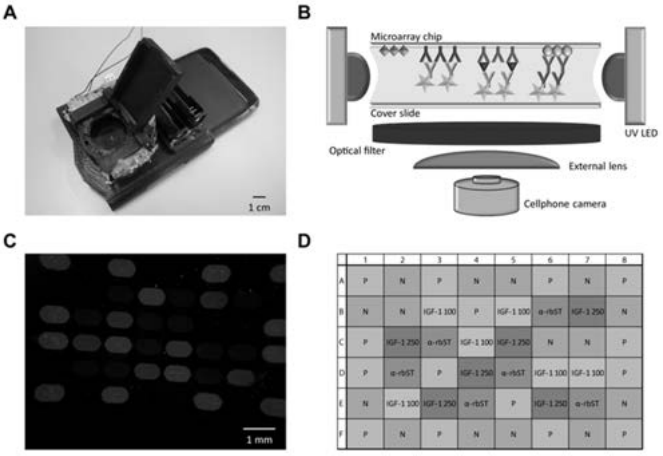
Protein microarray immunoassay for biomarkers in milk



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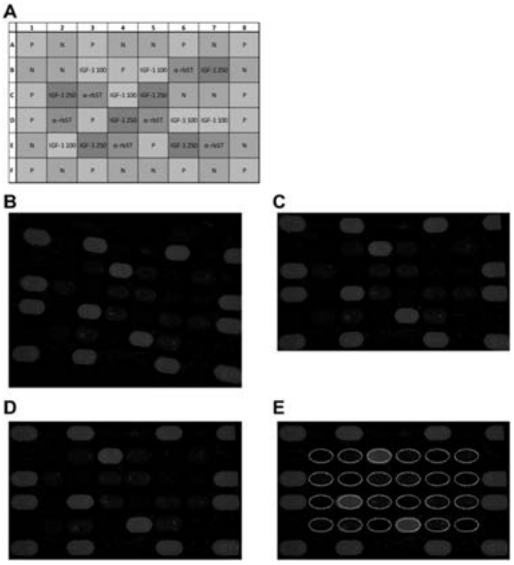
Processing software, App



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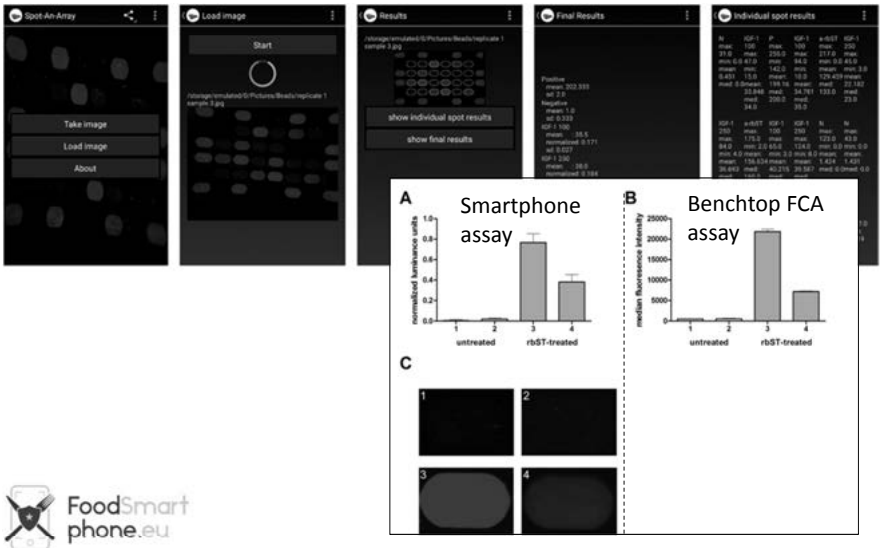
App development, results, benchmarking



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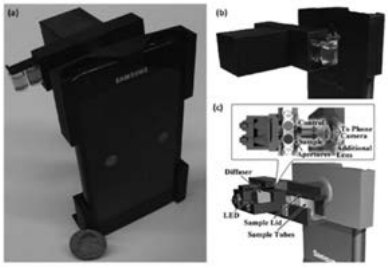
Smartphone-based diagnostics

Several major science and innovation gaps

- Miniaturization
- Flexibility/adaptability
- Validation, robustness
- Non-expert use
- Low-cost
- Analysis time



A personalized food allergen testing platform on a cellphone
Coskun at al., ***Lab Chip***, 2013, **13**, 636-640

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NIR-based food scanners

For example:

- http://tellspec.com/howitworks/
- https://www.spectralengines.com/products/food-scanner
- https://www.consumerphysics.com/myscio/scio/

https://www.youtube.com/watch?v=-PhVfCJg9Qw

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A glimpse of fancy simplicity (reability unknown)

For example:

- https://nimasensor.com/

https://youtu.be/PSQQ4ueEgAM

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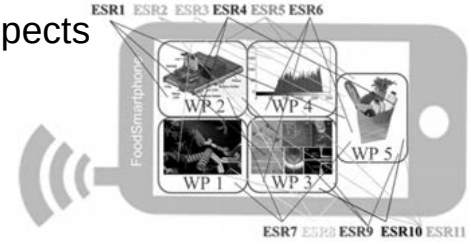
The FoodSmartphone objectives

- Smartphone-based (bio)analytical screening tools
- User-friendly, rapid, integrated sample prep
- Image data handling, communication, Apps
- On-site demonstrators: pesticides, allergens, mycotoxins, food spoilage, marine toxins
- Multidisciplinary trained researchers with improved career prospects



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Novel biorecognition concepts

- Aptamers (next to antibodies)
- DNA-directed immobilization strategies
- Immobilized enzymes

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Optical and electrochemical sensing

- Surface Plasmon Resonance (LSPR and iSPR)
- Labeling: fluorescent tags, QDs, carbon NPs
- Electrochemical signal transduction
- 3D-printed interfaces and attachments
- Interfacing assays with MS for lab confirmation

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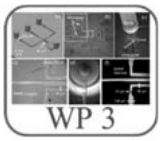
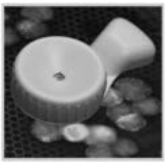
Integrated sample preparation



- Membrane-based devices (cross-flow)
- Microsieve-based devices
- 3D-printed lab-on-chip based devices
- Microfluidic paper-based analytical devices
- System integration



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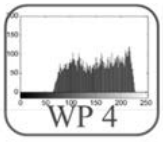
Data handling and software



- Prototype data handling solutions
- Apps development
- Secure stakeholder and cloud interfacing

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Demonstration of applicability

- Validated reference methods for pesticides, mycotoxins, allergens, antibiotics
- Protocols for within-lab and on-site testing
- On-site application
- Benchmarking versus lab-based methods

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The individual ESR projects

1. Multiplex smartphone assay for allergens
2. FoodSmartphone-MS confirmation
3. LSPR smartphone for marine toxins
4. LSPR smartphone for food spoilage organisms
5. Novel image analysis software
6. Enzyme paper assays for AChE inhibitors
7. DNA-addressable multiplex binding arrays
8. Electrode arrays for multiplex smartphone assays
9. Lab-on-chip for iSPR smartphone detection
10. Bioaffinity microsieves for integrated sample prep
11. Aptamer-based smartphone assays for aflatoxins

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The FoodSmartphone partnership

- RIKILT, Wageningen, NL
- Queens University Belfast, UK
- Univ. Chem. Tech., Prague, CZ
- CSIC, Barcelona, ES
- Linköping University, SE
- Aquamarijn Microfiltr., Zutphen, NL
- CSEM, Landquart, CH

- Barilla, Parma, IT
- Zeulab, Zaragoza, ES

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Hot Paper Studies

1. Read: *critically read the paper you got*
   (pdfs of all other papers are available on Blackboard)

2. Evaluate: *complete the evaluation form on page 2*

3. Share & discuss in your sub-group: *decide which of your sub-group papers is the most interesting to present plenary*

4. Present & debate plenary: *one 7 minutes presentation per sub-group* *(no presentation format, the sub-group can choose any form they prefer)*

Evaluation Form Hot Paper Studies

Your name: .....Your sub-group no.....

Paper title: .....

.....

Journal, volume, year, page: .....

Q1 what do you like most in this paper?

Q2 what is wrong and/or missing in this paper versus your ultimate aim of reliable smartphone-based food analysis?

Q3 what research do you propose to improve that situation (what would be the niche)?

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Summerschool- biorecognition

Monique Bremer
RIKILT Wageningen University and Research

Monique.Bremer@WUR.NL

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

Outline

- What is biorecognition?
- Biorecognition elements
- Poly- and monoclonal antibodies
- Characterisation of antibodies

Biorecognition

- Biorecognition: recognition and binding of a *specific analyte* by a *bioelement* such as an enzyme or antibody.
- Upon binding a change (e.g. in the structure of the biomolecule, mass or formation of a product) occurs.
- This change can be transformed into a signal and used for “detection”

- The bioelement is very specific to the target analyte. It does not recognise other analytes.

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Biorecognition elements

- Enzymes
- Cells
- Receptors
- Antibodies
- Nucleic acid
- Synthetic receptors: Aptamers

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Biorecognition elements: enzymes

- Large protein that acts as a catalyst in chemical reactions but remains unchanged at the end of the reaction

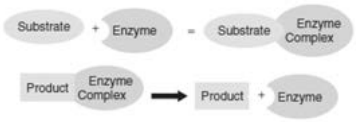

Fig. 5 Working principle of enzymes

- Enzymes are extremely specific in their action

- Example: glucose biosensor based on glucose oxidase
- used in diabetic self-monitoring of capillary blood glucose.

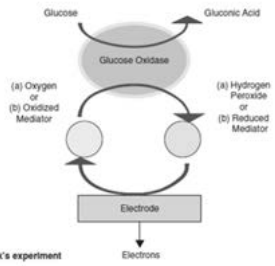

Fig. 7 Clark's experiment

IEEE Potentials (Volume: 25, Issue: 2, March-April 2006)

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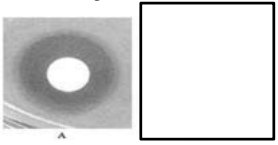
Biorecognition elements: Cells

- Cells are *detection devices* sensitive to surrounding environment and can respond to all kinds of stimulants
- Commonly used to detect global parameters like stress condition and toxicity and to monitor the treatment effect of drugs
- Example: microbial inhibition assay detects antibiotics residues in milk



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Biorecognition elements: aptamers

- Most common aptamers are **synthetic** single stranded DNA/RNA
- Typically 15 to 60 nucleotide bases in length
- Complex 3D structure in contrast to the idea of linear information holding structures
- Bind to the target with high affinity and specificity.
- Function as antibodies
- Targets: toxins, proteins, viruses etc.

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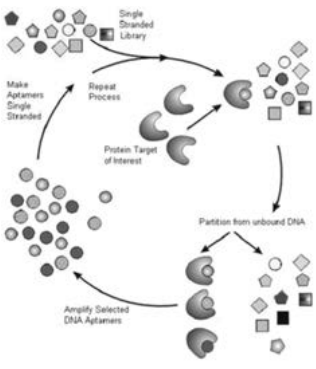
Biorecognition elements: aptamers

- Aptamers are selected via procedure called SELEX: Systematic Evolution of Ligands by EXponential enrichment
- *in vitro* selection of RNA/DNA from complex libraries of synthetic nucleic acids
- **Goal** of SELEX: to fish out aptamers and to make large amount of aptamers By chemical synthesis



SELEX: Systematic Evolution of Ligands by Exponential Amplification Tuerk, C. & Gold, L. (1990) Science 249, 505-510

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Biorecognition elements: aptamers

- Aptamers versus antibodies:

Antibodies	Aptamers
Requires the use of animals	No animals required
High affinity and specificity for the target	Medium high affinity and specificity for the target
Production difficult for toxic substances and lichaamseigen stoffen	Can be generated against toxins and molecules that do not elicit good immune response
Difficult to modify binding parameters on demand	Binding parameters could be modified
Reversible temperature denaturing	Irreversible temperature denaturing
Limited shelf life	Stable to long-term storage
Batch to batch variation	Little batch variation due to chemical synthesis
Labelling of antibodies can cause loss in affinity	Reporter molecules can be adjusted to aptamers at precise locations not involved in binding

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https://www.ifm.liu.se/edu/coursescms/tfya62/lectures/Biorecognition-elements-TFYA62.pdf

Biorecognition elements: antibodies

- **Antibodies (Ab)** are complex proteins (immunoglobulins) produced in response to a challenge by a foreign substance (**antigen**). Their function is to bind to the substance and identify it to the immune system that it should be removed from the body. Ab molecular weights range from 180kDa to 900kDa

- **Antigens** can be simple or complex molecules ranging in weight from **10kDa** to >1000kDa. When present they trigger an immune response which leads to the production of antibodies which bind to that antigen.

- **Epitope** is the specific part of the antigen to which an antibody binds

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Biorecognition elements: antibodies

- Antibody structure
- Fab: antigen binding fragment (2 similar sites)
- Fc: crystallisable fragment: binding to immune cells

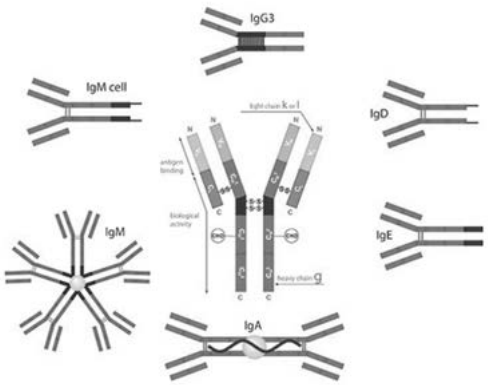

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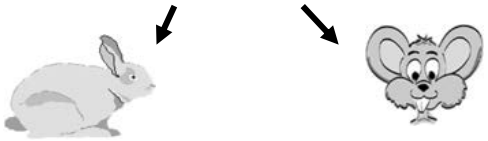
Figure 1. Antibody structure and isotypes
http://www.abcam.com/protocols/antibody-structure-and-isotypes

Biorecognition elements: antibodies

- **Production of antibodies**

Antigen+ adjuvant



Polyclonal antibody harvested from sera

Pool of antibodies with different characteristics

Monoclonal antibodies harvested following fusion and cell growth

From 1 single cell: 1 type of antibody all having same characteristics

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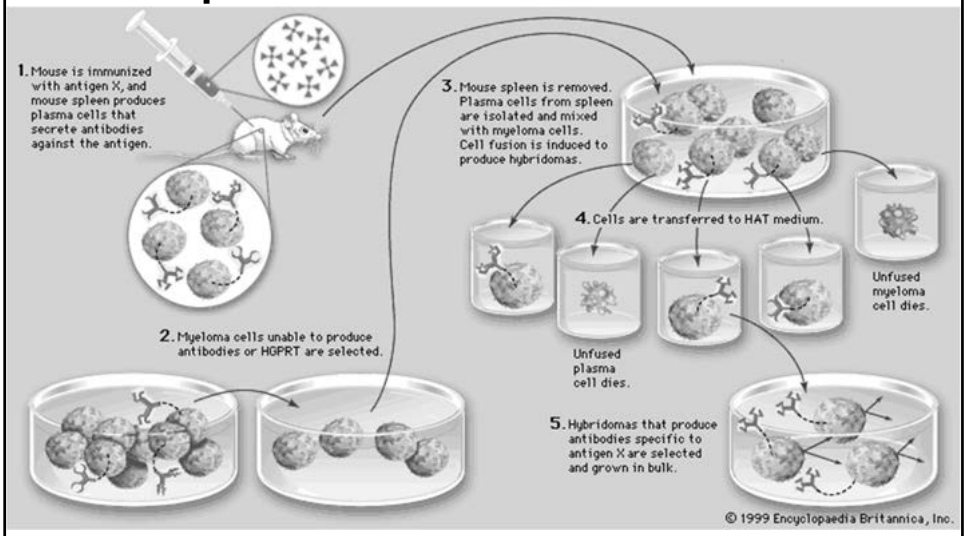
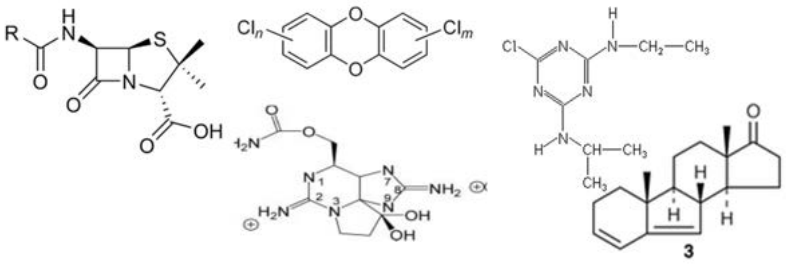
How to produce monoclonal antibodies?



Chemical Contaminant Targets



What do all these targets have in common?

All have molecular weights <1000 Da but to produce antibodies antigens must be >10,000 Da....

We have a problem.....

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How to induce antibodies against Small Molecular Weight Compounds (Haptens) ?

The Science of Biomolecular conjugation

Hapten-protein conjugate

Biorecognition elements: antibodies

- Antigens to produce antibodies:
- Body's own substance e.g. muscle proteins
  - Couple to foreign carrier protein (KLH)
  - Select peptide and couple to foreign protein
- Foreign substance
  - Conjugation depending on Mw
  - Toxic?

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Biorecognition elements: antibodies

- Characterisation of antibodies

Selection criteria

• **Final dilution of serum**	high dilution factor, good producing cells/high affinity antibodies
• **Working range in assay**	low limit of detection/high affinity antibodies
• **Cross-reactivity**	high specificity, no cross-reactivity

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Biorecognition elements: antibodies

- Characterisation of antibodies: cross reactivity
- Test binding to non-target compounds
- Example: 23 peanut monoclonal antibodies
- Application detection of peanut in food
  - Cross reactivity test with over 50 food components
    - Nuts, wheat, rye, barley, legumes, cacao, herbs, spices etc.

40 Mabs with 40 extracts simultaneous

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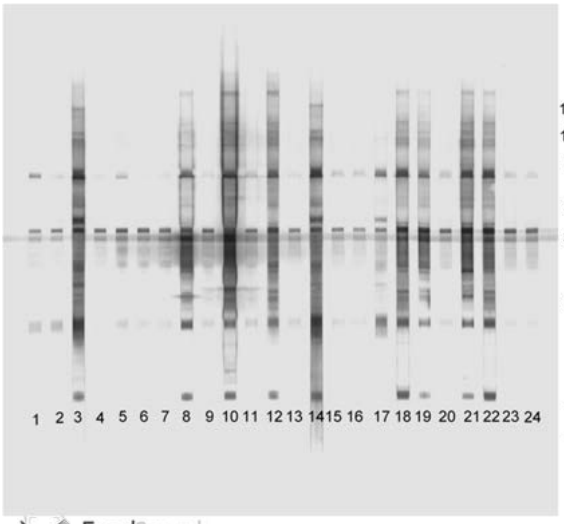
Biorecognition elements: antibodies



- SDS-PAGE
- Blotting on membrane
- Incubation with antibodies

Masterfood Peanut
1 49-2D11
2 49-4D4
3 51-1B6
4 51-2A12
5 51-2H5
6 51-3D12
7 51-3G7
8 51-4E10
9 51-5D3
10 51-5G9
11 51- 6F6
12 51-7C1
13 51-7G8
14 51-9F1
15 51-9G2
16 51-9H10
17 51-10C5
18 51-10F5
19 51-10G5
20 51-12D2
21 51-12D7
22 51-12E3

Arah2
23 52-1A11
24 55-3D5

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Summary

- Possible biorecognition elements
- Specific function of biomolecules can be used for biorecognition
  - Enzymes/substrate
  - Antibodies/antigen
- Development of poly- and monoclonal antibodies
- Characterisation of antibodies

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Summerschool- ligand binding assays

Monique Bremer
RIKILT Wageningen University and Research

Monique.Bremer@WUR.NL

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

Outline

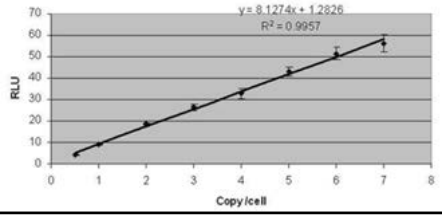
- Why are tests needed?
- Demands of screening assays
- How to design an immunoassay
- Detection methods
- Platforms and goals
- Comparison of methods

Why Are Tests Needed?

Measurement

- Verify the presence or absence of an analyte in a sample: **Qualitative Test**

- Quantify the amount of analyte in a sample: **Quantitative Test**



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Demands of a Screening Test

- **Rapid**
- **Reliable**
- **Low cost**
- **Low false positives**
- **No false negatives**
- **Safe**

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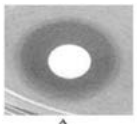
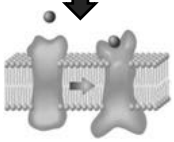
Ligand binding assays

Cell Based



Microbial inhibition assay detects antibiotics residues In milk

Receptor Based



Receptors assays used to detect contaminants in feed and food

Antibody Based

Antibody assays used to detect contaminants in feed and foods

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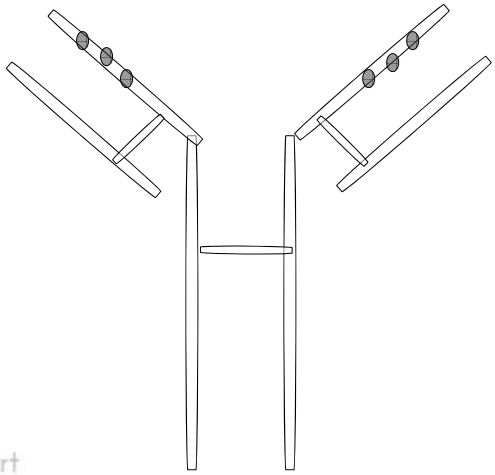
How to design an immunoassay?



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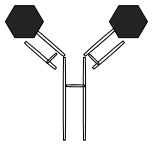
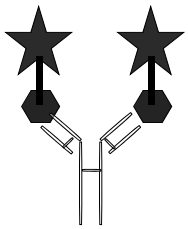
How to detect the binding ?





Label the analyte (or the antibody) with a detection molecule

Detection molecules can be a radioactive element, an enzyme, a fluorescent compound, ...

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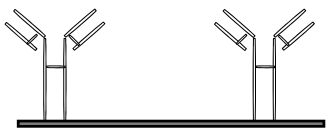
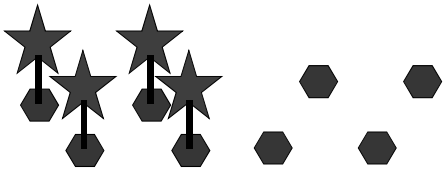
Measuring Competition of Interactions: low Mw

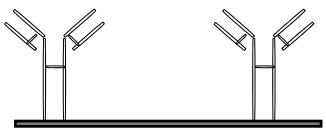
Example 1: No chemical present in sample



High signal

Example 2: Chemical present in sample





Low signal

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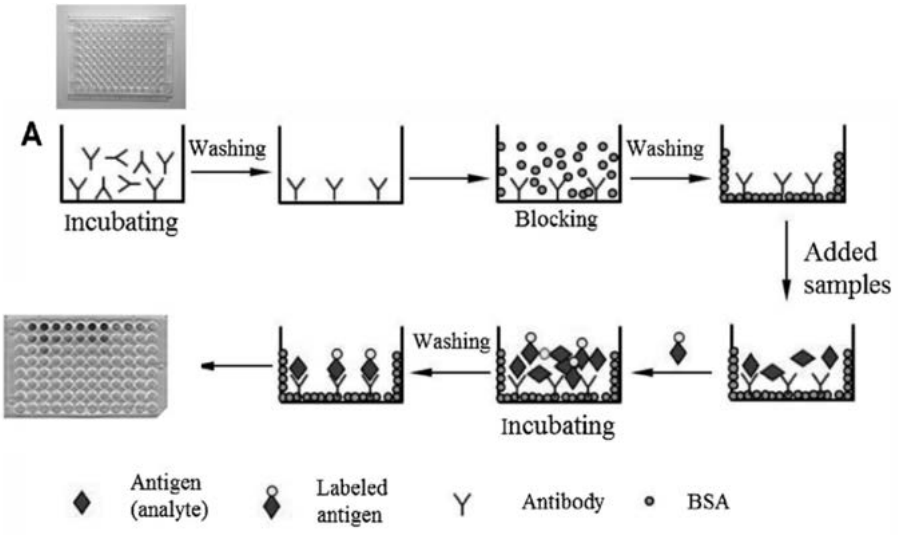
Competitive assays: low MW



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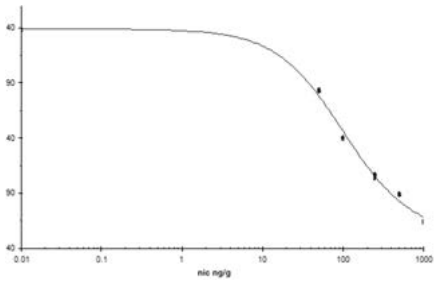
Quantitation in *Competitive* Binding Assays

High Signal = Low analyte concentration in sample

Low Signal = High analyte concentration in sample

Signal



Concentration

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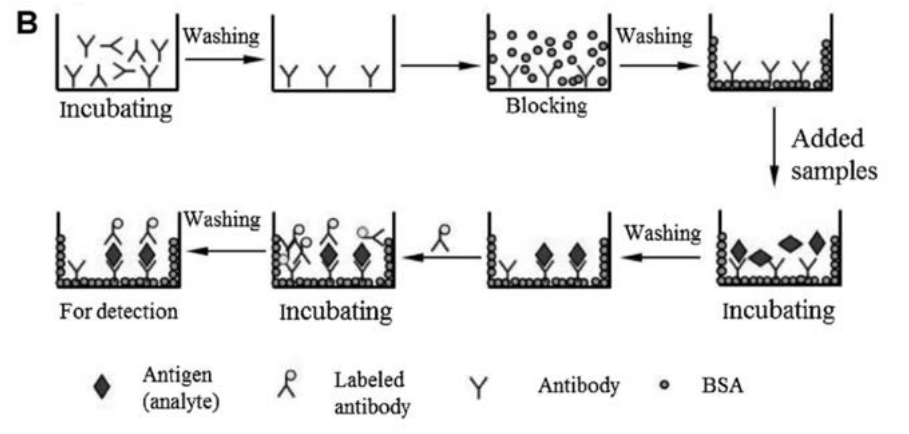
Non-competitive: high MW



- *"Sandwich" assays*
- *Analyte must be big enough to accommodate 2 Ab's*

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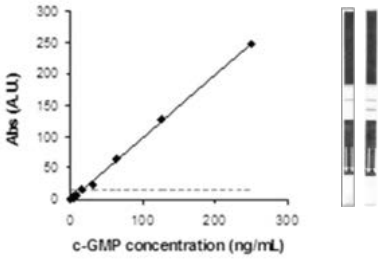
Quantitation in Non-*Competitive* Binding Assays

Low Signal = Low analyte concentration in sample

High Signal = High analyte concentration in sample

Signal



Concentration

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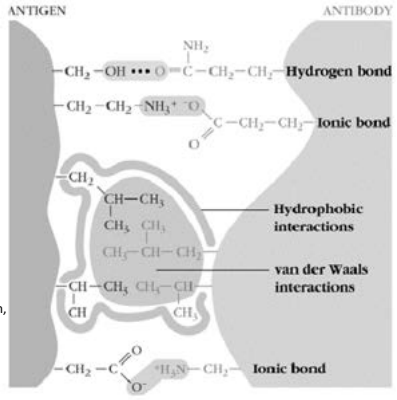
Binding forces

- Non covalent bonds: “weak interactions”
- Several weak bonds must be present: steric complementarity

Figure 1: The noncovalent interactions that form the basis of antigen-antibody (Ag-Ab) binding. [Adapted from Goldby et al. (2000)].



- Few aminoacids involved, surface area of few nm2
- Long range interactions: ionic hydrophobic bonds
- Water expelled
- Short range interaction: van der Waals forces

DOI: 10.1109/CEC.2003.1299409 · Source: IEEE Xplore
Conference: Evolutionary Computation, 2003. CEC '03. The 2003 Congress on, Volume: 4Su Dong Kim, Ki-Roo Shin,Byoung-Tak Zhang

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Binding forces

- Factors affecting binding forces???

- Ionic strength
- pH
- Organic solvents
- Temperature
- Conformational changes

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Extraction

- Simple as possible
- Physical chemical characteristics of target analyte
  - Solubility (hydrophilic hydrophobic)
  - Ch
  - Sta
  - Additives
- Factors affect binding forces!!

Compromise

- Interaction with matrix?
- Homogeneity sample?
- Removal of matrix particles

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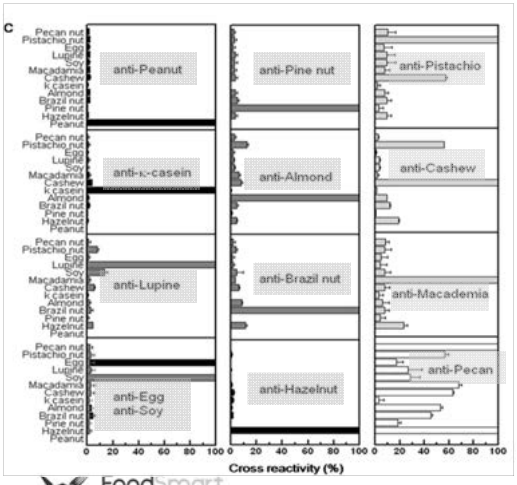
Cross-reactivity



Selective
(CR<1%)

Semi selective
(CR due to common epitopes)

Generic
(CR>10%)

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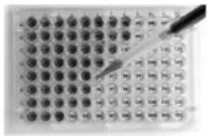
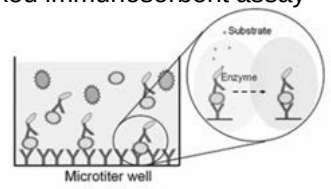
Detection methods
Routine methods

1 allergen

- ELISA- Enzyme linked immunosorbent assay

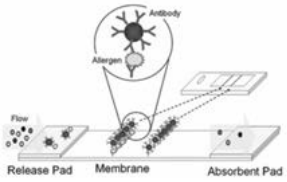




Lab

Batch control

- LFDs-Lateral Flow Devices



On-site

Cleaning control

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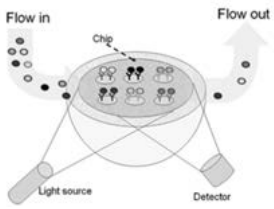
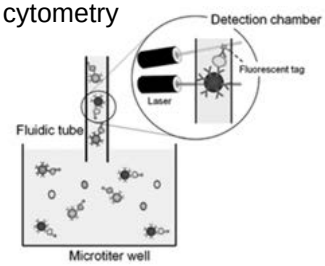
Detection: Screening methods
Multi-allergen methods

- iSPR Imaging Surface Plasmon resonance



- Microsphere flow cytometry



Multiple allergens

Multiplex at-line

fast
simple
cheap

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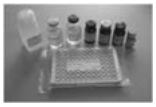
Immunoassay Platforms and goals



lab

Inspection services
Service labs

Biacore

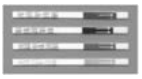
Luminex 100

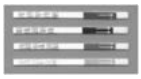
IBIS image

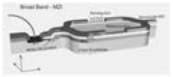
FlexMAP3D

singleplex

multiplex







Interferometer

Imaging SPR

MagPix

Patients

(Small) companies and restaurants

On site

Big companies

Inspection services
Service labs

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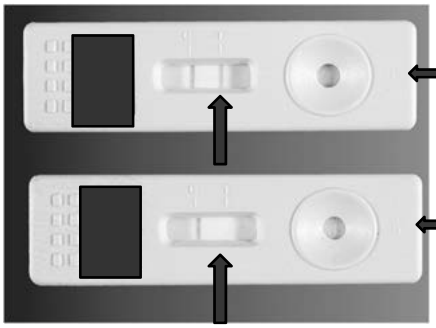
19

Measurement Devices

1.Dipsticks/strip tests/Lateral Flow Devices



Apply Sample

Result

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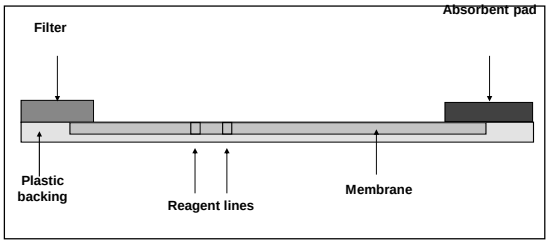
Inside a Lateral Flow Device



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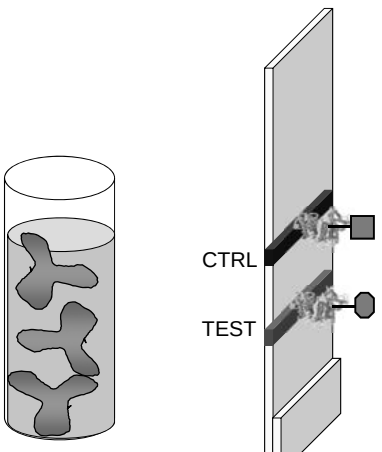
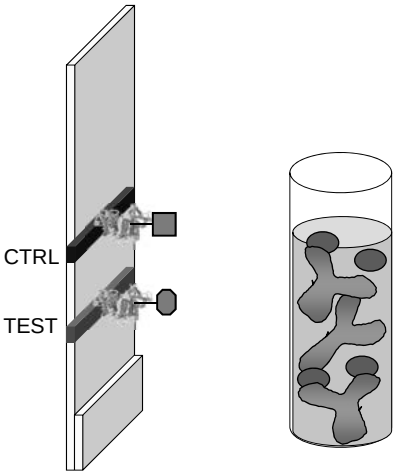
NEGATIVE SAMPLE



POSITIVE SAMPLE

(competition: <u>less labeled Ab available</u> for binding to the test line, **<u>so less color on Test Line</u>**)



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ELISA TESTS

Advantages
- Fast (~90 minutes)
- Low Cost equipment needed
- Simple to use
- Capable of testing high sample numbers
- Sensitive

Disadvantages
- Semi-Quantitative (usually)
- Can generate false positive results
- Can only detect a single analyte or family of analytes

Main Food Analysis Applications
- Mycotoxins in food and feed
- Veterinary drug residues in foods
- Allergens
- Marine biotoxins in shellfish
- Food adulteration
- Speciation

Lateral Flow Devices

Advantages
- Fast (~10 minutes)
- Low Cost
- Simple to use
- Portable

Disadvantages
- Qualitative (usually)
- Designed for testing low numbers of samples
- Sensitivity

Main Food Analysis Applications
- Mycotoxins in food and feed
- Antibiotics in milk
- Marine biotoxins in shellfish
- Food adulteration
- Speciation

Comparison screening methods

Strip test	ELISA (15 k€)	BIACORE (250 k€)	IBIS (90 k€)	LUMINEX (50 k€)	
10 min. 50 €	1-3 h. 200 €	25 min. 15 €	25 min. 15 €	1.5 h. 0.08 €	**1 Analyte 5 Samples**
4 h. 800 €	2-4 h. 1200 €	7 h. 240 €	7 h. 240 €	1.75 h. 1.30 €	**1 Analyte 80 Samples**
20 min. 100 €	2-4 h. 400 €	25 min. 15 €	25 min. 15 €	1.5 h. 0.16 €	**2 Analyte 5 Samples**
2.5 h. 750 €	days 3000 €	3 h. 75 €	25 min. 15 €	1.5 h. 1.2 €	**15 Analyte 5 Samples**
week 1200 €	days 18000 €	35 h. 1200 €	7 h. 240 €	1.75 h. 19 €	**15 Analyte 80 Samples**

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Summerschool- How to speed-up binding assays

Monique Bremer
RIKILT Wageningen University and Research

Monique.Bremer@WUR.NL

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

Kinetics of binding assays

- Biomolecular binding interaction:

$$R + A \underset{k_d}{\overset{k_a}{\rightleftharpoons}} RA$$

Binding is not all or nothing: a portion of A will be bound, a portion will be free

- k_a = association rate constant [$M^{-1}s^{-1}$]
- k_d = dissociation rate constant [s^{-1}]

- *association rate* $d[RA]/dt = k_a[R][A]$
- *dissociation rate* $d[RA]/dt = k_d[RA]$

Introduction to **Binding Assays**
Prof. Michel Nielen
RIKILT, Wageningen University & Research, Wageningen, NL
Prof. Chris Elliott
Queens University of Belfast, Belfast, NI, UK

R = biorecognition element
A = analyte
k_a = association rate constant [$M^{-1}s^{-1}$]
k_d = dissociation rate constant [s^{-1}]
M = Mol per liter

Kinetics of binding assays

- Reaction is in equilibrium when concentrations do not change:

$$d[RA]/dt = k_a[R][A] - k_d[RA]=0$$

- Equilibrium is reached when:

$$k_a[R][A] = k_d[RA]$$

R = biorecognition element
A = analyte
k_a = association rate constant [$M^{-1}s^{-1}$]
k_d = dissociation rate constant [s^{-1}]
M = Mol per liter

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Kinetics of binding assays

- Affinity of the binding: the strength of binding of a single molecule to its ligand. It is typically measured and reported by the equilibrium dissociation constant (K_D)
- Equilibrium association constant:
  $K_A= k_a/k_d=[RA] /[R][A]$ unit: M^{-1}
- Equilibrium dissociation constant:
  $K_D= 1/ K_A=k_d/k_a=[R][A] /[RA]$ unit: M
- In short, the smaller the K_D value the greater the affinity of the antibody for its target.

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Affinity of antibodies

K_D value	Molar concentration (sensitivity)	Affinity
10^{-4} to 10^{-6}	Micromolar (µM)	Most antibodies
10^{-7} to 10^{-9}	Nanomolar (nM)	10^{-9} High affinity antibodies
10^{-10} to 10^{-12}	Picomolar (pM)	Very high affinity antibodies

Applications of different affinities???

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Strong binders and weak binders...

Record holder?

RA = streptavidin-biotin , K_d = 10^{-14} Mol/l

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How to speed-up binding ?

$$R + A \underset{k_d}{\overset{k_a}{\rightleftharpoons}} RA$$

High Ligand coverage ($1.7 - 2.2 \times 10^{10}$ molecules/mm²) ≈1500-2000 interactions of Ab_2- proteins per magnetic particle

by shifting the equilibrium !

$$R + A \overset{k_a}{\rightharpoonup\!\!\!\!\leftharpoondown} RA \quad (k_d)$$

Result: $K_d = 10^{-15}$ Mol/l (instead of 10^{-8})

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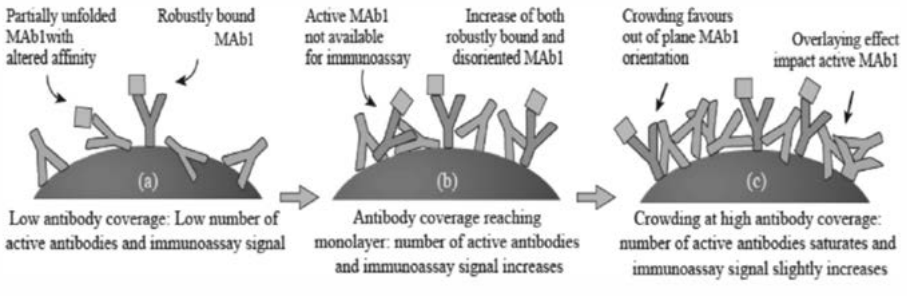
V. Mani et al., Anal. Chem., 84 (2012) 10485-10491

Not too many binders, steric issues!



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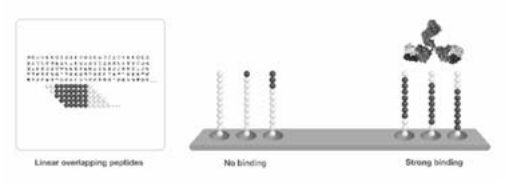
B. Saha et al., Anal. Chem., 86 (2014) 8158-8166

Peptides and conformation epitopes



The target linear sequence is converted into a library of all overlapping linear peptides directly synthesized on a proprietary solid support called "mini-card". Binding of antibodies is quantified using an automated ELISA-type read-out. Constructs containing right amino acid sequence in the correct conformation best bind the antibody.

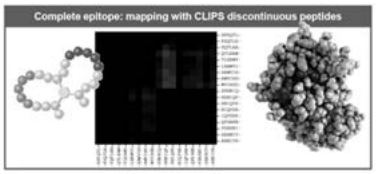


Figure 2: Identification of a secondary binding region of the discontinuous epitope of daratumumab using double-looped combinatorial CLIPS peptides. The binding results are shown as a heat map. Each coordinate on the map combines two protein fragments (shown at the axes). For each combined peptide, the observed binding is plotted on a color scale between back and red. The newly identified region is shown in red on the structure visualisation.

https://www.pepscan.com/precision-epitope-mapping/hisense-linear-epitope-mapping

Mixing and diffusion

- Mass transport limitation to a surface!

$$A_{bulk} \underset{k_m}{\overset{k_m}{\rightleftharpoons}} A_{surface} + R \qquad A_{surface} + R \underset{k_d}{\overset{k_a}{\rightleftharpoons}} RA$$

K_m : mass transfer rate constant

In microtiter well:
mixing: equilibrium is reached in less than 10 min
without mixing: hours to reach equilibrium

diffusion rate limits the rate of the reaction when no mixing is used

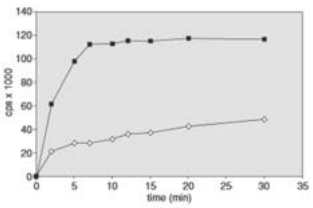


Fig. 4. The effect of mixing on the first incubation in a two-step non-competitive assay of hTSH. 50 µl hTSH standard (9 µIU/ml) and 50 µl of assay buffer was incubated with continuous mixing (■) or without mixing (○) at room temperature. After the first incubation was completed, the wells were washed and 100 µl of labelled antibody solution (200 ng/well) were added to the wells and mixed continuously for a fixed time.

https://www.perkinelmer.com/lab-solutions/resources/docs/APP_DELFIAHowToOptimizeRapid.pdf

Mixing and diffusion

- Mixing is key!

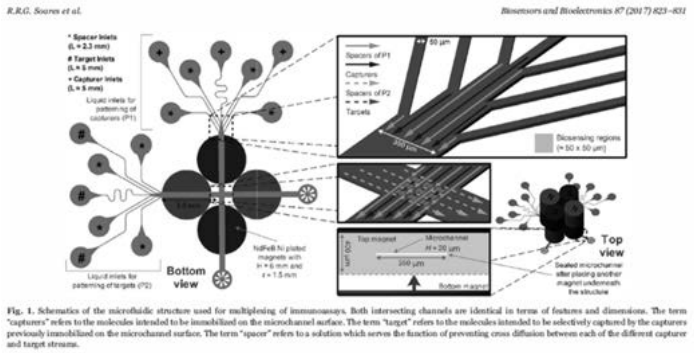


Fig. 1. Schematics of the microfluidic structure used for multiplexing of immunoassays. Both intersecting channels are identical in terms of features and dimensions. The term "capturers" refers to the molecules intended to be immobilized on the microchannel surface. The term "target" refers to the molecules intended to be selectively captured by the capturers previously immobilized on the microchannel surface. The term "spacer" refers to a solution which serves the function of preventing cross diffusion between each of the different capturer and target streams.

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Temperature

- In general: reaction rate increases at least twofold when the temperature increases by 10°C.

- How to deal with it using smartphones??

- Careful of denaturing proteins!

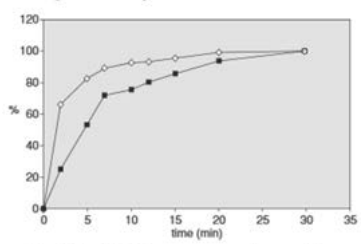

Fig.1. The effect of temperature on the second incu-bation step (labelled antibody) of a two-step non-competitive assay of hTSH. The total assay volume was 100 µl and the tracer concentration 200 ng/well. The standard concentration was 9 µIU/ml. The assays were done at 25° C (■) and 35° C (□), respectively.

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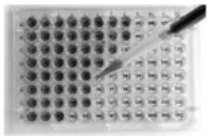
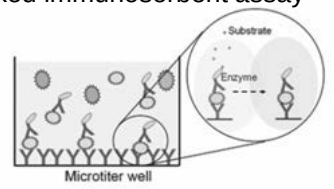
https://www.perkinelmer.com/lab-solutions/resources/docs/APP_DELFIAHowToOptimizeRapid.pdf

Detection methods: singleplex

- ELISA- Enzyme linked immunosorbent assay



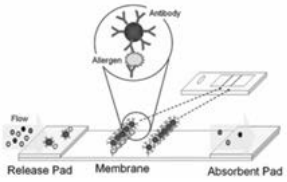


1 allergen

Lab

Batch control

- LFDs-Lateral Flow Devices



On-site

Cleaning control

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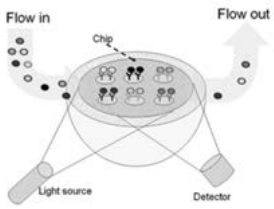
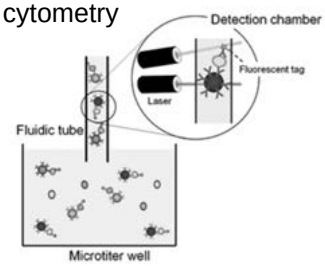
Detection: multiplex

- iSPR Imaging Surface Plasmon resonance



- Microsphere flow cytometry



Multiple allergens

Multiplex at-line

fast
simple
cheap

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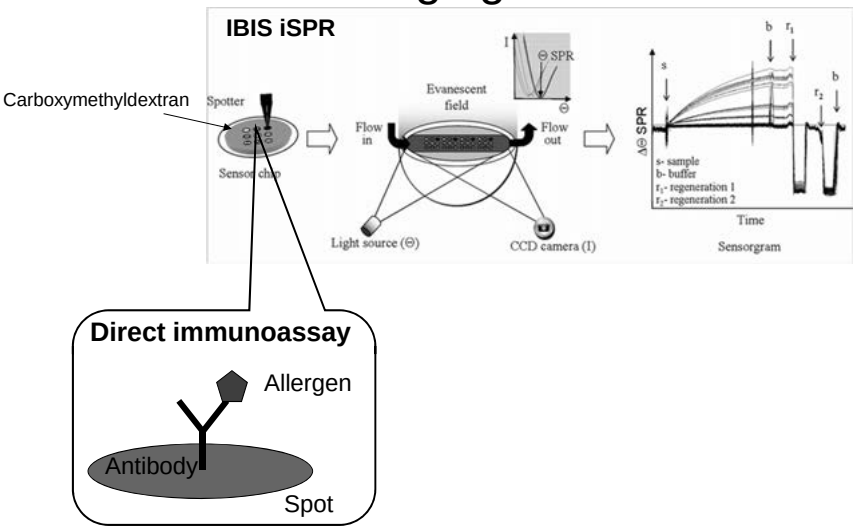
Workflow with imaging SPR



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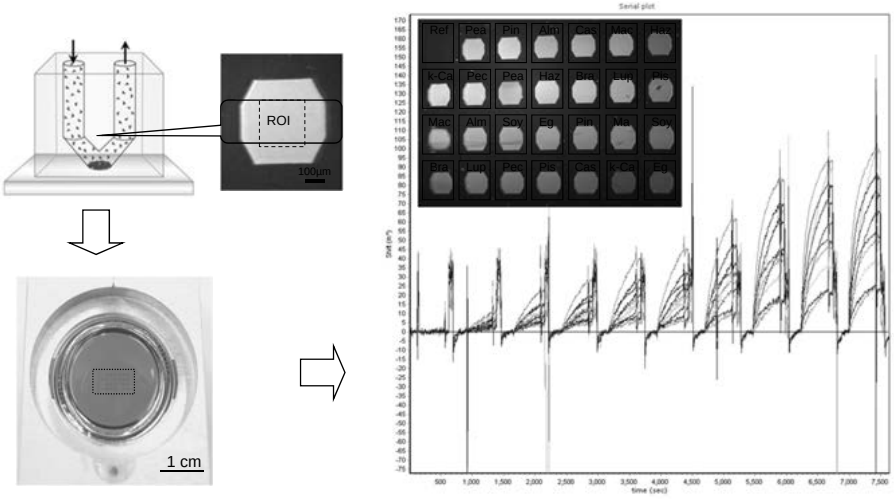
Sensor chip & Sensorgrams



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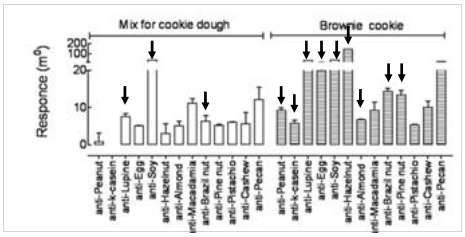
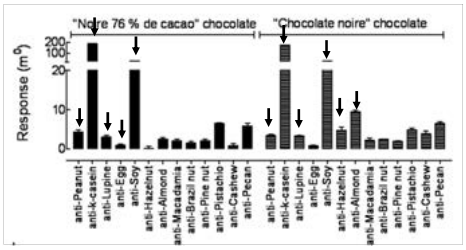
Food samples - profiling



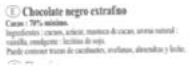


Labels:

Soy

Soy, egg, milk
May contain peanuts

Soy
Traces of egg gluten and milk



Soy
Traces of peanuts, milk, hazelnuts, almonds

Pistache, cashew pecan macademia non specific

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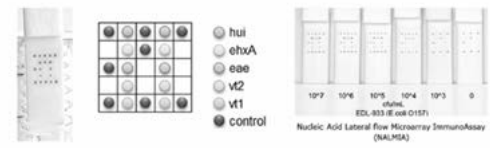
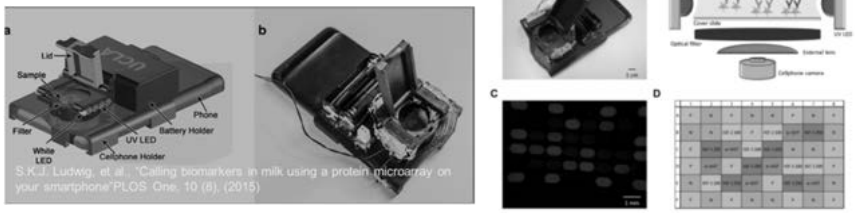
↓ Correctly declared
↓ Not declared
↓ Incorrectly declared

Novel platforms



Aart van Amerongen WUR



S.K.J. Ludwig, et al., "Calling biomarkers in milk using a protein microarray on your smartphone"PLOS One, 10 (8), (2015)

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Comparison screening methods

Strip test singleplex	ELISA singleplex	BIACORE multiplex	IBIS multiplex	LUMINEX multiplex	
10 min.	1-3 h.	25 min.	25 min.	1.5 h.	**1 Analyte 5 Samples**
4 h.	2-4 h.	7 h.	7 h.	1.75 h.	**1 Analyte 80 Samples**
20 min.	2-4 h.	25 min.	25 min.	1.5 h.	**2 Analyte 5 Samples**
2.5 h.	days	3 h.	25 min.	1.5 h.	**15 Analyte 5 Samples**
week	days	35 h.	7 h.	1.75 h.	**15 Analyte 80 Samples**

In the Communication Workshop we will use Kahoot!
Please download the free app on your device before the workshop.

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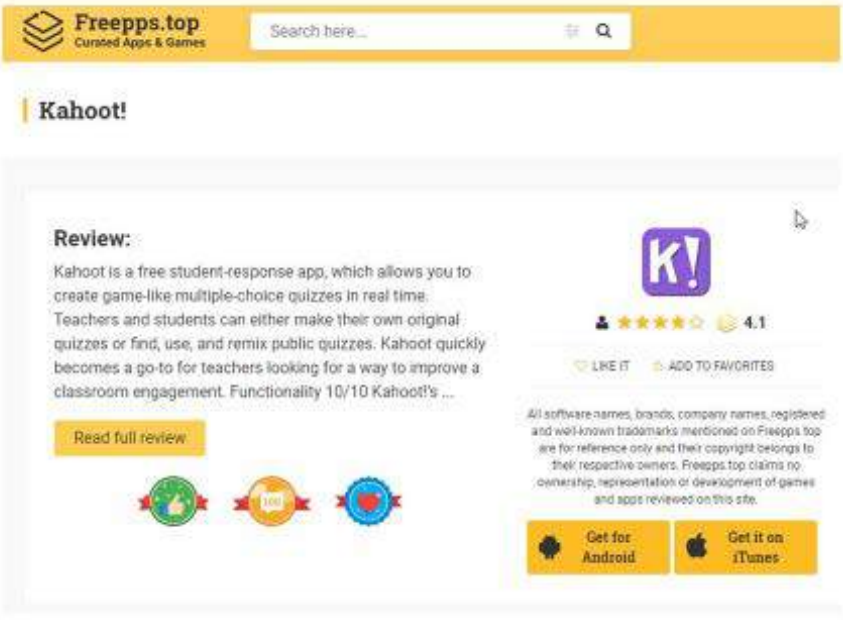
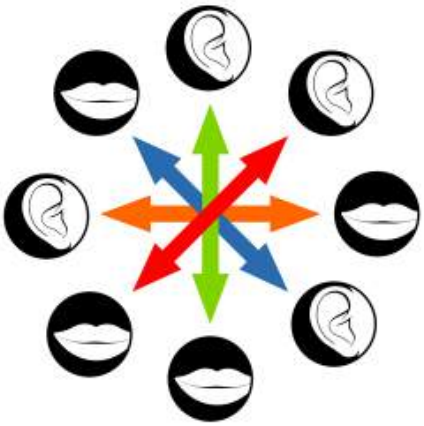


2017 WORKSHOP
CROSS CULTURAL COMMUNICATION



About the trainers

Marcella Bos (1963) is facilitator, trainer and mentor of teams and teamleaders.
Marcella is founder of Bos Matchworks BV (2001), associate partner of Engaging Leadership Group and talent banker at MasterPeace. Marcella is an expert in creating meaningful meetings and events, with a strong focus on the experience of participants. In 2008 she launched the 5 Wheel Drive concept and released the book 'Events en Beleven' in collaboration with Johan Rippen. Before she started her own company Marcella worked in the Banking Industry, in Healthcare, Education and as a Journalist. She studied Social Studies at Hogeschool van Amsterdam.
Marcella is facilitator of teams and their leaders: trainer in personal **GROWTH** and leadership development and organizer of meaningful events

Hanneke van Marle (1977) for many years held, among others, the position of Global Talent Manager at Rabobank International. Nowadays Hanneke shares her experiences and expertise in human resources and human behavior, and she shares her strategic and analytic talent in training courses, masterclasses and workshops, both as trainer and as facilitator.

Marcella and Hanneke both work for Bos Matchworks on various programs about personal leadership, personal and team effectiveness, team connection, flow, group culture and (cross-cultural) communication.

You can contact us via marcella@bosmatchworks.nl

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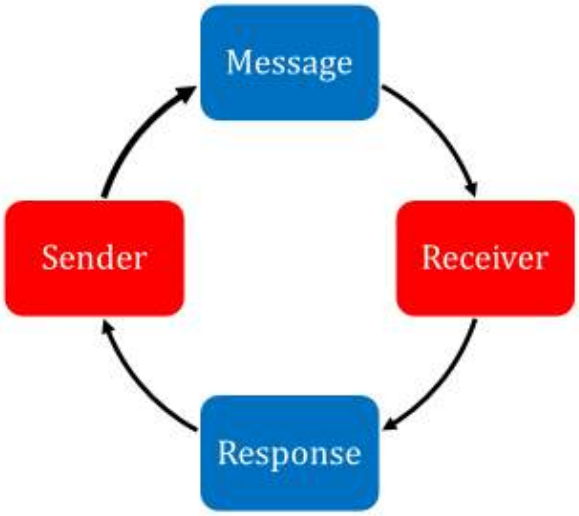
Effective Communication Cycle



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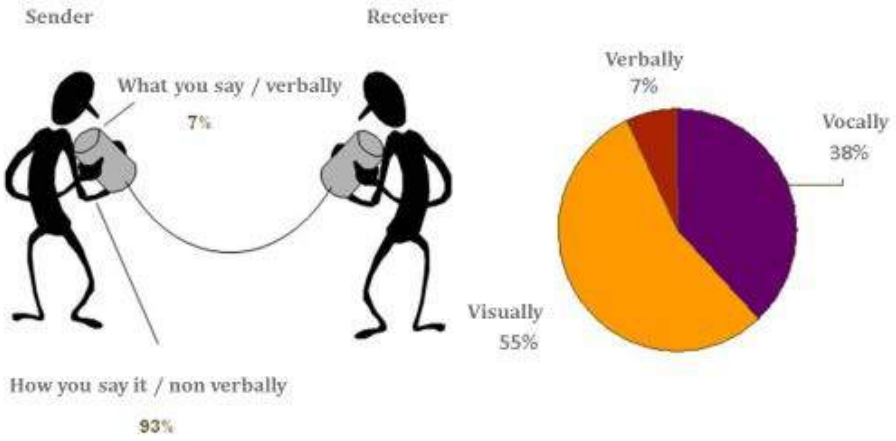
The impact of our communication



8 ways of listening

5

8 ways of listening, described

Pretending
You give the impression that you're listening, say 'yes' and nod every once in a while, but meanwhile you mainly invest in your own thoughts.
Responsive listening
Hearing sound, ignoring contents. You immediately know the solution and answer the other.
Selective listening
You only hear certain things, often what you expected or what you wanted to discuss.
Autobiographic listening
You're listening while filtering everything through your own experiences and paradigms. You hear your own story.
Disputing listening
You only listen to the other to find the weak spots in his argument. As soon as you get the opportunity, you interrupt him/her and rush to explain why you're right.
Attentive listening
You pay close attention to what someone says and allow the words to get through to you.
Reflective listening
You repeat what the other says to show them that you've heard them. The risk is that this may become a technique of copying.
Empathic listening
You're listening with the intention to understand the other. You suppress your own judgment, answers, solutions and interests. You try to put yourself in the shoes of the other. You ask questions until you truly understand the other.

6

The Lewis Model © Richard D.Lewis

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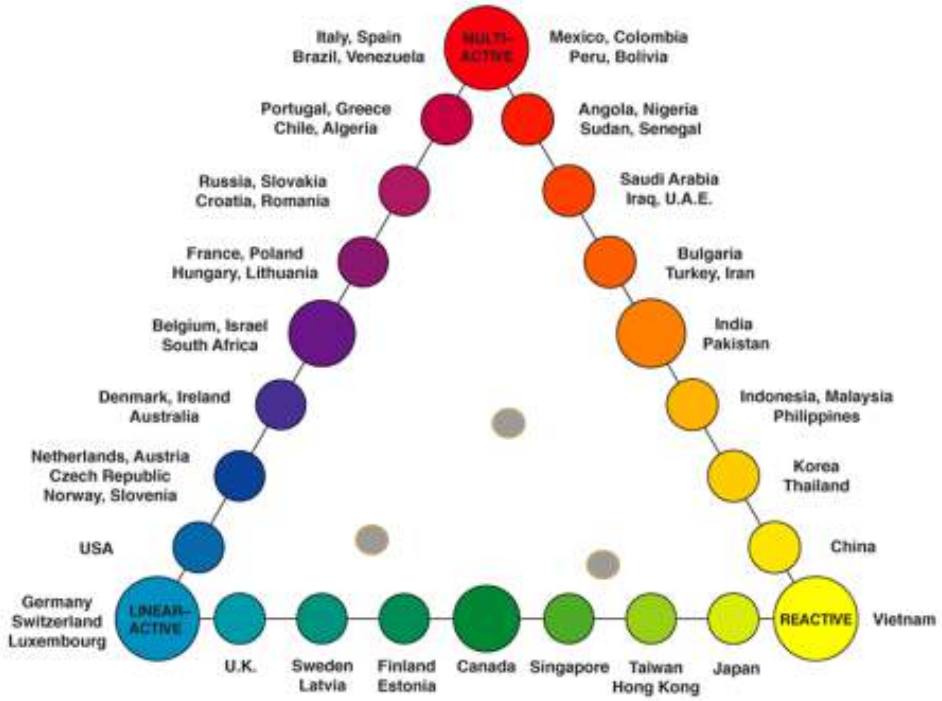

The Lewis Model © Richard D.Lewis

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Chief characteristics of the three categories

Linear-active	Multi-active	Reactive
Talks half the time	Talks most of the time	Listens most of the time
Does one thing at a time	Does several things at once	Reacts to partner's action
Plans ahead step by step	Plans grand outline only	Looks at general principles
Polite but direct	Emotional	Polite, indirect
Partly conceals feelings	Displays feelings	Conceals feelings
Confronts with logic	Confronts emotionally	Never confronts
Dislikes losing face	Has good excuses	Must not lose face
Rarely interrupts	Often interrupts	Doesn't interrupt
Job-oriented	People-oriented	Very people-oriented
Sticks to facts	Feelings before facts	Statements are promises
Truth before diplomacy	Flexible truth	Diplomacy over truth

Lineair Active people like the questions
What? When? How many?....

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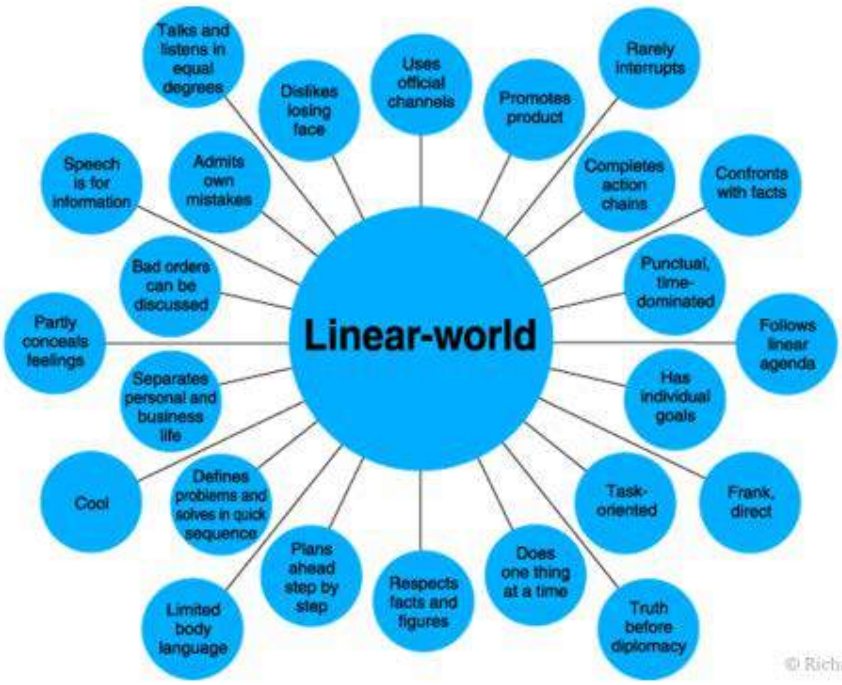
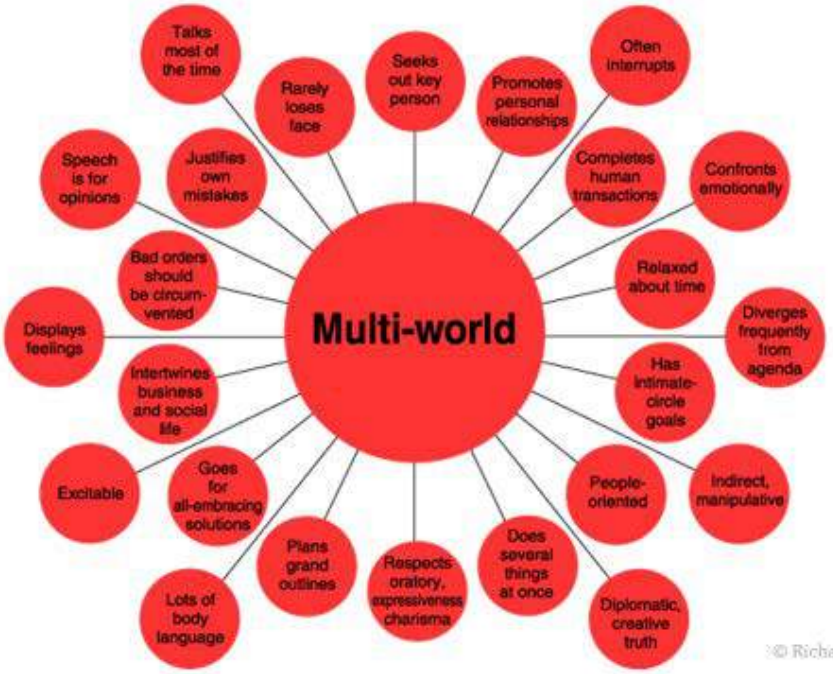


Multi Active people are creative and interested in
How people communicate and **relate** to each other



Reactive people will be convinced by
Who says it, and their authority, experience and expertise

Introduction Surface Modification

From non-interacting to romantic surfaces

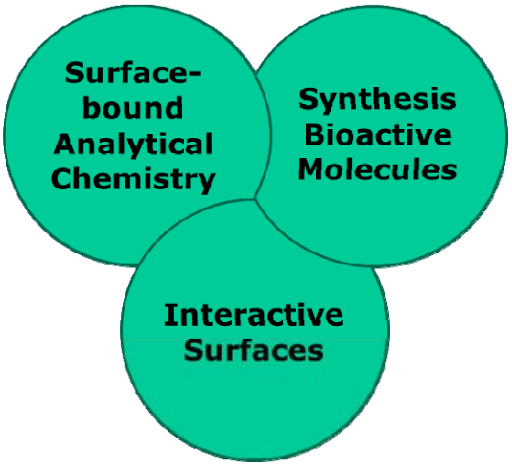
Han Zuilhof

Laboratory of Organic Chemistry
Wageningen University

Novel, antifouling surfaces & Novel surface analysis technique

Bio-organic Surface Chemistry

Surface-bound biological moieties find increasing use – however, resulting surfaces are highly complex



Organic Surface Chemistry

Couple relevant properties of substrate
(fluorescence / robustness
non-toxicity / conductivity)

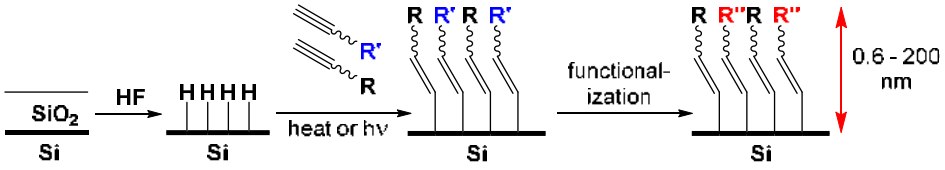
via covalent bonds

to tailor-made functionality
(biological activity / surface conductivity/ ...)

⇒ Novel attachment chemistry (new substrates, milder, faster)
⇒ Extending possible functionalities
(Al_2O_3, CrN, Si, SiC, PtOx, SiN, ITO, …)

General review on SAMs:
Angew Chem Int Ed. **2014**

Covalently Bound Coatings



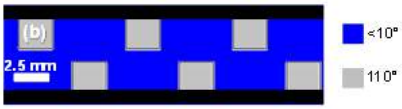
Review:
Langmuir **2012**, 9920
Most recent improvement:
JACS **2017**, ASAP

Three basis roles:
- Functional Monolayers
- Primer layers
- Micrometer-thick coatings

Challenge: Smart coatings that combine antifouling & sensing properties

Functional Surfaces
One molecule at a time....

Pushing the limits:
functional surfaces of just
1 molecule thick!

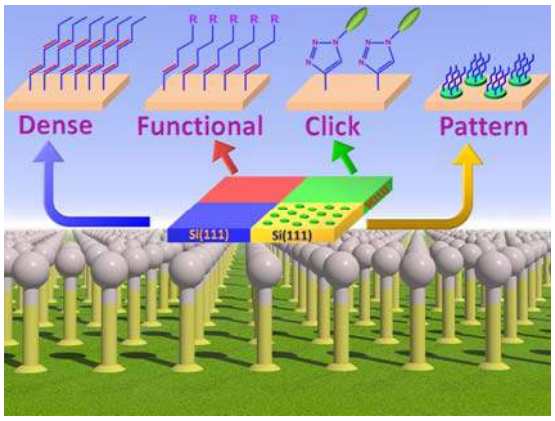


Example:
1.5 nm monolayer can
direct 100,000 nm flow!

⇒ Can we also stop fouling by thin layers?

Langmuir **2016**, 2389

Organic Monolayers: Relate to them!



The Chemical Marriage:

"make surfaces beautiful,
develop strong bonds,
look at them in detail
& put them to work"

Zuilhof and co-workers:
Invited Feature Article *Langmuir* **2012**, 9920.

Romantic or Slippery Surfaces

Long-term stability

Specific Recognition

Bao Bao & her mom

Romantic or Slippery Surfaces

Long-term stability

Specific Recognition

Bao Bao & her mom

Versus: Optimized Non-contact:
(bonus material slides 45-51:
Self-healing antifouling Teflon-like surfaces)

Click Chemistry for Surface Functionalization

Ideal reactions: 1) take two mild reagents
2) mix them at room temperature in water
3) go away and drink some coffee
4) done!

⇒

Simple, well/chosen building blocks

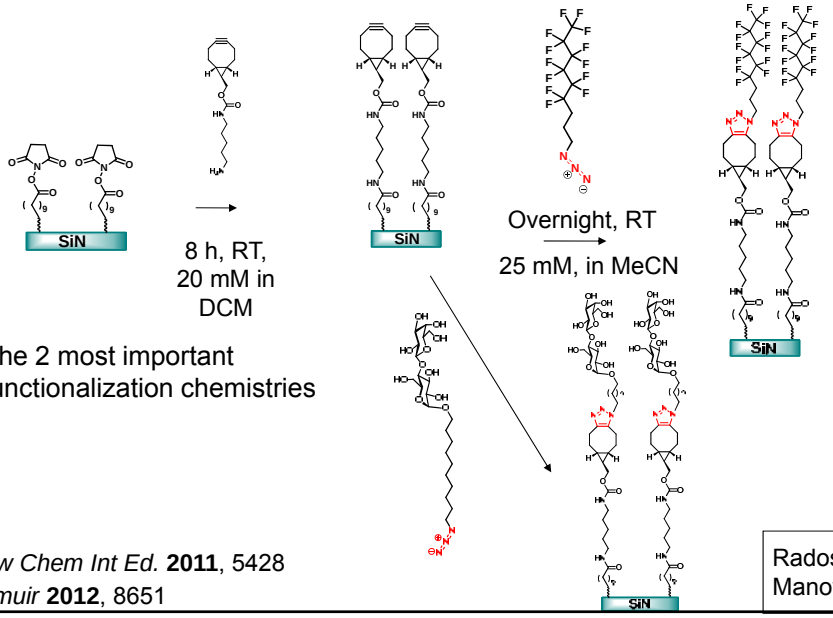
Complex structure that can perform a job

Typically: 2-component addition reactions: azide-alkyne
thiol-ene, thiol-yne
SuFEx chemistry

Angew Chem Int Ed **2011**, 5428
Adv Mat Interfaces **2015**, 1500135

Complex Functionalization of Surfaces

Activated Ester & Copper-free Click Chemistry



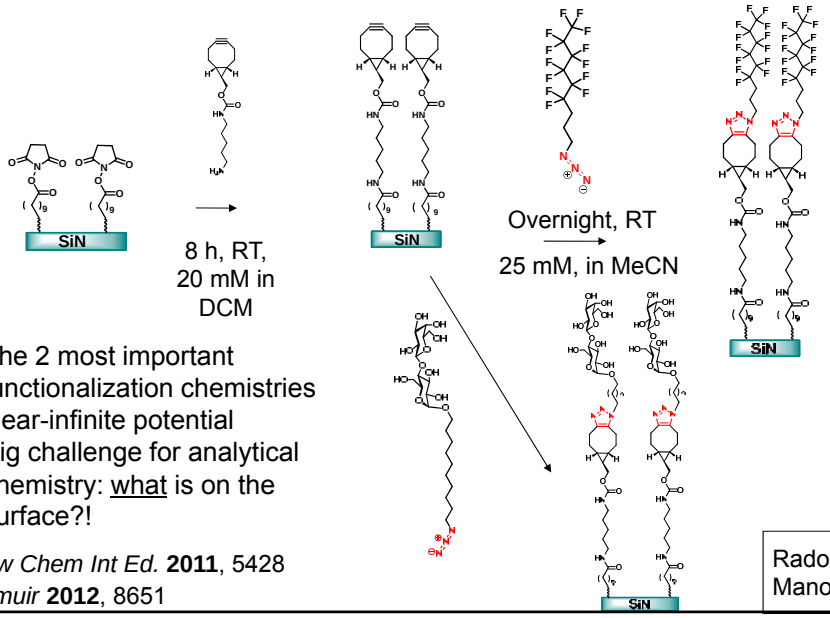
- The 2 most important functionalization chemistries

Angew Chem Int Ed. **2011**, 5428
Langmuir **2012**, 8651

Radostina Manova

Complex Functionalization of Surfaces

Activated Ester & Copper-free Click Chemistry



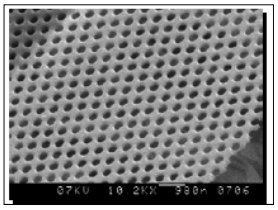
- The 2 most important functionalization chemistries
- Near-infinite potential
- Big challenge for analytical chemistry: what is on the surface?!

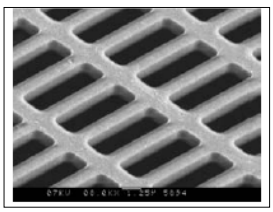
Angew Chem Int Ed. **2011**, 5428
Langmuir **2012**, 8651

Radostina Manova

Use of Si_xN_4: Advanced Filtration & Capture

Functionalization to Control Surface Properties

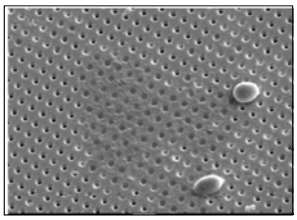




Microsieves

high-flux filtration devices

Ai Nguyen

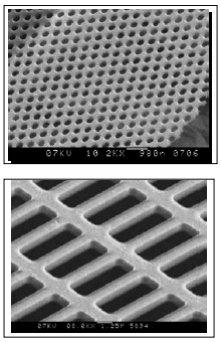


Biofouling serious problem
⇒ protein-repelling coating required

Bedacht door
WUR-hgl
Cees van Rijn
☺

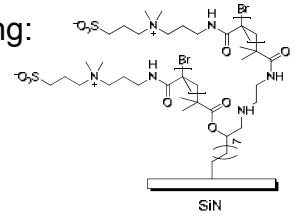
Zwitterionic Polymer Brushes

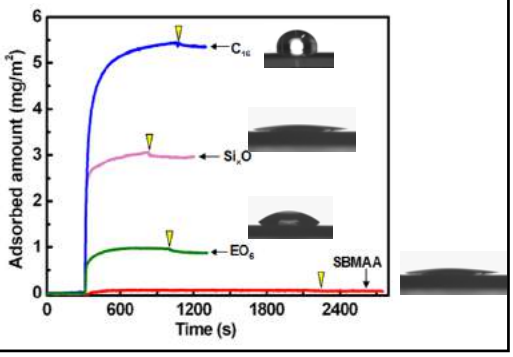
Surface Functionalization for Protein Repellence



Microsieves

Polar zwitterionic coating:
Highly polar,
Highly stable,
high protein repellence!

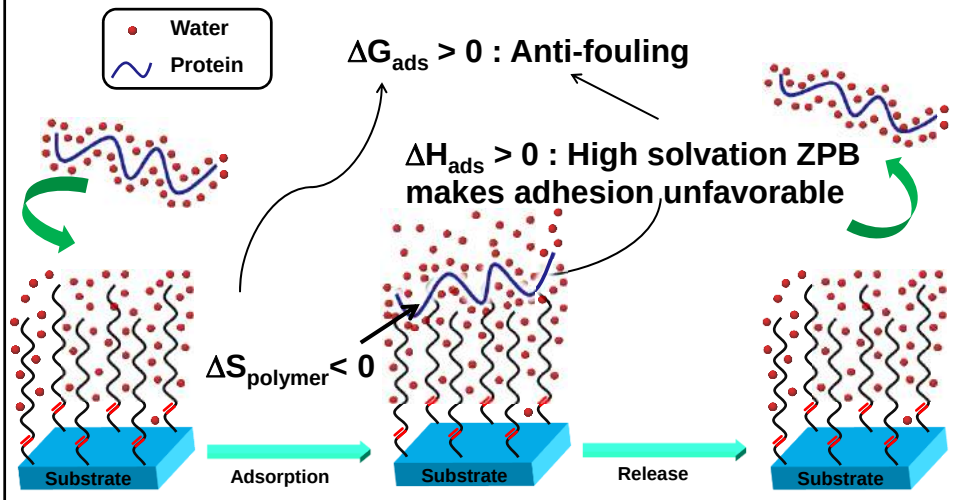




Ai Nguyen *Langmuir* **2011**, 2587

Anti Polymer-Fouling Coatings

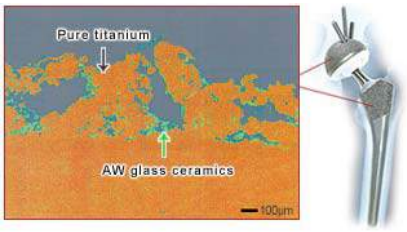
Mechanistic Considerations behind succes of ZPB

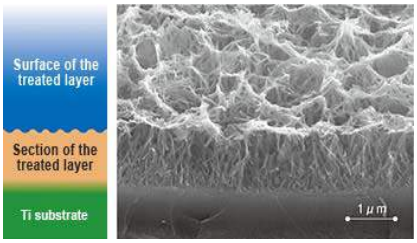


Upon adsorption: loss of $\Delta S_{polymer}$, loss of solvation $\Delta H_{polymer}$ & ΔH_{brush} (not much change ΔS_{brush}) $\Rightarrow$ only possible compensation: $\Delta H_{polymer\text{-}brush}$

Use of Zwitterionic Polymer Brushes

Non-Immunogenic Implants

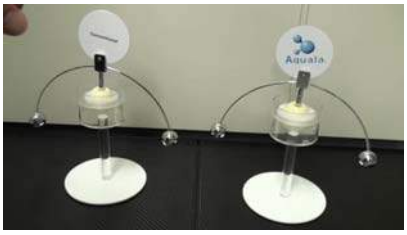




Surface of implants:

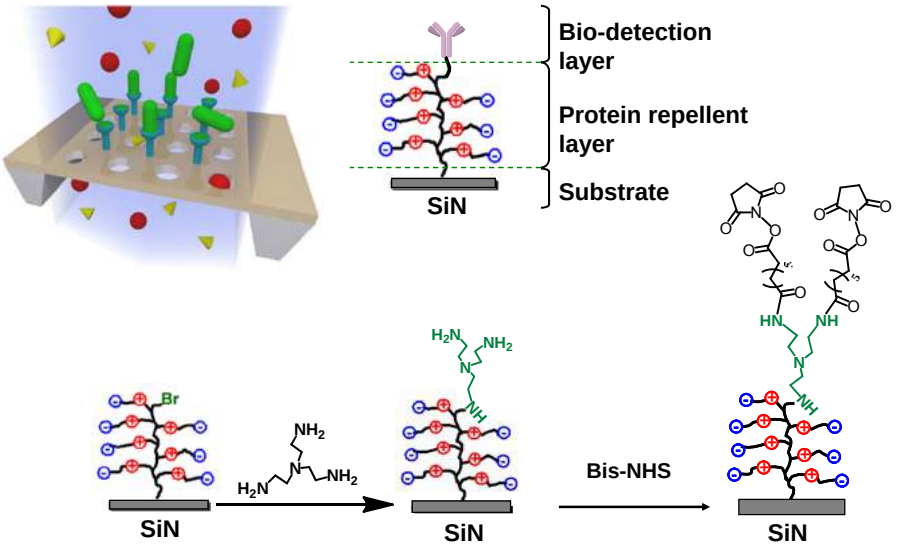
- porous, to facilitate deep penetration of living bone tissue & enhance binding
- coated with bio-active ZPB to reduce immune response (body sees 'just water')

Aquala® Zwitterionic Polymer-coated Implants



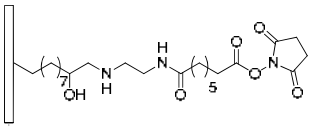
150 nm ZPB yields significantly reduced friction

Abs on SiN: Efficient Bacterial Detection

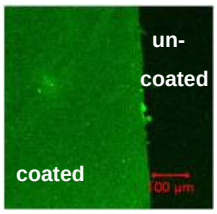


Efficiency of protein capture: Two routes

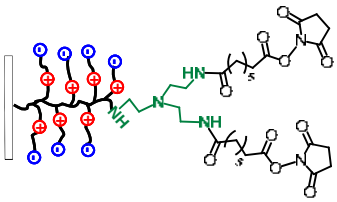
Epoxide-derived NHS-monolayer SiN



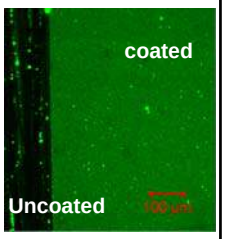
1) H_2N~Biotin
2) Steptavidin~Fluorophore



NHS-Zwitterionic-polymer SiN



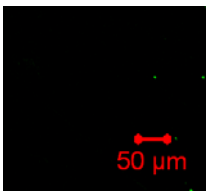
1) H_2N~Biotin
2) Steptavidin~Fluorophore



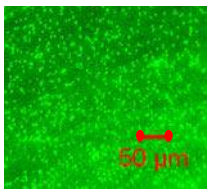
⇒ Both approaches yield effective coupling to protein of choice..
.... but is it True Love ("binding to one above anyone else...")??

Salmonella detection without blocking buffer

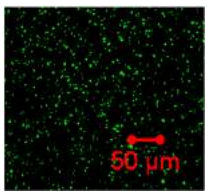
Binding of fluorescent salmonella via abs on surface



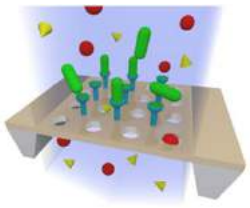
Control: Bare SiN



SiN - linker - antibody



SiN - SBMA -linker - antibody



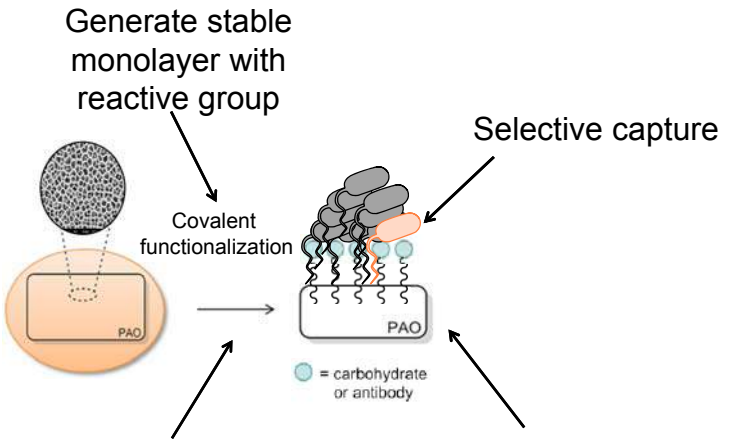
Langmuir **2012**, 604

⇒ specific detection with minimal background noise

⇒ stellar performance ☺

Selective Capture of Bacteria on Porous Al_2O_3

Sugar-coated, Bacteria-recognizing Surfaces



⇒ goal: immobilize only desired bacteria for detection or bio-catalysis

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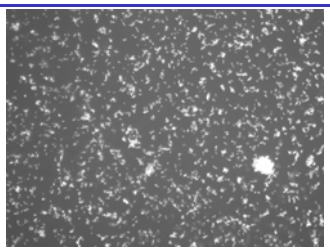
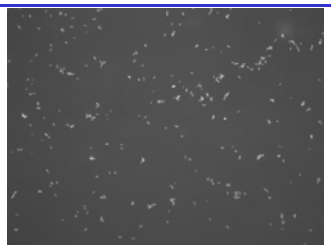
Selective Capture of Bacteria on Porous Al_2O_3

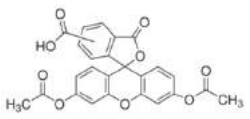
OH termination: non-Bacteria-recognizing Surface



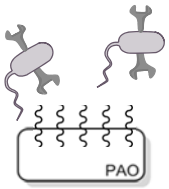
OH – before washing – 200x
11.8% colored surface

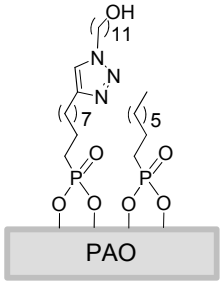


OH – after washing – 200x
1.6% colored surface



Bacteria stained with cFDA

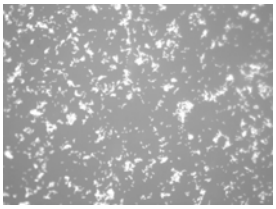




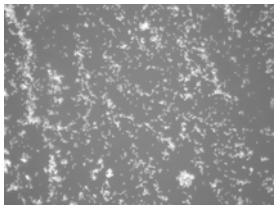
20

Selective Capture of Bacteria on Porous Al_2O_3

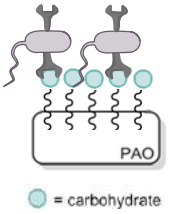
Mannose termination: Bacteria-recognizing Surface

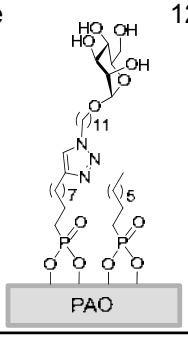


Man – before washing – 200x
13.3% colored surface



Man – after washing – 200x
12.6% colored surface

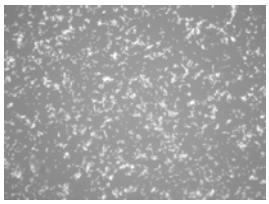




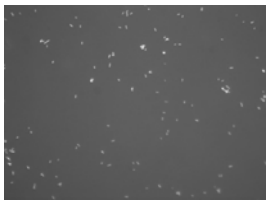
Langmuir **2014**

Selective Capture of Bacteria on Porous Al_2O_3

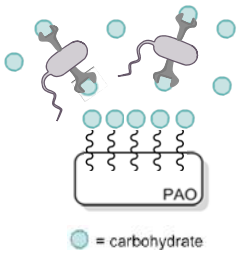
Mannose Competition: On-Surface & in solution

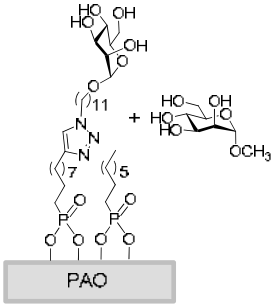


Man@surf – before washing
13.9% colored surface



Man@surf – after washing with Man sol
0.7% colored surface





Sweet surface recognizes & binds bacteria
& releases with mild trigger as well!

Langmuir **2014**

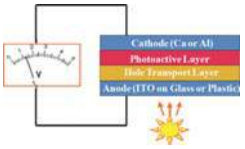
Biofunctionalization on any surface?!

Romance on Indium Tin Oxide (ITO)

- Optical transparency
- Electrical conductivity
- Biocompatible

LCD displays

Solar cells



- **Stable functionalization for life science applications?!**
- **Interaction point for ´smart ITO-based electr0n1cs'**

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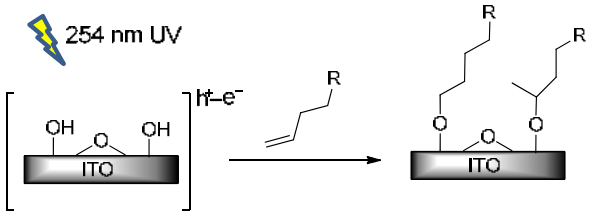
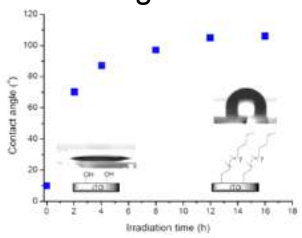
Photochemical Grafting on ITO Substrate

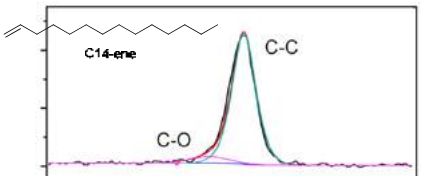


Water contact angle: 15º → 103º



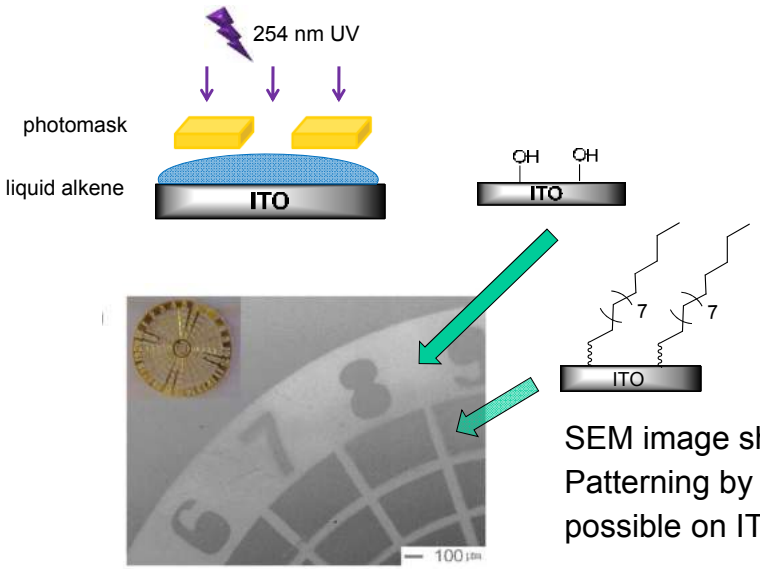
⇒ gradual formation of 3 -10 nm hydrophobic organic layer

XPS Modified ITO: C1s



⇒ **1** C atom from **C14-ene** bound to surface: C−O−In(Sn) bond formed!

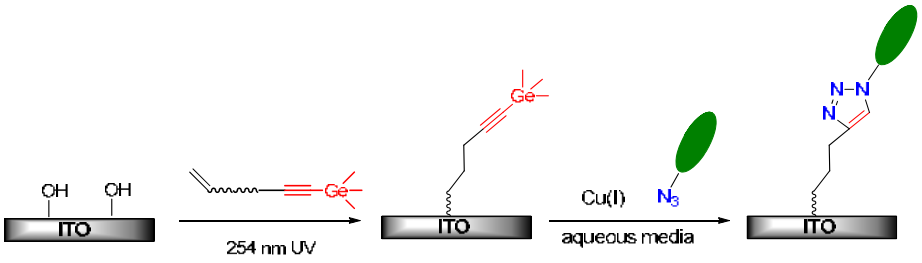
Photochemical patterning on ITO Substrate



SEM image shows:
Patterning by light possible on ITO!

25

"Clickable" Organic Layer for Further Functionalization (I)



"One-pot" deprotection/click reaction!

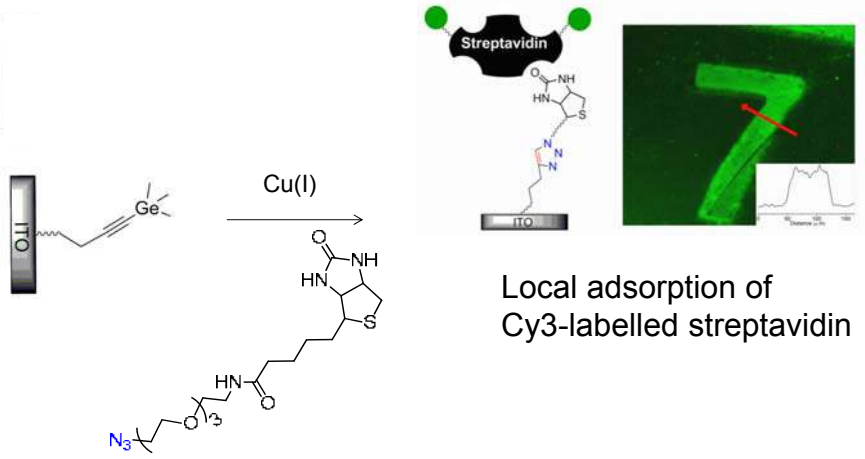
Ideal alkyne protecting group:
- survives during UV irradiation
- displays distinctive signal in XPS

26

Langmuir **2012**, 5350

Yan Li

"Clickable" Organic Layer for Further Functionalization (II)



Local adsorption of Cy3-labelled streptavidin

Langmuir **2012**, 5350

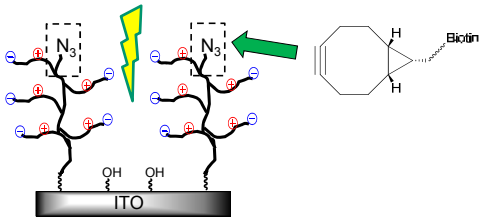
Top-functionalized Zwitterionic Polymers on ITO
Looking for the Right One!

<u>Overall aim:</u>

Combine
generic protein repellence
& specific bioaffinity

⇒ Top-functionalized Zwitterionic Polymers

Light-induced patterned functionality



Top-functionalized Zwitterionic Polymers on ITO
Looking for the Right One!

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⇒ Top-functionalized
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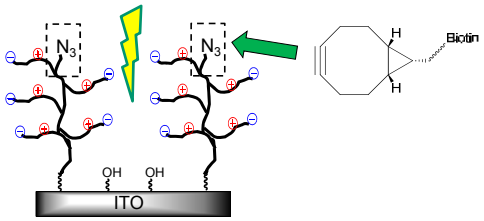
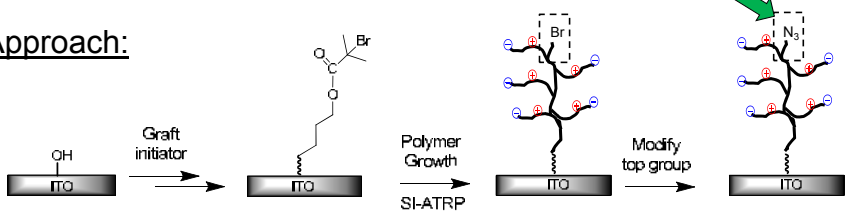
Light-induced patterned functionality



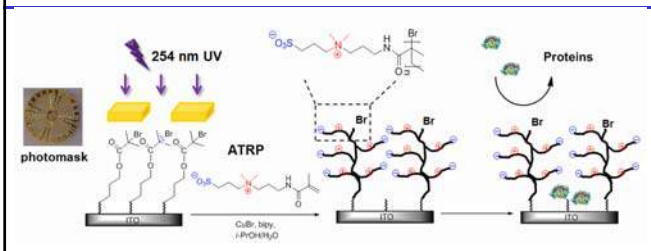
Handle for SPAAC reaction
& further biofunctionalization

<u>Approach:</u>



29

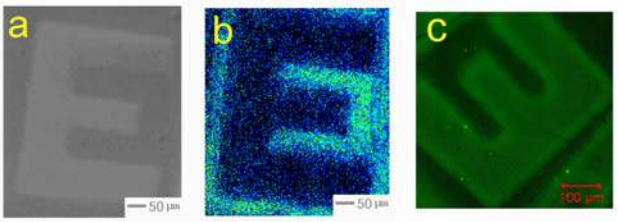
Step 1: Patterning Zwitterionic Polymers on ITO



Photomask

Letter 'E':
No polymer

Outside of 'E':
Polymer



SEM

N mapping with Auger spectroscopy

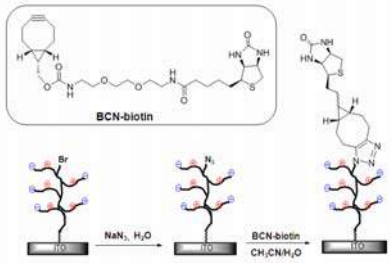
Nonspecific adsorption of FITC-BSA (not on polymer!)

Yan Li

Langmuir **2012**, 12509

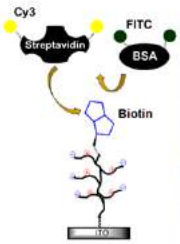
Step 2: Biological Push-Pull Games on ITO

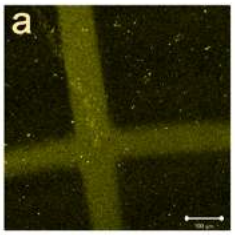
Cu-Free Click Functionalization Top of Polymer Brush



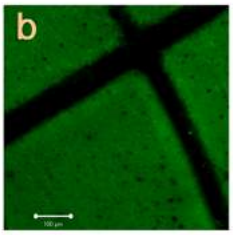
looking for the Right One: strictly monogamous interactions:

Patterned polymer attracts 1 protein & repels all other





Streptavidin: on cross



BSA: exclusively outside cross

Langmuir **2012**, 12509

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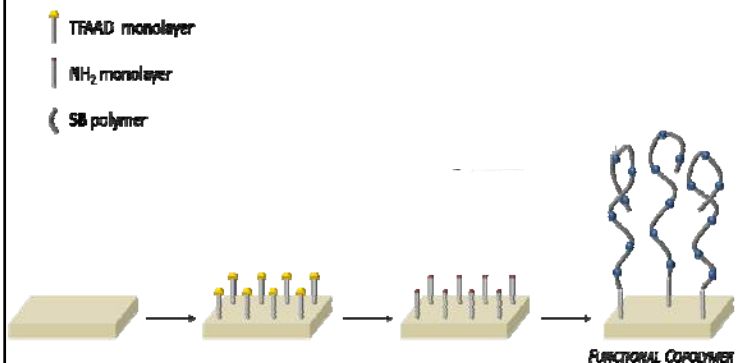
Towards 3D Romance

Polymer brush: “cooked rather than uncooked spaghetti” ⇒
With increasing thickness polymer end less available ⇒
3D approach

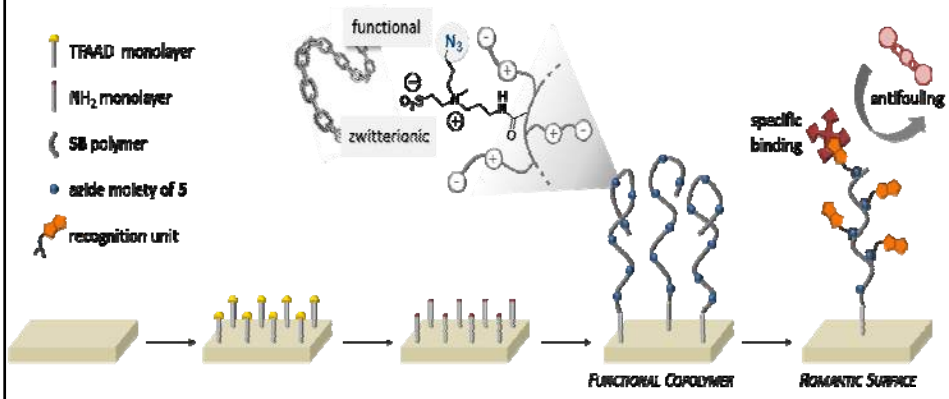


Stefanie Lange
Esther van Andel

33

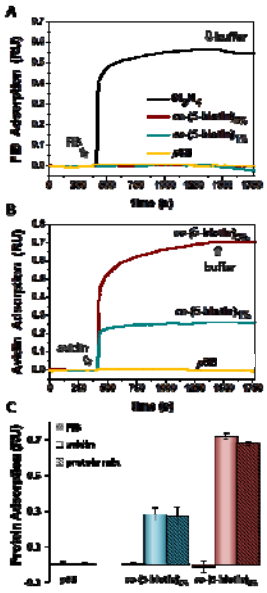
Towards 3D Romance

Ideal antifouling polymer: - all monomers zwitterionic
- # available functional groups known



34

Step 3: Thickness-Independent Romance



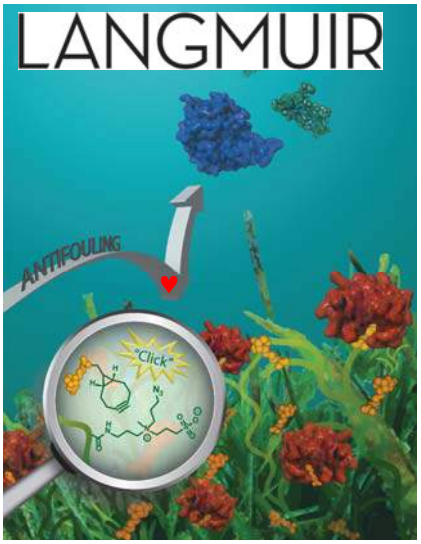
With few % functional monomer:

- Excellent antifouling of zwitterionic brushes retained
- Efficient capture of target….
- ….also in protein mixture

35

Clickable Brush: Mode of Presentation

versus



Esther van Andel

♥ Cover Issue #40

♥ ACS Editor's Choice

Langmuir **2016**, 10199

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Self-healing Antifouling Surfaces

Concept Generation 1

Zwitterionic Polymer Brushes:
Excellent antifouling
Tailor-made functionality
But:
How to repair?

?

Chemical damage of top
Mechanical scratch
….

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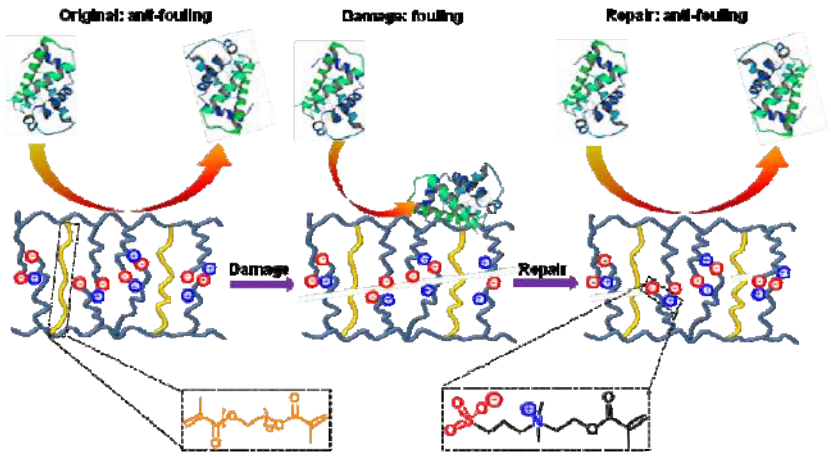
Self-healing Antifouling Surfaces
Concept Generation 1



Zwitterionics: antifouling & self-interacting;
cross-linker for stability

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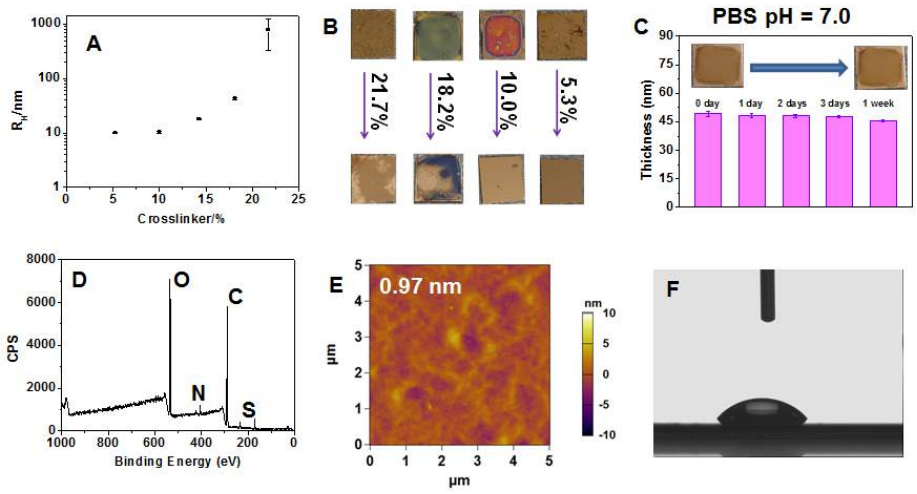
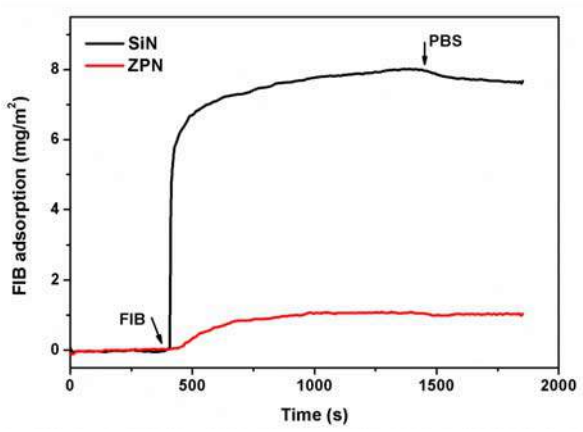
Self-healing Antifouling Surfaces
Composition



14.3% cross-linker: stable & flat, wetting films

39

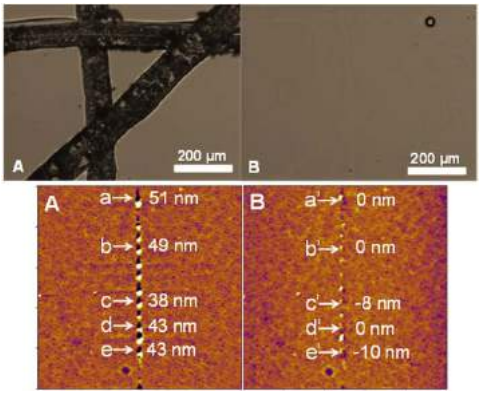
Self-healing Antifouling Surfaces
Antifouling



Good antifouling characteristics
(but not yet excellent; more generations needed...)

40

Self-healing Antifouling Surfaces
Self-repair



Optical & Tapping Mode AFM images of ZPN coatings.
(A) mechanically damaged and
(B) after immersion into water for 1 min and drying by argon

Also: recovery of mechanical strength (not shown here)

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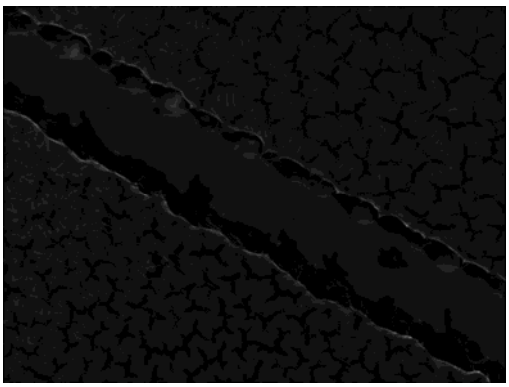
Self-healing Antifouling Surfaces

Self-repair in real time: Just add water…



Water-induced self repair only takes minutes!

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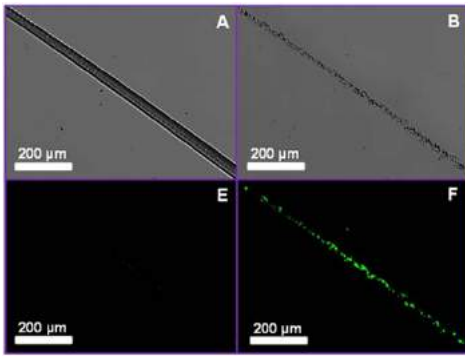
Self-healing Antifouling Surfaces

Self-repair also recovers antifouling character!

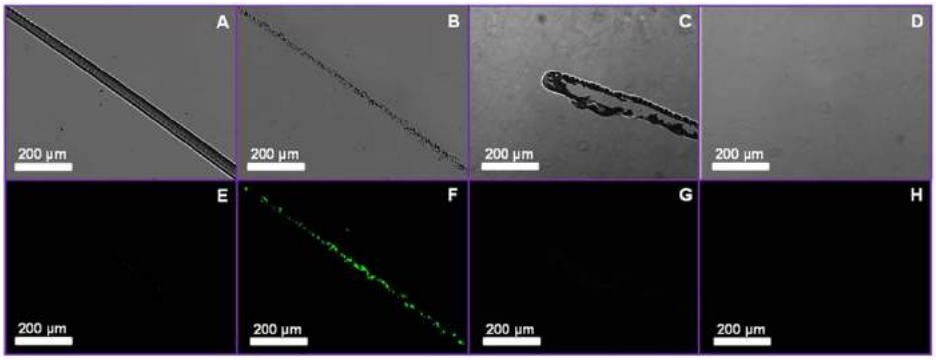


A/E: sample with scratch placed in soln with fluorescent protein ⇒ biofouling ⇒ fluorescence along damaged scratch (B/F)

43

Self-healing Antifouling Surfaces

Self-repair also recovers antifouling character!



A/E: sample with scratch placed in soln with fluorescent protein ⇒ biofouling ⇒ fluorescence along damaged scratch (B/F)

C/G: different sample with scratch; first repair (1 min in water), then placed in soln with fluorescent protein ⇒ D/H: no biofouling/ fluorescence ⇒ self-repair recovered antifouling character!

Take-home messages

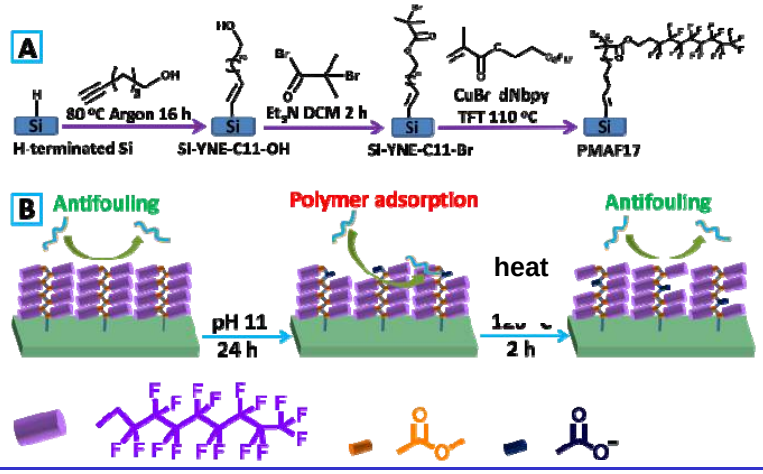
1) Increasing number of chemical tools available for tailor-made surface functionalization
2) Surface analytical chemistry faces interesting challenges (do NOT believe the cartoons!)
3) Self-healing Coatings: Not perfect, but pretty good
4) Romantic Surfaces: Combining Generic Protein Repellence with Specific Bio-Affinity
5) Materials get smarter & smarter, e.g. inclusion of self-healing properties.

Acknowledgements

Zhanhua Wang
Cees van Rijn
Michel Nielen
Maarten Smulders

Fluoro-Polymer Brush: Polymer Antifouling Properties?!

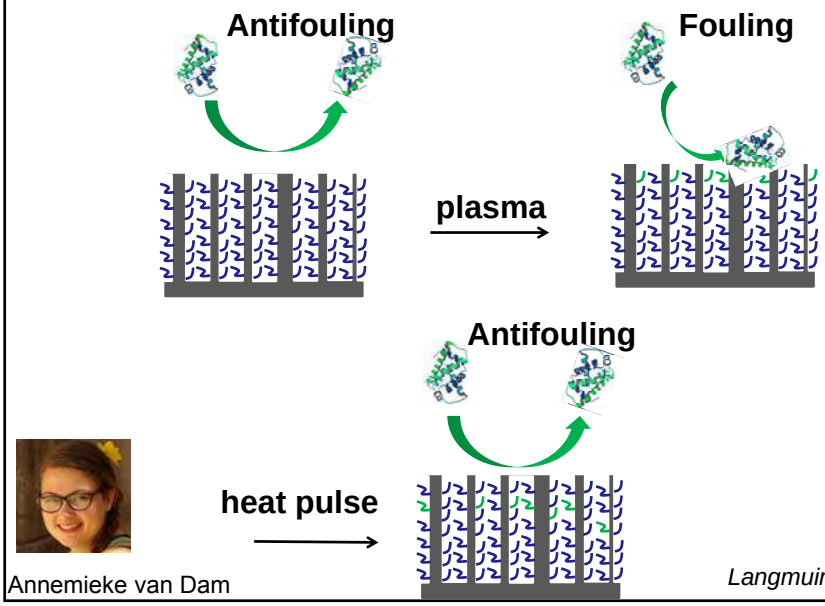
Grow polymer coating – antifouling – damage ⇒ still antifouling?



Goals: better anti-fouling, improved stability & self-healing

Self-healing superhydrophobic anti-fouling surfaces

Combine chemistry & nanostructuring

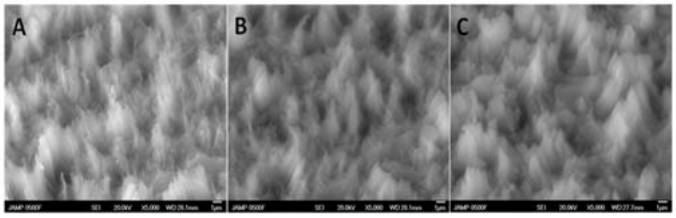


Annemieke van Dam

Langmuir **2016**, 6310

Self-healing superhydrophobic anti-fouling surfaces

1) Prepare Nano-rough surface (here: Si, with Ag etching)



2) Grow polymers from there.

Self-healing superhydrophobic anti-fouling surfaces

3) Self-healing superhydrophobicity:
Top row: just in air

Si	Flat Si with **F17**-brush	Ns-Si with **F17**-brush	Ns-Si with **F17**-brush after O_2 plasma	Ns-Si with **F17**-brush after O_2 plasma & repair

Self-healing superhydrophobic anti-fouling surfaces

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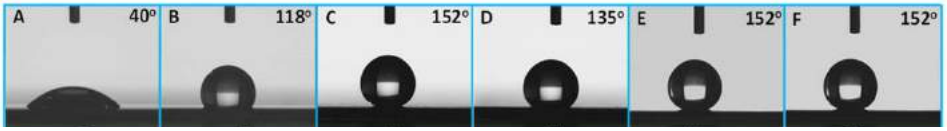

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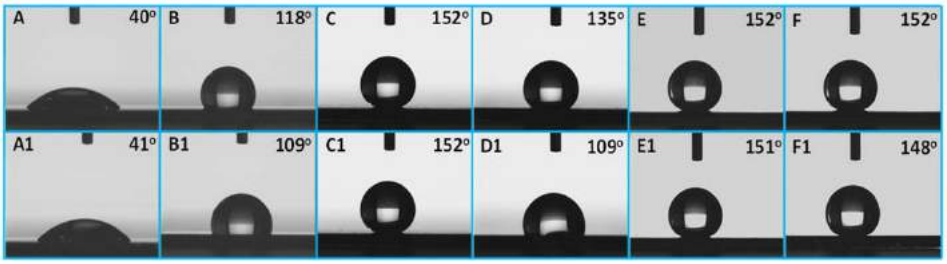
Self-healing superhydrophobic anti-fouling surfaces

3) Self-healing superhydrophobicity:
Top row: just in air
Bottom row: dip in protein-containing solution, rinse & measure



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Self-healing superhydrophobic anti-fouling surfaces

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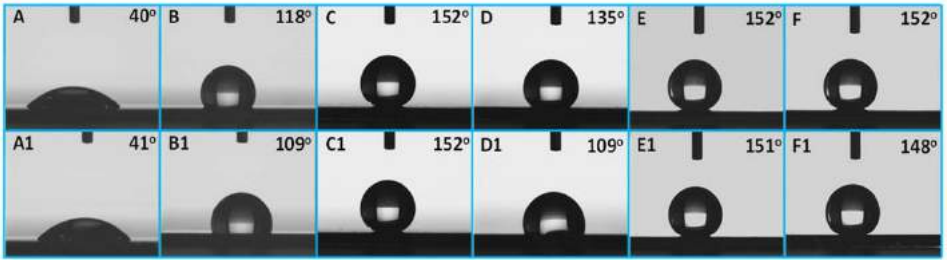

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Langmuir **2016**, 6310

AQUAMARIJN
FUTURE TECHNOLOGY, ENGINEERED FOR NOW

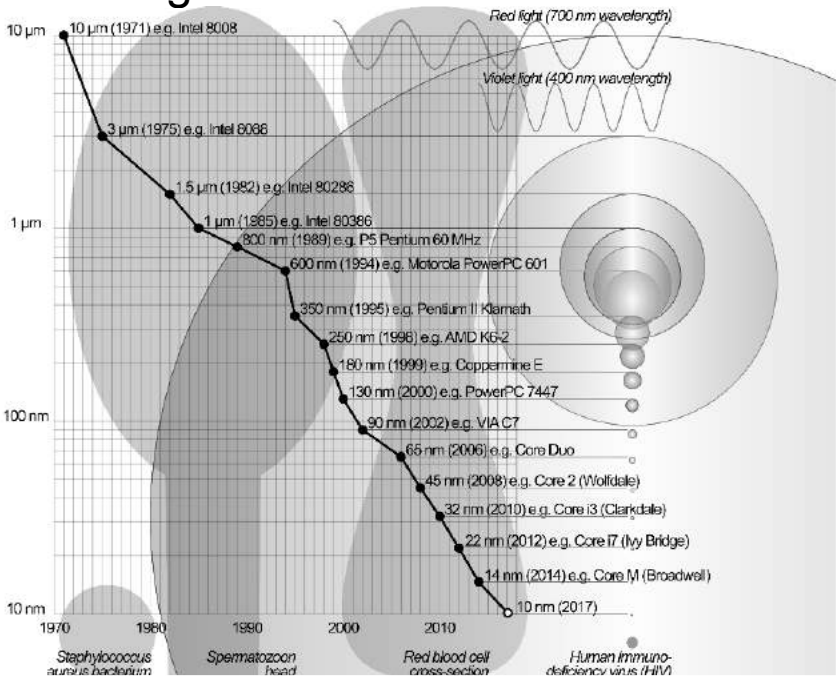
Nano and Micro Engineered Membrane Technology (Microsieves)

FoodSmart phone.eu

Wageningen 28 June 2017

Who are we?

founded in 1994
silicon micromachining experts
innovative products based on microengineered membranes




Cees van Rijn


Jacob Baggerman


Tong Duy Hien


Ai Nguyen

Outline

- Microengineered membrane technology
- Applications
  - Filtration
  - Microbial detection
  - Cell capture
  - Emulsification
  - Spraying

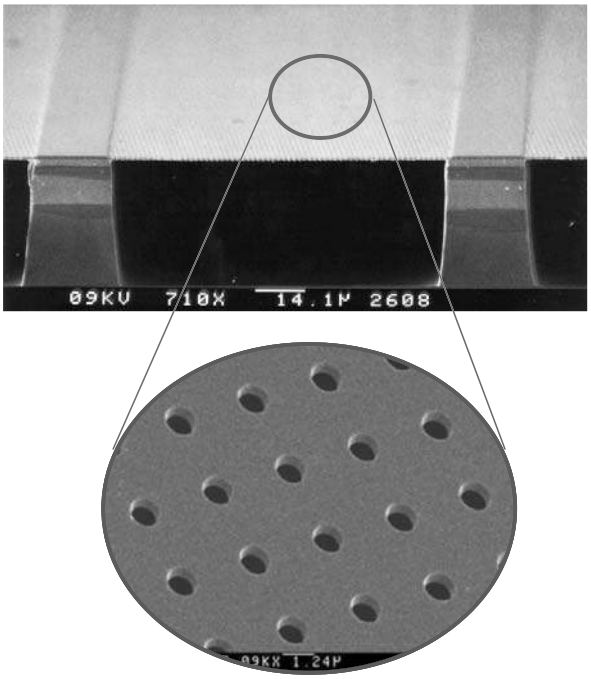
Sieves…

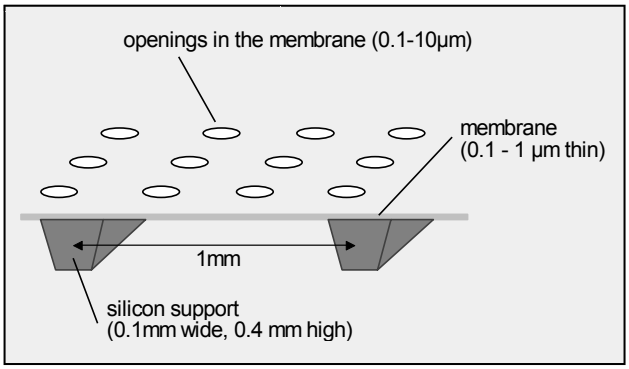
Pore size

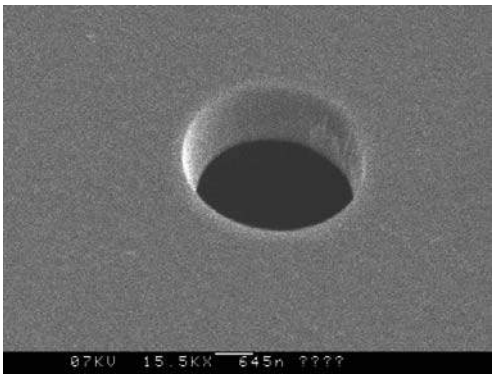
“Millifiltration”

Ultrafiltration

Microengineered membranes: Microsieves

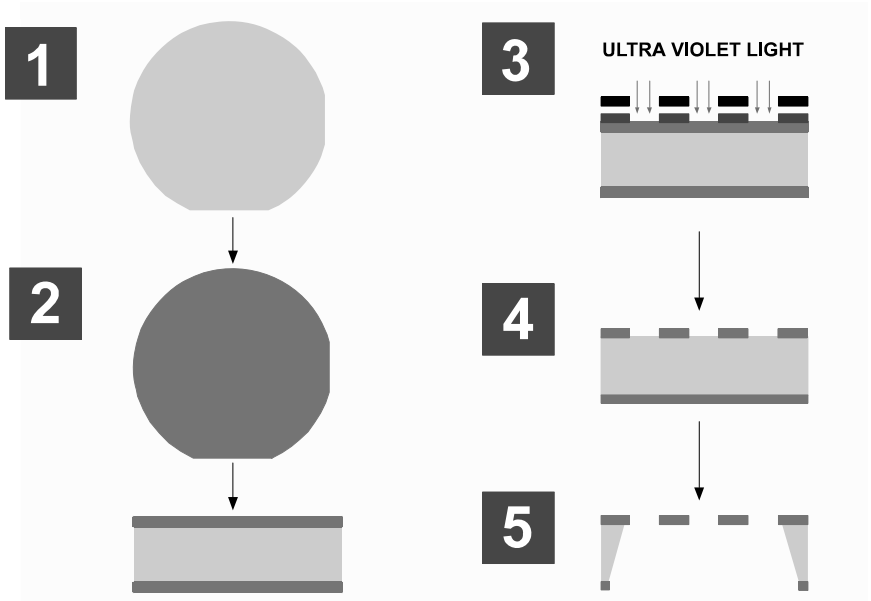


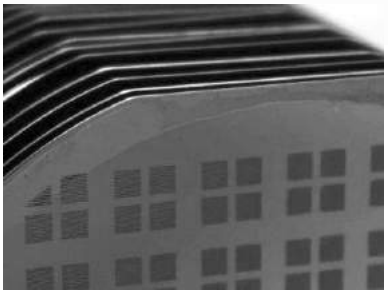


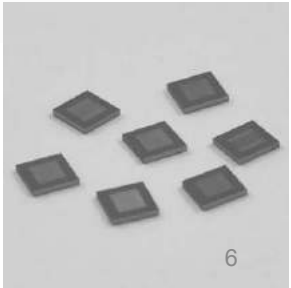


C. J. M van Rijn, Nano and Micro Engineered Membrane Technology
Elsevier, The Netherlands, 2004. ISBN 0444514899.

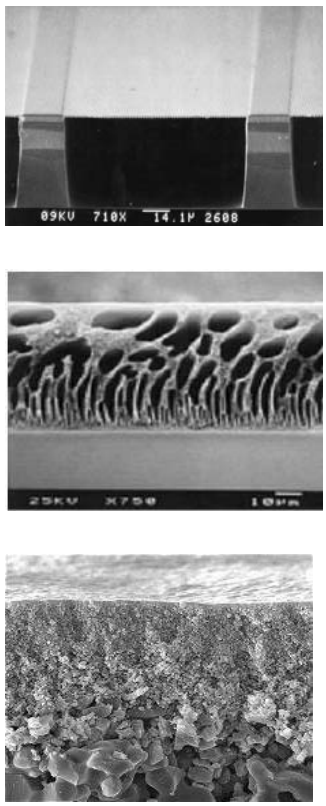
Microfabrication of microsieve



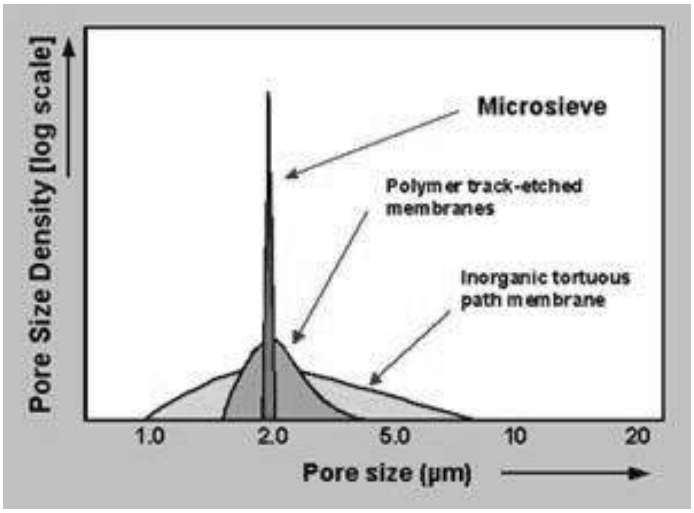




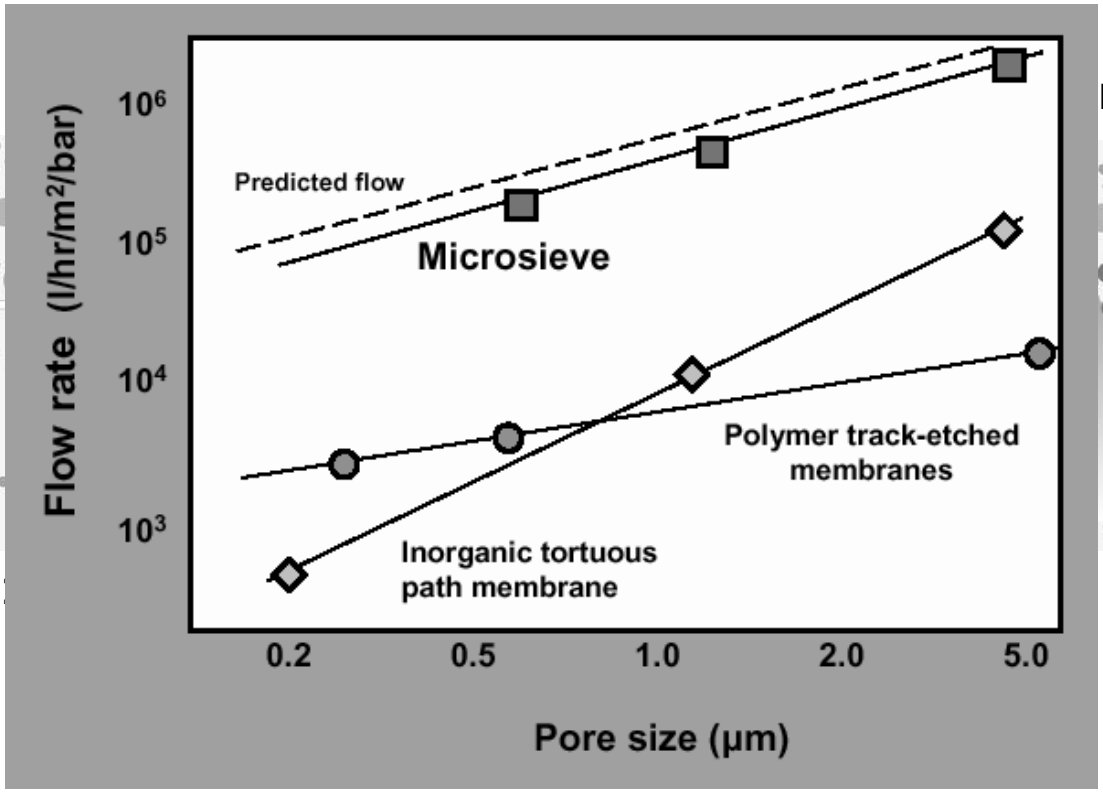
Homogenous pore size



- ✓Chemical inertness
- ✓High mechanical strength
- ✓Highly homogeneous pore size distribution
- ✓Capability of surface functionalization



Low Trans-membrane Pressure High Flux Performance

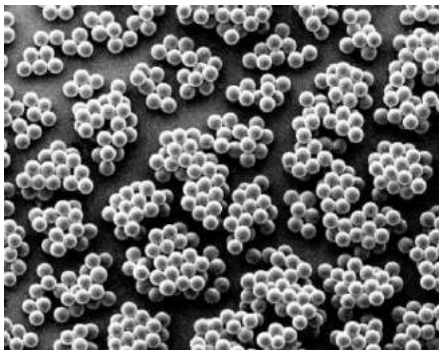


ne

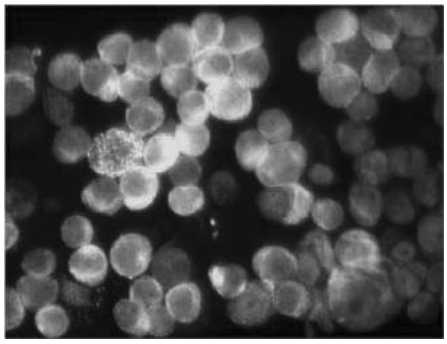
TMP
Flux:

Applications of microsieves

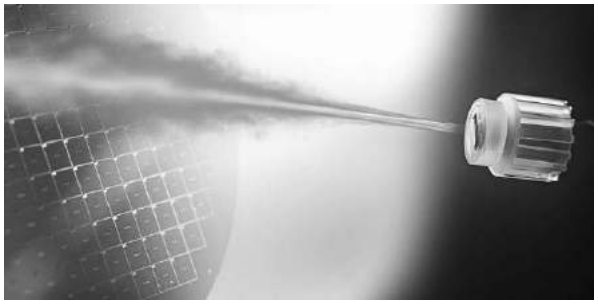
Filtration



Emulsification

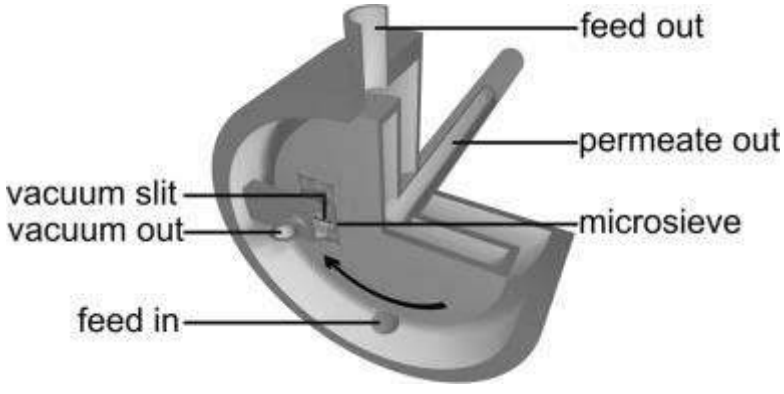


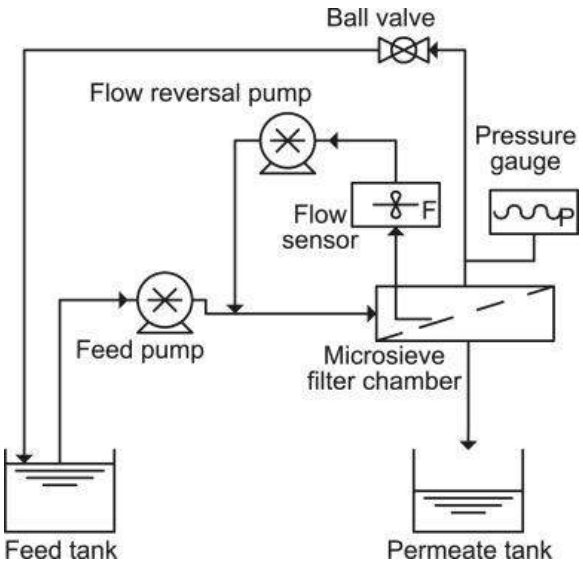
Cell Detection



Sprays

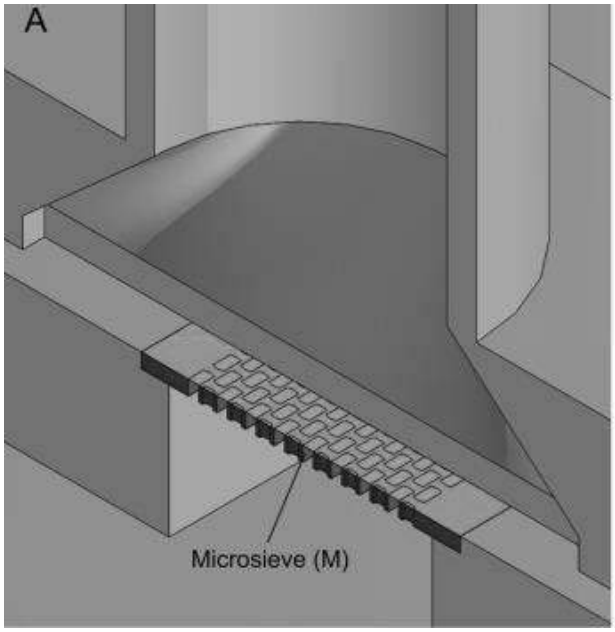
Rotating Microfiltration system

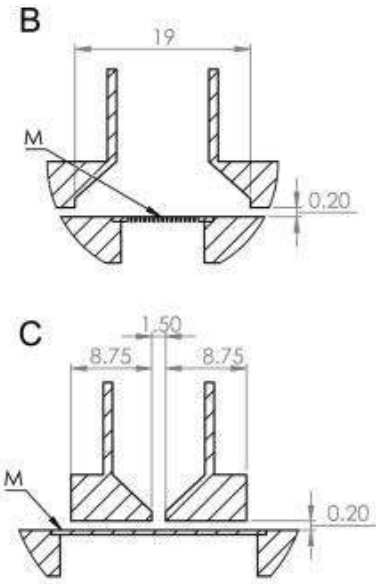




Journal of Membrane Science, 494, **2015**, pp121–129

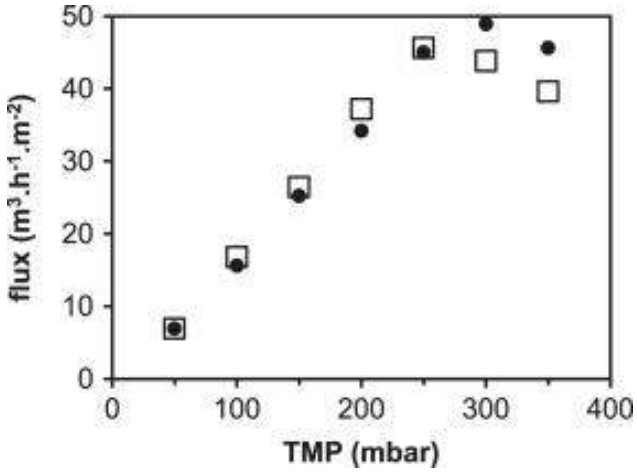
Vacuum Slit



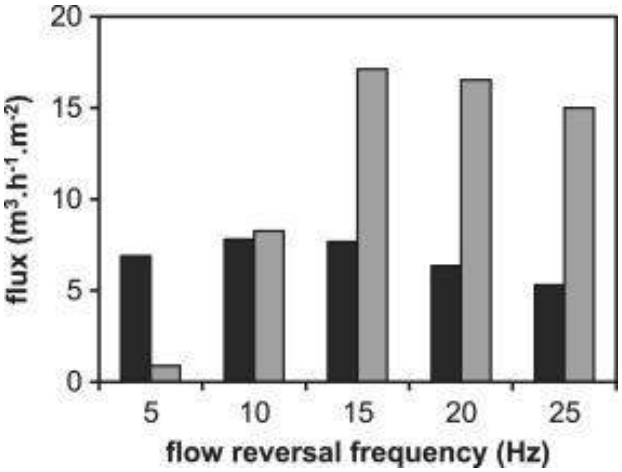


Suction flow (l/min)	ΔP (bar)
1.4	0.07
2.2	0.12
2.9	0.17
3.4	0.21
3.6	0.23

Filtration Characteristics: Pressure and Rotation Frequency



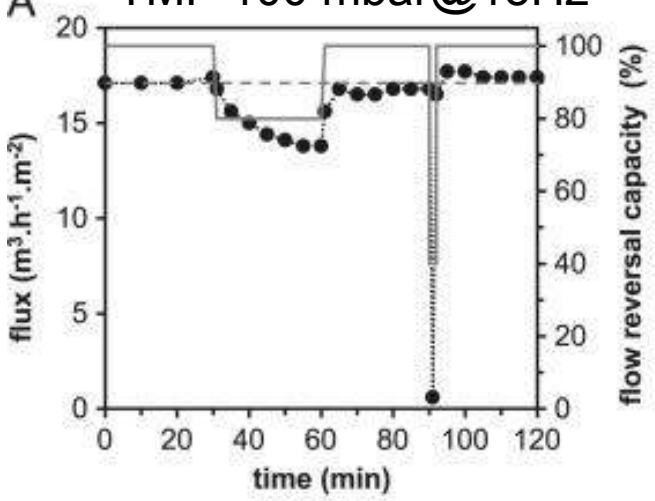
Frequency 20 Hz



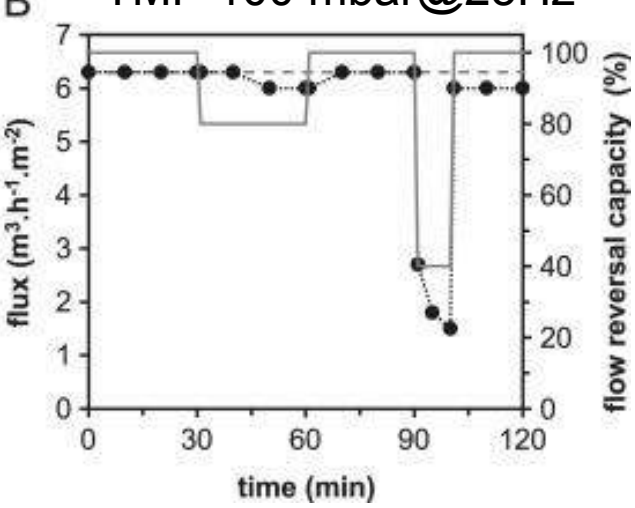
TMP
Black: 50 mbar
Gray: 100 mbar

Flow Reversal

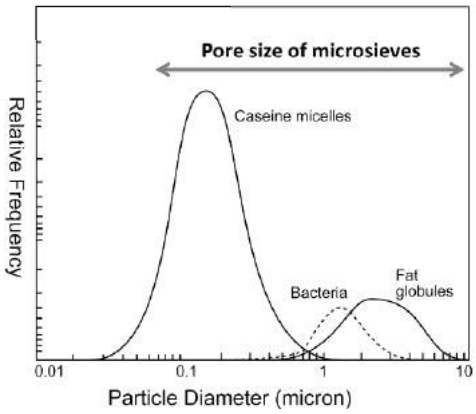
skimmed milk
TMP 100 mbar@15Hz

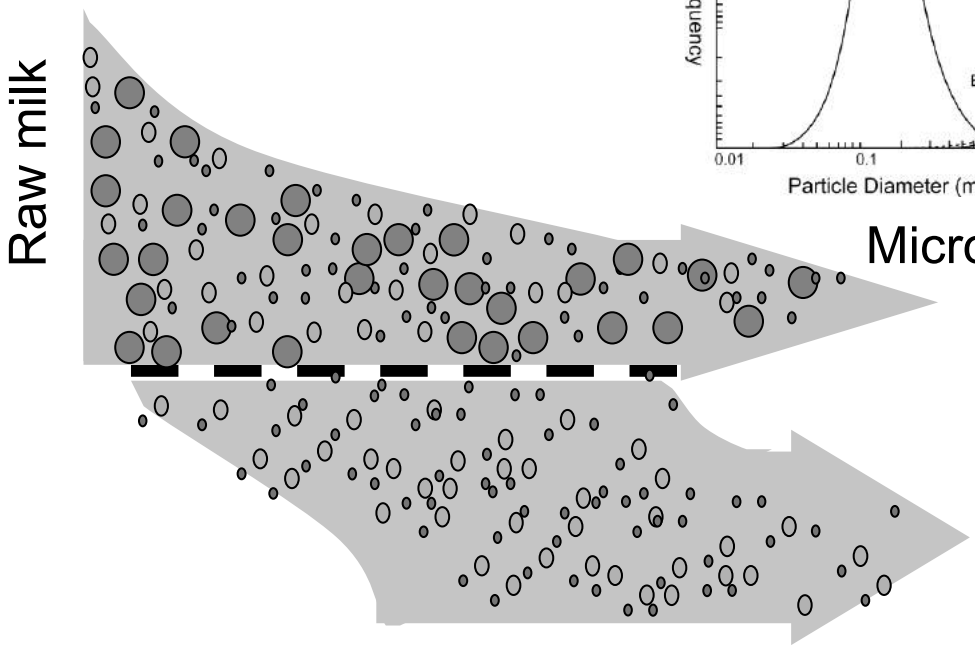


whole milk
TMP 100 mbar@25Hz

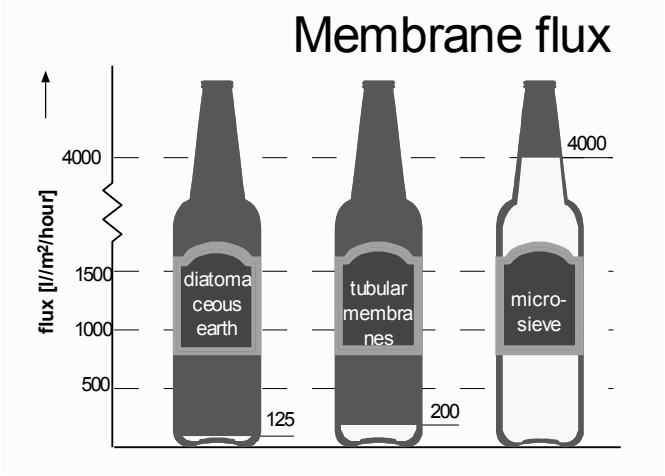


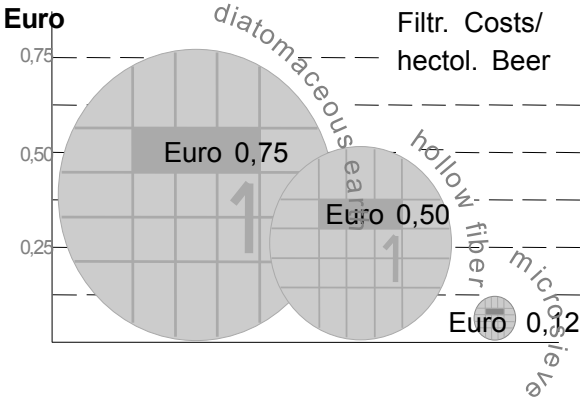
Milk filtration





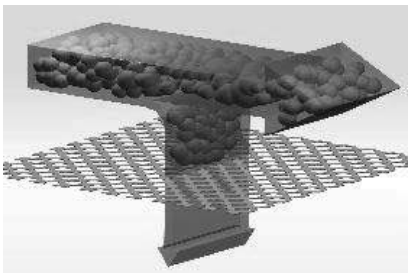
Beer Filtration





Conditions:

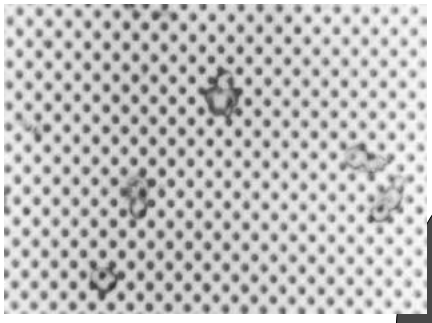
- cross-flow filtration rate < 2 m/s
- transmembrane pressure < 0.2 bar
- back shock frequency < 5Hz

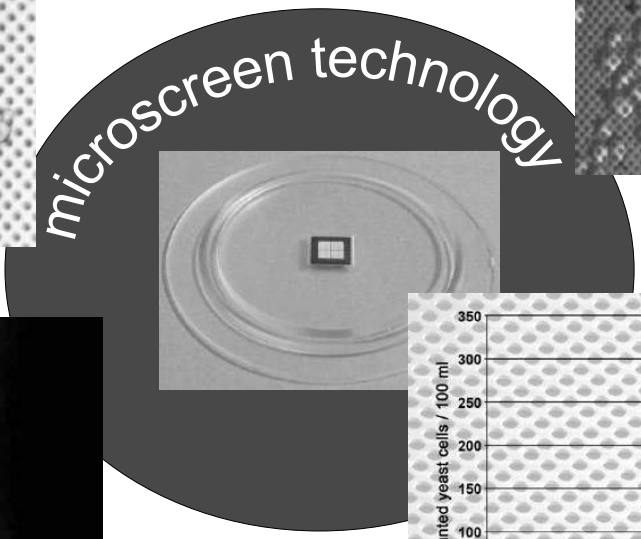


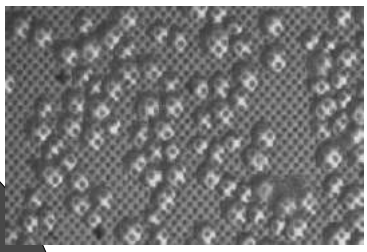
Microfiltration Applications

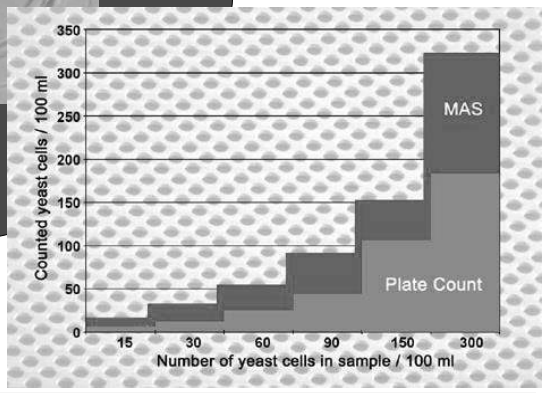
- Sterilization of food and pharmaceutical fluids
- Purification wash water for integrated circuits
- Clarification of juices and beer
- Membrane bioreactor for waste water treatment

Micro-biological Analysis



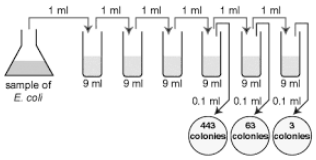
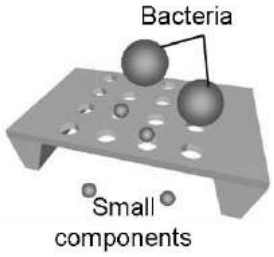
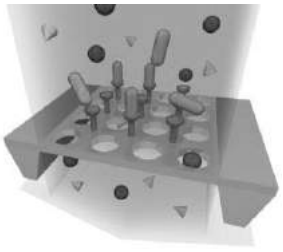
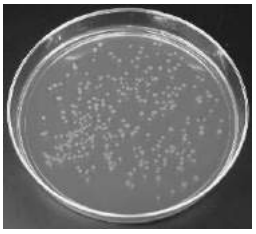
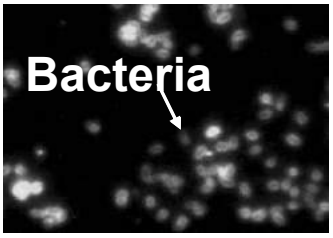
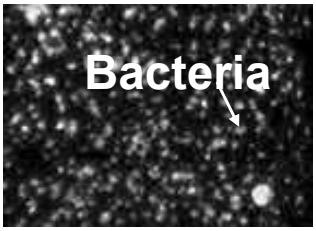




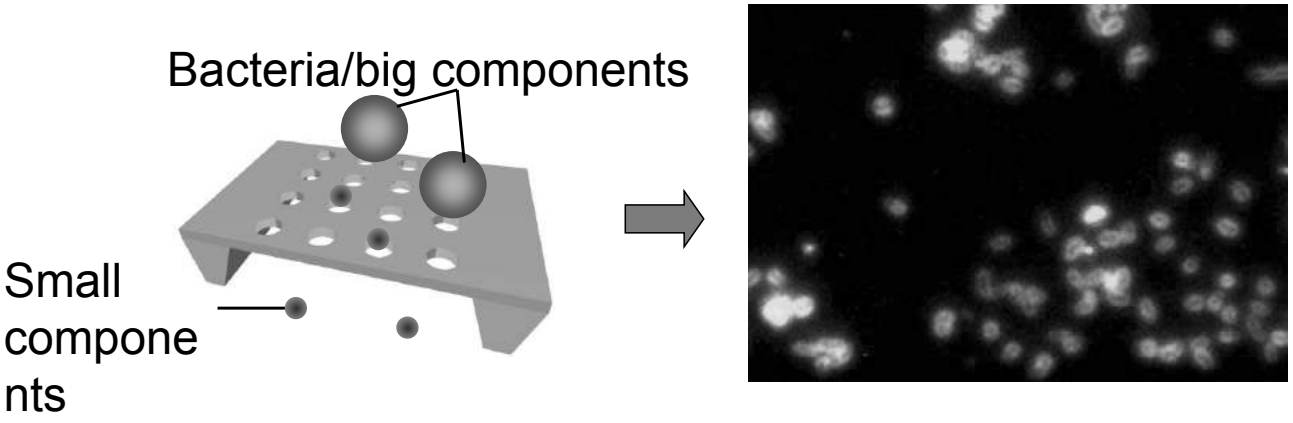


microscreen vs plate count

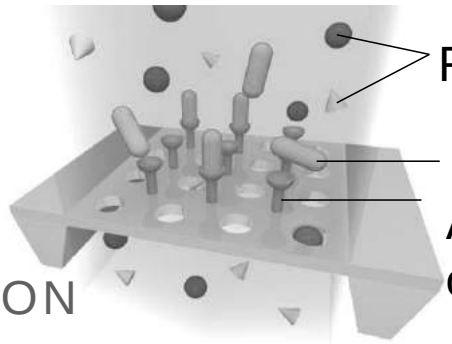
Microbial detection

Techniques	Plate count	Microsieve-based Detector (new)	Antibody-coated Microsieves (new)
Principle			
Sensitivity	✓High	✓High	✓High
Incubation time	×Long	✓No need	✓No need
Washing steps	×Cumbersome	✓Fast	✓Fast
Sample volume	×Small	×Small	✓Large
Output			

Bacteria capture by size

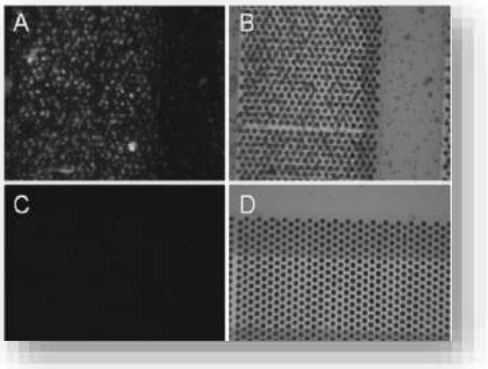


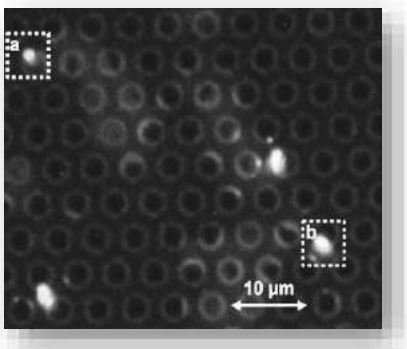
SURFACE CHEMISTRY & MICROFILTRATION

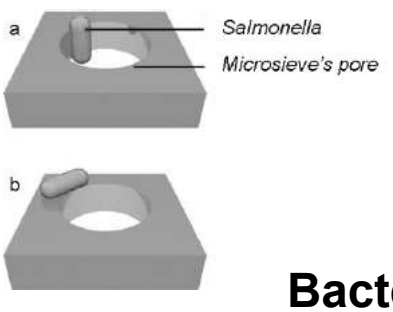


Salmonella detection on microsieves

Bacteria captured by Antibody-coated microsieves



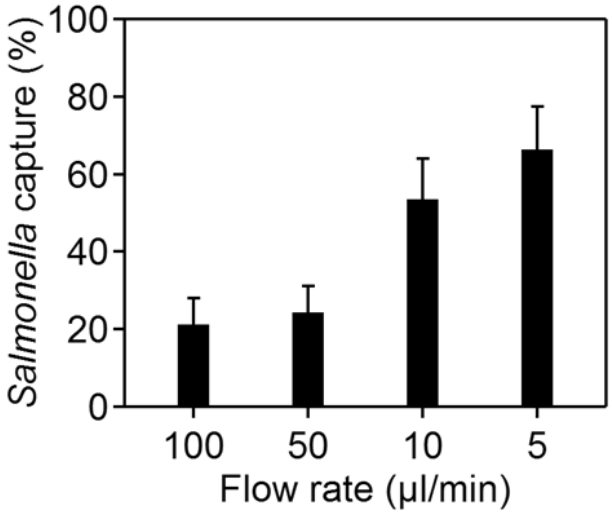




Bacteria captured on the surface and inside the pore

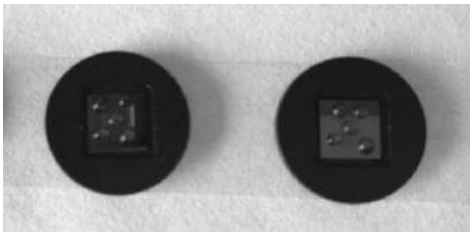
Capture efficiency vs Flowrate

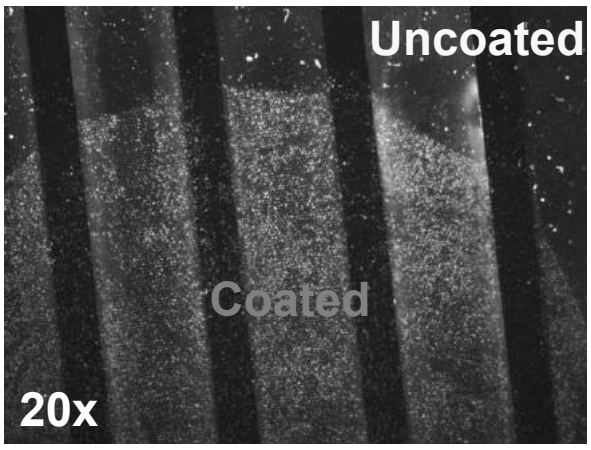
Capture efficiency of 3.5-µm antibody-coated microsieves compared to 0.45-µm microsieves as positive control (100% capture)

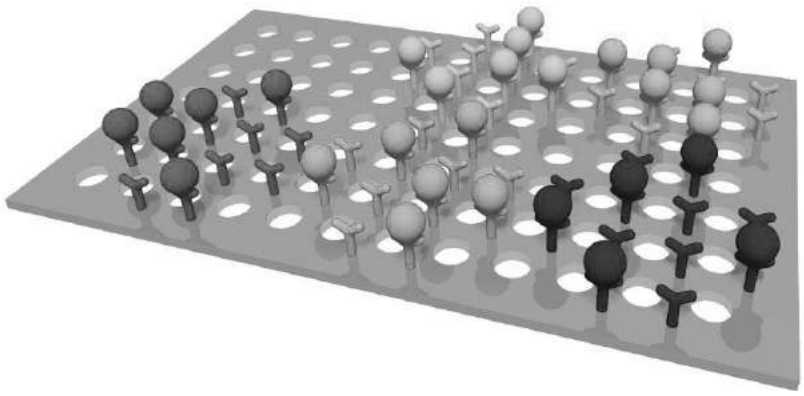


slower flow → more capture

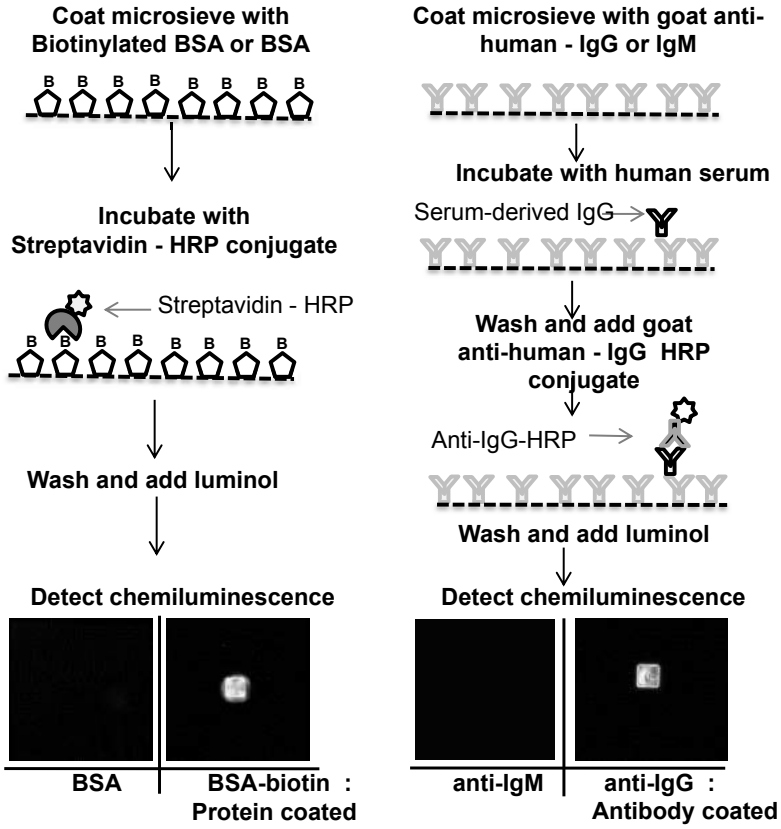
Possible microarray on microsieves





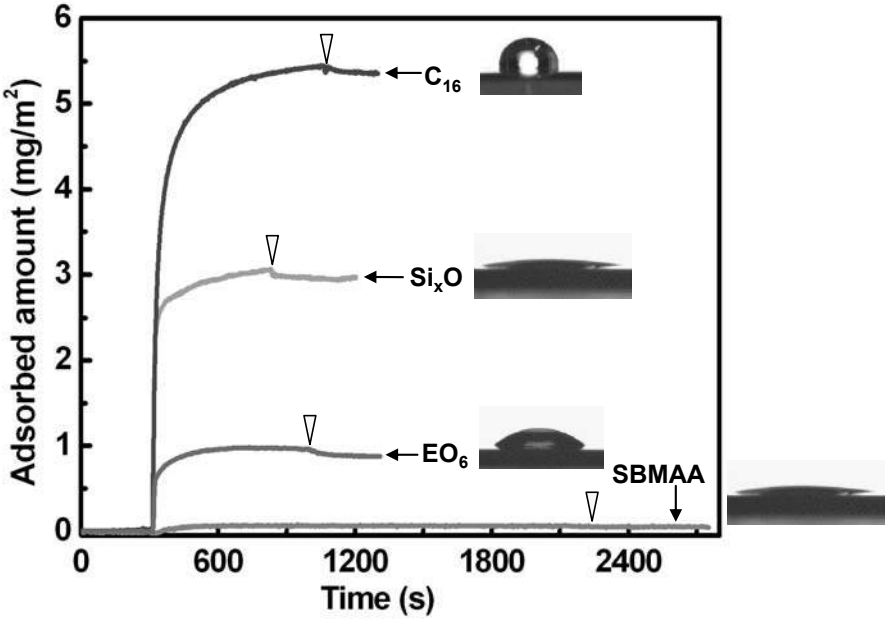


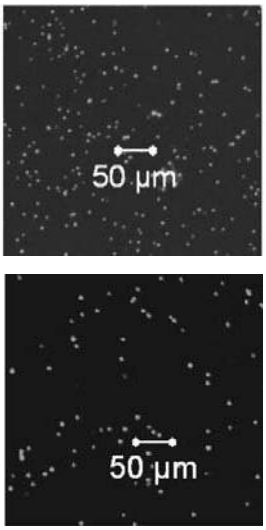
Immunoassays on microsieves



FoodSmartphone.eu

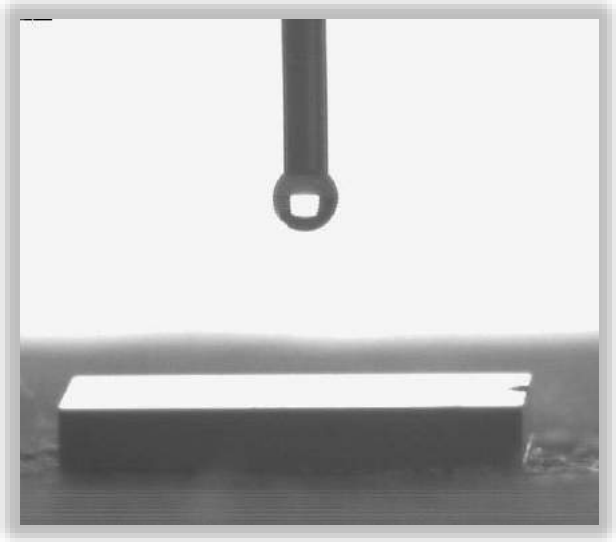
Protein-repellent microsieves



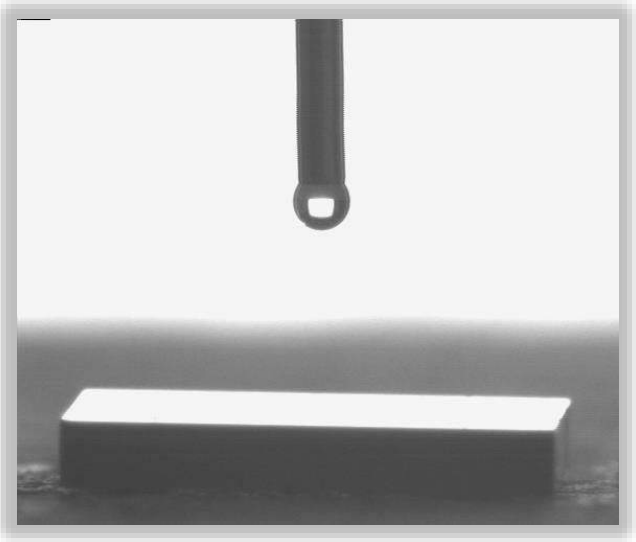


Improve wettability of microsieves

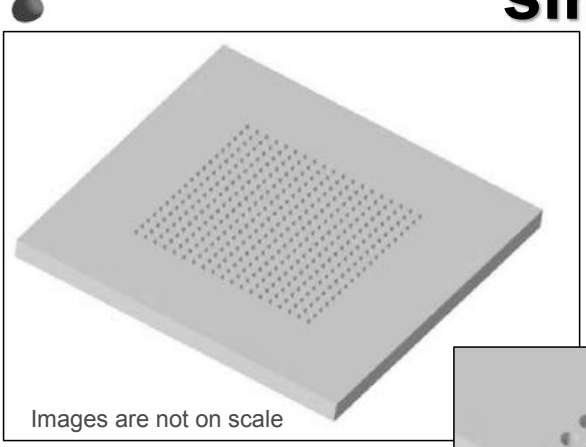
UNCOATED



Zwitterion-COATED



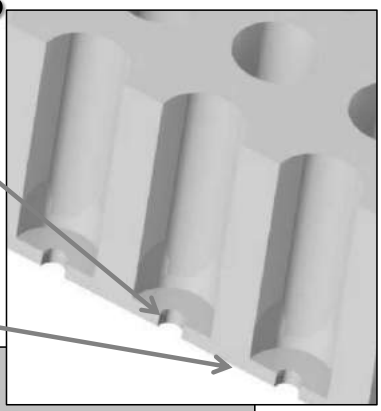
Microwell plate for single cell in single wells

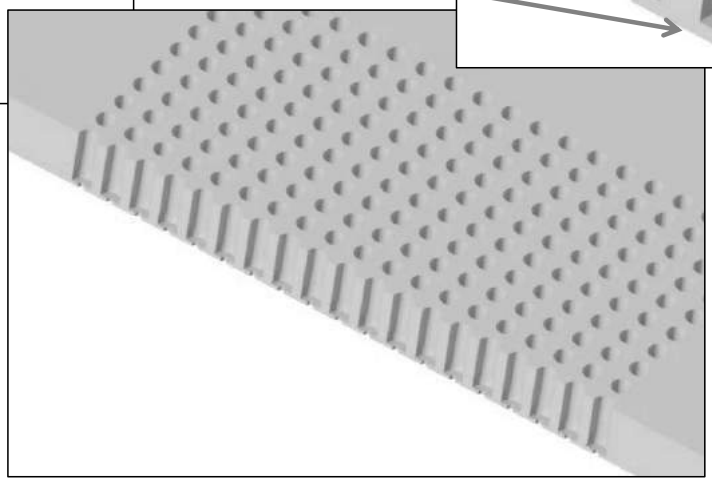


Images are not on scale

Single pore per well

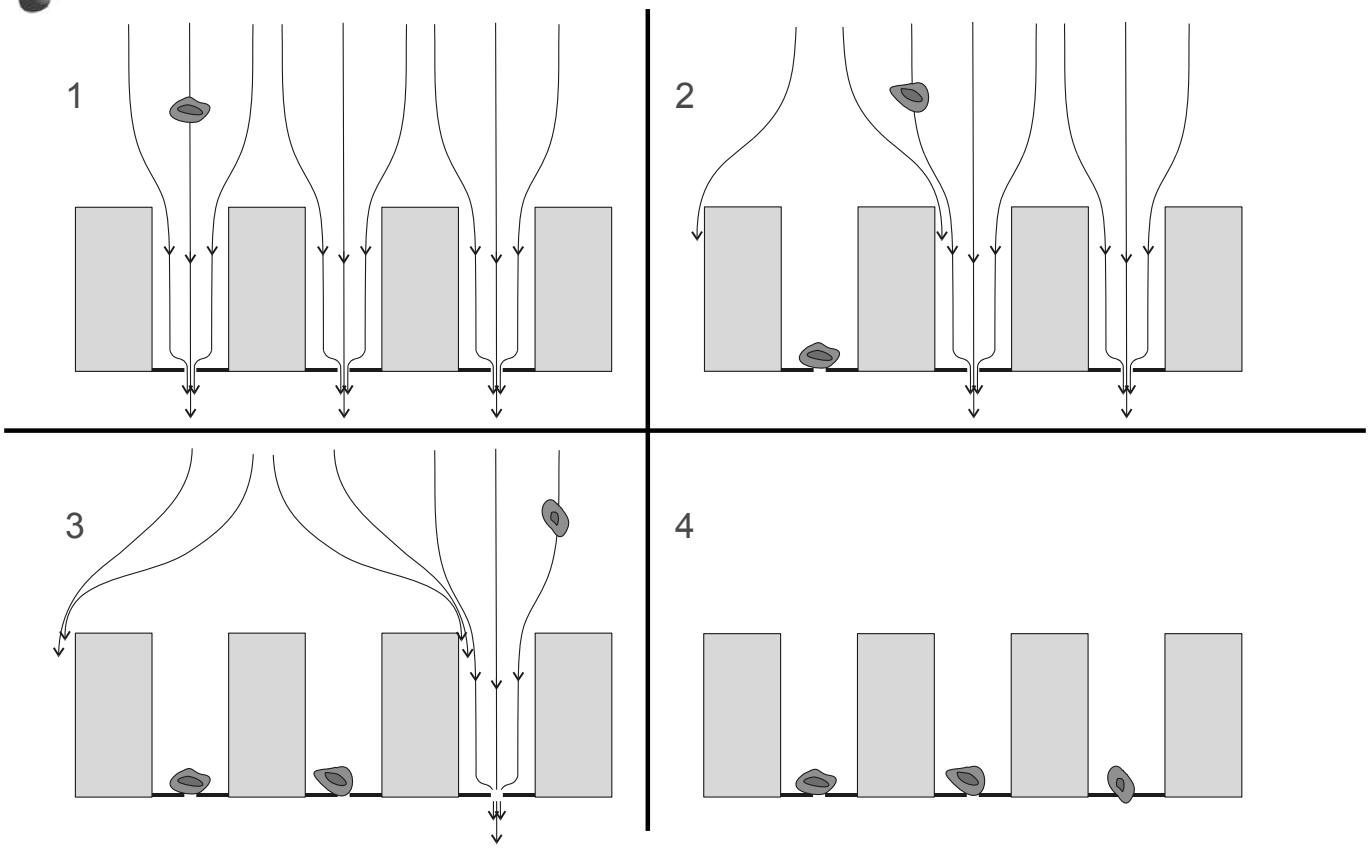
Silicon nitride membrane



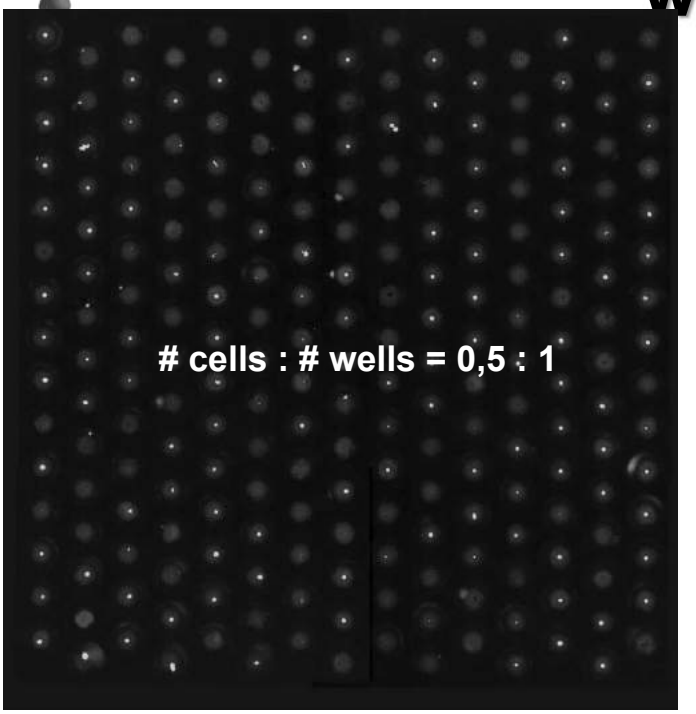


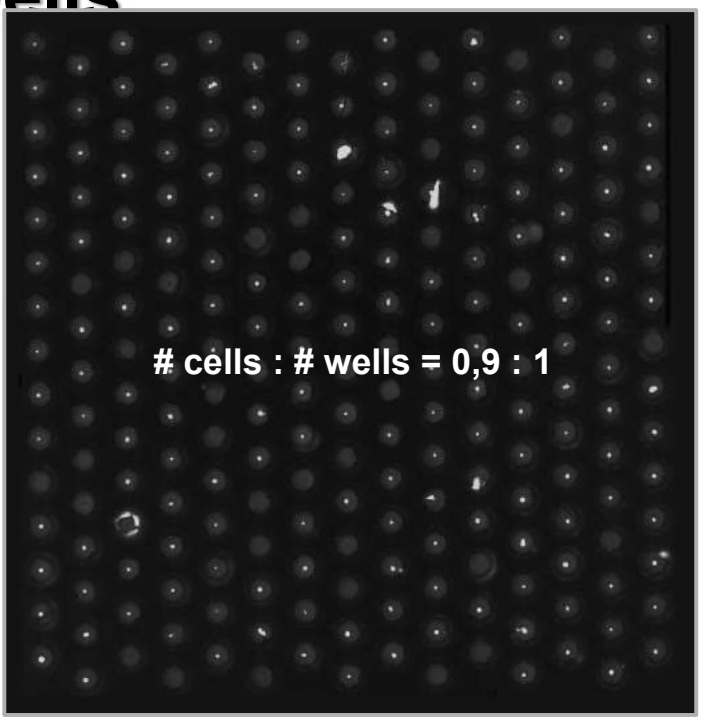
Cup diameter 120 microns
Height 380 microns

Single sorting principles



seeding: Single cells in individual wells





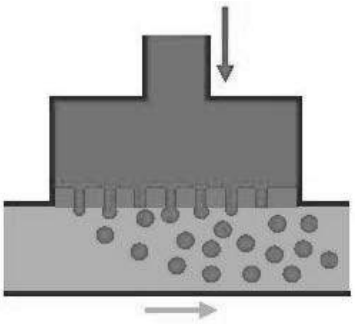
Very high fill grade with single cells per well

After capture / seeding of single cells in individual wells

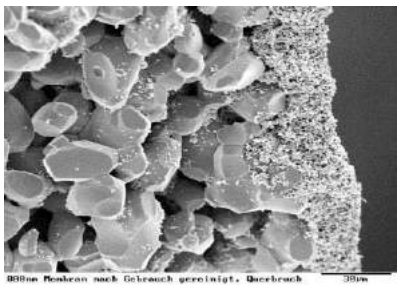
- Add reagents to the well plate and monitor the response of individual cells
  - Pharmacology, drug testing, drug screening
- Transfer individual cells to a next analysis platform
  - RT-PCR for gene analysis, point-mutations
  - Sequencing of the whole genome
- Transform the well content to next analysis platform
  - Analyze the products produced by the individual cells

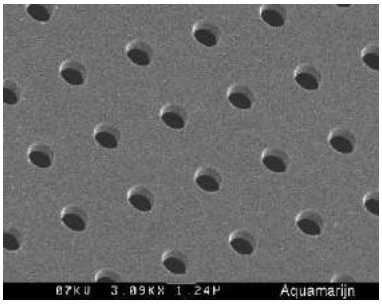
Membrane Emulsification

current technology

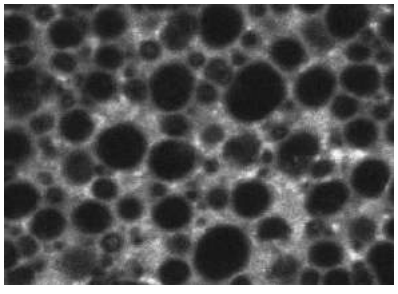


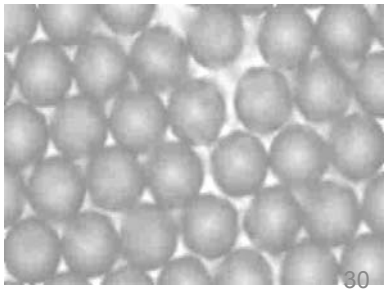
microsieve technology





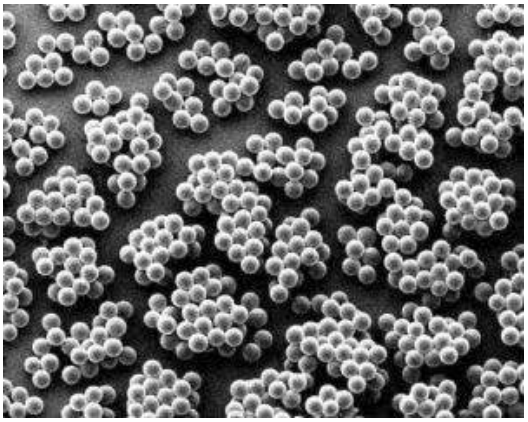
More stable

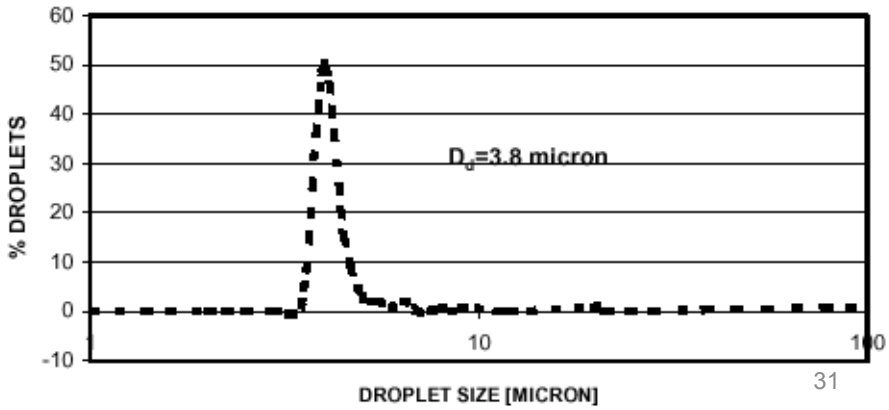




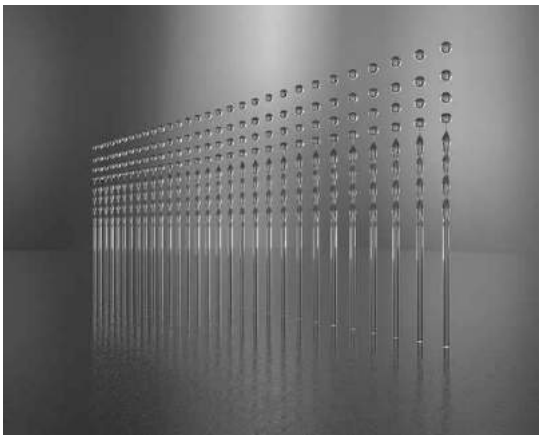
Microsieve emulsification

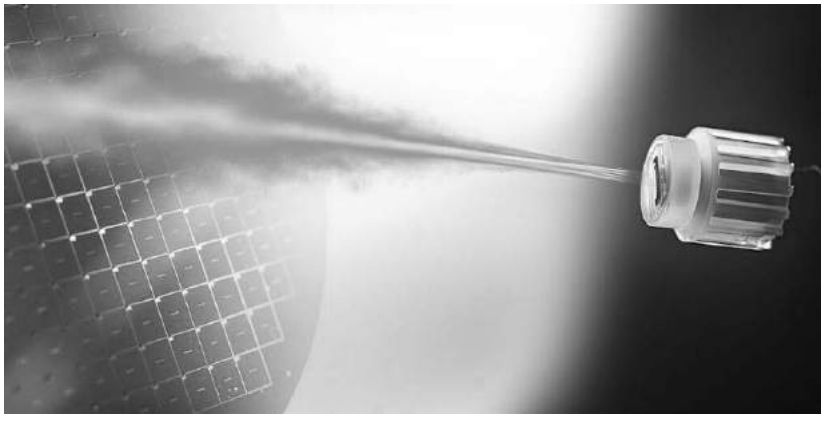
- optimal droplet/particle size
- droplets 2 – 100 μm, particles 50nm – 150 μm
- monodisperse: C.V. ≈ 5 %
- reproducible, robust and scalable
- encapsulation/double emulsions





Ultra fine Rayleigh sprays





Summary

- Micro engineering yields new filter types
- Micro engineered membranes show improved performance at lower cost
- Micro engineered membranes provide new solutions for microbiology and cell biology
- Combining bottom-up nanotechnology with top-down micro-engineering for better performance

1st Summer School on Smartphone-based Food Analysis
Wageningen, The Netherlands, 26-30 June 2017

Electrochemical detection

Louis de Smet [1]

The vast growth of smartphone use has opened opportunities to develop portable smartphone-based sensor (SPS) systems for field and point-of-care (POC) applications.[1] Electrochemical (bio)sensor platforms represent an attractive type of sensors for SPS development in a variety of settings, including medical, water for in-field environmental monitoring,[2] and food analysis.[3]
In this lecture, first some basics on electrochemistry, electrical equipment and electrodes and voltammetry will be discussed. Given its widespread use in electroanalytical chemistry focus will be given on cyclic voltammetry and several of its modes of operation. Next, three recently published cases will be discussed, with an emphasis on the surface chemistry and the chemical reactions involved in the detection mechanisms.
Case I covers work on an electrochemical immunosensor for the detection of food allergens.[4] The allergen of choice was β-casein, one of the main milk proteins. The enzymatic activity was amperometrically detected by adding hydrogen peroxide and a suitable redox mediator. With Hepatitis C core antibody as the analyte of interest, case II is more related to the medical field,[5] but the study nicely 1) shows the use of yeast cell lines to produce dual-affinity constructs and 2) gives a comparison of the optical (fluorescent) and electrochemical detection of antibodies. Such a biosensor-based approach is illustrative for the detection of other pathogens as well, including food-related applications.[6] Also Case III deals with two parallel approaches,[7] *i.e.* the colorimetric (visible light) and voltammetry-based electrochemical detection of bacteria using substrates specific to enzymes produced by each species.
The presented cases reflect the main recent developments in combining electrochemical approaches with smartphone technology. Finally, some opportunities for further research and a brief outlook will be presented.

Suggestions for further reading:

[1] Smartphone-Based Sensors, Gao, X; Wu, N. Electrochem. Soc. Interface 2016, 25, 4, 79-81 [doi]
[2] Universal mobile electrochemical detector designed for use in resource-limited applications, Nemiroski, A.; Christodouleas, D.C.; Hennek, J.W.; Kumar, A.A.; Maxwell, E.J.; Fernández-Abedul, M.T.; Whitisides, G.M. PNAS, 2014, 111, 33, 11984-11989 [doi]
[3] 'Electroanalytical Techniques and Instrumentation in Food Analysis' by Carlos M. Pereira and Rubin Gulaboski, Chapter 17 in the Handbook of Food Analysis Instruments (Ed: Otles), CRC Press, 2008 [doi]
[4] Case I: Electrochemical immunosensor for the determination of β-casein, Molinari, J.; Moina, C.; Ybarra, G. J. Electrochem. Sci. Eng. 2015, 5, 1, 9-16 [doi]
[5] Case II: Detection of Hepatitis C core antibody by dual-affinity yeast chimera and smartphone-based electrochemical sensing, Aronoff-Spencer E.; Venkatesh A.G.; Sun A.; Brickner H.; Looney D.; Hall D.A. Biosens. Bioelectron., 2016, 86, 690–696 [doi]
[6] a) Electrochemical biosensors for fast detection of food contaminants – trends and perspective, Rotariu L.; Lagarde F.; Jaffrezic-Renault N.; Bala C.; Trends in Analytical Chemistry 2016, 79,80–87 [doi]; b) 'Electrochemical Biosensors for Food Security: Allergens and Adulterants Detection' by Campuzano, S. et al. in Biosensors for Security and Bioterrorism Applications (Nikolelis and Nikoleli), Springer, 2016 [doi]

[1] E-mail; louis.desmet@wur.nl; Wageningen University & Research, Organic Chemistry, Wageningen, The Netherlands

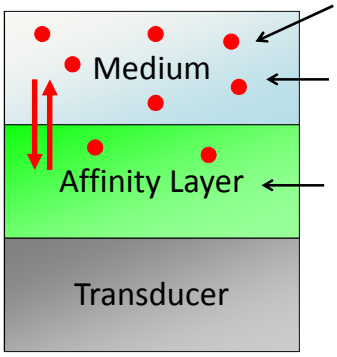
[7] Case III: Colorimetric and Electrochemical Bacteria Detection Using Printed Paper- and Transparency-Based Analytic Devices, Adkins J.A.; Boehle K.; Friend C.; Chamberlain B.; Bisha B.; Henry C.S. Anal. Chem. 2017, 89, 3613−3621 [doi]

Dr Louis C.P.M. de Smet
Wageningen University
Laboratory of Organic Chemistry
Stippeneng 4
6708 WE Wageningen
E louis.desmet@wur.nl
W www.louisdesmet.nl

Outline

1. Chemical Sensors & Transducers
2. Electrochemistry
3. Equipment, Electrodes & Voltammetry
4. Electrochemical SmartPhone-based Sensors (SPS)
   - Case I: Casein (allergen)
   - Case II: Antibody (virus)
   - Case III: Enzyme (bacteria)
5. Concluding Remarks

1 Chemical Sensors



Biomolecules, metal ions, Volatile organic compounds, …

Water, gas, …

Polymer films, organic monolayers, metal oxides, metal-organic frameworks, …

1 Selected Transducers

Thermal Sensors / Microcalorimeters (ΔH)

Mass Sensors / Cantilevers (Δm)

Optical Transducers (Δn, fluorescence)

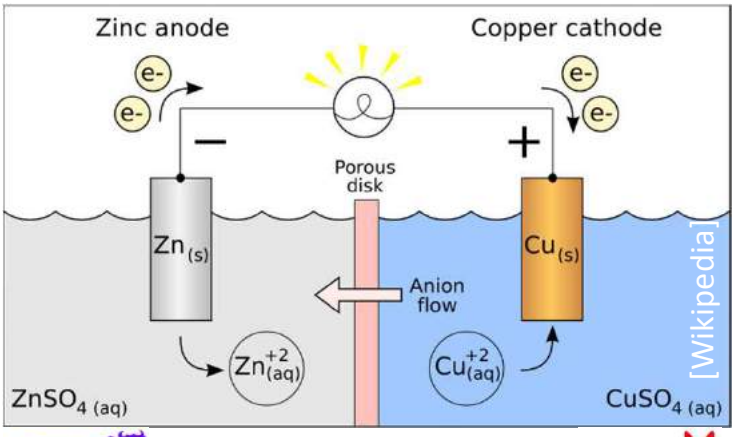
Piezo-electric Devices (Δm)

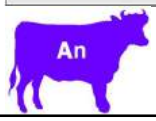
Electrochemical Transducers:

- Potentiometric
- Voltammetric
- Conductometric
- Impedance (capacitance)
- Transistor-based Sensors

2 Electrochemistry

chemical changes produced by electricity & production of electricity by chemical changes





Reduction = gaining electrons
Oxidation = losing electrons

2 Electrochemistry

$Zn(s) \rightarrow Zn^{2+}(aq) + 2e^-$ $\qquad$ $Cu^{2+}(aq) + 2e^- \rightarrow Cu(s)$

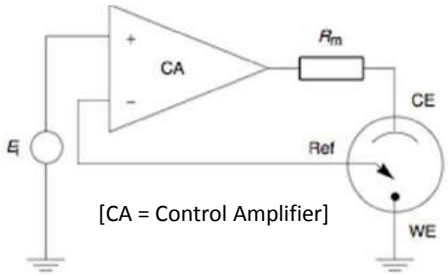
$$Zn(s) \,|Zn^{2+}(aq) \,\|\, Cu^{2+}(aq) \,|\, Cu$$

$E^0_{cell} = E^0_{cathode} - E_{0anode}$

$E^0_{cell} = +0.340V + (-0.763V) = 0.423V$

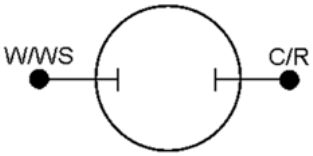
$\Delta G = -n \times F \times E_{cell}$

3 Potentiostat: Control & Measure

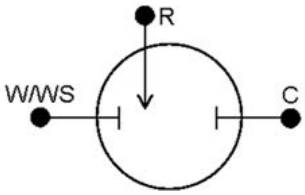


- Control of ΔE (CE & WE) to have a well-defined ΔE (WE & REF)
- ΔE (WE & REF) and I (CE & WE) are continuously measured

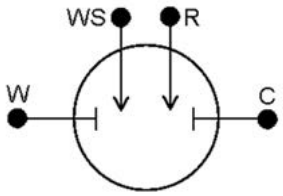
3 # Electrodes



To measure the current when applying potential



Current: W ⇔ C
Potential: W ⇔ R



Separate pairs of current-carrying (W ⇔ C) and voltage-sensing electrodes(WS ⇔ R)

3 Reference Electrodes (1/2)

Standard hydrogen electrode (SHE)

$2\ H^+\ (aq) + 2\ e^- \rightarrow H_2\ (g)$ + Pt electrode $E = 0$ V

More practical & often used: Ag|AgCl electrode

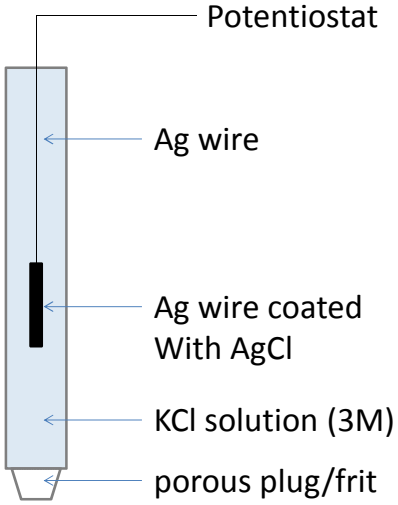
Equilibrium is between the Ag and AgCl:

$$\left.\begin{array}{l} Ag^+\ (aq) + e^- \rightarrow Ag(s) \\ AgCl\ (s) \rightarrow Ag^+\ (aq) + Cl^-\ (aq) \end{array}\right\} \quad AgCl\ (s) + e^- \rightarrow Ag\ (s) + Cl^-\ (aq)$$

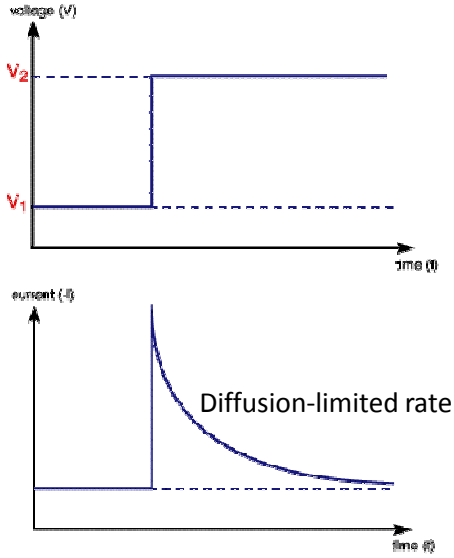
$$E = E^0 - \frac{RT}{F} \ln a_{Cl^-}$$

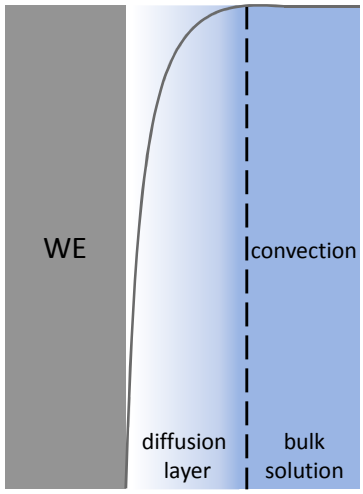
$E = 0.23$ V

3 Reference Electrodes (2/2)



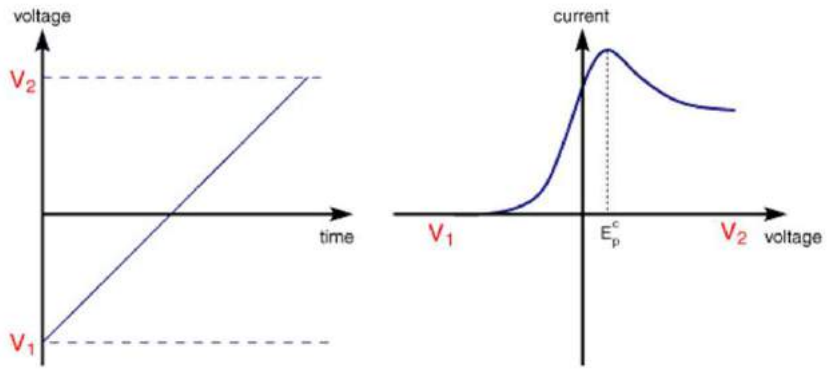
3 Voltammetry: Potential step





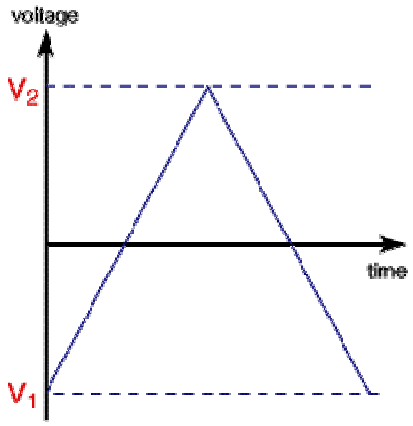
3 Voltammetry: Linear sweep

Linear sweep



www.ceb.cam.ac.uk/research/groups/rg-eme/teaching-notes

3 Cyclic Voltammetry: Principle (1/2)



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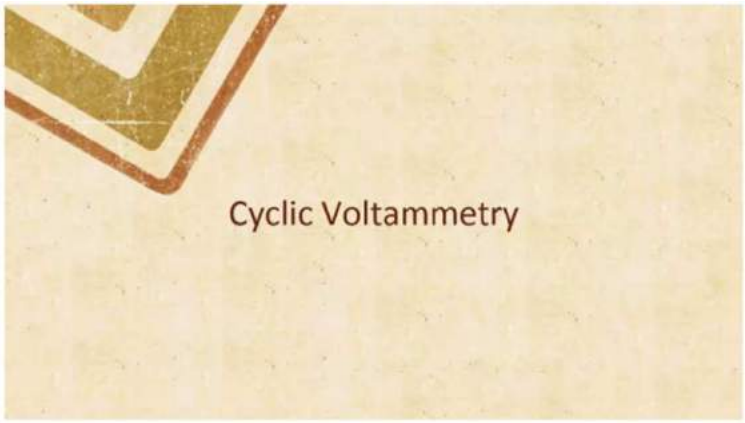
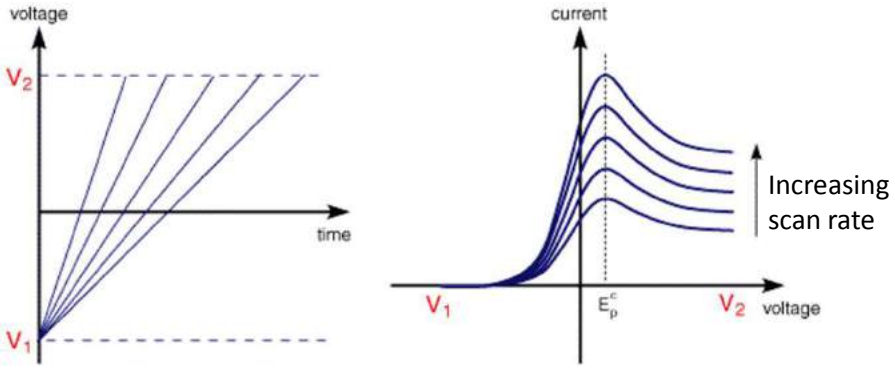
3 Cyclic Voltammetry: Principle (2/2)



https://www.youtube.com/watch?v=SRKgJHHbEIQ

3 Different Scan Rates



www.ceb.cam.ac.uk/research/groups/rg-eme/teaching-notes

3 CV Characteristics

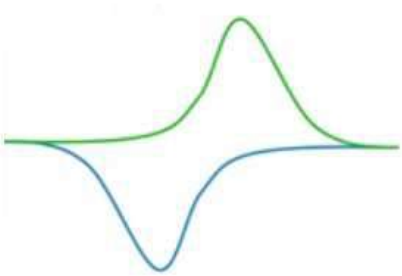
CVs of reversible electrochemical reactions have well-defined characteristics:

1) $\Delta E = 59/n$ mV

2) Positions peak voltage independent of scan rate

3) Peak current ratio = 1

4) Peak currents ~ (scan rate)^{½}



4 Electrochemical SmartPhone-based Sensors (SPS)

1. Chemical Sensors & Transducers
2. Electrochemistry
3. Equipment, Electrodes & Voltammetry
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   - Case I: Casein (allergen)
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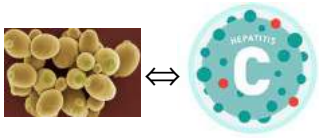
5. Concluding Remarks



4 Case 0: uMED

11984–11989 | PNAS | August 19, 2014 | vol. 111 | no. 33

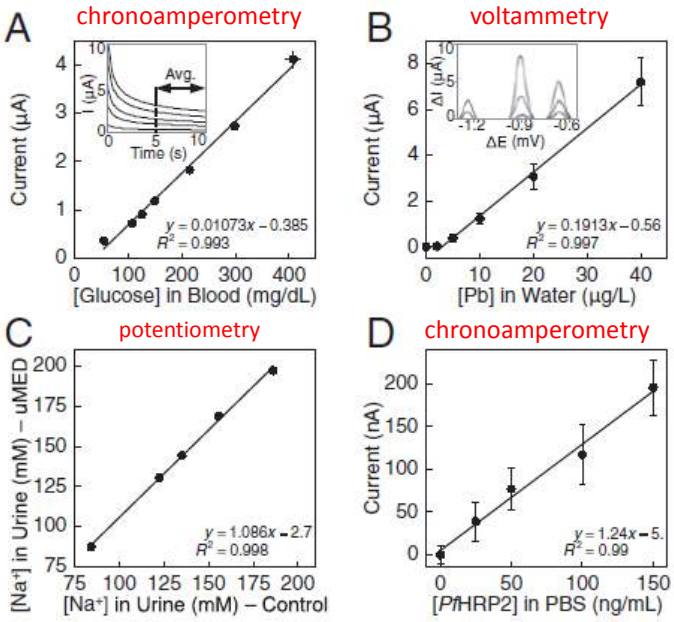
Universal mobile electrochemical detector designed for use in resource-limited applications

Alex Nemiroski[a], Dionysios C. Christodouleas[a], Jonathan W. Hennek[a], Ashok A. Kumar[b], E. Jane Maxwell[a], Maria Teresa Fernández-Abedul[c], and George M. Whitesides[a,d,e,1]

[a]Department of Chemistry and Chemical Biology, [b]School of Engineering and Applied Sciences, [d]Wyss Institute for Biologically Inspired Engineering, and [e]The Kavli Insitute for Bionano Science, Harvard University, Cambridge, MA 02138; and [c]Departamento de Química Física y Analítica, Universidad de Oviedo, 33006 Oviedo, Asturias, Spain

- Glucose in blood
- Heavy Metals in water
- Sodium in urine
- Malaria (antibody)

4 Case 0: uMED



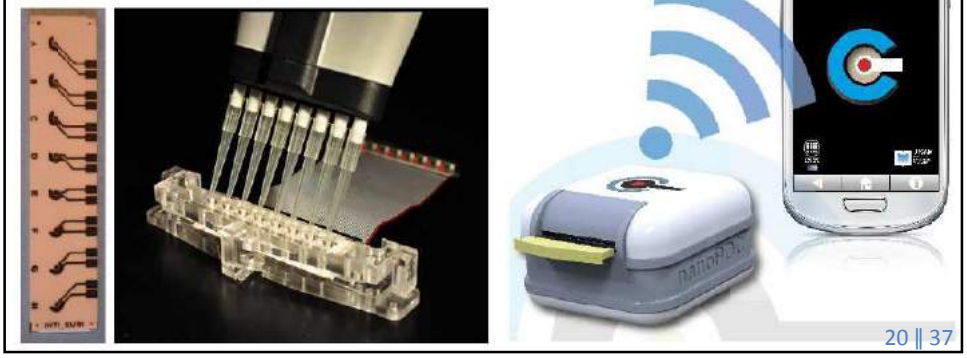
4 Case I: Casein

Electrochemical immunosensor for the determination of β-casein

Judith Molinari, Carlos Moina and Gabriel Ybarra

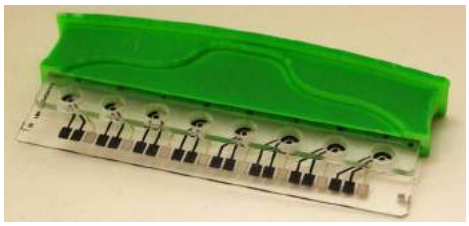
Unidad Técnica Nanomateriales, INTI-Procesos Superficiales, Instituto Nacional de Tecnología Industrial, C.C. 157, B1650WAB San Martín, Argentina

J. Electrochem. Sci. Eng. **5(1)** (2015) 9-16; doi: 10.5599/jese.2015.0072



4 Case I: Casein

NanoPOC
= portable potentiostat



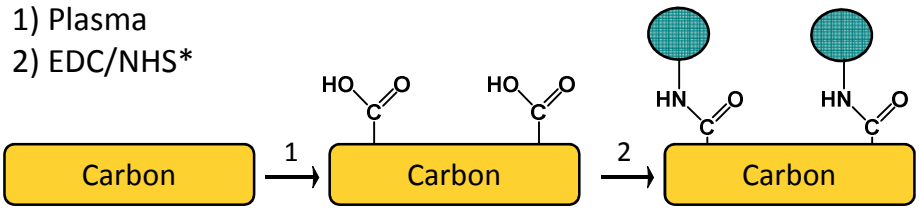
8 electrochemical cells:

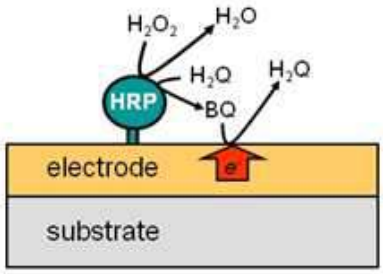
Ag/AgCl (RE)
2 carbon electrodes (WE and CE)
⇕
Neodynium magnets

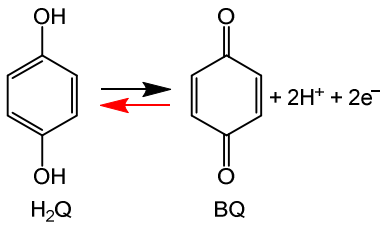
Salomón et al. *IEEE 9th Ibero-American Congress on Sensors*, Bogotá, Colombia, 2014, 1-4

4 Case I: Casein

1) Plasma
2) EDC/NHS*



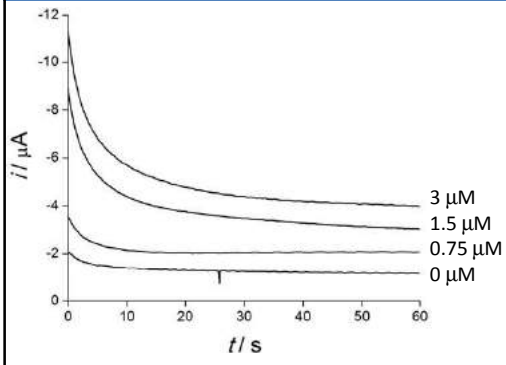


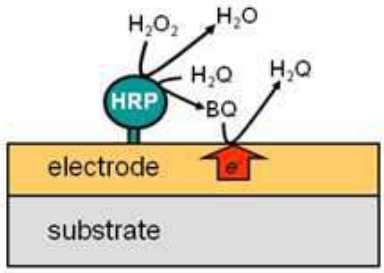


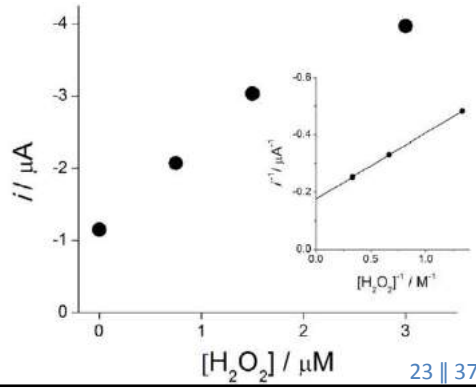
$H_2O_2 + 2H^+ + 2e^- \longrightarrow 2H_2O$

* See lecture Zuilhof

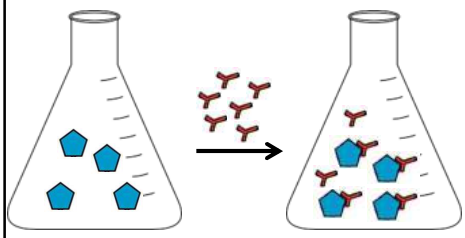
4 Case I: Casein

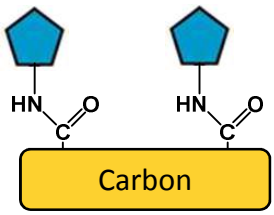


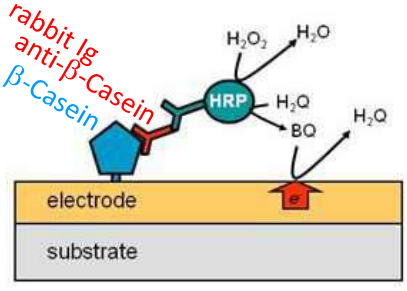


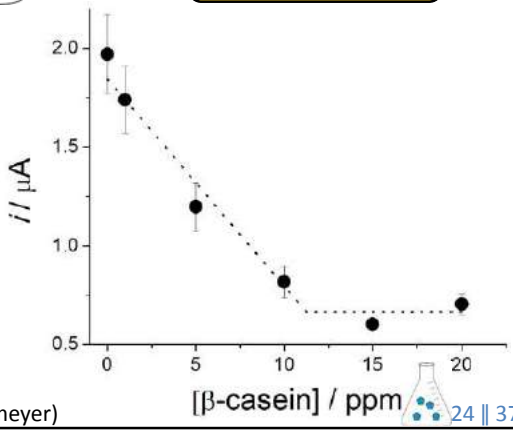


4 Case I: Casein









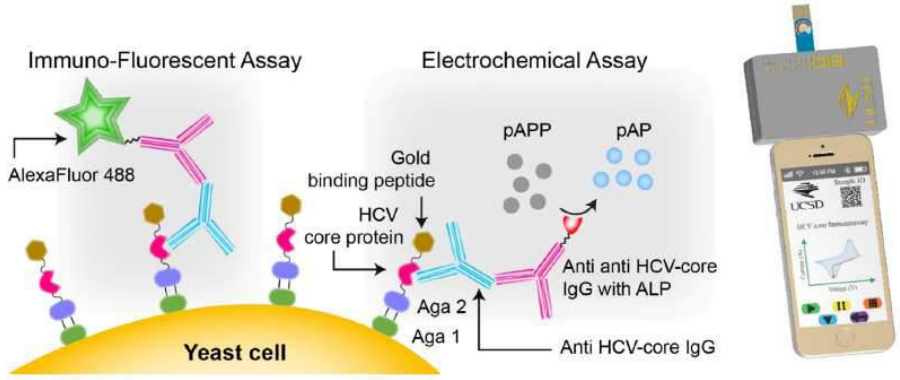
Elisa-like approach (lectures Bremer & Niemeyer)

4 Case II: Antibody

Detection of Hepatitis C core antibody by dual-affinity yeast chimera and smartphone-based electrochemical sensing

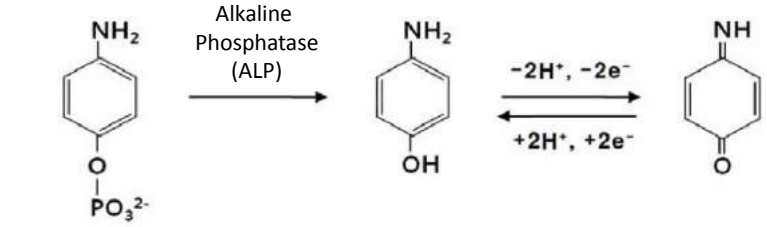
Eliah Aronoff-Spencer [a,*,1], A.G. Venkatesh [b,1], Alex Sun [b], Howard Brickner [a], David Looney [a,c], Drew A. Hall [b]

[a] School of Medicine, University of California, San Diego, La Jolla, CA, 92093 USA
[b] Department of Electrical and Computer Engineering, University of California, San Diego, La Jolla, CA, 92093 USA
[c] VA San Diego Healthcare System, San Diego, CA, 92161 USA



Biosensors and Bioelectronics 86 (2016) 690–696

4 Case II: Antibody

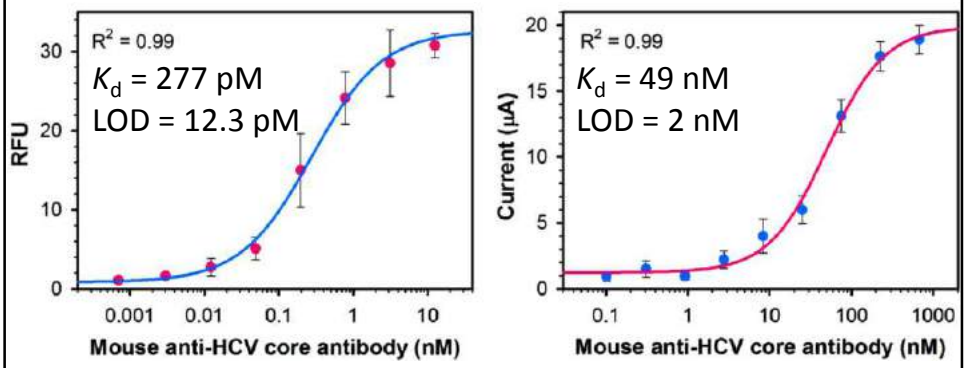


p-aminophenyl phosphate (*p*APP)

p-amino phenol (*p*AP)

p-quinone imine (*p*QI)

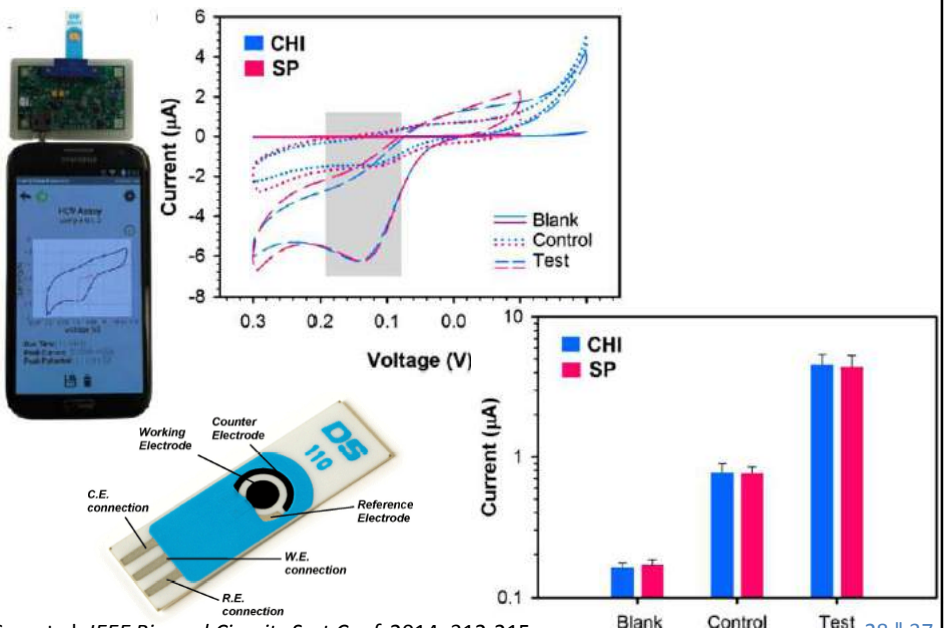
4 Case II: Antibody



Future work aim to reduce differences:

- reduce distance between yeast surface (monolayer) and WE
- attachment of yeast-free biobricks

4 Case II: Antibody



Sun et al. *IEEE Biomed Circuits Syst Conf.* 2014: 312-215

4 Case III: Enzyme

analytical chemistry

Anal. Chem. 2017, 89, 3613−3621

Colorimetric and Electrochemical Bacteria Detection Using Printed Paper- and Transparency-Based Analytic Devices

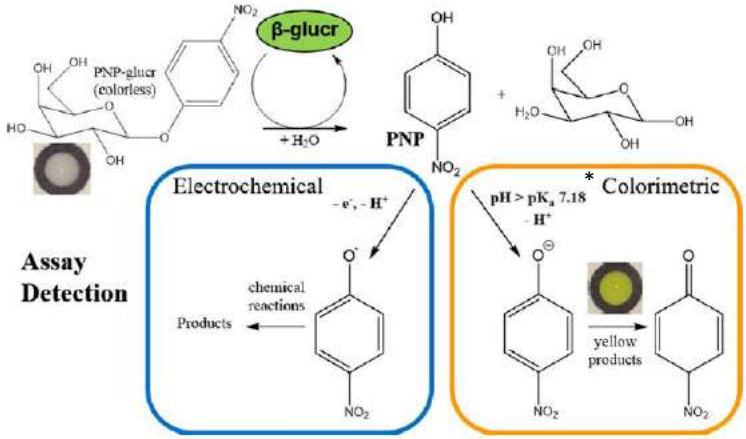
Jaclyn A. Adkins,[†] Katherine Boehle,[†] Colin Friend,[‡] Briana Chamberlain,[§] Bledar Bisha,[∥] and Charles S. Henry*[,†,‡,§]

[†]Department of Chemistry, [‡]School of Biomedical Engineering, and [§]Chemical and Biological Engineering, Colorado State University, Fort Collins, Colorado 80523, United States

[∥]Department of Animal Science, University of Wyoming, Laramie, Wyoming 82071, United States

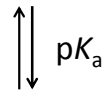
4 Case III: Enzyme

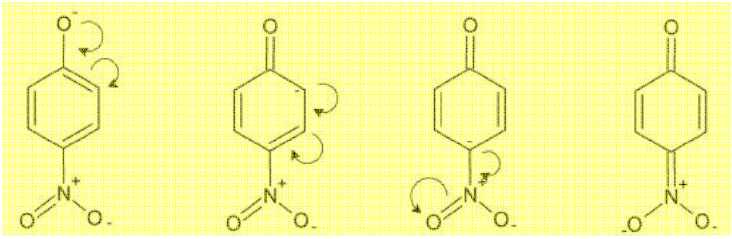
Glycoside hydrolase



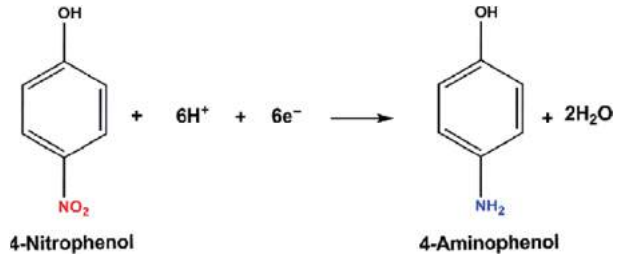
* Colorimetric / optical detection (lecture Nielen)

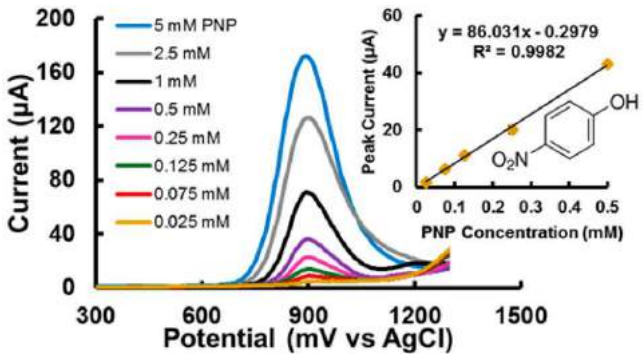
4 Case III: Enzyme



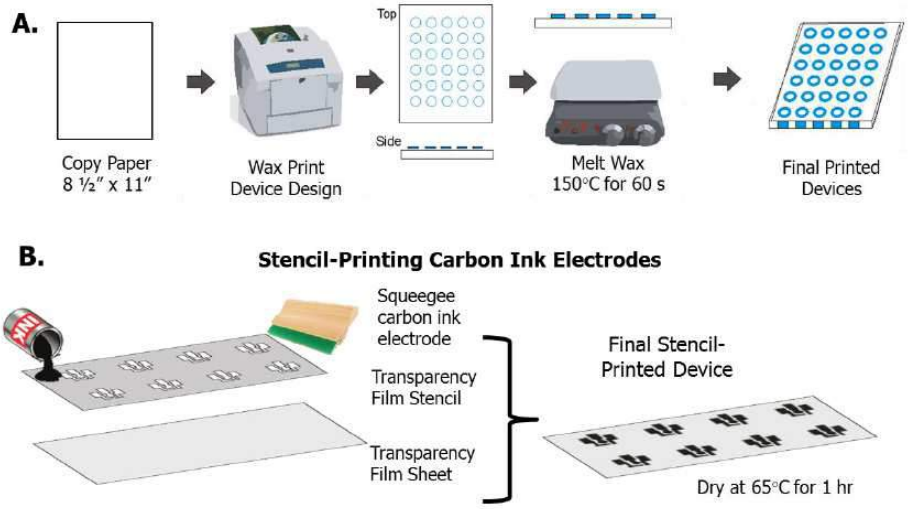


4 Case III: Enzyme

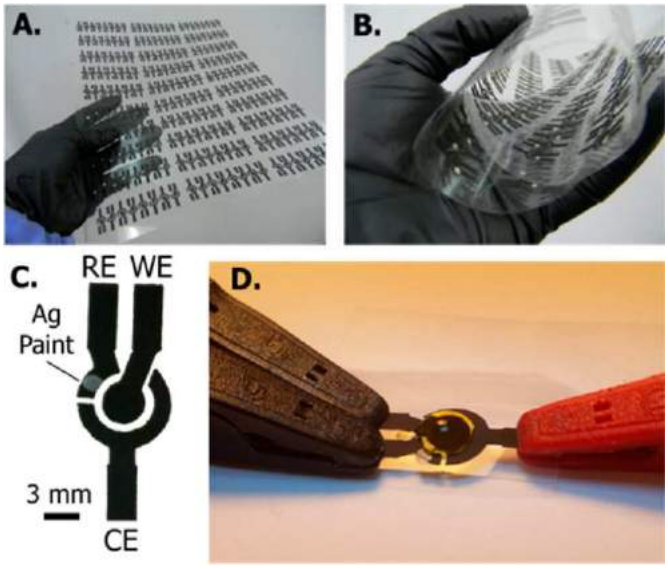




4 Case III: Enzyme

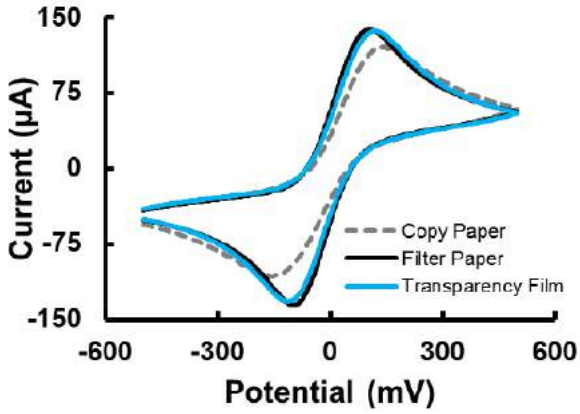


4 Case III: Enzyme



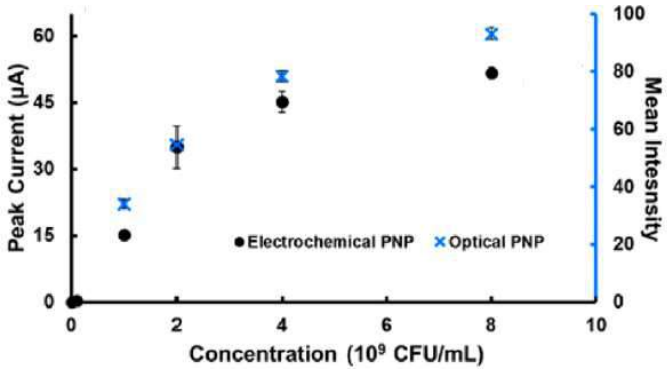
4 Case III: Enzyme

Comparison $K_3Fe(CN)_6/K_2Fe(CN)_6$ redox chemistry for different electrode substrates



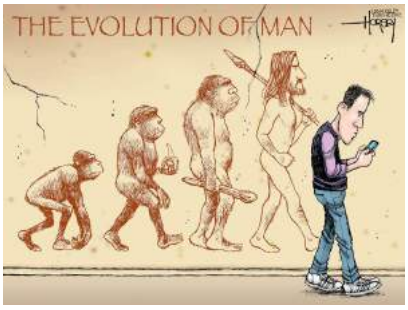
4 Case III: Enzyme

Electrochemical + colorimetric response

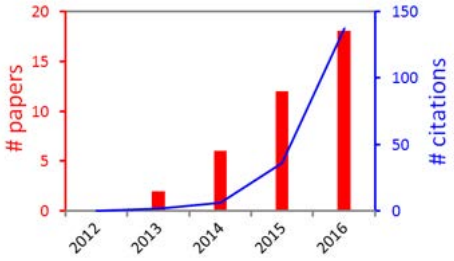


Colony-**F**orming **U**nit

5 Concluding Remarks



"electrochemical" and "smartphone" as topics
(source: ISI Web of Science, June 15, 2017)



- Electrochemical detection: from medical to food analysis
- Selectivity (in complex media) and stability important issues
- Smart integration electr(ochem)ical sensors

Redox chemistry!
e.g. capacitive, transistors

Optical Detection

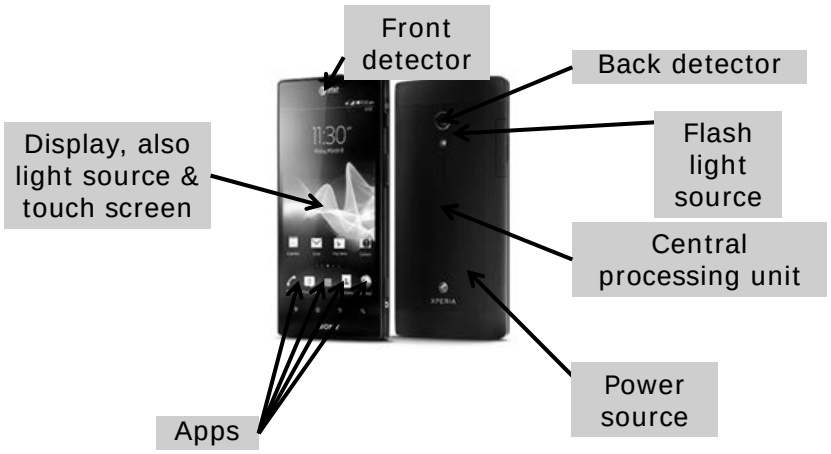
Michel Nielen, FoodSmartphone coordinator
RIKILT, Wageningen Research, NL

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

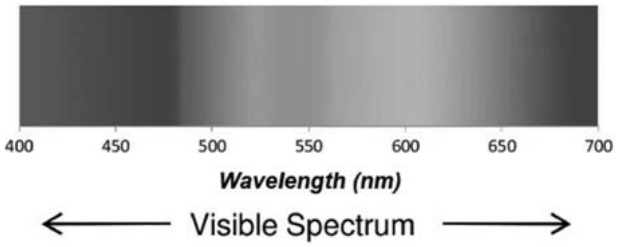
How to detect a biorecognition event

- Using a label, that is detected via
  - Absorbance (Vis-range) / reflectance
  - Fluorescence
  - Chemiluminescence
  - Electrochemistry (→ *lecture Louis de Smet*)
  - others
- Label-free
  - SPR
  - others

Available on your smartphone



- *Vis illumination with white light: some wavelengths are absorbed, our eyes observe the reflected complementary colors !*



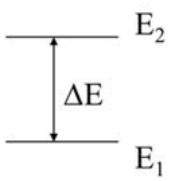
Electronic excitation in molecules

Theory of electromagnetic radiation:

-Maxwell: general theory about light, electricity, magnetism.

-Quantum mechanics: energy states of matter and electromagnetic radiation are quantized

-Electrons in molecules move in orbitals at discrete energy levels, defined by quantum theory



- EM radiation: $E_{PHOTON} = h\nu = hc/\lambda$
- Resonance Requirement: $\Delta E = E_2 - E_1 = h\nu = hc/\lambda$
- Probability of transitions differs
- Excitation: outer shell electrons move to higher energy antibonding orbital, energetically only possible in UV-Vis

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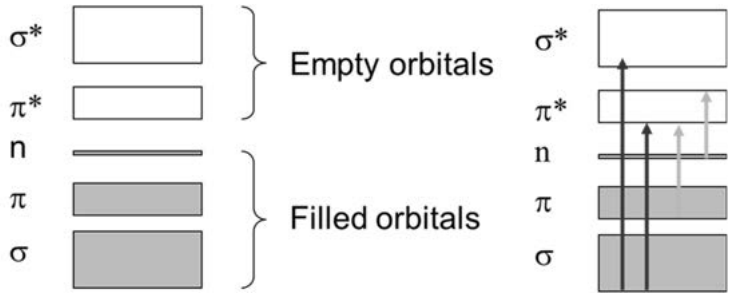
See also in: McMurry, "Organic Chemistry", 9th Int. Edition, p. 369

Electronic energy levels

- Large molecules have many electrons in many orbitals
- Each orbital has its own energy, so there are many discrete energy levels present in a molecule



- Transitions σ or σ* less relevant: ΔE too big, λ< 200 nm

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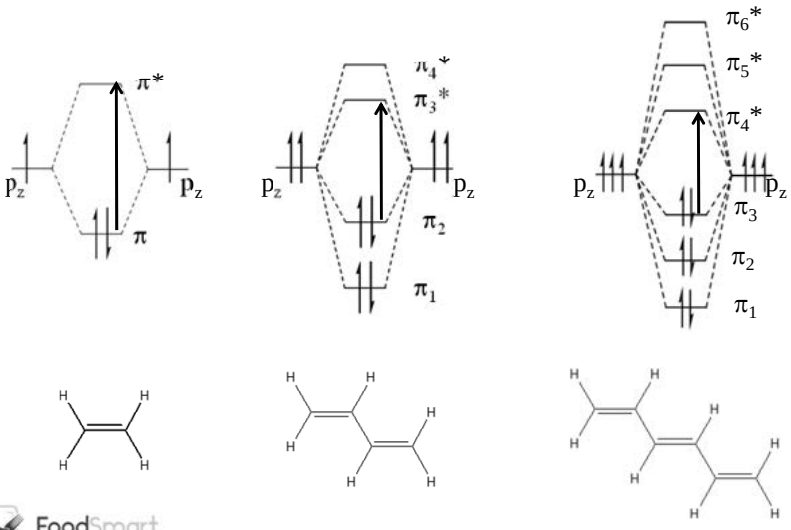
See also in: McMurry, "Organic Chemistry", 9th Int. Edition, p. 369

π-π* transitions longer polyenes



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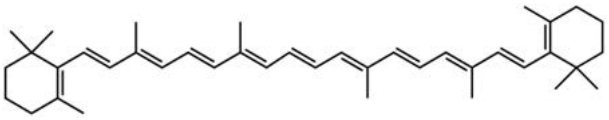
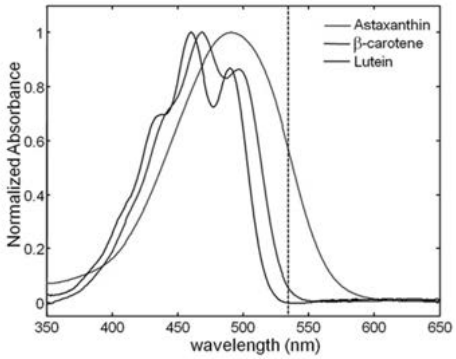
© McMurry (9th) p 439

π-π* transitions longer polyenes



β-carotene: 11 conjugated double bonds
longer wavelength, higher intensity



PLoS ONE, **2011**, *6(9)*, e24302

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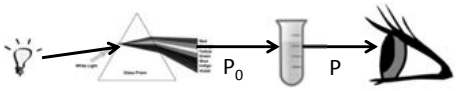
UV-Vis absorption spectroscopy

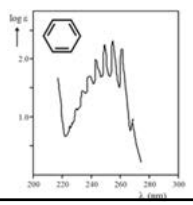
- Lambert-Beer law

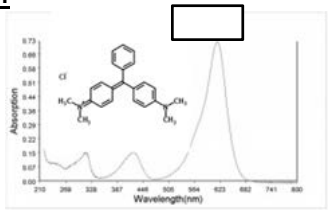


$A = -\text{Log } T$ and T is P/P_0, the fraction of light that passes through the sample; at a specific <u>wavelength</u> and for <u>dilute solutions</u>:

$A = \varepsilon_\lambda bc$ *and ε is the molar absorptivity or molar extinction coefficient [liter/(Mol*cm)], b is the path length [cm] and c is the concentration [Mol/liter]*

- A and ε are wavelength dependent: absorption spectrum depends on <u>molecular substructures:</u>





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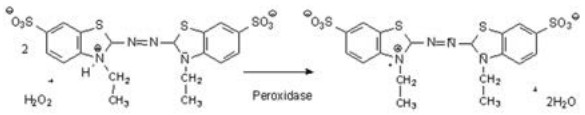
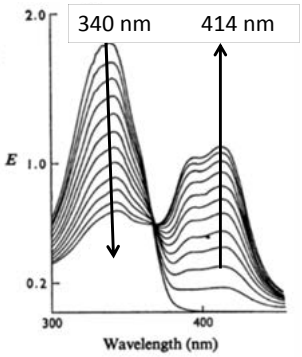
UV-Vis absorption spectroscopy

- Transmission or reflection mode?
  - pathlength much shorter in reflection mode
- Enclosed or under ambient light?
  - ambient possible, provided adequate reference
- *Example: colorimetric immunoassay*
  - Detection Ab covalently linked to enzyme
  - Add substrate (ABTS, TMB), Vis signal

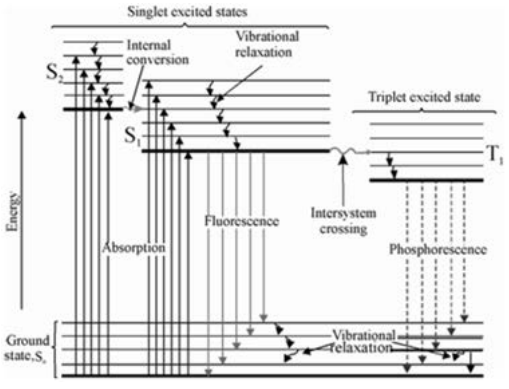


A_2BTS



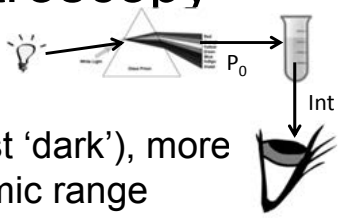
Luminescence versus absorption

- Enclosed or under ambient light?
  - Preferably enclosed (measurement against dark!)
- Jablonski diagram:



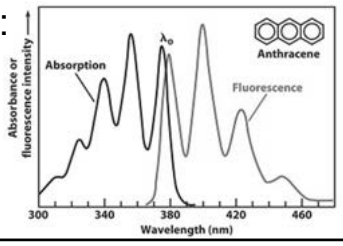
Fluorescence spectroscopy

- Emission of light from an excited state of a molecule
- More sensitive (light against 'dark'), more specific, larger linear dynamic range



at low concentrations: $Int = kP_0c$ and k is a constant that includes ε, c is the concentration [Mol/liter], P_0 is the incident irradiance

- Excitation spectrum similar as UV-Vis absorption spectrum. Emission spectrum:
- Selection of λ_{ex} and λ_{em}.



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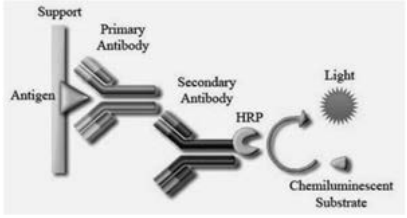
Chemiluminescence

Emission of light arising from a chemical reaction:

- No background from excitation light scattering
- Example: luminol + H_2O_2 ⟶ blue light
- Highly relevant reaction for amplified biosensing
  - Glucose: using glucose oxidase yields H_2O_2
  - HRP catalyses the oxidation of luminol to 3-aminophthalate (particularly in the presence of iodophenol enhancer)



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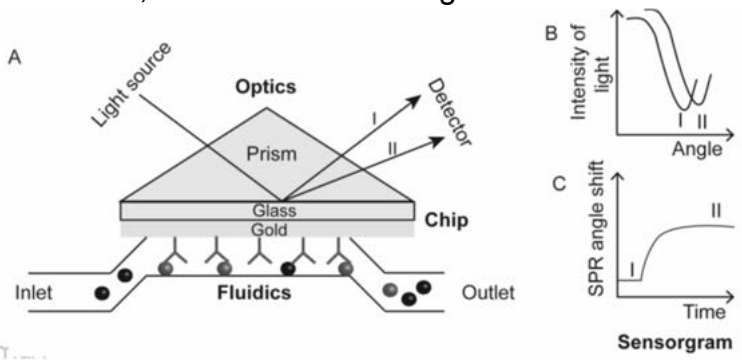
Surface Plasmon Resonance

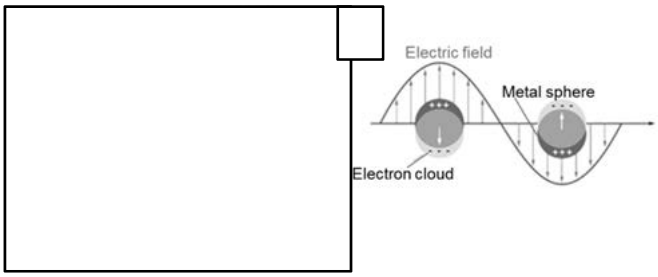
- Light strikes boundary: evanescent wave generated
- If gold (metal) film, then evanescent wave interacts with free electron clouds in gold layer: propagating plasmons
- Intensity minimum: SPR angle, function of refractive index near the surface, acts as a "biosensing antenna"



https://www.youtube.com/watch?v=sM-VI3alvAI

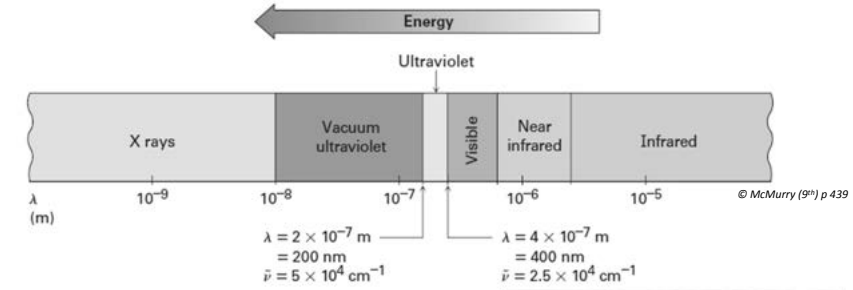
Localized Surface Plasmon Resonance

- LSPR: simpler instrumentation, no optics, just room light
- Gold (metal) nanoparticles or nanostructures
- *Note: gold nanoparticles widely used as a label in dipstick assays (red to purple color changes when gold particles aggregate)*



https://www.youtube.com/watch?v=QLT1vrnJXWI

UV-Vis absorbance lab setup



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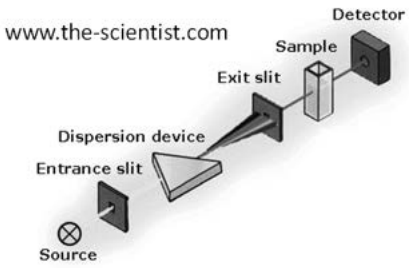
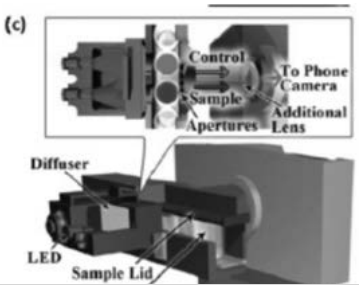


Absorbance detection setup 1

Example of dual transmission mode on smartphone

- Independent of (fluctuating) ambient light and manual alignment
- 2x LED 650 nm (Δλ 15 nm) as light source
- 2x diffuser for uniform illumination of tubes
- 7x demagnification lens: both tubes within one image

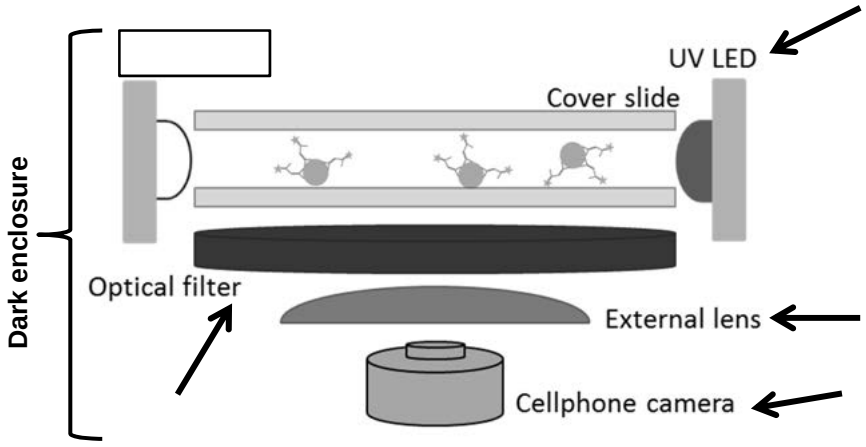


Coskun at al., Lab Chip, 2013, 13, 636-640

Absorbance detection setup 2

Example of reflection mode under white light

Fluorescence detection setup 1

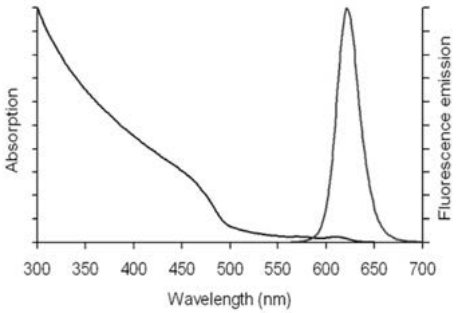


Excitation – Emission spectrum

- Fluorescence label: Quantum Dots 625

12x

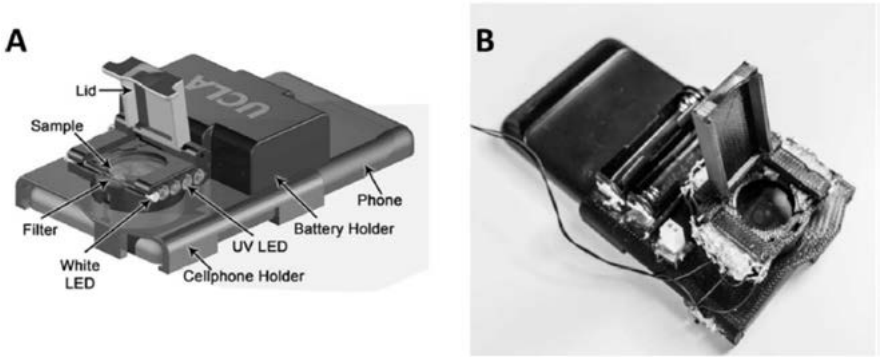
380 nm



1x

610 nm

Fluorescence detection setup 1



Ludwig *et al*., *PLOS ONE*, 10(8): e0134360 (2015)

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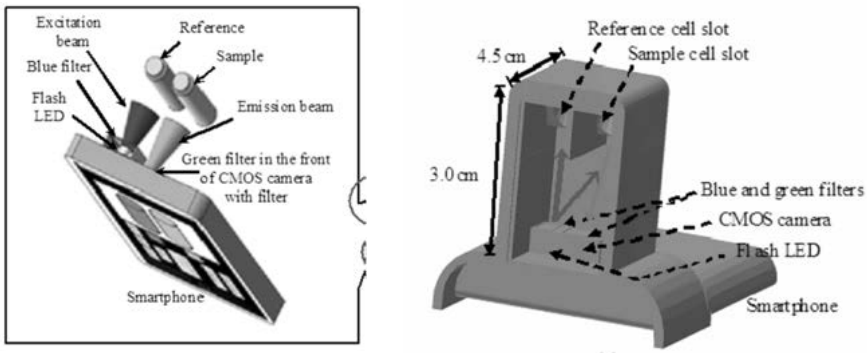
Fluorescence detection setup 2

Use your flash light LED as excitation source!

- White light, 400-700 nm
- Requires excitation filter, for example blue filter 450 nm (Δλ 21 nm)
- Simplified set-up, less power consumption



Hossain *et al*., *Photonic Sensors*, 5(4), 2015: 289-297

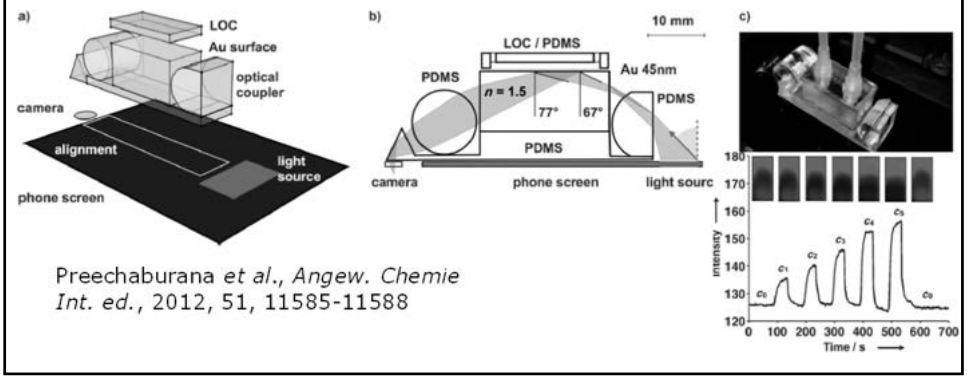
Chemiluminescence setup

- As in fluorescence set-up, but without excitation light source, no excitation filter
- Only emission light filter required
- But more complex (bio)reagents supply

SPR detection setup

Use your display as light source!

- Easily programmed to desired color (wide spectral band)
- Wide-angle illumination for angle-resolved SPR
- Not limited by poor dynamic range of camera, only the position of the SPR-dip has to be determined (camera spatial resolution)



Preechaburana *et al.*, *Angew. Chemie Int. ed.*, 2012, 51, 11585-11588

Camera image processing

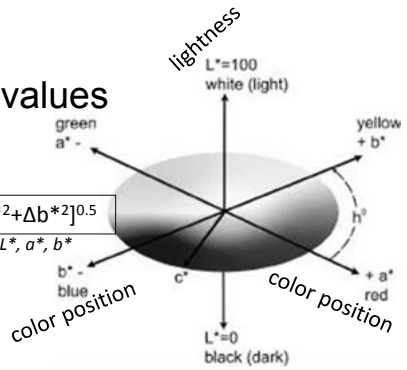
-Measurement of colors: hue (color) and depth (color intensity)

-Multiple options, convert your image into

- grayscale values
- RGB values
- CIE L*a*b* color space values
- ...*etc*....

$$\Delta E^* = [\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}]^{0.5}$$

Δ versus standard L, a*, b**



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Summary

- Biorecognition assays exploit usually optical spectroscopy as a readout system
- Same principles apply for smartphone approach, but often reflection mode measurements
- Smartphone assay challenges with respect to reagent storage and incubation time(s)
- Only low-cost smartphone modifications required

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1st Summer School on Smartphone-based Food Analysis
Wageningen, The Netherlands, 26-30 June 2017

Microfluidics / Lab-on-a-chip

Daniel Filippini [1]

The miniaturization and automatization of chemical analyses carried out in microfluidic and lab-on-a-chip (LOC) configurations can be associated with a number of advantages such as producing less waste, using less reagents and demanding smaller samples.

The smaller dimensions involved in LOC shortens diffusion distances and maximizes the area to volume ratio, which affect in the flow regime and overall behaviour of the systems.

In this lecture, the motivations for LOC based analyses are presented together with the conceptual background to understand the microfluidic behaviour, and simple tools to analyse and predict the operation of fluidic systems.

State or the art LOC solutions are presented to highlight the contrast with autonomous LOC solutions aimed at complementing cell phones for chemical detection. Examples of such implementation are discussed in detail as well as the consideration of LOC detection principles that optimize the readout resources available in cell phones.

Suggestions for further reading:

[1] W.Tian, E. Finehout Eds., Microfluidics for Biological Applications, Springer (2008).
[2] Science 288 (2000), 113.
[3] Science 290 (2000), 1536.
[4] Lab Chip 13 (2013), 415.
[5] Lab Chip 9 (2009), 417.
[6] Anal. Chem. 80 (2008), 6206.
[7] Nat. Comm. 3 (2012), 1283.
[8] PNAS 105 (2008),19606.
[9] Lab Chip 9 (2009), 2286.
[10] Lab Chip 13 (2013), 51.
[11] Biosens. & Bioelectron. 77 (2016), 1153.
[12] Angewandte Chemie 54 (2015), 8708.
[13] Trends in Biotechnology 32 (2014), 351.
[14] Angewandte Chemie 52 (2012), 11585.

[1] E-mail; danfi@ifm.liu.se; Optical Devices Laboratory, Linköping University, Linköping, Sweden

FoodSmartphone.eu

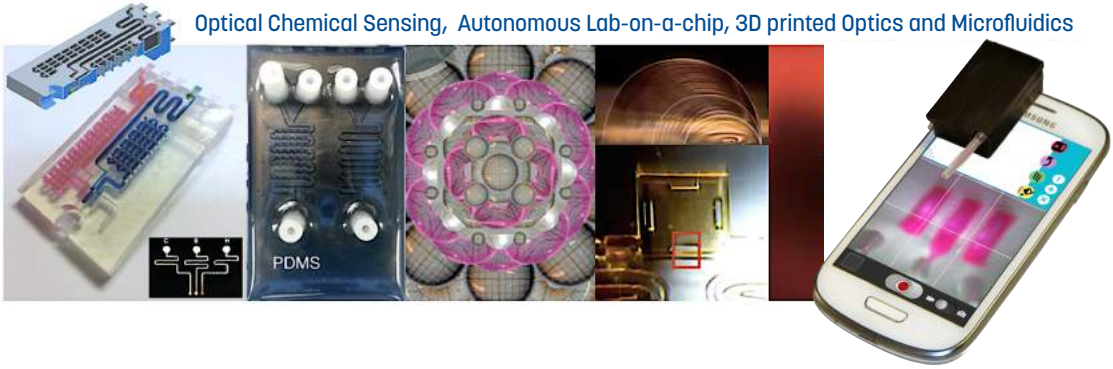
Summer School on Smartphone-based Food Analysis,
RIKILT, Wageningen, The Netherlands, 26-20 June 2017

LINKÖPING UNIVERSITY

Microfluidics / Lab-on-a-chip

Daniel Filippini
Professor in Applied Physics
Optical Devices Laboratory (ODL)
Division of Sensor and Actuator Systems (SAS), Linköping University, Sweden

Optical Chemical Sensing, Autonomous Lab-on-a-chip, 3D printed Optics and Microfluidics

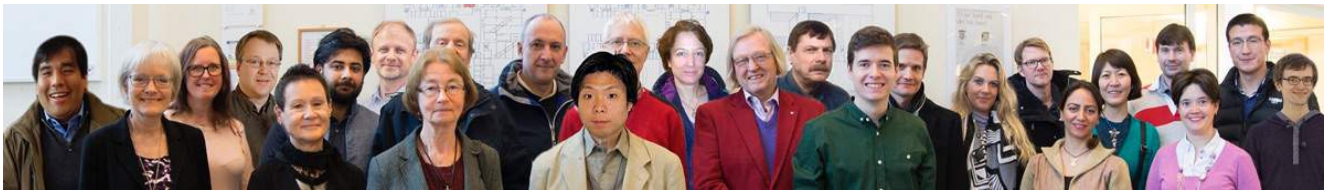


LINKÖPING UNIVERSITY

Division of Sensor and Actuator Systems - SAS

Department of Physics, Chemistry and Biology - IFM

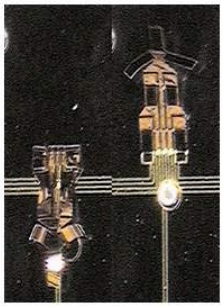
Graphene sensors; Soft microrobots and actuators; Biosensors; 3D printed optics and Autonomous lab-on-a-chip; Chemical imaging; Data mining; Integrated detection systems



Applied Sensor Science

- Graphene and SiC-FET gas sensors
- Graphene and monolayers for gas sensors
- Emissions monitoring and control
- Particle sensors
- Smart Sensing / Data Mining

Bionics and Transduction Science

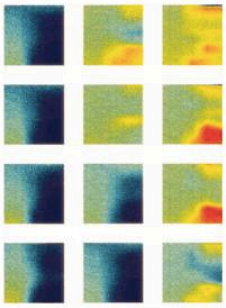


- Electroactive surfaces and scaffolds
- On-chip mechanostimulation of cells
- Polymer (micro-)actuators
- Soft microrobotics

Biosensors and Bioelectronics

- Integrated Biosensor Platform
- Membrane based lateral flow tests
- Encapsulated catalytic and biocatalytic system
- Processable materials for printed bioelectronic devices

Chemical Sensor Systems



- Electrochemical sensing
- Basic electrochemistry
- Gas sensing
- Drinking water monitoring sensor systems

Optical Devices Laboratory

- Optical Chemical Sensing
- Autonomous Lab-on-a-chip
- 3D printed fast prototyping of Optics and Microfluidics
- Distributed Detection Systems and Apps

LINKÖPING UNIVERSITY

Division of Sensor and Actuator Systems - SAS

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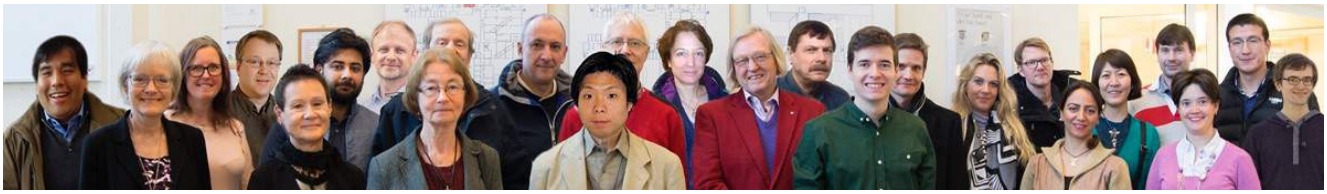
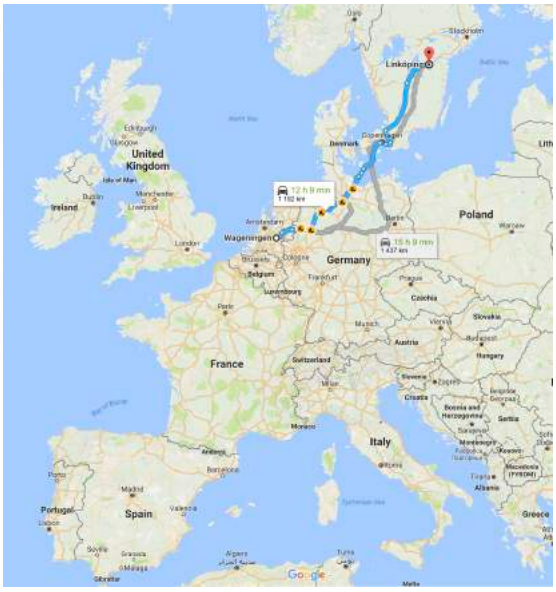
Graphene sensors; Soft microrobots and actuators; Biosensors; 3D printed optics and Autonomous lab-on-a-chip; Chemical imaging; Data mining; Integrated detection systems





Sahl Sadeghi

Anke Suska

Daniel Filippini

ESR9 / PhD Applied Physics
Lab-on-chip devices for smartphone imaging Surface Plasmon Resonance (iSPR) detection

WP3
Integrated sample preparation devices

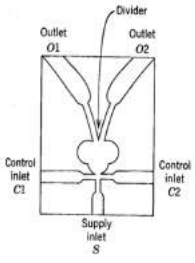
Optical Devices Laboratory

- Optical Chemical Sensing
- Autonomous Lab-on-a-chip
- 3D printed fast prototyping of Optics and Microfluidics
- Distributed Detection Systems and Apps

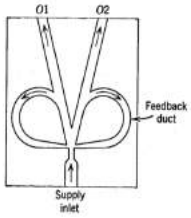
Overview

- Background / Motivations
- Lab-on-a-chip (LOC) solutions
- Autonomous LOC
- Cell phone LOC readout

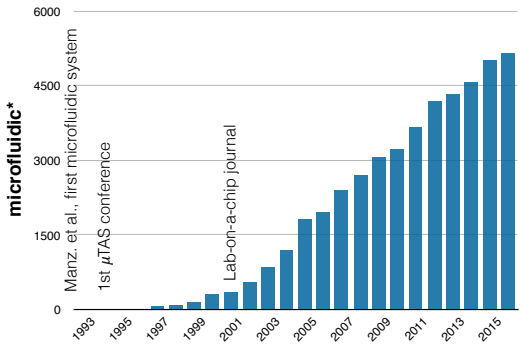
Jet deflection amplifier



Oscillator

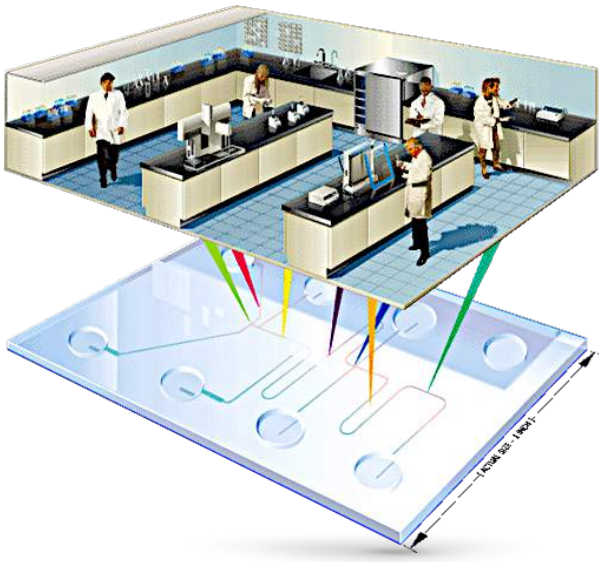


Fluidics for components in pneumatic computers
S. Colin, Microfluidics, Wiley (2004)
B.W. Anderssen, The Analysis and design of pneumatic systems, Wiley (1967)



Background / Motivations

- **Small volumes** (less waste, lower reagents costs and smaller sample volumes for diagnostics)
- **Faster analysis** and response times due to short diffusion distances, fast heating, high surface to volume ratios, small heat capacities
- **Better process control**, compactness and automatization allowing parallelization and high-throughput analyses
- Compatible with mass production technologies leading to cost-effective disposable chip configurations

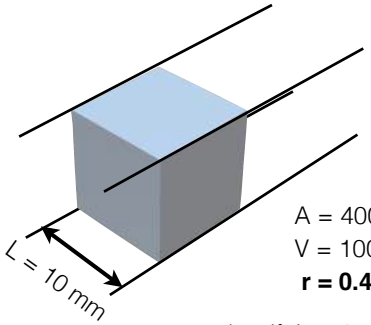


Background

- **Surface / volume ratio inversely proportional to size**
- Reynolds number (Re) is small
  ➔ laminar flow

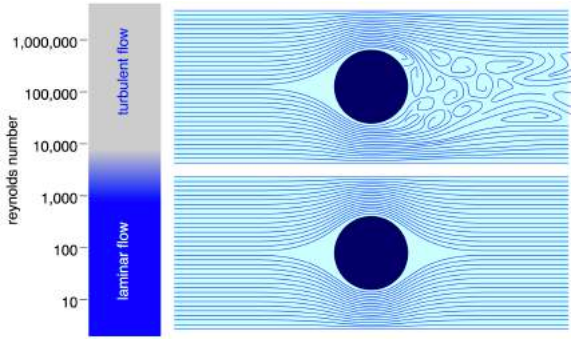
$$\text{Re} = \frac{\text{inertial forces}}{\text{viscous forces}} = \frac{\rho v^2/L}{\eta v/L^2} = \frac{\rho L v}{\eta}$$

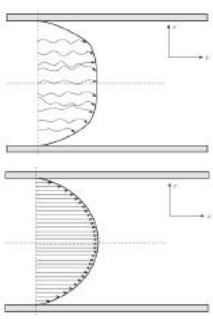
ρ = density, η = viscosity
water at 1 mm/s in a 50 µm microchannel Re = 0.05

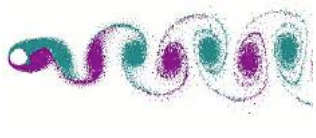


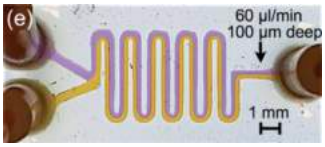
A = 400 mm²
V = 1000 mm³
r = 0.4 mm⁻¹

but if L = 1 mm
r = 4 mm⁻¹









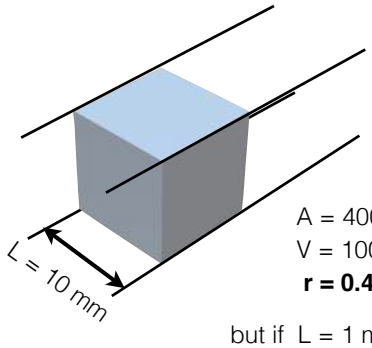
Background

- Péclet Number (Pe)

$$Pe = \frac{diffusion\ time}{convection\ time} = \frac{L^2/_D}{L/_v} = \frac{v \cdot L}{D}$$

Pe >> 1 ➔ diffusion length (L_d), L_d << L

L = characteristic size, D = diffusion coefficient, v = flow velocity



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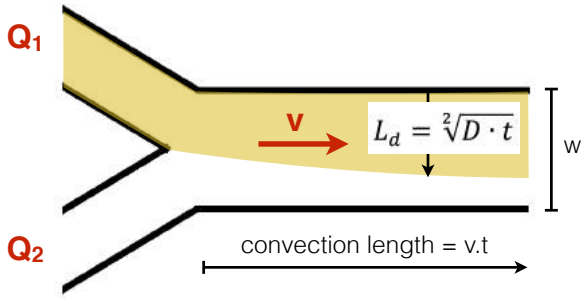


$t_{mix} \approx w^2/D$

$L_{mix} \approx v \,.\, t_{mix} = v \,.\, w^2/D =$ **Pe . w**

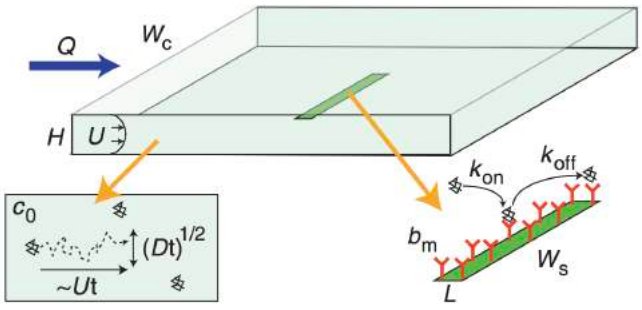
D ≈ 1000 μm²/s
w ≈ 100 μm
v ≈ 1 cm/s

➔

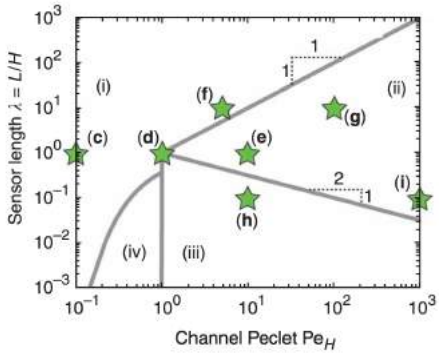
t_{mix} = 10s
Pe = 1000
L_{mix} = 10 cm

Mixing requires long channels

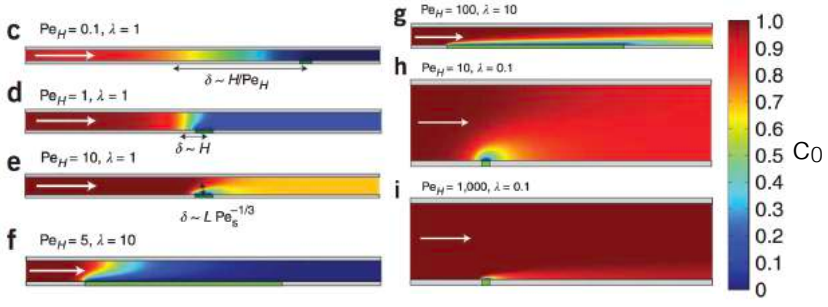
Background



"Phase diagram" of mass transport and steady-state flux



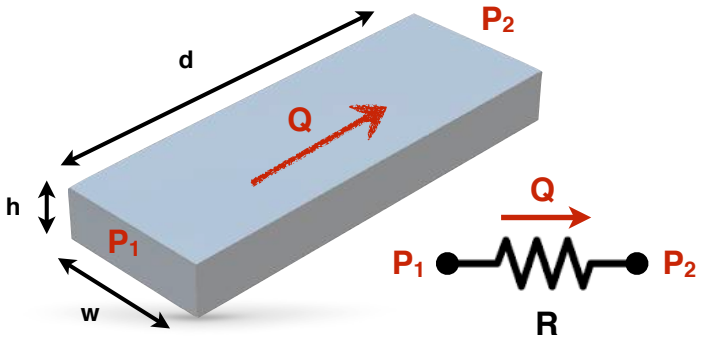
c_0 = target concentration
U = velocity
Q = volumetric flow rate
H = channel of height
W_c = channel width
L = sensor of length
W_s = sensor width
b_m = receptors per unit area.
k_{on} & K_{off} = kinetic rate constants for the (first-order) binding reaction
D = diffusivity of the target molecules



TM Squires, RJ Messinger, SR Manalis, Making it stick: convection, reaction and diffusion in surface-based biosensors, Nat. Biotech. 26, 417 (2008)

Background

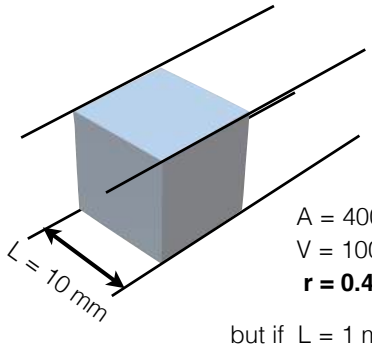
- Hydraulic resistance inversely proportional to dimension



$$R = \frac{12 \cdot \eta \cdot d}{w \cdot h^3} \qquad \Delta P = R \cdot Q \qquad \Delta U = R \cdot I$$

Q = 100 µL/hr
d = 2 cm
w = 100 µm
η = 1 mPa.s

h = 100 µm ➔ ΔP ~ **1 mbar**
h = 10 µm ➔ ΔP ~ **1 bar**



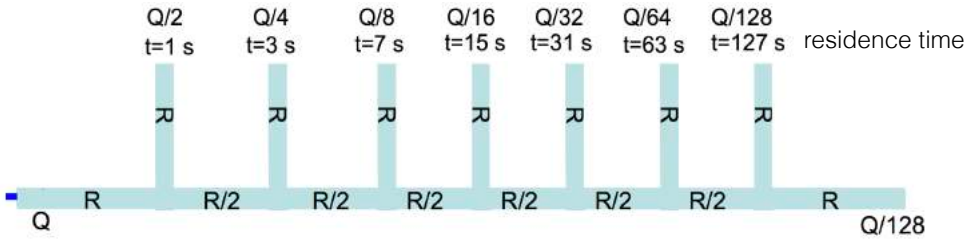
A = 400 mm^2
V = 1000 mm^3
r = 0.4 mm^{-1}

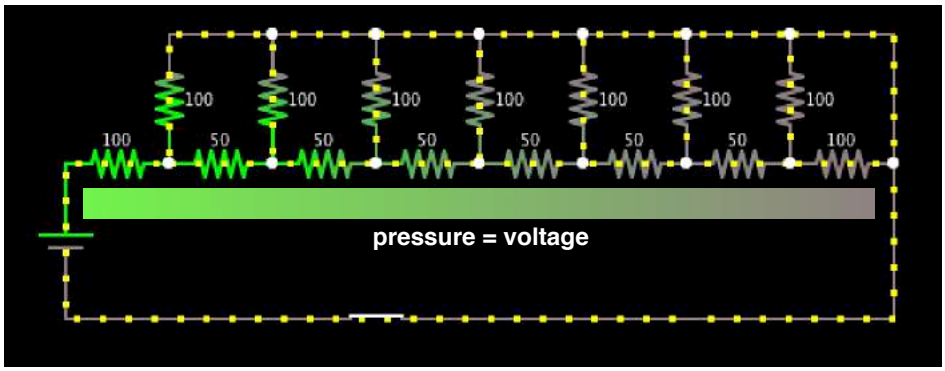
but if L = 1 mm
r = 4 mm^{-1}

Classical microfabrication
h ≳ 100 µm

- high pressure drops
- leakages
- low flow rates (µL/hr)

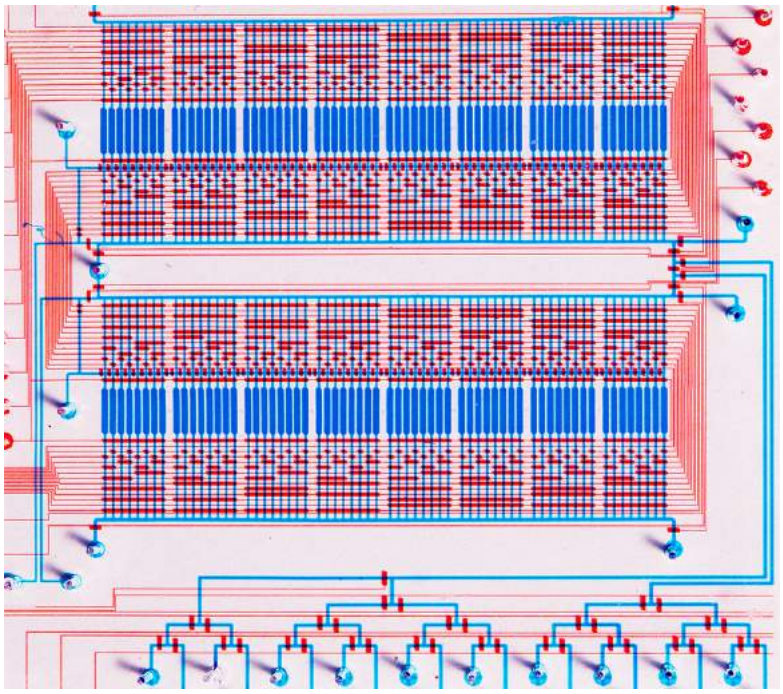
Simple Analogue Simulation

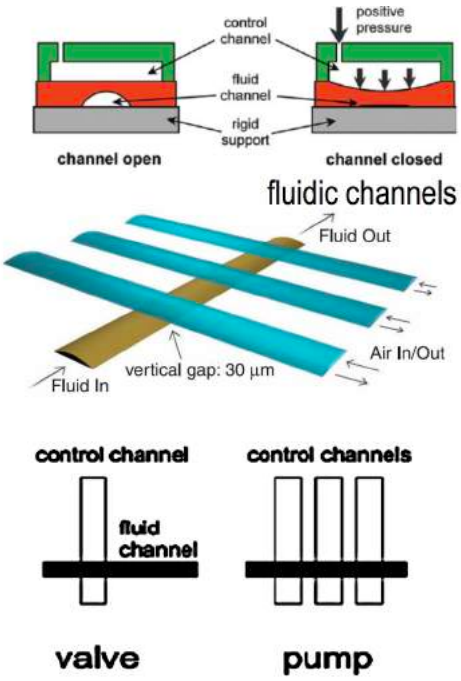




www.falstad.com

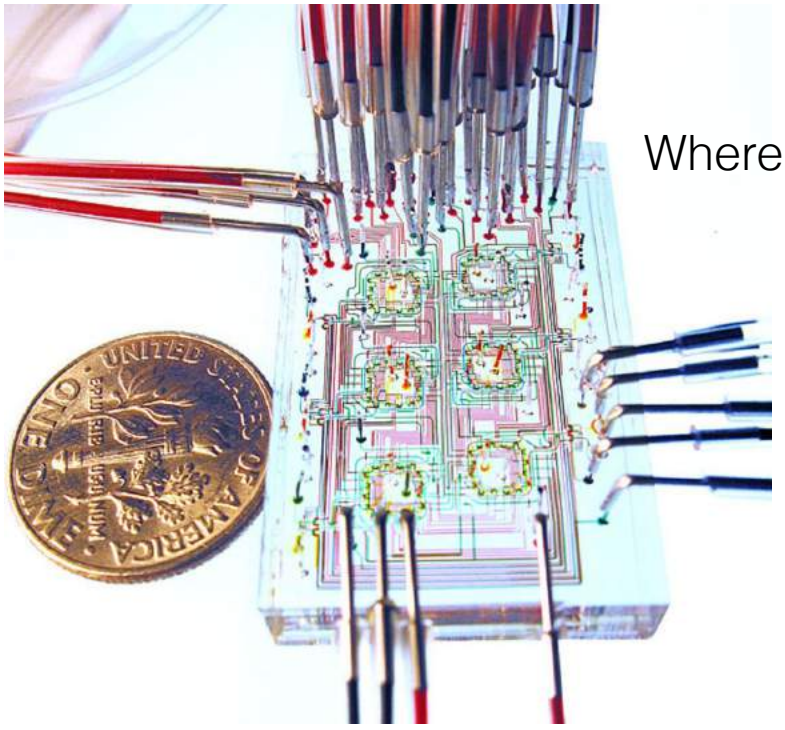
Lab-on-a-chip / Large-scale integration



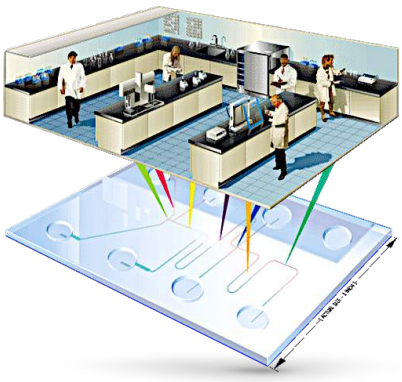


Unger MA, Chou HP, Thorsen T, Scherer A, **Quake SR**, "Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography", Science 288: 113-116 (2000)
Quake SR and Scherer A, "From Micro to Nano Fabrication with Soft Materials", Science 290: 1536-40 (2000).
X. Wu, N, Schneider, A Platen, I Mitra, M Blazek, R Zengerle, R Schüle, M. Meier, PNAS, 113, E4143-50 (2016)

Chip-in-the-lab



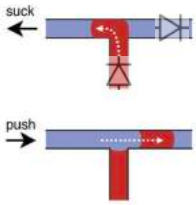
Where do these tubes go?



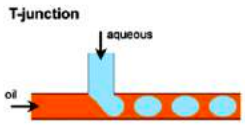
Main functions

- Volume metering

Lab Chip, 2012, 1587

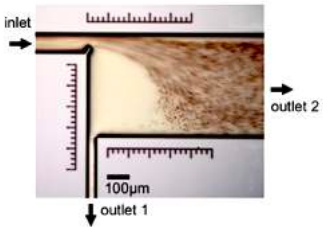


Appl. Phys. Lett. 2003 , 83, 4664

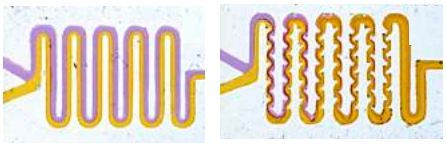


- Separation

Micromachines 61, 2015, 239



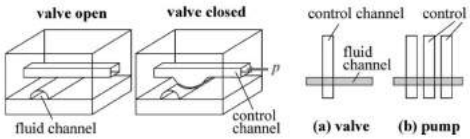
Lab Chip, 2014, 424

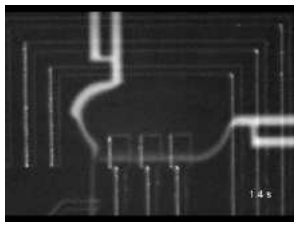


- Mixing

- Transport / Valves

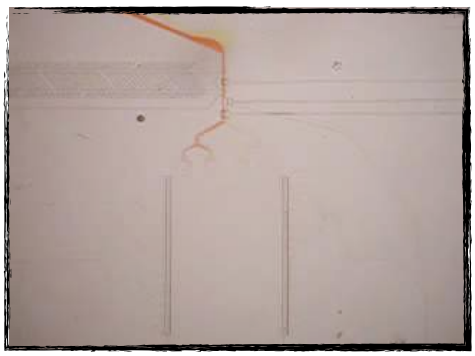
Lab Chip, 2007, 1094



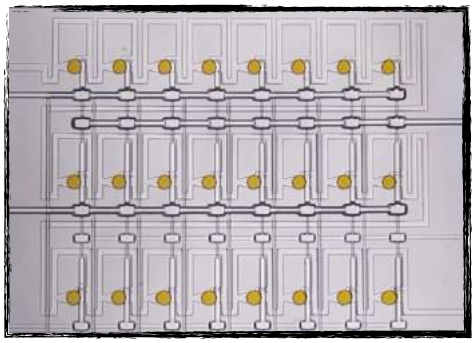


Lab Chip, 2013, 415

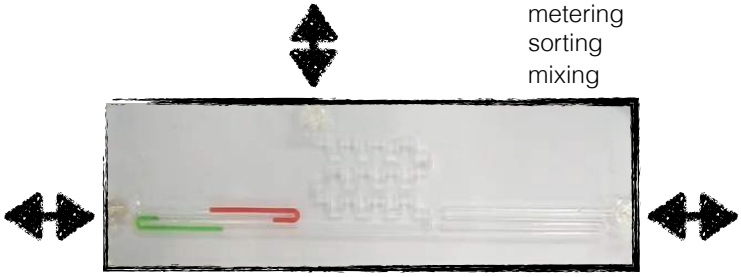
Control in Laboratory Configurations



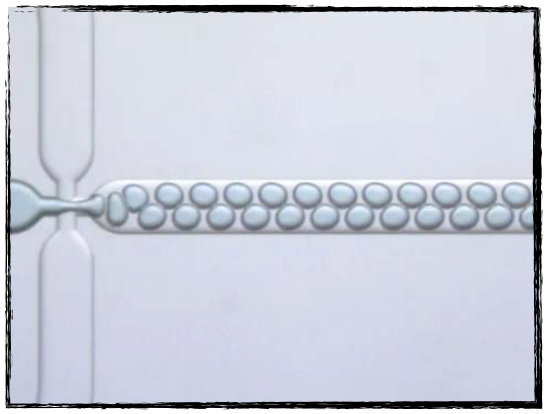
Lab Chip 2009, 417



Lab Chip 2016, 1698



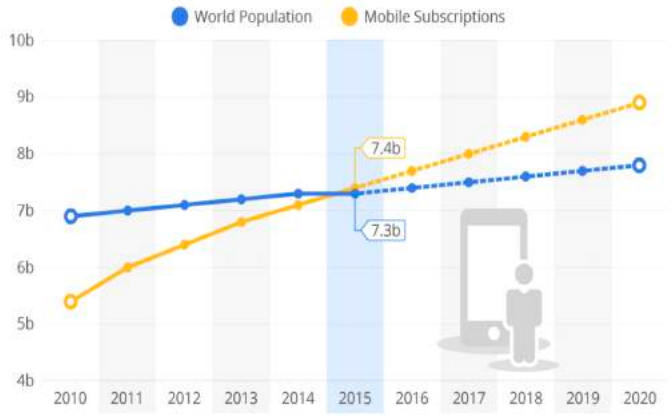
Anal. Chem., 2008, 80, 6206–6213



www.dolomite-microfluidics.com/droplets

Chemical sensing with cell phones

- Cell phone are the **most ubiquitous** technical infrastructure
- Available **for free** for chemical sensing if properly interfaced
- Autonomous increase of sophistication
- Wireless and portable communication platform
- Equipped with sophisticated physical transducers: cameras, screens, accelerometers, magnetic and capacitive sensors, etc.



United Nations, Ericsson sources / from Statista.com

Interfacing chemical sensing to cell phones

Computer controlled instruments

Jing Li, NASA's Ames Research Center for Cell-All DARPA
IOIO Mint - Portable Android Development Kit

Reusable accessory solutions

Lab on a Chip 13 (2013), 51
JoVE (2013)DOI:10.3791/50451

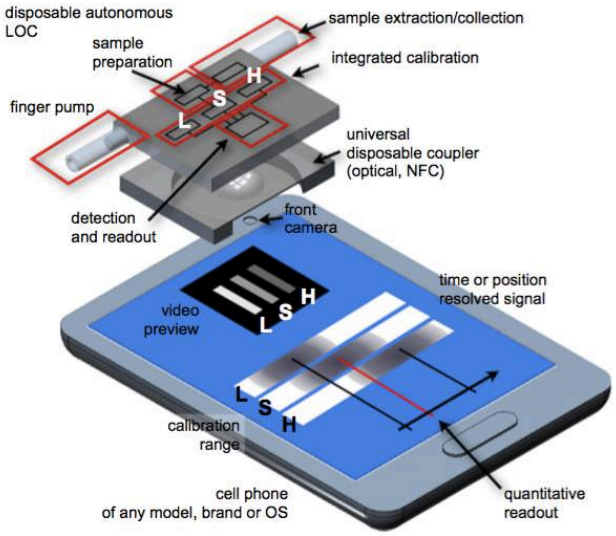
Disposable accessory solutions

Biosens. & Bioelectron. 77, 2016, 1153
Angewandte Chemie 54, 2015, 8708
Trends in Biotechnology 32, 2014, 351
Angewandte Chemie 52, 2012, 11585

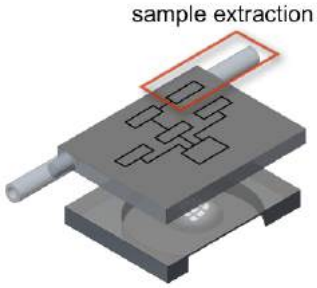
Not Ubiquitous → **Ubiquitous**

Device Requirements

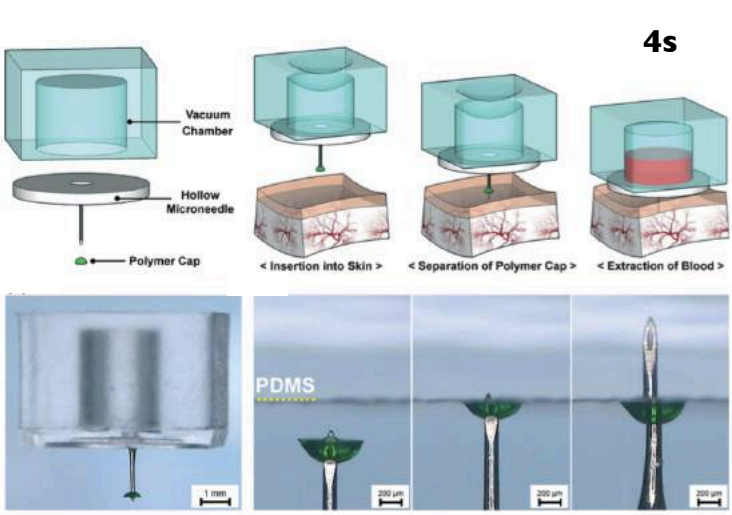
- **Disposable**
  Selective and Low cost
- **Generic**
  Detection principles and Architectures
- **Universal interfacing**
  All phone models, brands, OS and generations (default OS resources)
- **Autonomous**

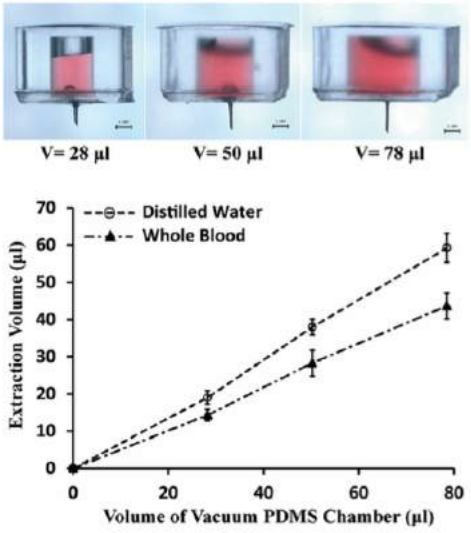


G. Comina, A. Suska, D. Filippini, Biosensors and Bioelectronics 77, 2016, 1153
P. Preechaburana, A. Suska, D. Filippini, Trends in Biotechnology 32, 2014, 351



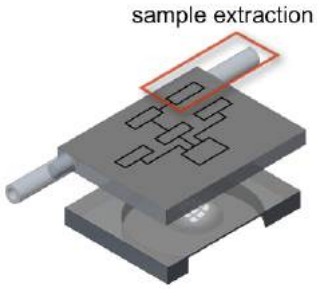
Sample extraction /collection



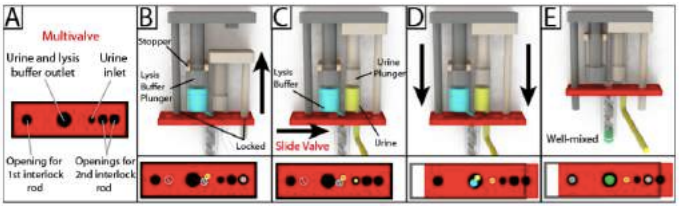


C. Li, et al., Lab Chip, 2015, 15, 382–390

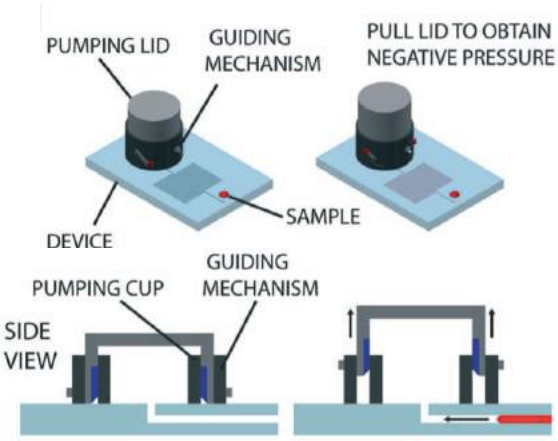
Sample extraction /collection

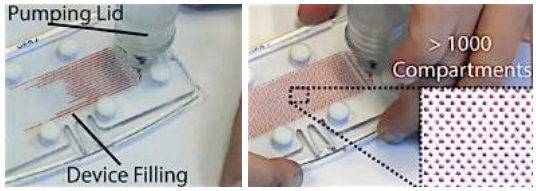


A. F. Coskun, et al., Lab Chip, 2013, 13, 4231–4238



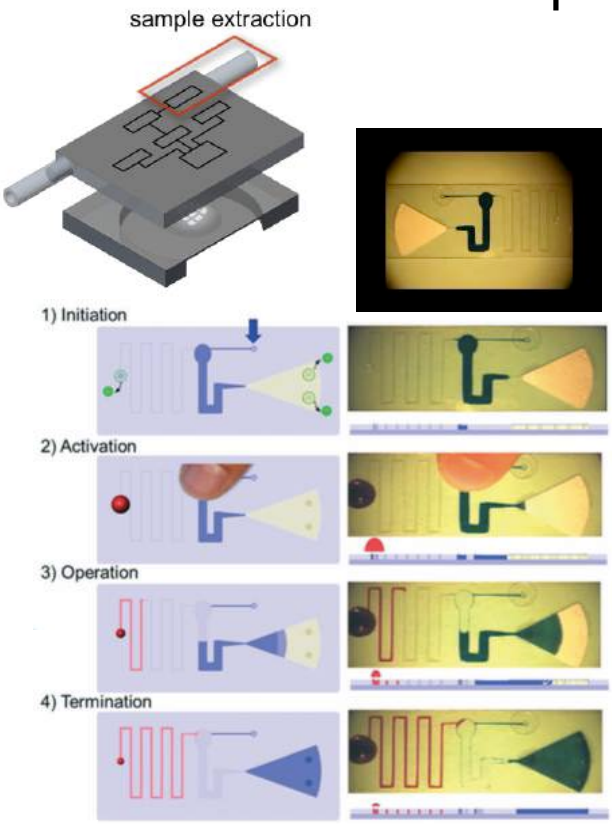
E. Jue, et al., Lab Chip, 2016



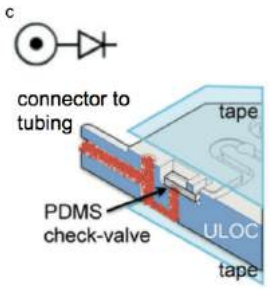


S. Begolo, et al., Lab Chip, 2014, DOI: 10.1039/c4lc00910j

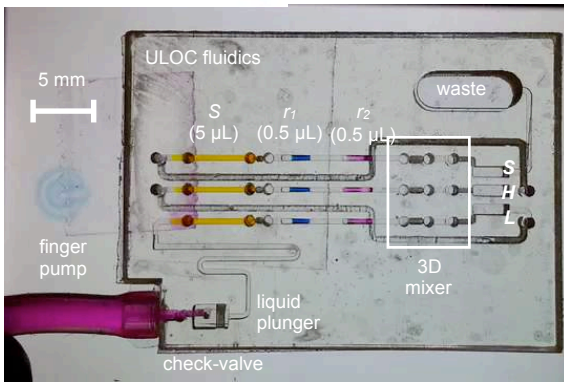
Sample collection and transport



T. Kokalj, et al., LabChip,2014,14,4329–4333



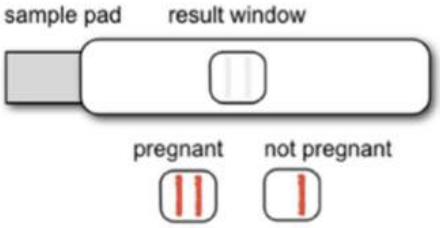
- Unibody-LOC
- 0.5 US$/device
- *H,L* calibration range
- integrated pump
- integrated mixer
- conditioning = 6s



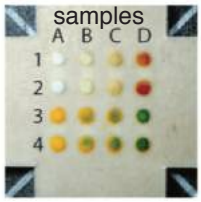
G. Comina, et al., Angew. Chem. Int. Ed. 2015, 54, 8708 –87

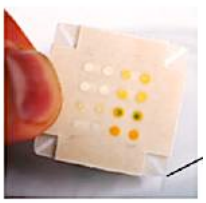
Sample collection and transport

Lateral flow devices

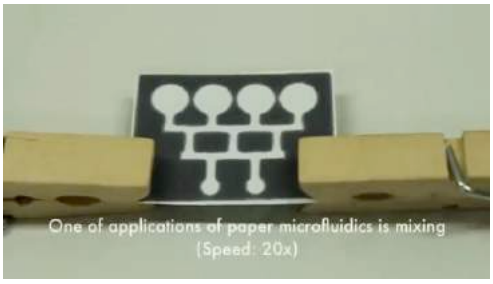


3D paper fluidics / Paper Analytical Devices

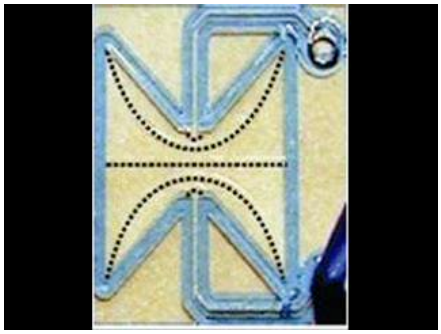




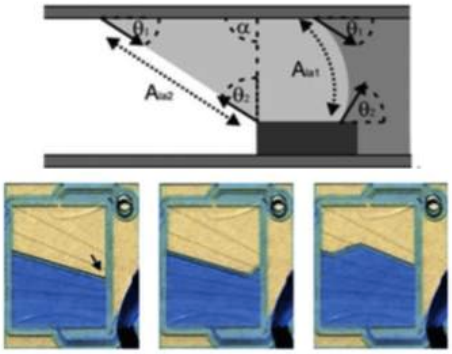
A. W. Martinez, S. T. Phillips, G. M. Whitesides,PNAS 105, 2008,19606–19611



MIT Lab 5 / Paper Microfluidics, https://www.youtube.com/watch?v=J5LwNGm0tbw



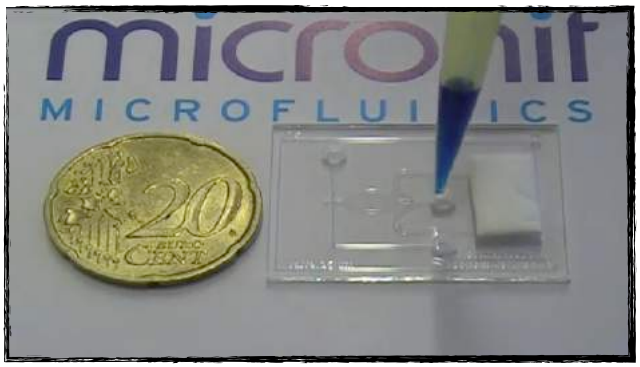
Phaseguides



P. Vulto, et al., Lab Chip 11, 2011, 1596-1602

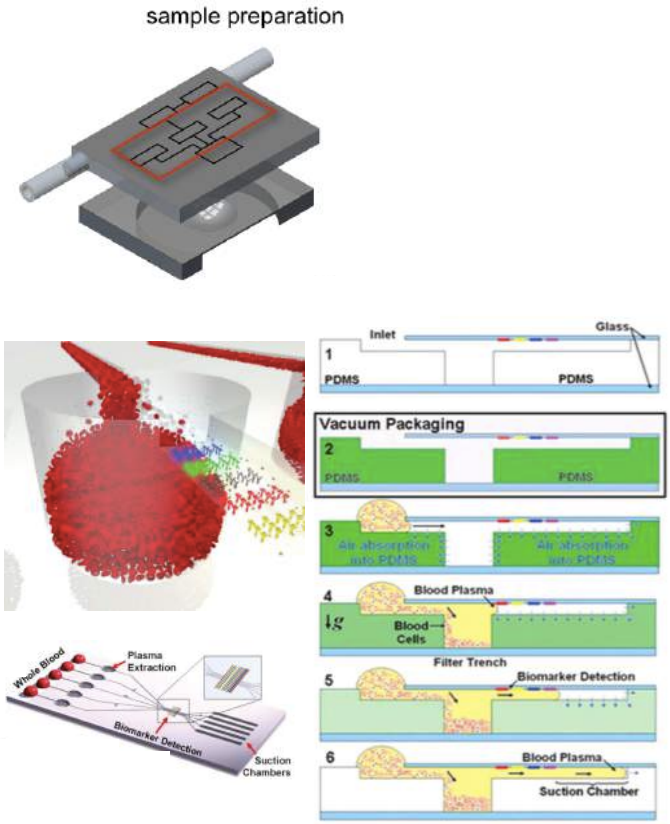
Sample collection and transport

Lab-on-a-disk, Capillary valves

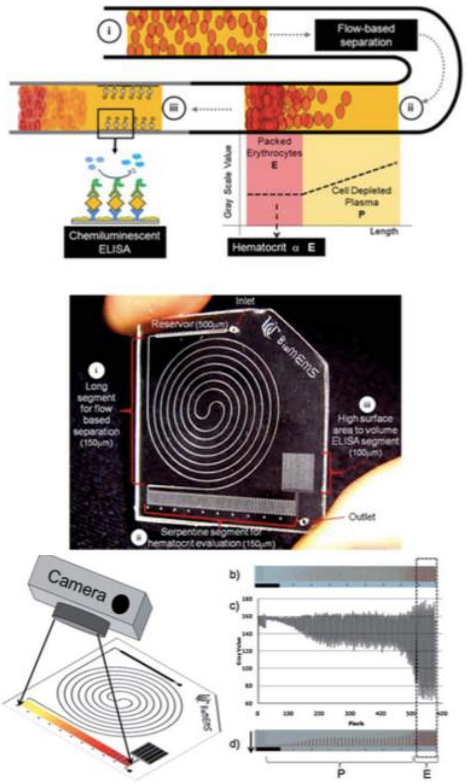


M. La, et al. ,Biomicrofluidics (2015)

Separation

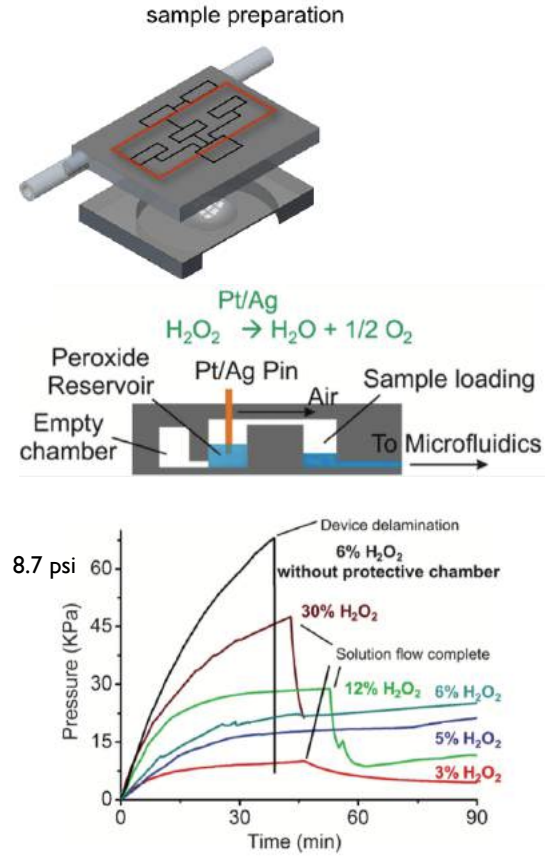


I. Dimov, et al. ,Lab Chip, 2011, 11, 845–850

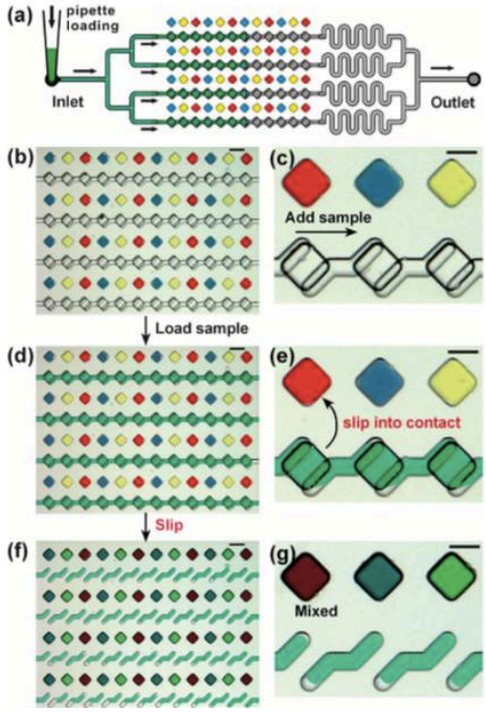


A. Browne, et al., Lab Chip, 2011, 11, 2440

Transport and conditioning



J. Wang, et al., Lab Chip, 2010, 10, 3157–3162



W. Du, et al., Lab Chip, 2009, 9, 2286–2292

Detection

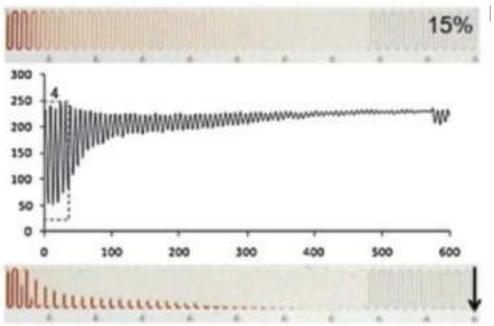
Cell Phone Readout: Intensity vs. Position and Time

Intensity

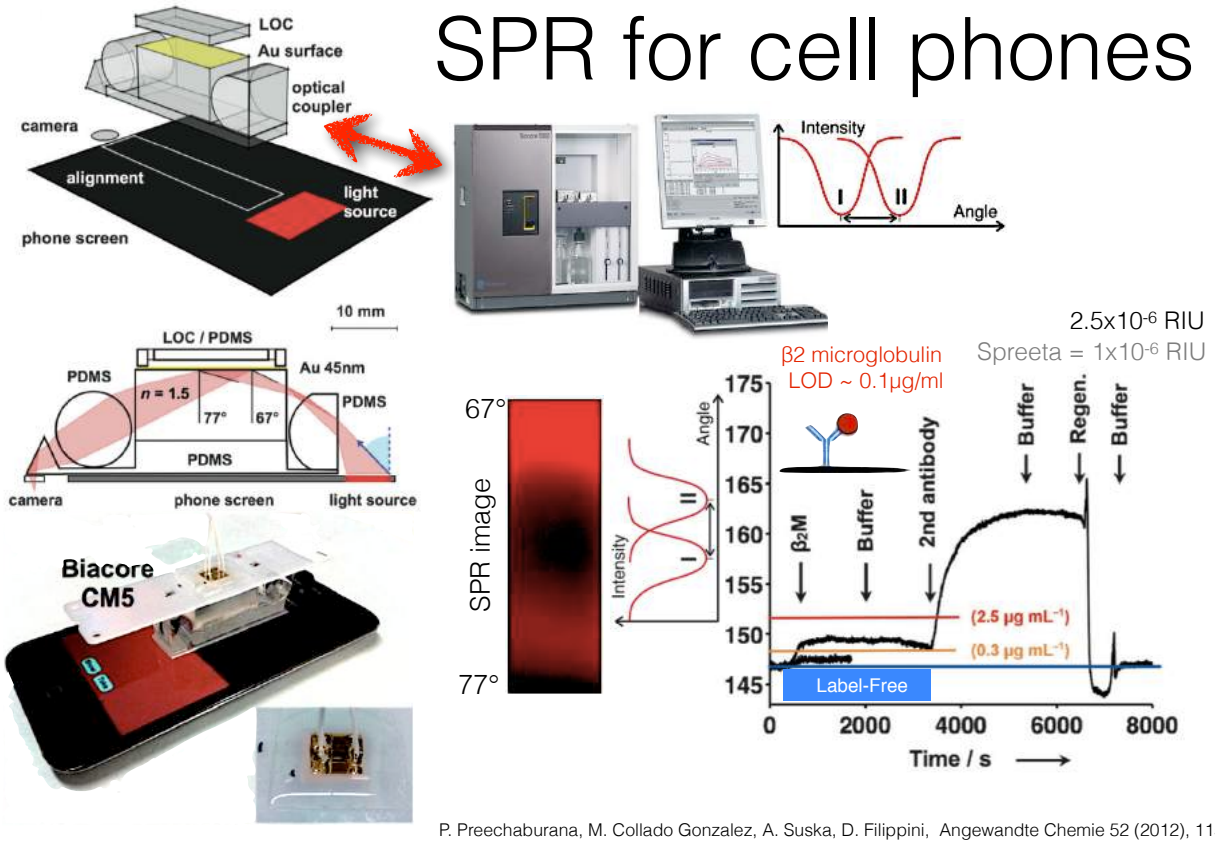
- 256 levels/channel
- Limited dynamic range
- Unchanged specifications
- Quantitative value affected by illumination

Pixels and video frame rate

- 5MP front cameras (~2500x1900)
- 12 MP rear cameras (4000x3000)
- 30 fps to 240fps in slow motion
- Continuously evolving



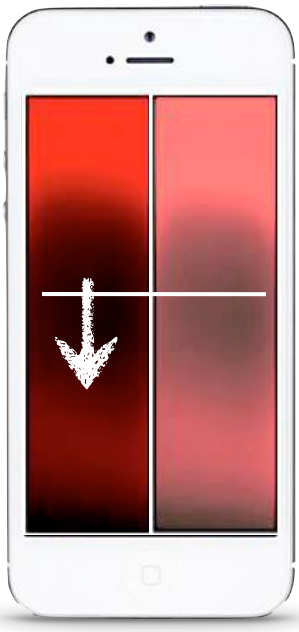
Disposable Angle Resolved SPR for cell phones



P. Preechaburana, M. Collado Gonzalez, A. Suska, D. Filippini, Angewandte Chemie 52 (2012), 11585

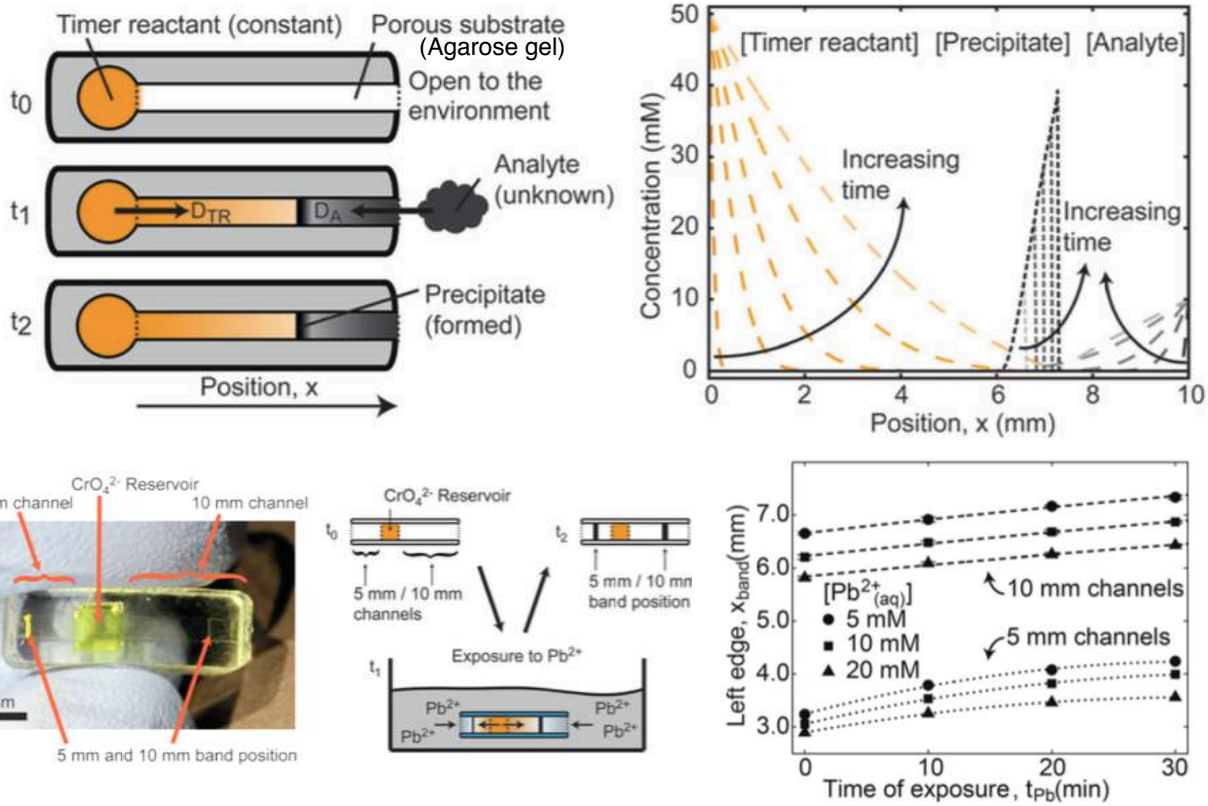
SPR detection advantages

- **Displacement detection**
  - 1920x1080 pixels (HD video) vs. 256 levels intensity resolution (>7x)
  - Robust to ambient light contamination
- **Time resolution**
  30 fps standard (240 fps slow motion)
- **Limited signal contrast**
  well within cameras dynamic range
- Screen luminance (>200 nits) enough as light source.
- Generic principle / supports label-free detection



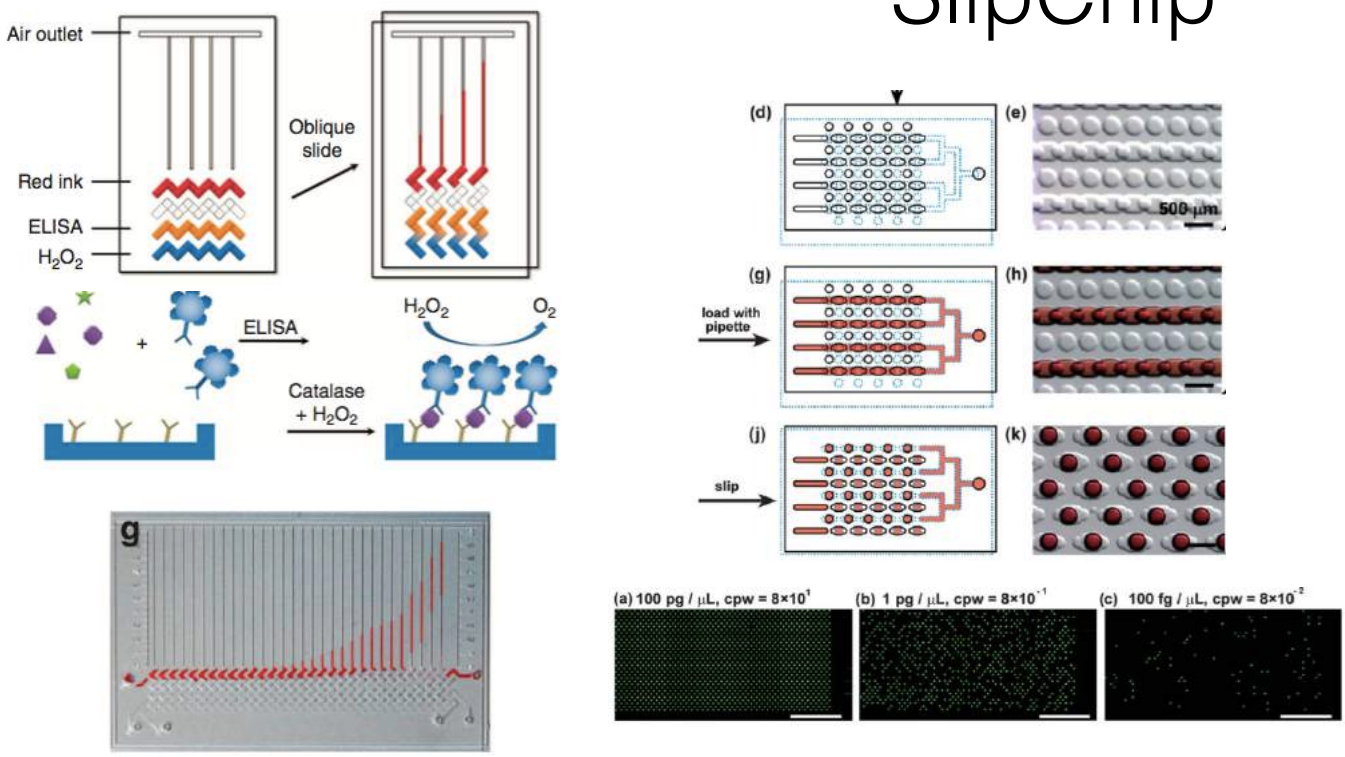
P. Preechaburana, M. Collado Gonzalez, A. Suska, D. Filippini, Angewandte Chemie 52 (2012), 11585

Diffusion–reaction



L. Gerber, L. Rosenfeld, Y. Chen, S. Tang, Lab Chip, 2014, 14, 4324–4328

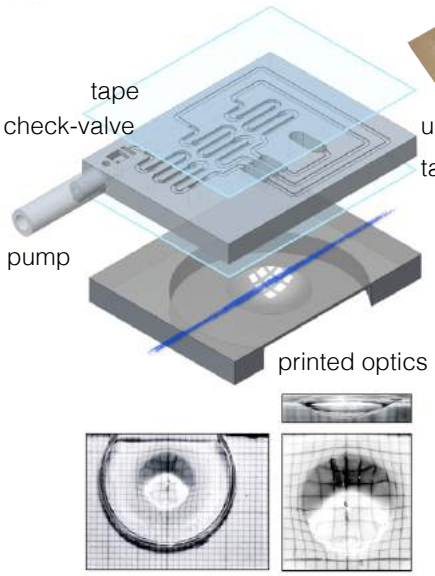
Bar-chart-chip and Digital SlipChip



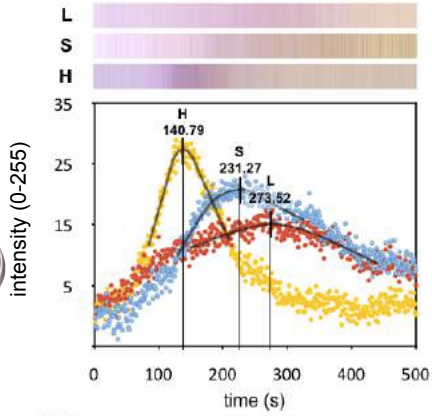
Y. Song, et al., *NATURE COMMUNICATIONS | 3:1283 , 2012*

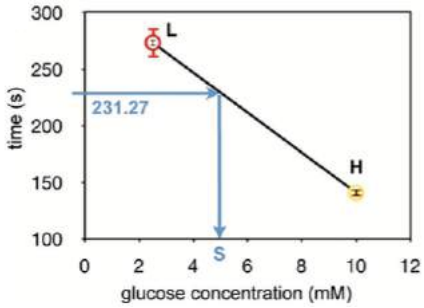
F. Shen, et al., *Lab Chip, 2010, 10, 2666–2672*

Quantitative Glucose Unibody for **Any** cell phone



- 256 intensity levels vs. 30 fps
- robust to ambient illumination





G. Comina, A. Suska and D. Filippini, Angew. Chem. 54, 2015, 8708.

Summary

- Classical microfluidic/LOC configurations offer an extensive catalog of analytical solutions, which are best configured for laboratory operation or in dedicated instruments.
- Autonomous LOC for cell phone detection imply adapting numerous microfluidic principles to operate beyond laboratories.
- Optical responses are best configured for cell phone detection using position and time response than the absolute value of intensity or color.

Optical Devices Laboratory
Division of Sensor and Actuator Systems

LINKÖPING UNIVERSITY

Computer Aided Design (CAD) & 3D Printing

Daniel Filippini [1]

Digital manufacturing entails the automatic conversion of a computer aided design (CAD) into its materialization as a physical object. Dominant industrial techniques such as computer numeric controlled drilling are subtractive methods, where a block of material is carved out to produce the desired CAD. Alternatively, modern additive manufacturing (AM) techniques aim at only contributing the material necessary to create the design. Beyond such economy of building materials, AM enables the generation of monolithic hollow geometries not possible with subtractive methods.

In this lecture, main AM technologies are presented, with special emphasis on AM fabrication of lab-on-a-chip (LOC) and optical devices. Practical advantages for low-cost fast-prototyping, especially relevant during the development cycle of any particular LOC or optical design are illustrated, and the Unibody-LOC design principle is discussed.

Examples of Unibody-LOC mixers, unidirectional valves, pumps and autonomous LOC are considered, as well as the use of the same workflow for the fabrication of optical components.

The lecture concludes with the illustration of a CAD for Unibody-LOC generation using minimum resources and free software.

Suggestions for further reading:

[1] https://formlabs.com/3d-printers/form-2
[2] http://www.nanoscribe.de/en/
[3] https://www.3dhubs.com
[4] Lab Chip 11 (2011), 288.
[5] https://www.stratasysdirect.com
[6] Nature Materials 16 (2017), 303.
[7] Nature 536 (2016), 451.
[8] Nature Materials 15 (2016), 815
[9] Lab Chip 14 (2014), 424.
[10] Lab Chip 14 (2014), 2978.
[11] Anal. Methods, 2016, 8, 6135.
[12] Angewandte Chemie 54 (2015), 8708.
[13] https://www.autodesk.com/products/fusion-360/overview
[14] Edmund Optics, The making of an Aspheric Lens, https://www.youtube.com/watch?v=JK1auletTfg
[15] M. Bass Ed., Handbook of Optics, Vol I, Chapter 40 and 41, McGraw-Hill, (1995)
[16] I. Gibson, D. W. Rosen, B. Stucker, Additive Manufacturing Technologies. Rapid Prototyping to Direct Digital Manufacturing, Springer, (2010).
[17] M. Schaub, J. Schwiegerling, E. C. Fest, A. Symmons, R. Hamilton Shepard, Molded Optics Design and Manufacture, CRC Press, (2011).

[1] E-mail; danfi@ifm.liu.se; Optical Devices Laboratory, Linköping University, Linköping, Sweden

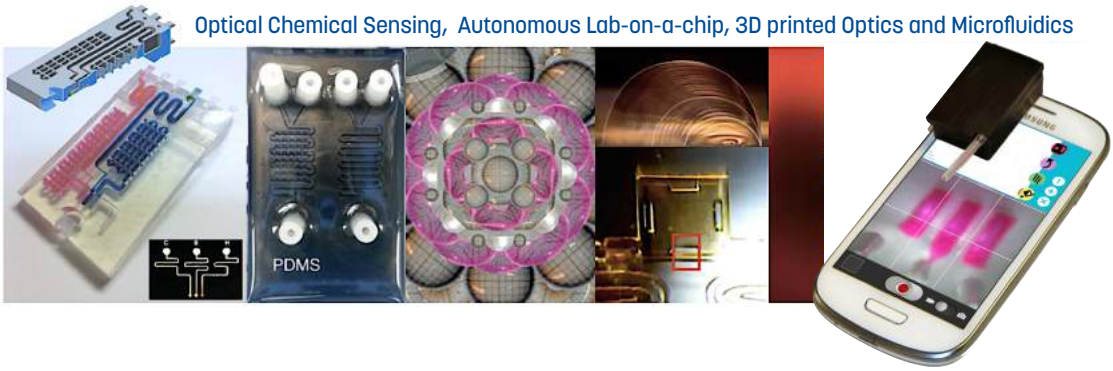
FoodSmartphone.eu

Summer School on Smartphone-based Food Analysis,
RIKILT, Wageningen, The Netherlands, 26-20 June 2017

LINKÖPING UNIVERSITY

Computer Aided Design (CAD) & 3D Printing

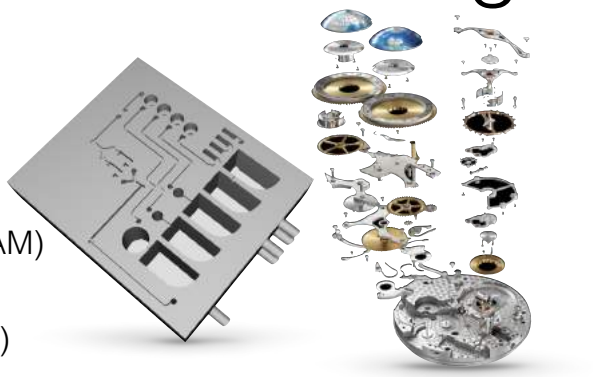
Daniel Filippini
Professor in Applied Physics
Optical Devices Laboratory (ODL)
Division of Sensor and Actuator Systems (SAS), Linköping University, Sweden



Overview

- Digital microfabrication
- Additive Microfabrication Principles
- Motivations for additive microfabrication of Lab-on-a-chip (LOC) devices and optics.
- Examples of 3D printed fluidics and optics

Digital Manufacturing



- Computer Aided Design (CAD)
- Computer Aided Manufacturing (CAM)
  - Computer Numeric Control (CNC)

Subtractive Drills, Milling Machines, Plasma Cutters, Water Jet Cutters, Laser Cutters.

Additive 3D printer.

Patek Phillipe
Grandmaster Chime
2.6 m€
https://youtu.be/FdxbysUSSAM

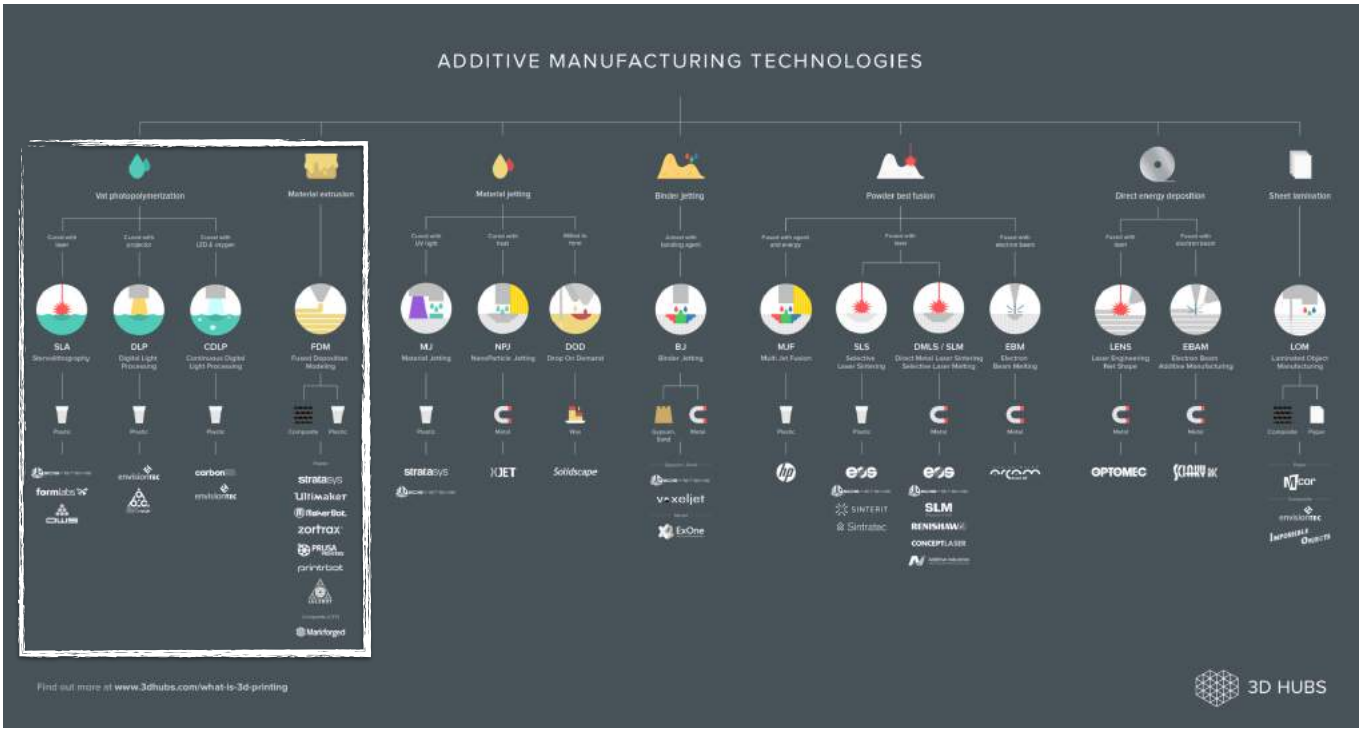
CNC vs. Additive Manufacturing (AM)

Selective Laser Sintering (SLS)

- Conceived to work with metals
- Result is a part in a process / involves multiple manufacturing steps
- Expensive platforms
- Excellent surface finish
- Accuracy within 50 μm
- Significant waste

- Conceived to work with polymers
- Single step manufacturing
- Less expensive platforms
- Poorer surface finish
- Accuracy worse than 500 μm
- Minimal waste (Sustainable)
- Complex monolithic architectures cannot be made by CNC

Additive Manufacturing (AM)

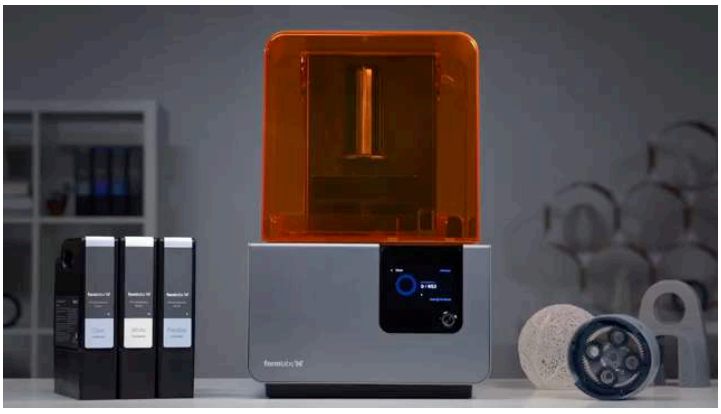


Vat photopolymerization

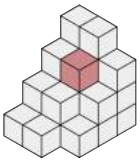
Stereolithography (SLA) uses a single point laser that maps a cross sectional area (layer) of a design through the bottom of the tank solidifying the material. Then he platform lifts up and lets a new layer of resin to be processed.

Direct light processing (DLP) uses a digital light projector screen to flash a single image of each layer all at once.

- Several platforms under 5000€
- Low surface roughness
- Voxel ~ 100 x 250 x 250 μm^3



https://formlabs.com/3d-printers/form-2



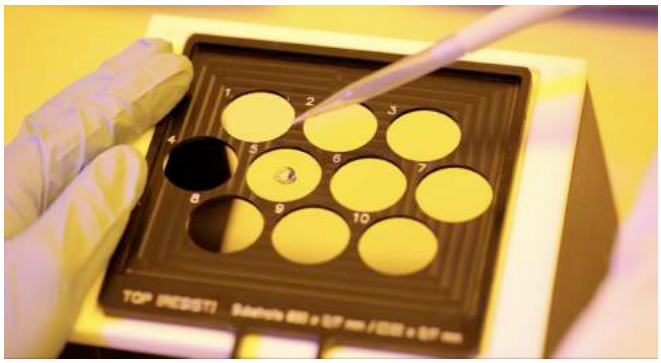
Optical Devices Laboratory - LiU

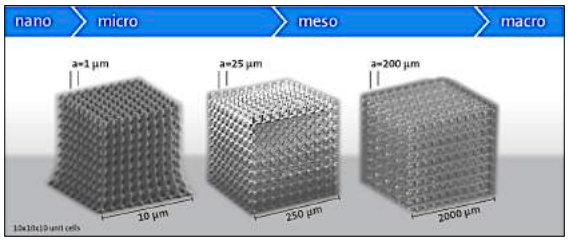
2 photon polymerization - Direct Laser Writing

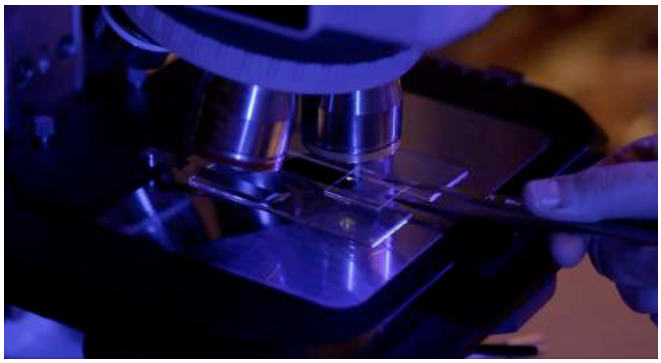
nanoscribe

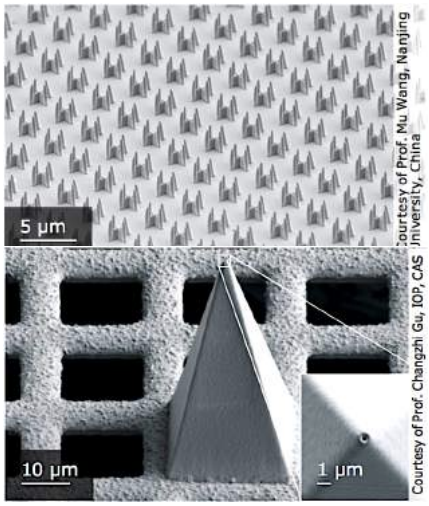
~400 000 €

~100 nm resolution

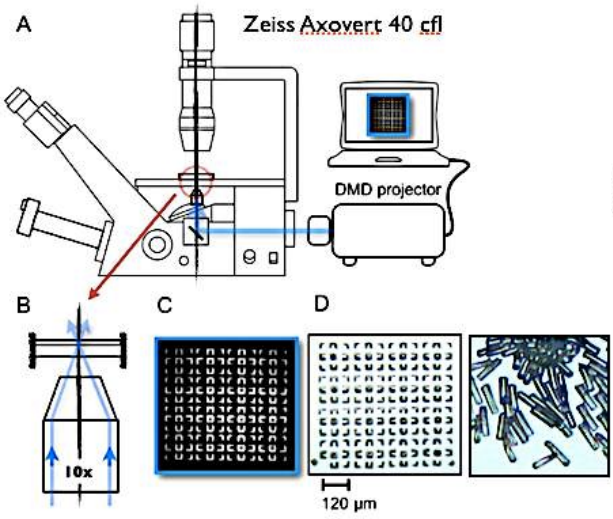


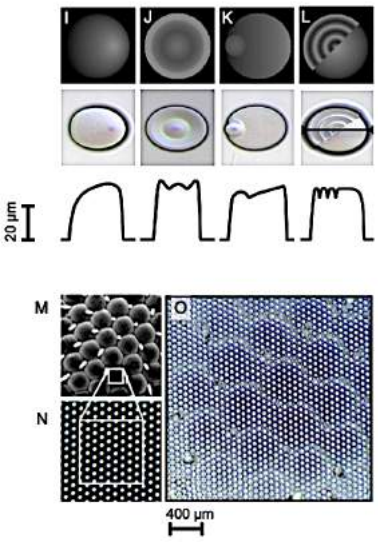


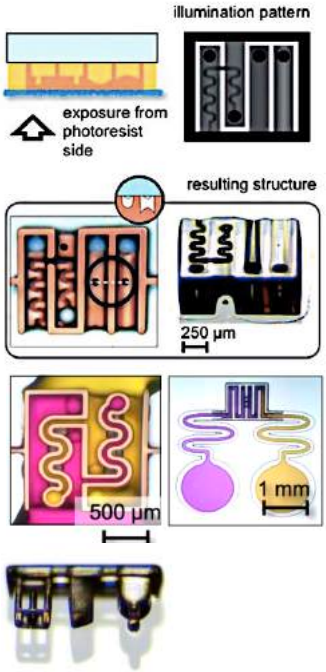




Microscope Projection Lithography Systems - MPLS





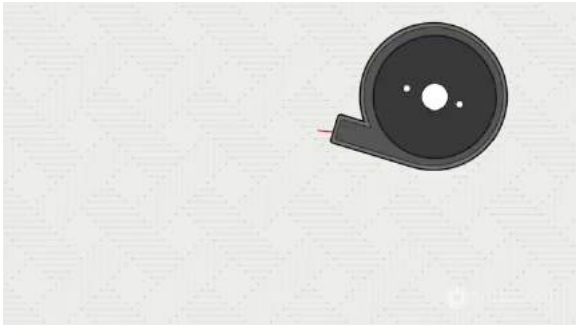


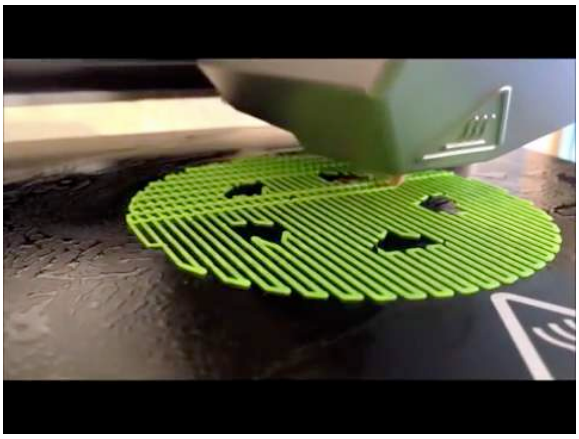
P. Prechaaburana, D. Filippini, Lab Chip, 2011, 11, 288–295

Material extrusion

Fused Deposition Modeling (FDM) uses uses a string of solid thermoplastic material (filament), pressed through a heated nozzle. The nozzle is scanned to deposit the melted material at precise locations, where it cools down and solidifies.

- From 100€
- Poor surface roughness
- Voxel ~ 100 x 500 x 500 μm^3





Material extrusion

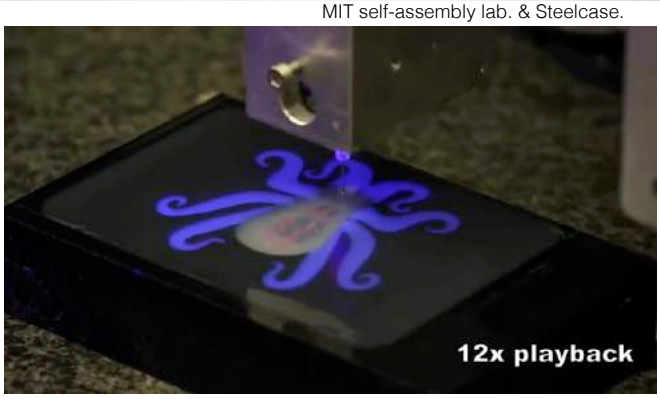
- 3D Bioprinters
- 20 000 - 250 000€
- Numerous materials
- Full control of the printing path
- Resolution similar to FDM

Nature Materials 16 (2017), 303.

Liquid Printer

- It does not print layer by layer
- Direct 3D path supported in a polyurethane gel.
- After gel removal free printout
- Faster than layer by layer
- Control of surface tension and channels geometry.
- Not commercial

MIT self-assembly lab. & Steelcase.



Nature 536 (2016), 451.

Material jetting

Material jetting dispenses a photopolymer from hundreds of tiny jets in a printhead to build up a part layer by layer. As the droplets are deposited they are cured by UV light. The model requires support.

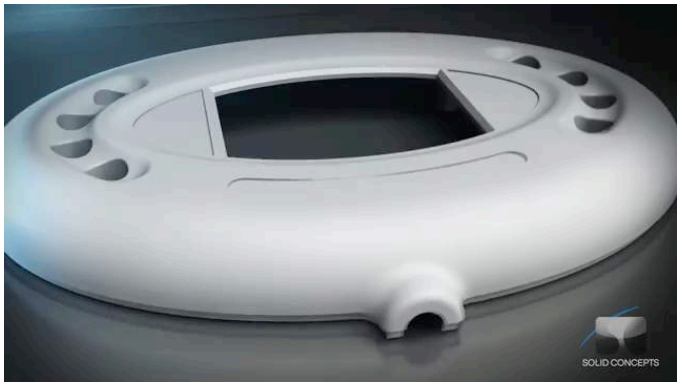
- 50 000 - 750 000€
- Industrial standard
- Resolution ~ 25μm^3

stratasys

Multi-color and multi-material bio-model.
Technology: PolyJet

Selective Laser Sintering (SLS)

SLS uses a laser to sinter thin layers of powdered material one layer at a time to create a solid structure. The process begins by spreading an initial layer of powder over a build platform. The cross section of the part is then sintered by the laser at which point the build platform drops down one layer thickness



- From 10 000USD (Fuse 1)

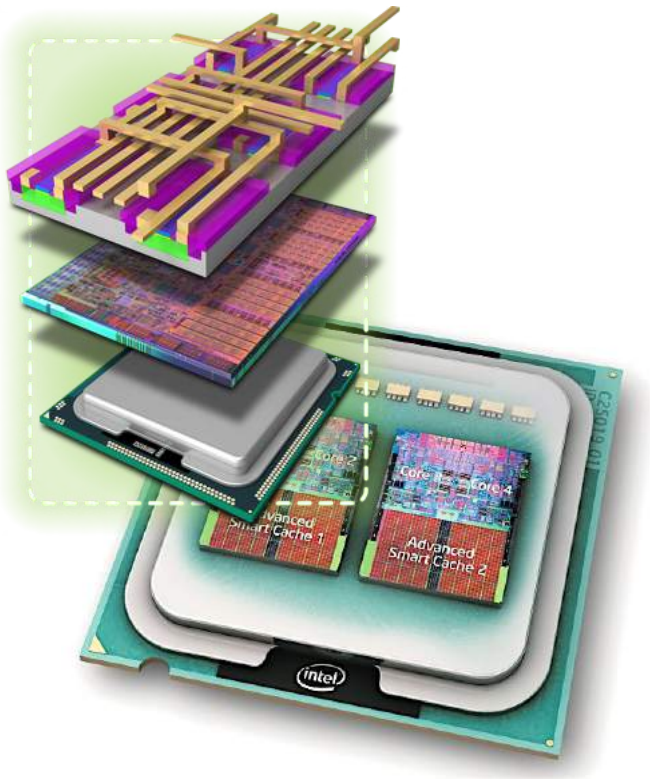
stratasys

Nature Materials 15 (2016), 815

Lab-on-a-chip Microfabrication

Photolithography / Integrated Circuit Fabrication

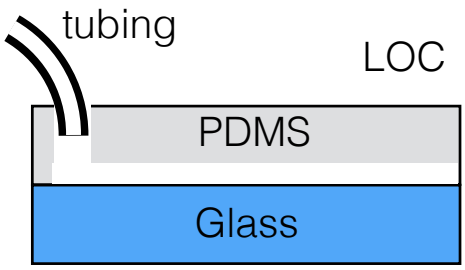
- High reproducibility and throughput
- Parallel processing
- Automatic process
- Expensive equipment (~billion €)
- Expensive running costs / Clean room environment
- XY resolution 22-16 nm
- Z resolution ~Å

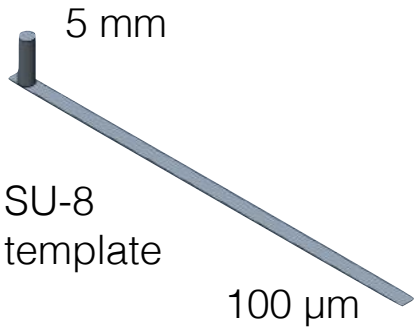


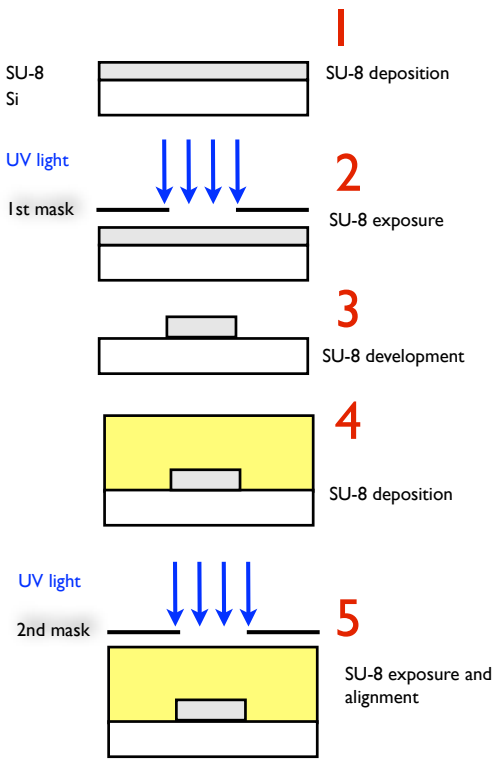
Motivations for 3D printed LOC

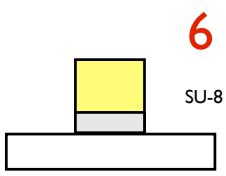
- Classical LOC microfabrication method is inherited from IC fabrication.
- It requires specialized skills (learning process) and special facilities (clean rooms).
- 3D structuring introduces additional fabrication steps, including additional permanent masks and alignment procedures.
- Flexibility to introduce changes is limited and costly.
- Thickness is typically limited to under 100μm.
- 3D printed LOCs explore a low-cost fast-prototyping concept, which does not require clean rooms, specialized facilities, or training in fabrication.

Classical micro fabrication









Classical

6 fabrication steps

2 masks

1 alignment

3D Printed

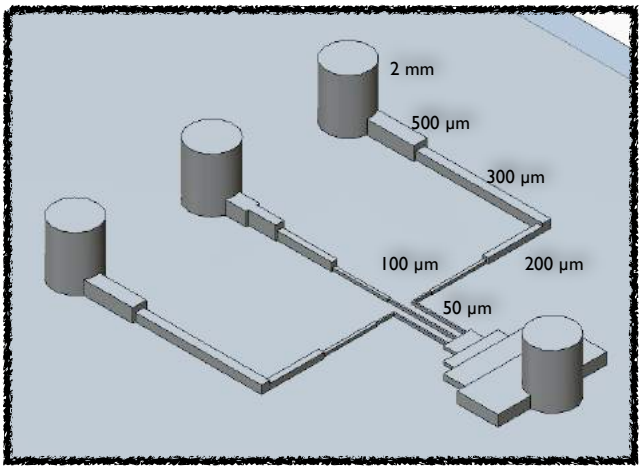
1 fabrication step

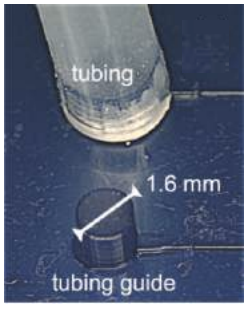
Fabrication made by the printer

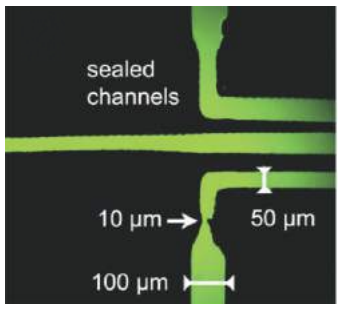
Easy modifications

No problem with 5 mm feature

3D Printed templates for PDMS Lab-on-a-chip





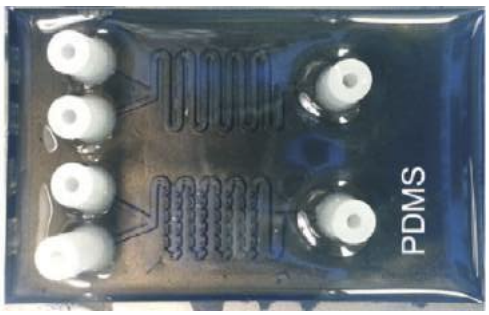


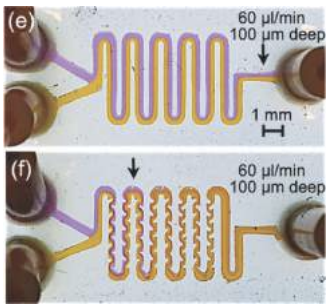
3D vs classical

1 vs 18 steps

0 vs 6 masks

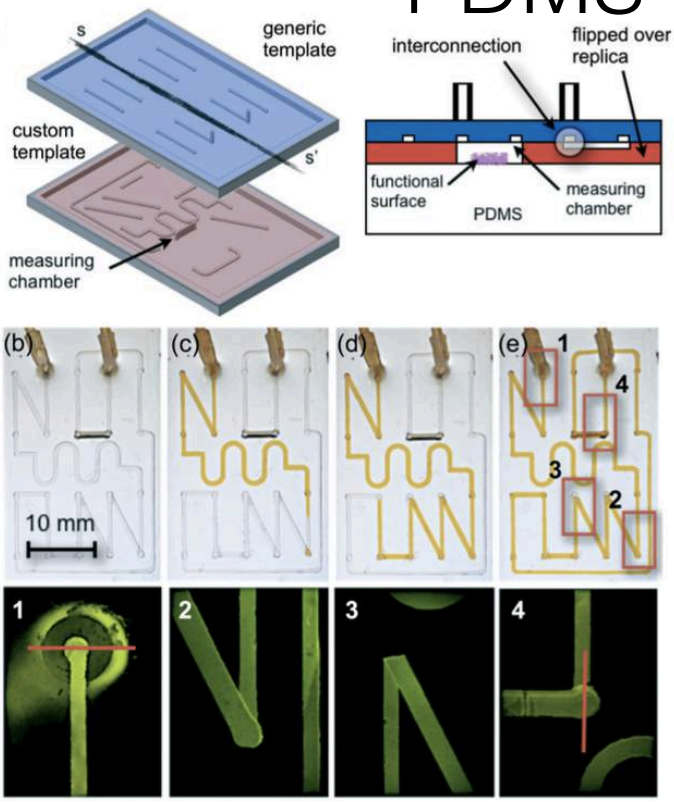
0 vs 5 align.





G. Comina, A. Suska and D. Filippini, *Lab Chip* 14, 2014, 424.

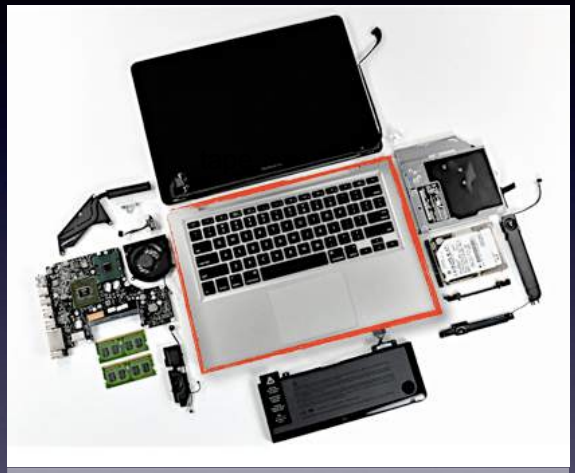
3D Printed templates for PDMS Lab-on-a-chip

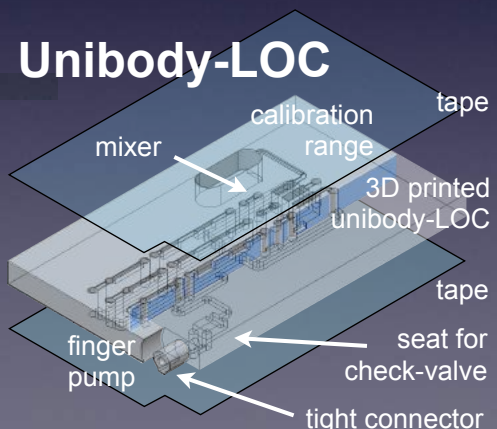


- Flexible to introduce changes (~4 iterations/day)
- Low cost (~0.5 USD/device)
- Fast (~20 min/device)
- Fabrication skills transferred to printer
- Any number of 3D features / without depth limitations
- Outside clean room

G. Comina, A. Suska and D. Filippini, Lab Chip 14, 2014, 424.
G. Comina, A. Suska and D. Filippini, Lab Chip 14, 2014, 2978.
G. Comina, A. Suska and D. Filippini, Proc. of SPIE 10061 (2017).

Apple - Unibody Design





~0.5 US$/prototype
~20 min./prototype
fabrication skills replaced by printer
any # of modifications / iteration

G. Comina, A. Suska and D. Filippini, *Lab Chip* 14, 2014, 2978

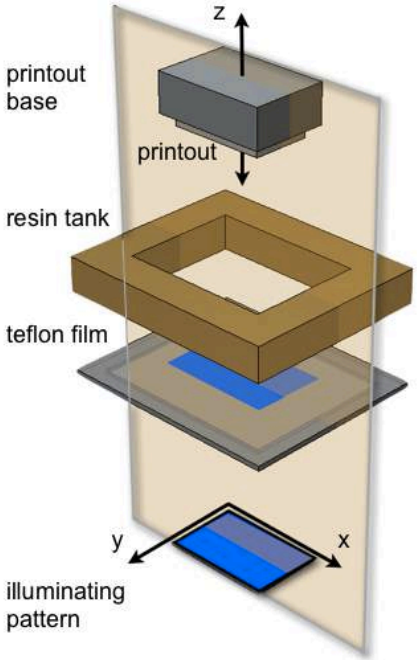
Fabrication platform

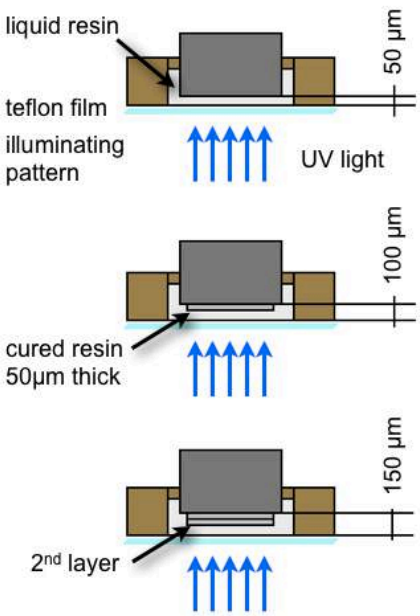
- Consumer grade stereolithography (SL-DLP) 3D printer (Miicraft, 2299 US$) vs. (clean room 1p/year ~3600 US$)
- Prototype fabrication time under 20min
- Materials cost ~0.5 US$/prototype
- Resolution ~ 50 x 50 x 50 µm
- Surface roughness under 200 nm
- Working volume: 40 x 30 mm x 180 mm
- Proprietary resin, undisclosed composition. MSDS: Acrylate Monomer + Modified Acrylate Oligomer + Epoxy Monomer + Photoinitiator.

G. Comina, A. Suska and D. Filippini, *Lab Chip* 14, 2014, 424.
G. Comina, A. Suska and D. Filippini, *Lab Chip* 14, 2014, 2978.

background: 72 prototypes / month ~ 40 US$

Stacking Plane Roughness





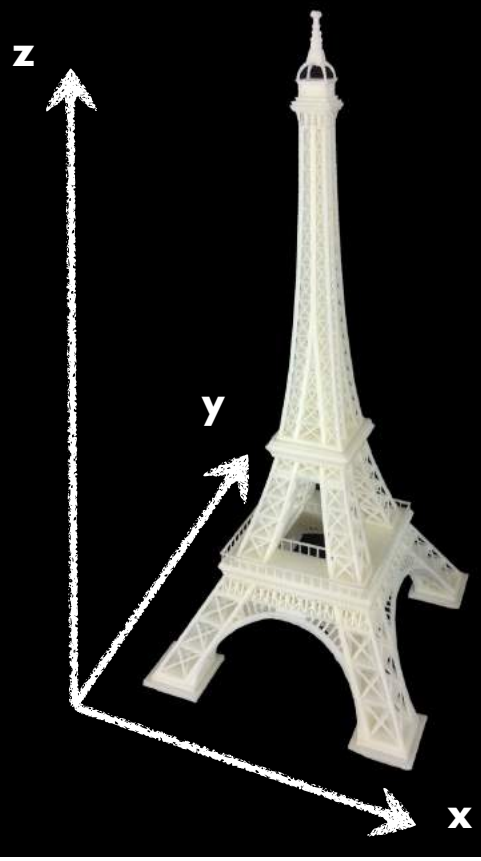
G. Comina, A. Suska and D. Filippini, *Micromachines* 6, 2015, 437.

Fabrication platform

- Consumer grade stereolithography (SLA-laser (405nm)) 3D printer (Form1+, 2400 US$) vs. (clean room 1p/year ~3600 US$)
- Prototype fabrication time about 20min
- Materials cost ~0.5 US$/prototype
- Resolution ~ 250 x 250 x 25 µm
  50 x 50 x 50 µm
- Surface roughness around 1 µm
- Working volume: 125 x 125 mm x 165 mm
  40 x 30 mm x 180 mm
- Proprietary resin, undisclosed composition. MSDS: Acrylate Monomer + Modified Acrylate Oligomer + Epoxy Monomer + Photoinitiator.

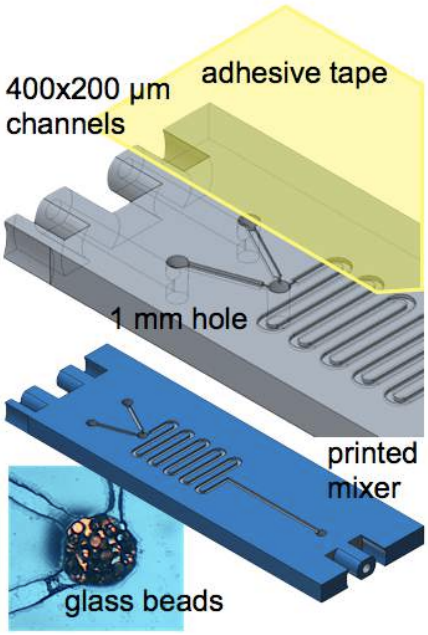
G. Comina, A. Suska and D. Filippini, *Lab Chip* 14, 2014, 424.
G. Comina, A. Suska and D. Filippini, *Lab Chip* 14, 2014, 2978.

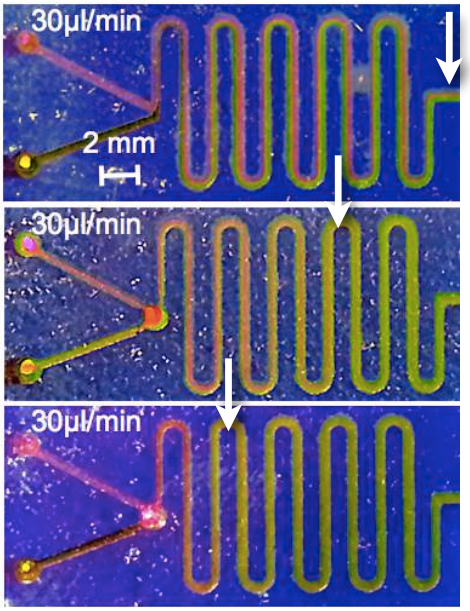
Use of z-dimension with Form1+ 3D Printing



- ✓ Complete freedom to configure geometry
- ☹ Increase printing time but allows simultaneous printouts
- ✓ Easier to avoid closed structures
- ✓ Wall roughness is better than Miicraft and enables 4 configurable sides

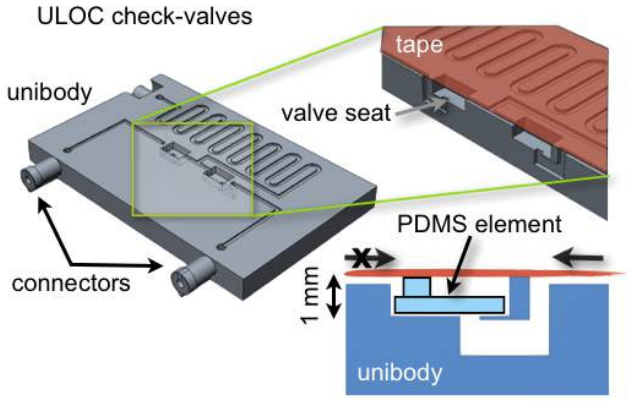
Unibody Mixers

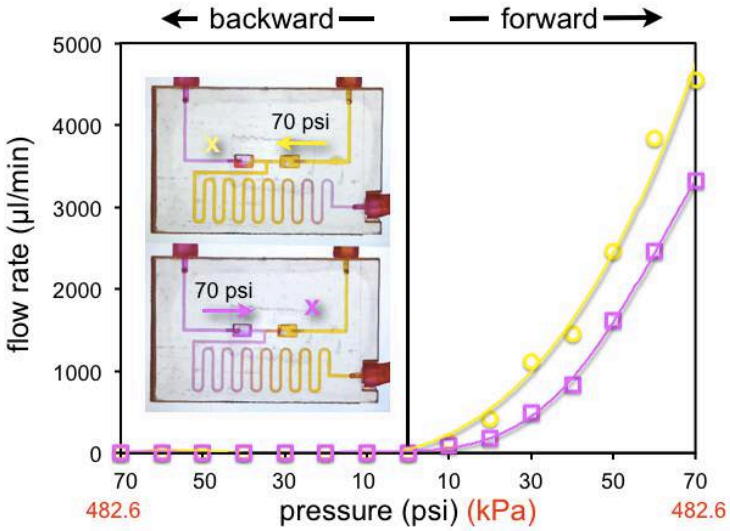




G. Comina, A. Suska and D. Filippini, *Lab Chip* 14, 2014, 2978.

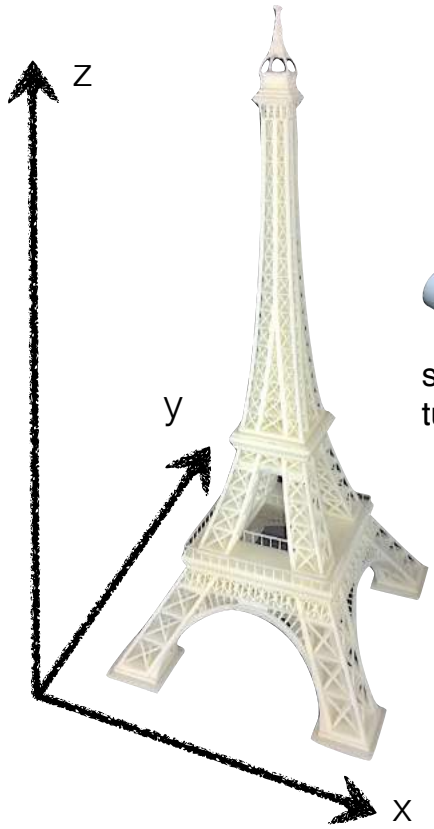
Unibody-LOC unidirectional valves

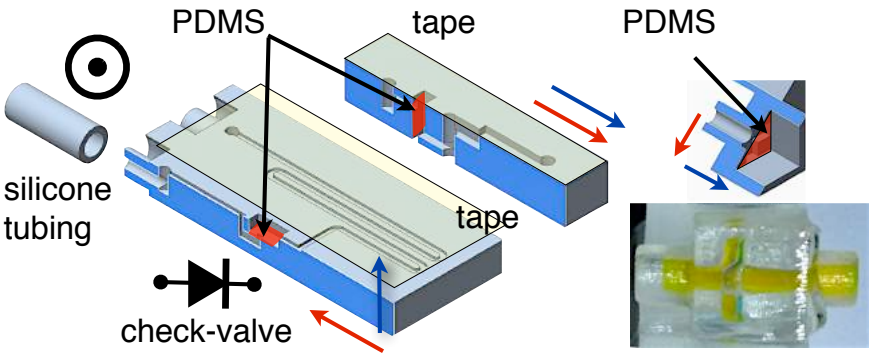




G. Comina, A. Suska and D. Filippini, *Angew. Chem.* 54, 2015, 8708.
G. Comina, A. Suska and D. Filippini, *Micromachines* 6, 2015, 437.

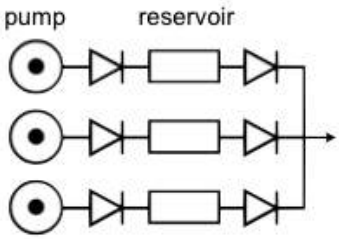
Same CAD in different printers

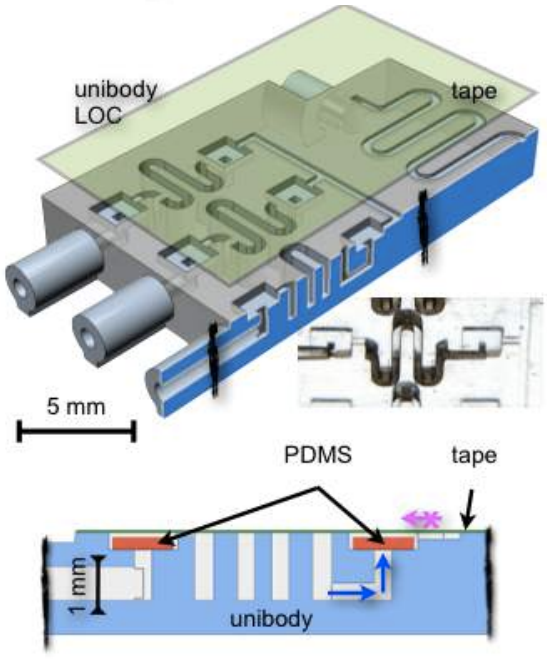




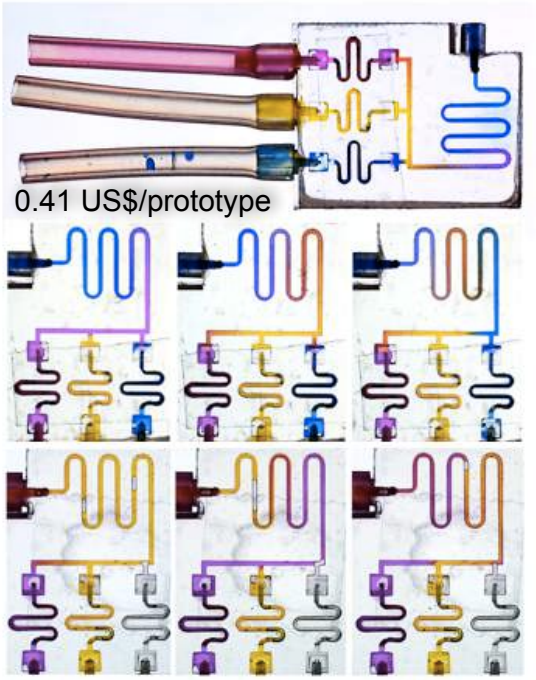
Miicraft
Form1+

Suska, A.; Comina, G; Filippini, D. SPIE BIOS, San Francisco, 28-31January 2017.



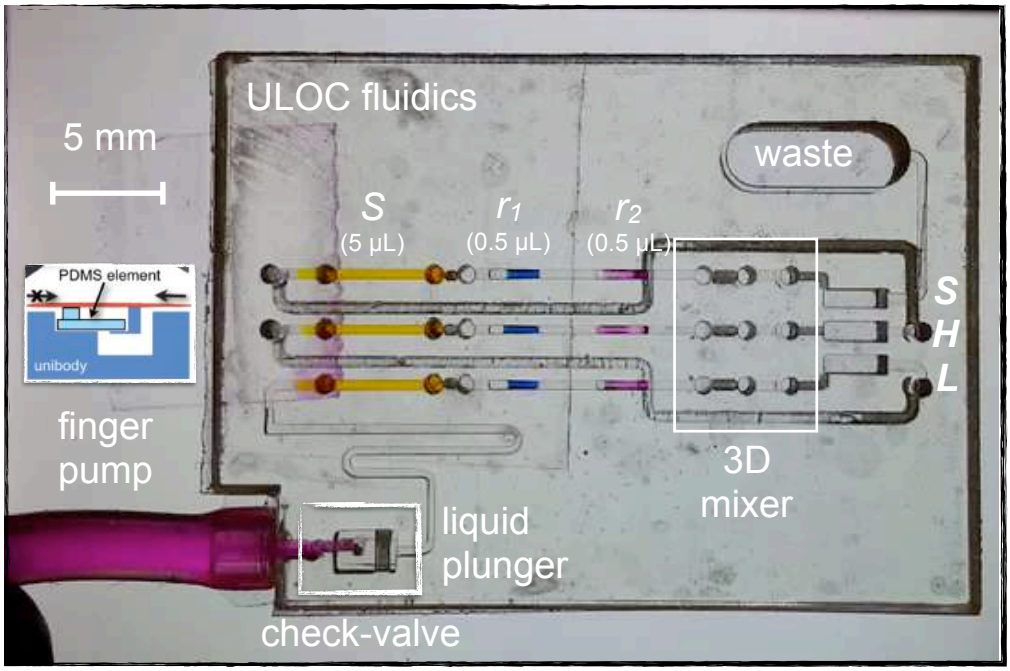


Unibody injector with manual pumps



Suska, A.; Comina, G; Filippini, D. Proceedings of *MFHS 2014 – 2nd International Conference on MicroFluidic Handling Systems*, **2014**.

Unibody-LOC for enzymatic detection

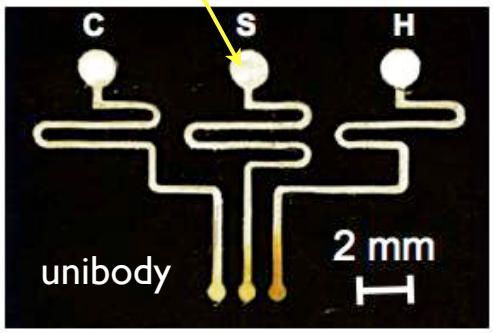


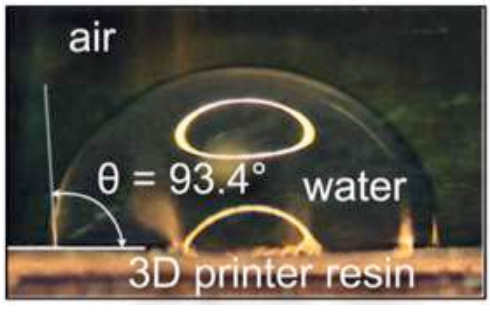
- H,L calibration range
- Integrated pump
- Integrated mixer
- Deep features for reagent capture
- Optical readout
- Conditioning = 6s
- Glucose
- Glutamate

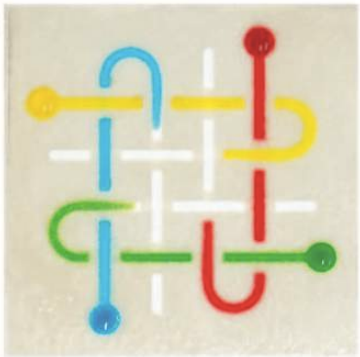
G. Comina, A. Suska and D. Filippini, Angew. Chem. 54, 2015, 8708.
G. Comina, A. Suska and D. Filippini, Anal. Methods, 2016, 8, 6135.

Unibody Paper Fluidics

cellulose paste







G. Comina, A. Suska and D. Filippini, *Lab Chip* 14, 2014, 2978.

A. Martinez, S. Phillips, G. Whitesides , *PNAS 105, 2008,19606.*

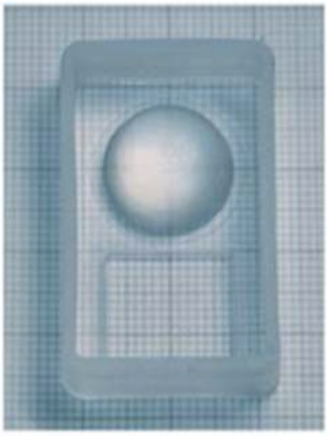
Fabrication of Optical Components

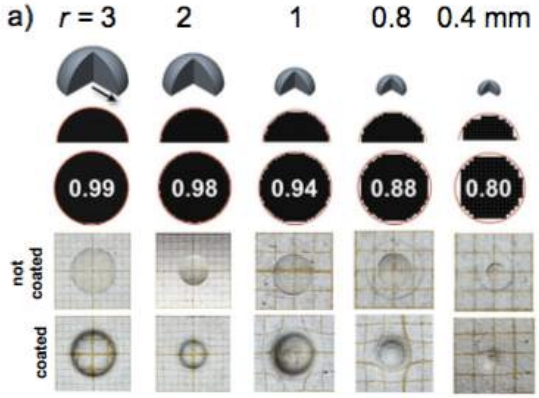
Classical Fabrication of Lenses

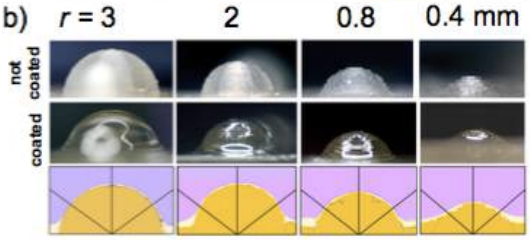
- Demands expensive industrial equipment
- Multiple manufacturing steps
- Requires trained operators
- Hard to customize beyond solids of revolution

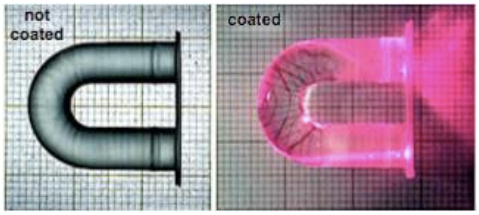
Edmund Optics

3D printed optics







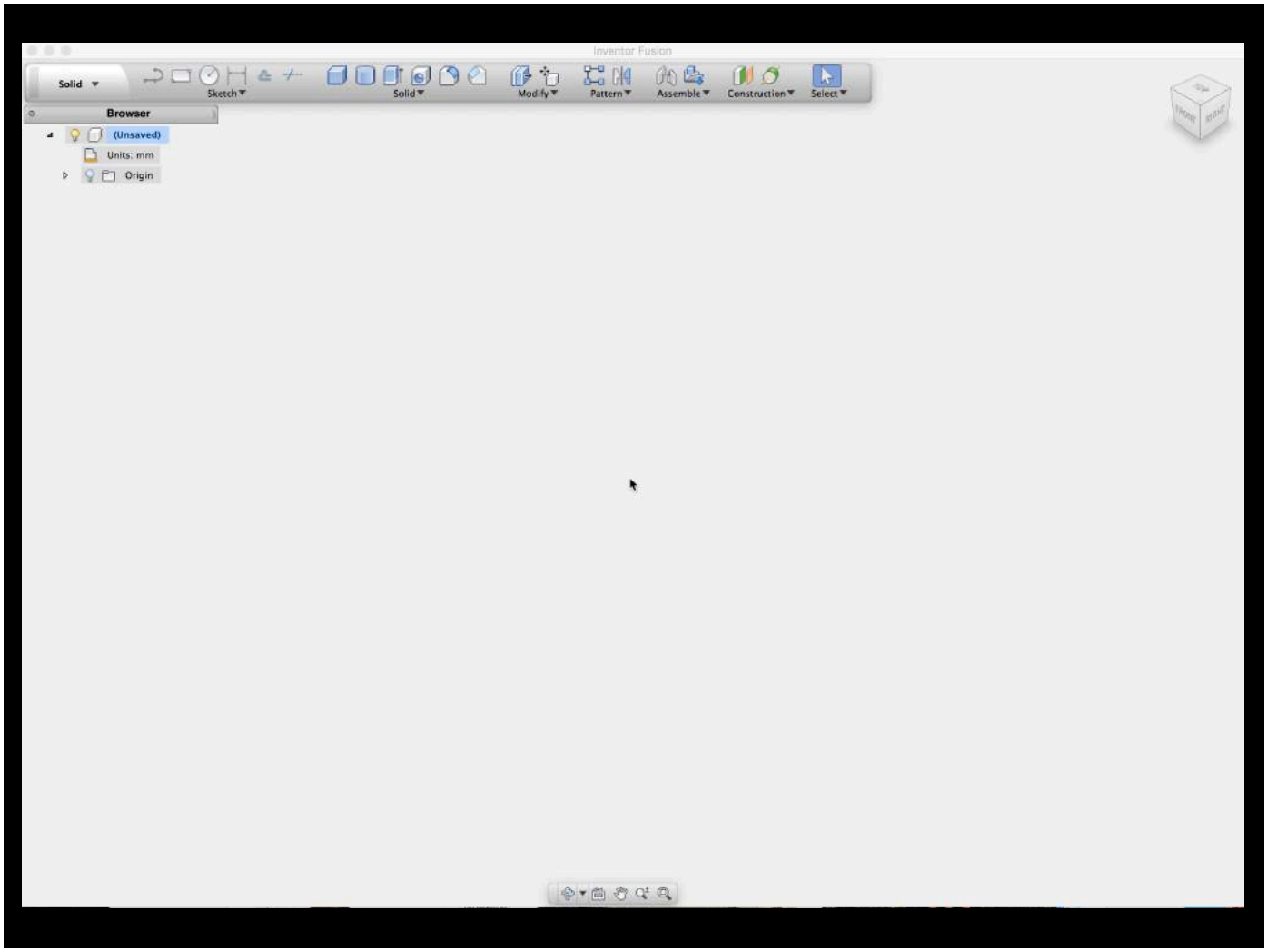


G. Comina, A. Suska and D. Filippini, *Angew. Chem.* 54, 2015, 8708.
G. Comina, A. Suska and D. Filippini, *Anal. Methods, 2016, 8, 6135*

Computer Aided Design (CAD)

Autodesk Inventor Fusion

Autodesk Fusion 360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Sketch
Solid
Modify
Pattern
Assemble
Construction
Select
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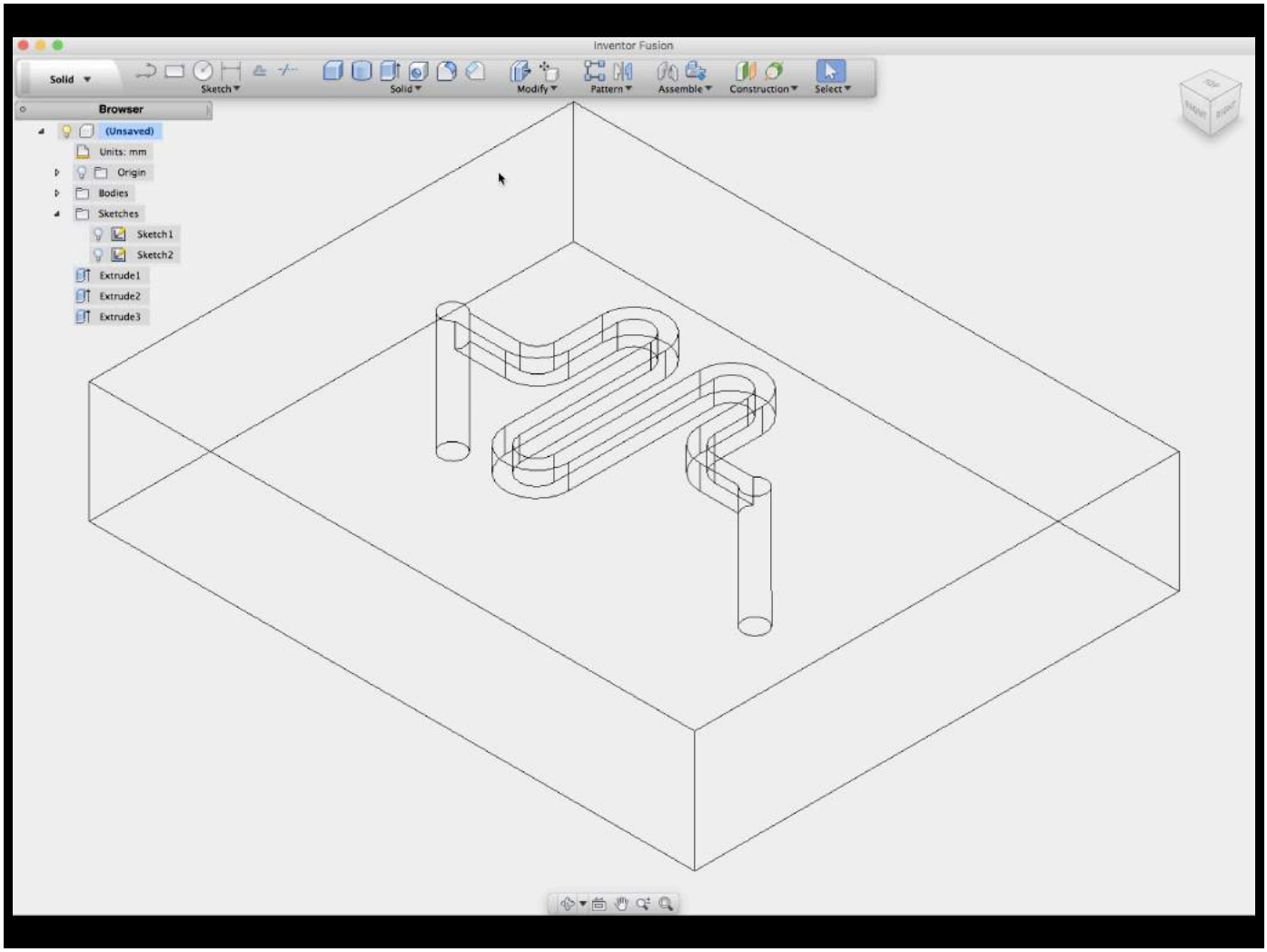
Units: mm
Origin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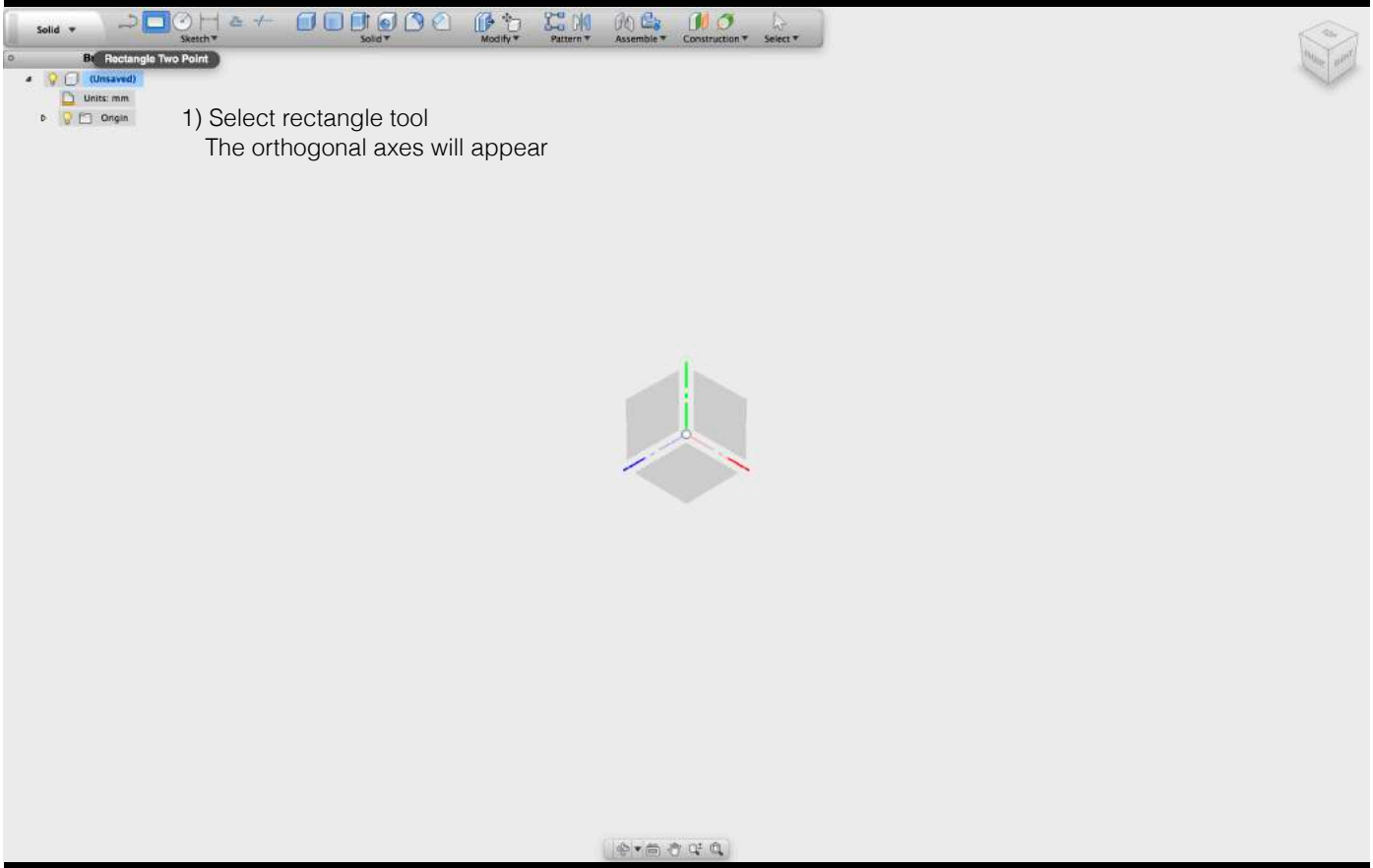
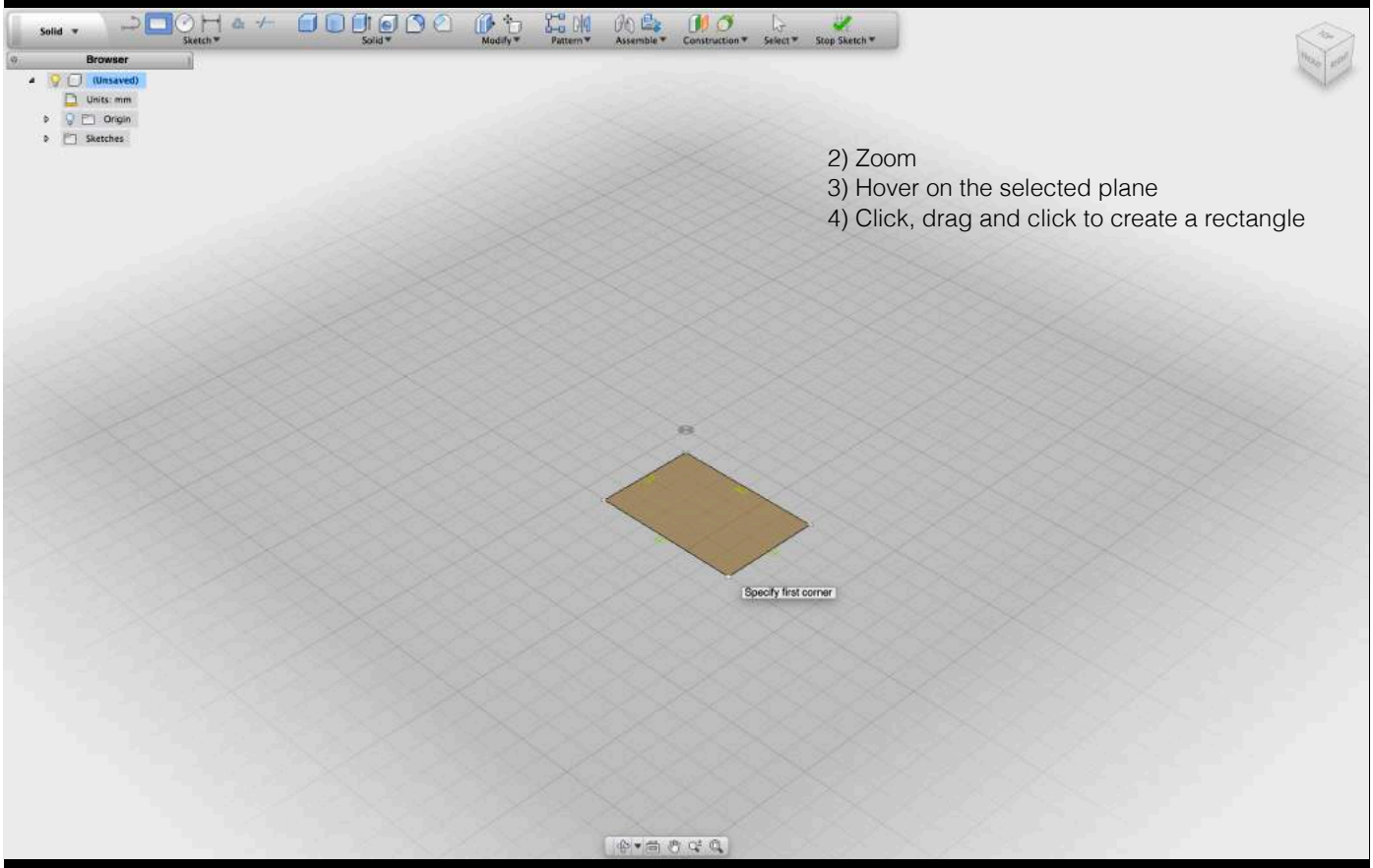
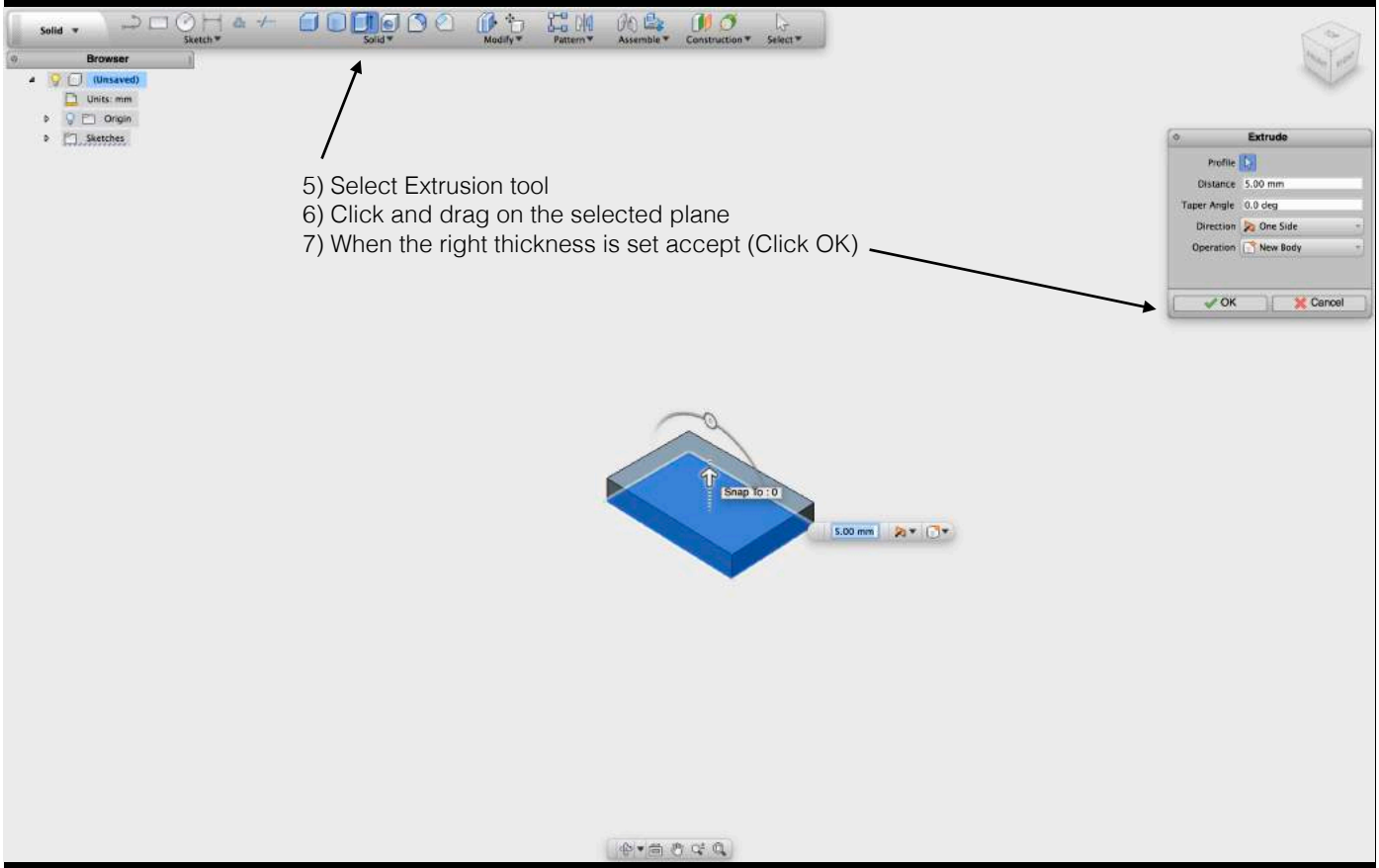
Origin
Bodies
Sketches
Sketch1
Sketch2
Extrude1
Extrude2
Extrude3



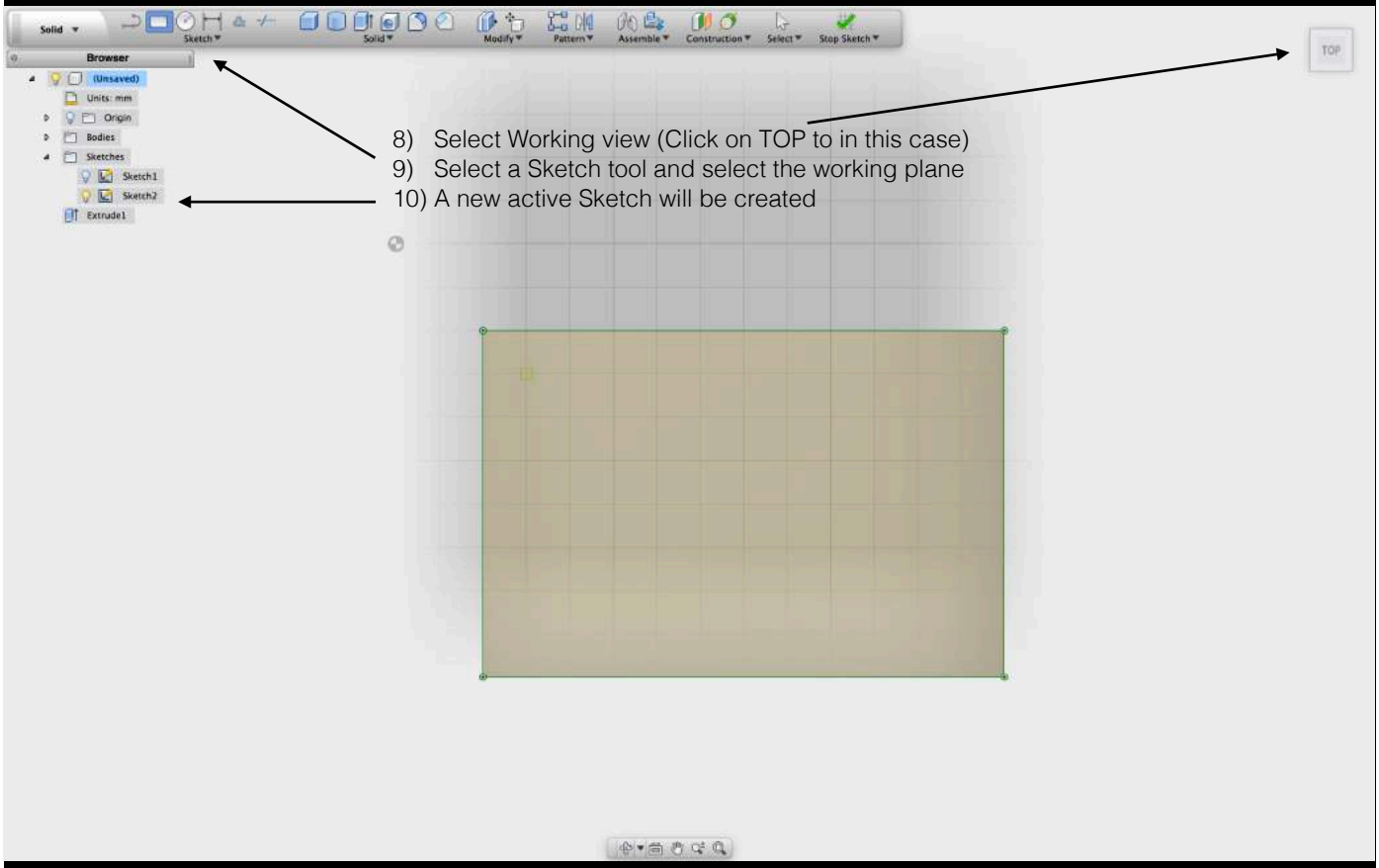
1) Select rectangle tool
The orthogonal axes will appear



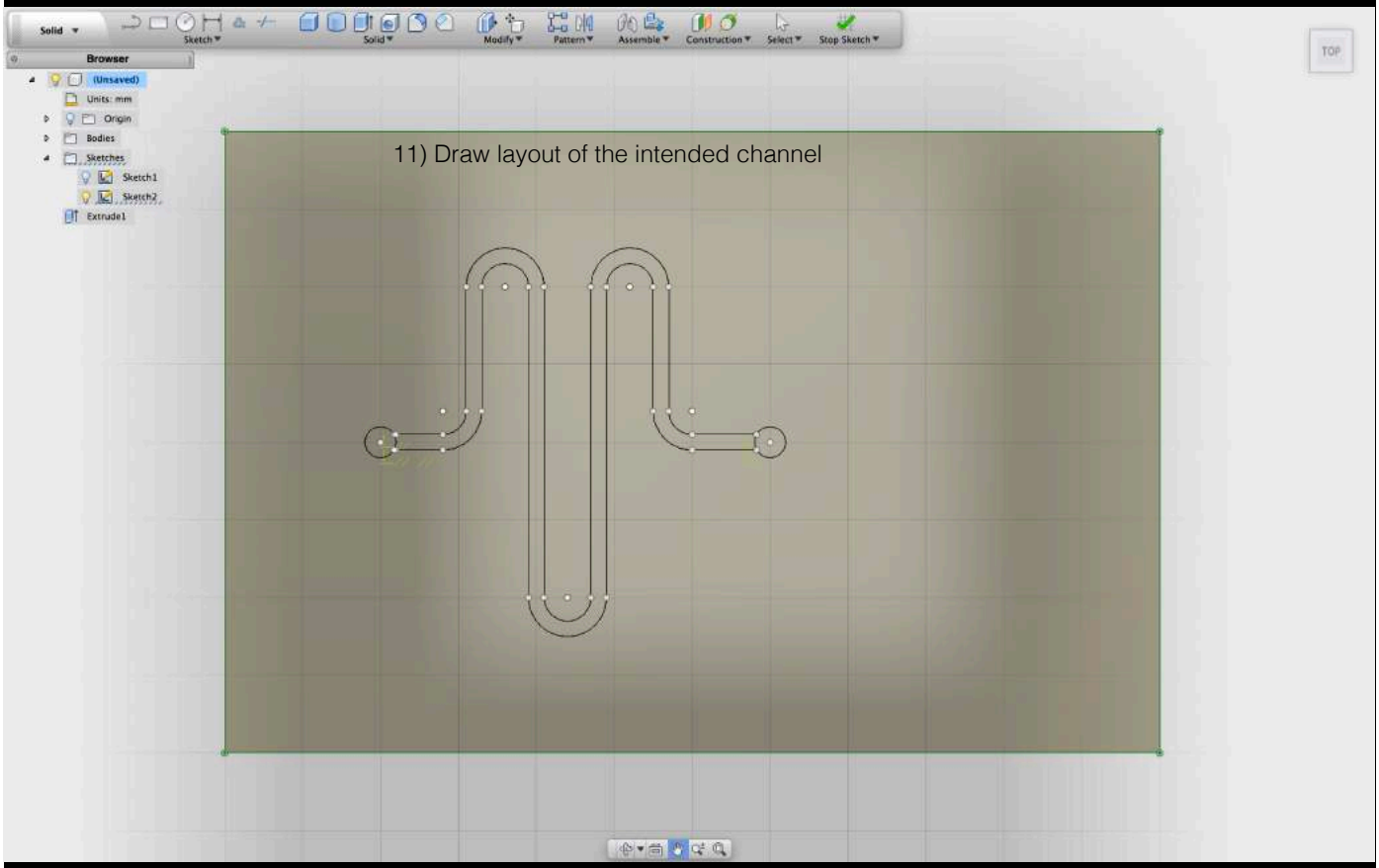
2) Zoom
3) Hover on the selected plane
4) Click, drag and click to create a rectangle

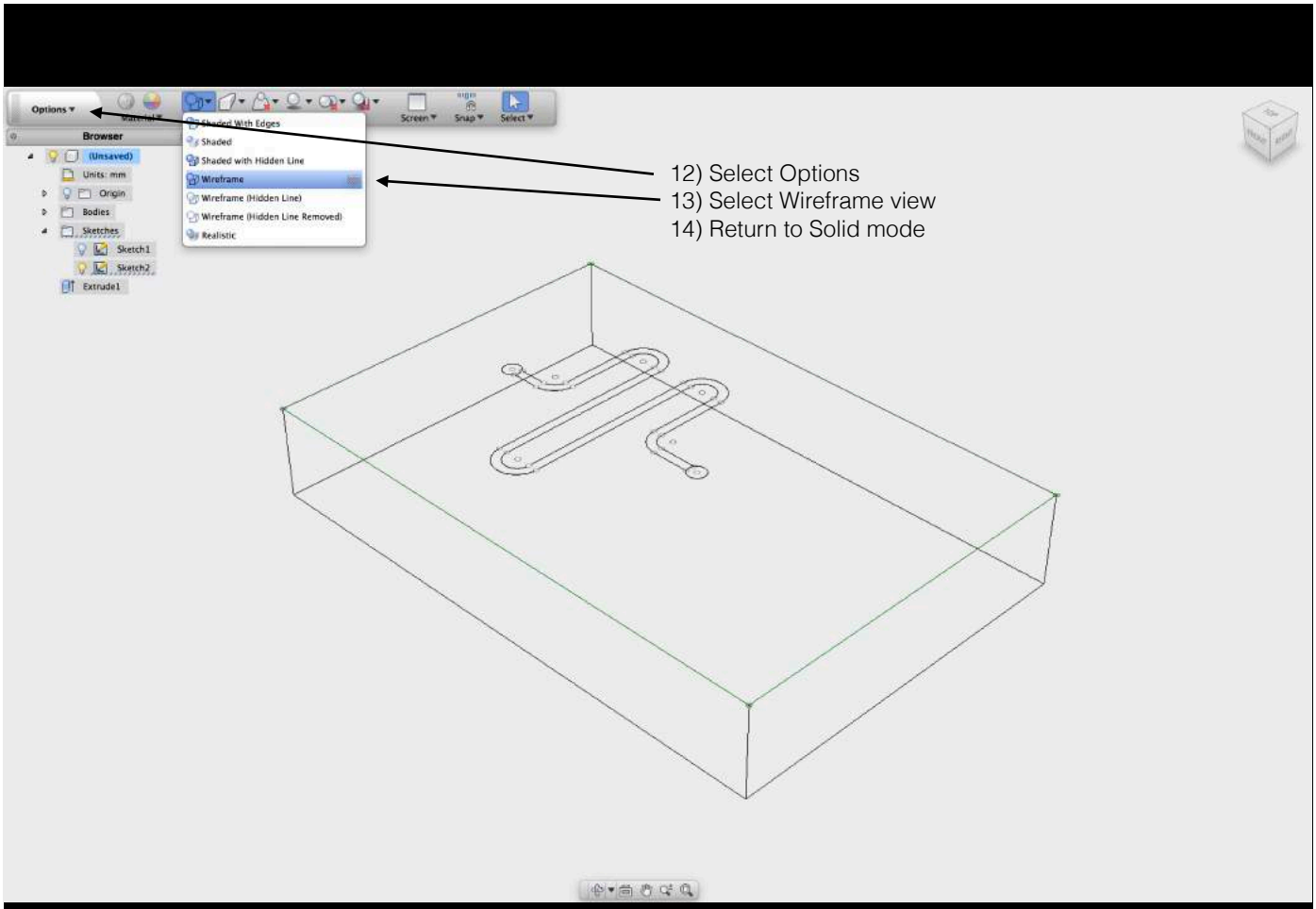


5) Select Extrusion tool
6) Click and drag on the selected plane
7) When the right thickness is set accept (Click OK)

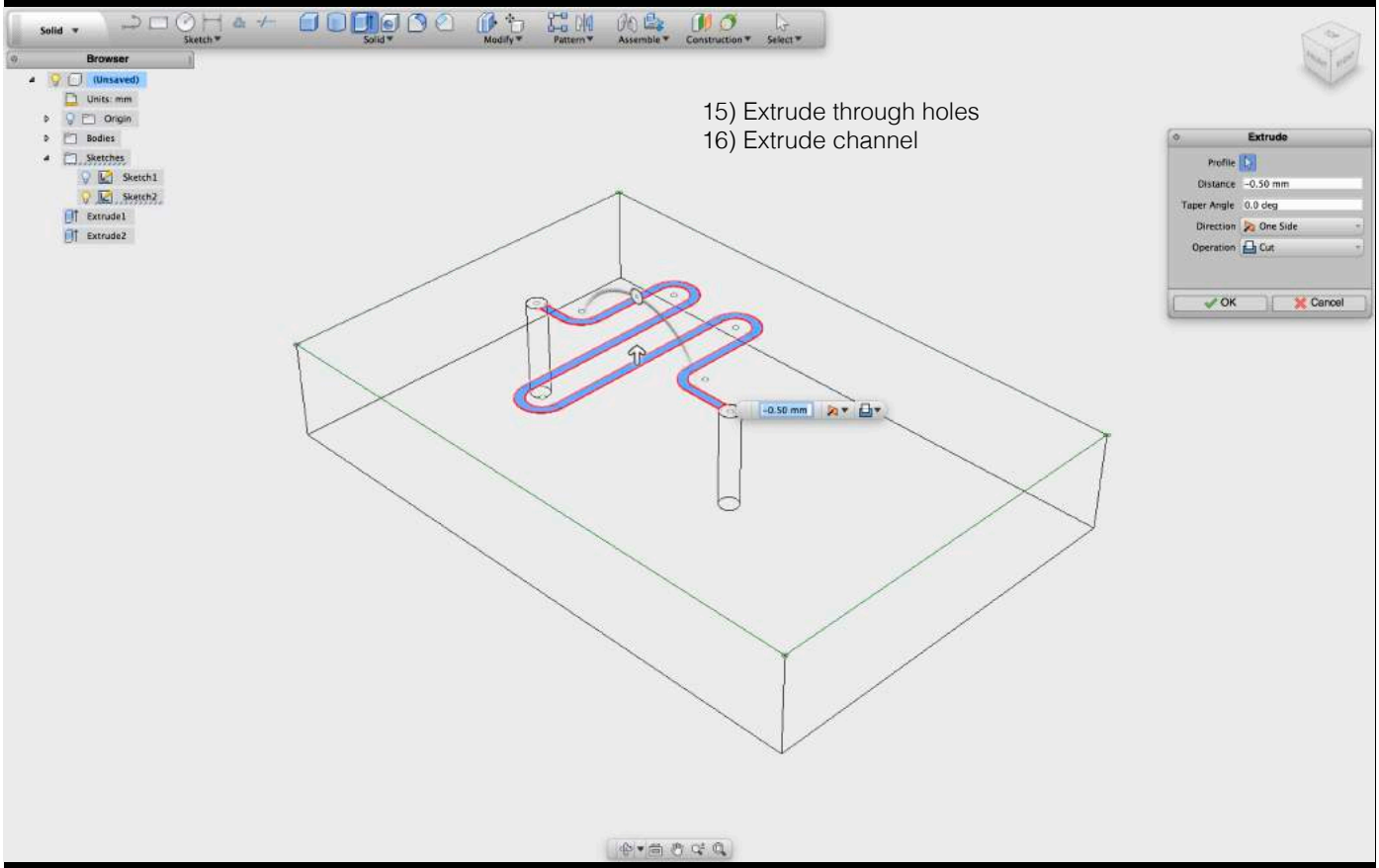


8) Select Working view (Click on TOP to in this case)
9) Select a Sketch tool and select the working plane
10) A new active Sketch will be created

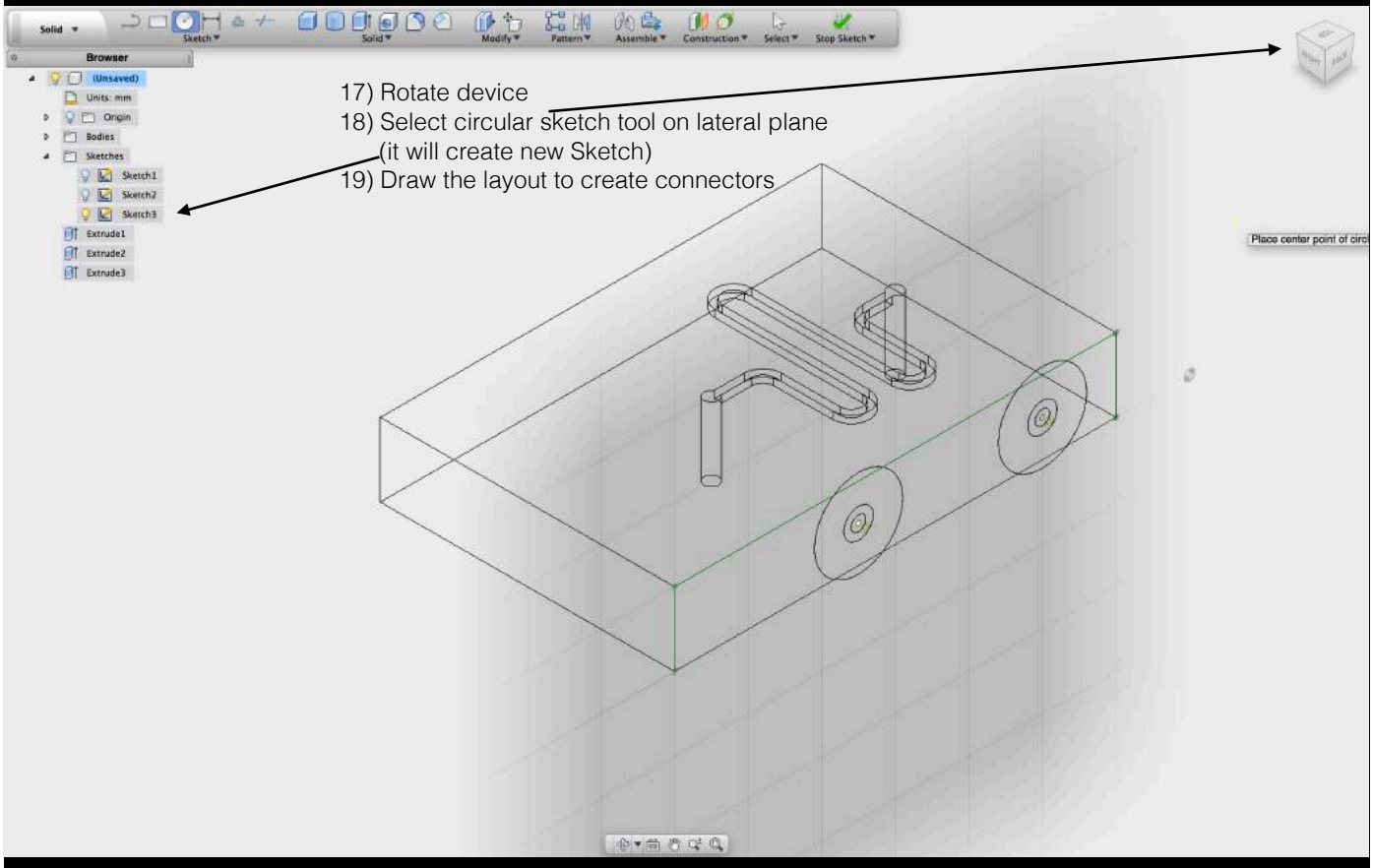

Solid
Sketch
Solid
Modify
Pattern
Assemble
Construction
Select
Stop Sketch
Browser
(Unsaved)
Units: mm
Origin
Bodies
Sketches
Sketch1
Sketch2
Extrude1
TOP
11) Draw layout of the intended channel

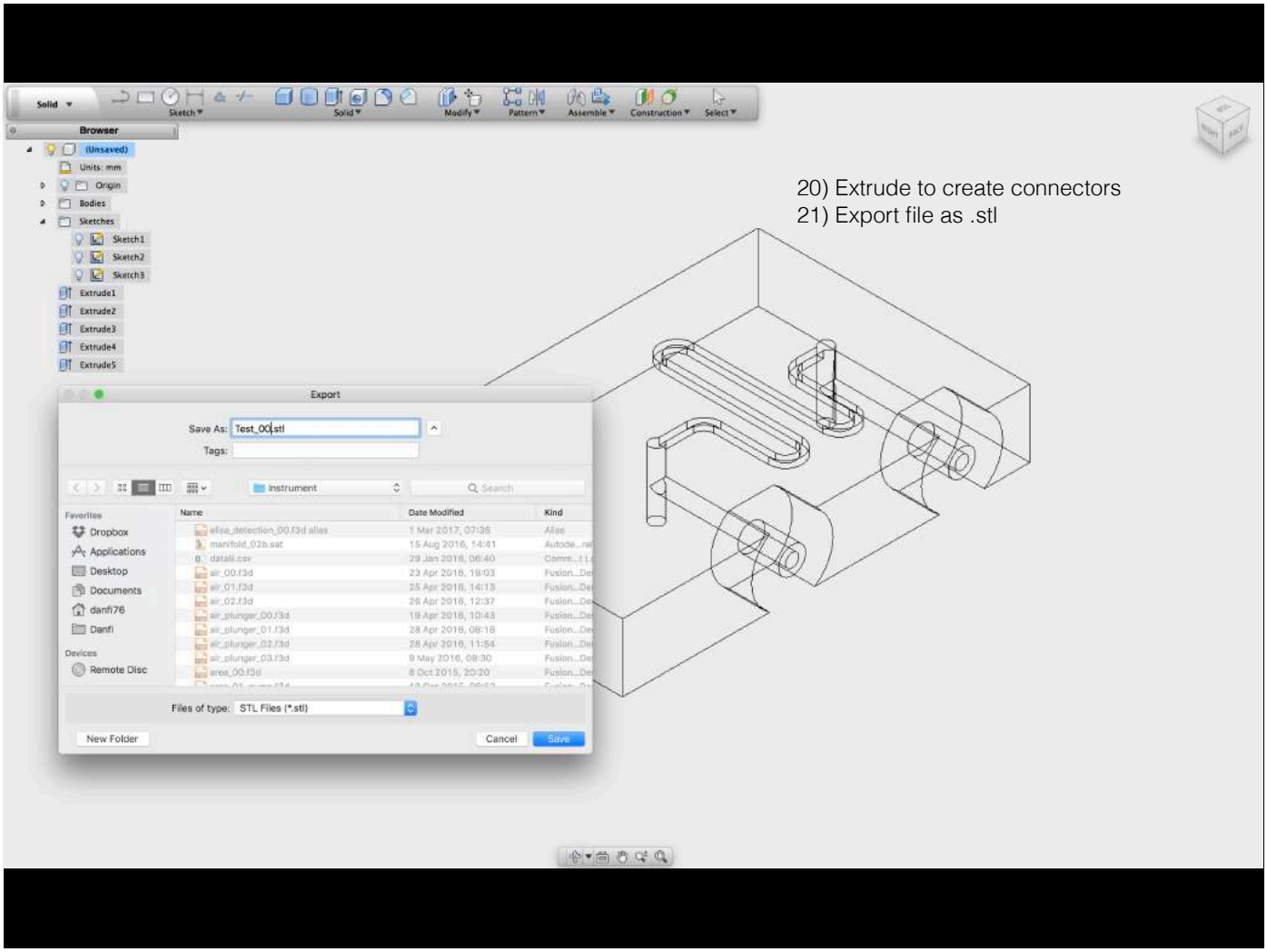

Options
Browser
(Unsaved)
Units: mm
Origin
Bodies
Sketches
Sketch1
Sketch2
Extrude1
Shaded With Edges
Shaded
Shaded with Hidden Line
Wireframe
Wireframe (Hidden Line)
Wireframe (Hidden Line Removed)
Realistic
Screen
Snap
Select
12) Select Options
13) Select Wireframe view
14) Return to Solid mode

15) Extrude through holes
16) Extrude channel

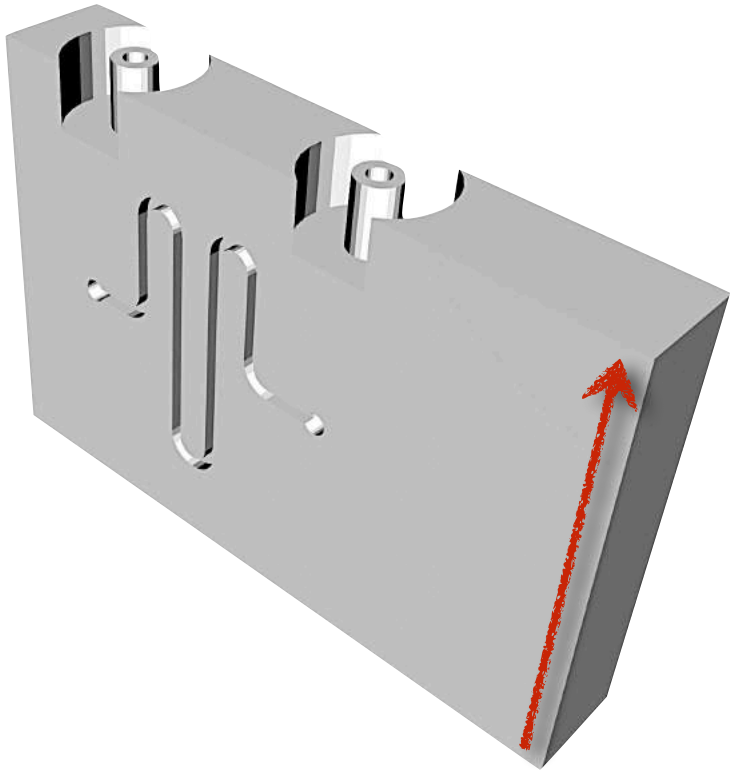


17) Rotate device
18) Select circular sketch tool on lateral plane
(it will create new Sketch)
19) Draw the layout to create connectors





20) Extrude to create connectors
21) Export file as .stl



22) Open .stl file with the printer software
23) Print in the indicated direction

Summary

- Affordable SLA and DLP 3D printers are well adapted for low-cost fast-prototyping of disposable optics and microfluidics.

- Additive techniques not only facilitate numerous affordable iterations during the development process but also allow architectures not easily achievable by classical methods.

- Releases the resources normally devoted to routine fabrication labor for more creative design and optimization tasks.

Optical Devices Laboratory
Division of Sensor and Actuator Systems
LINKÖPING UNIVERSITY

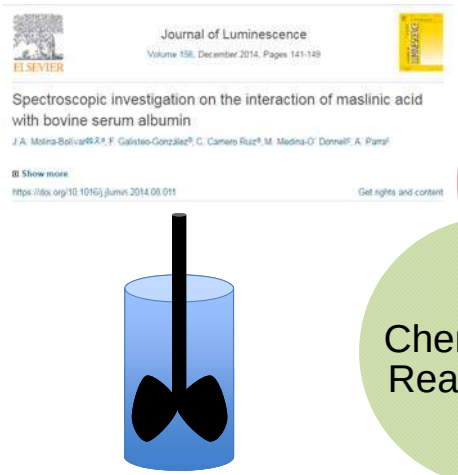
Smartphone Data Handling

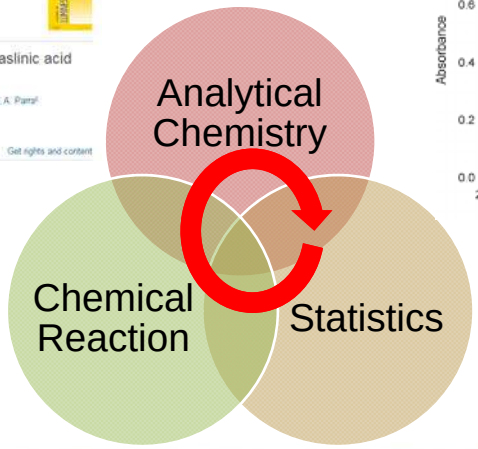
Jeroen Jansen
Assistant Professor / acting department head

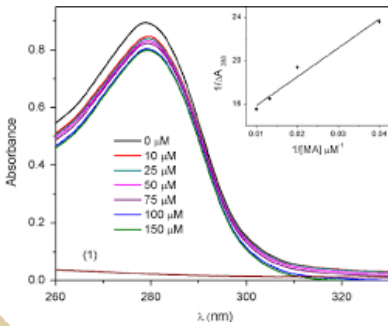
Analytical Chemistry and Chemometrics

Radboud University

Chemometrics: bringing together three worlds







$$\log_{10}\frac{I_o}{I} = \varepsilon\, l\, c$$

Greek letter, epsilon

concentration of solution (mol dm^{-3})

length of solution the light passes through (cm)

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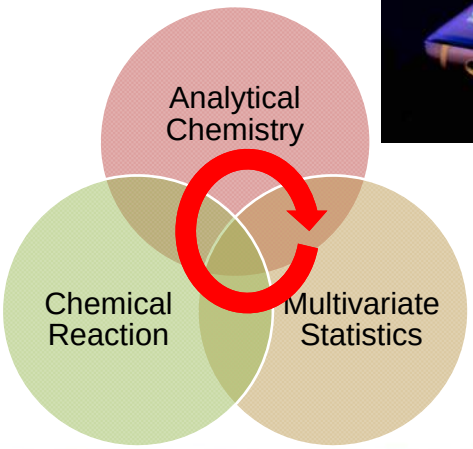
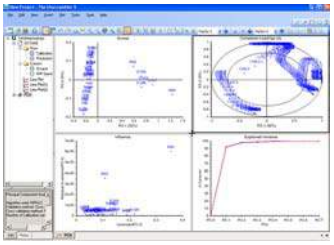
Chemometrics: bringing together three worlds





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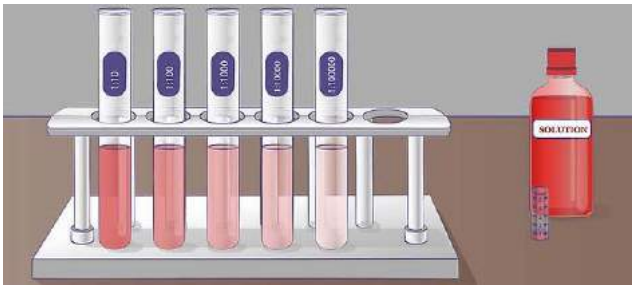
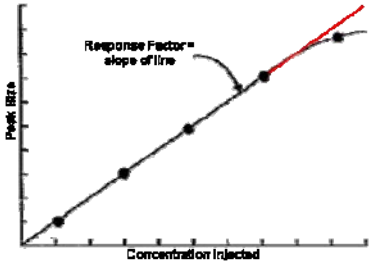
Spectroscopy for calibration

- Spectroscopy observes the dilution of a chemical compound





- Peak size = Color Intensity ~ analyte concentration
- Lambert Beer law

$$\log_{10}\frac{I_0}{I} = \varepsilon\, l\, c$$

(Greek letter, epsilon) — concentration of solution (mol dm^{-3}) — length of solution the light passes through (dm)

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Chemical Measurement with smart phones

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Short communication

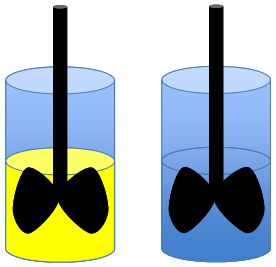
A smartphone algorithm with inter-phone repeatability for the analysis of colorimetric tests

Ali K. Yetisen[a,*,1], J.L. Martinez-Hurtado[a,*,1], Angel Garcia-Melendrez[b], Fernando da Cruz Vasconcellos[a], Christopher R. Lowe[a]

[a] Department of Chemical Engineering and Biotechnology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QT, United Kingdom
[b] Department of Engineering, University of Cambridge, Trumpington Street, Cambridge CB2 1PZ, United Kingdom

From Reaction to color to Value

- Peak size = Color Intensity ~ analyte concentration



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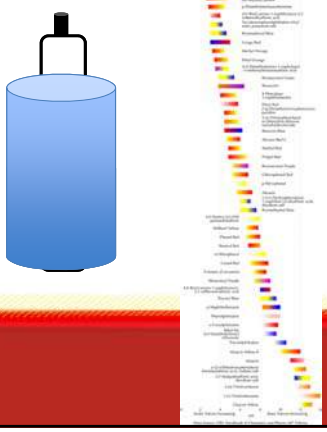
From Reaction to color to concentration



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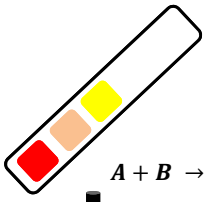
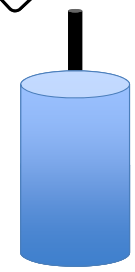
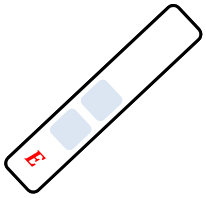
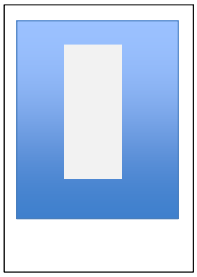
What happens in this process?

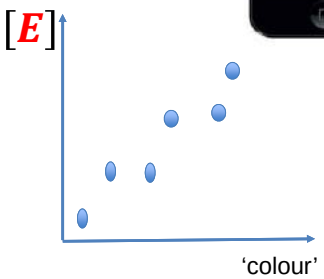


$A + B \rightarrow C \rightarrow D + E$









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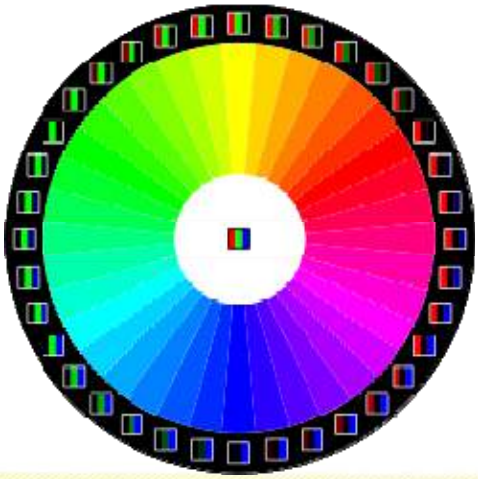
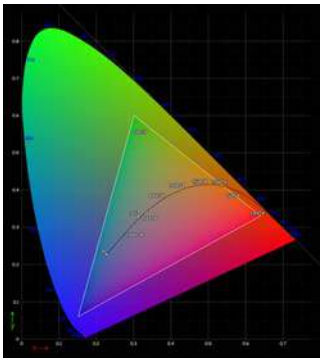
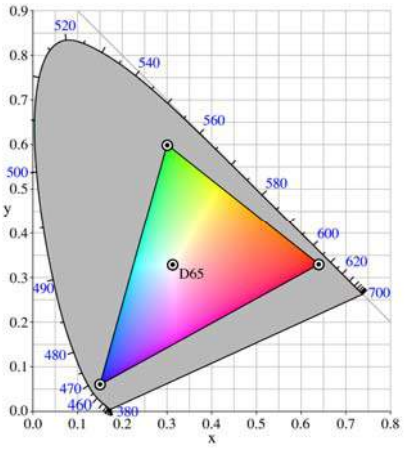
What is ‘colour’?







Every colour is a coordinate in RGB space

https://upload.wikimedia.org/wikipedia/commons/thumb/c/c5/RGB_color_wheel_10.svg/250px-RGB_color_wheel_10.svg.png

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Calibration with colorimetry

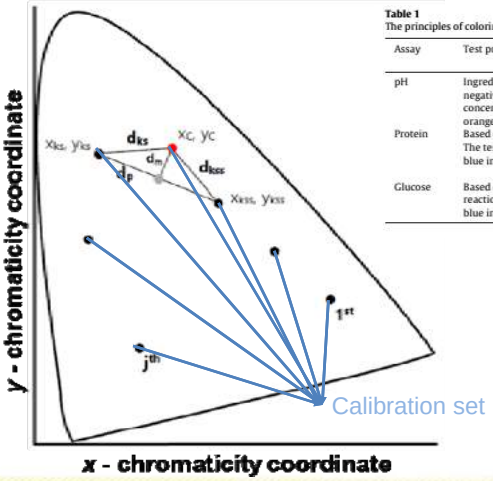
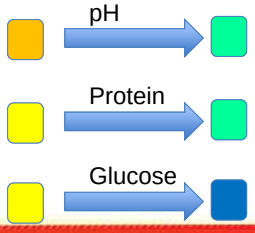


Table 1
The principles of colorimetric reactions in cobas® Combur3 Test®, Roche.

Assay	Test principle	Reactive ingredient (per 1 cm² patch area)	Range	Detection limit	Operating temp. (°C)
pH	Ingredients specifically react with H^+; the pH is the negative common logarithm of the H_3O^+ concentration. The tests pad change color from orange to greenish blue as the pH increase.	Bromothymol blue (13.9 µg), methyl red (1.2 µg) and phenolphthalein (8.6 µg)	5–9	N/A	Stable up to boiling point
Protein	Based on protein error principle of a pH indicator. The test pad changes color from yellow to greenish blue in the presence of albumin.	3′,3″,5′,5″-Tetrachlorphenol-3,4,5,6-tetrabromsulfophthalein (neutral form) (13.9 µg)	0–100 mg/dL	6 mg albumin/dL	Stable up to boiling point
Glucose	Based on glucose oxidase/peroxidase reaction. The reaction pad changes color from yellow to dark blue in the presence of glucose.	Tetramethylbenzidine (103.5 µg), glucose oxidase (6 U) and peroxidase (35 U)	0–300 mg/dL	40 mg/dL	<55



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Placing colors onto a common scale

$$R_l = \left(\frac{0.055 + R_c}{1.055}\right)^{2.4} \quad (1)$$

$$G_l = \left(\frac{0.055 + G_c}{1.055}\right)^{2.4} \quad (2)$$

$$B_l = \left(\frac{0.055 + B_c}{1.055}\right)^{2.4} \quad (3)$$

Next, linear RGB values are converted to tristimulus values, *X*, *Y*, *Z* by the following relationships [16]:

$$X = 0.1805B_l + 0.3576G_l + 0.4124R_l \quad (4)$$

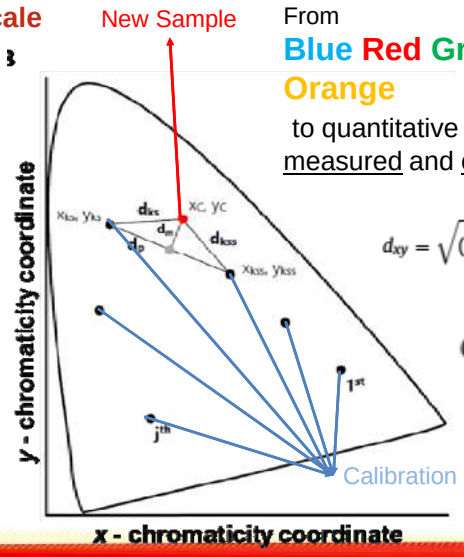
$$Y = 0.0722B_l + 0.7152G_l + 0.2126R_l \quad (5)$$

$$Z = 0.9505B_l + 0.1192G_l + 0.0193R_l \quad (6)$$

Finally, *X*, *Y*, *Z* tristimulus values are converted into the 2D (*x*, *y*) CIE 1931 chromaticity space [17] using:

$$x_j = \frac{X}{X+Y+Z} \quad (7)$$

$$y_j = \frac{Y}{X+Y+Z} \quad (8)$$



From
Blue Red Green Purple Orange
to quantitative values that can be measured and compared

$$d_{xy} = \sqrt{(x_{ks} - x_{kss})^2 + (y_{ks} - y_{kss})^2}$$

$$C_m = \frac{d_p d_C}{d_{xy}}$$

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From color transition to pH readout

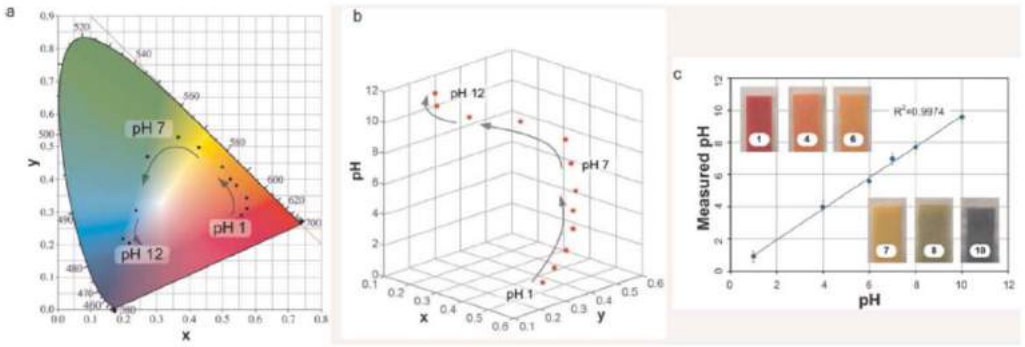


Fig. 3 (a) pH paper colors in CIE 1931 color space. (b) The 3-D view of the relation between *xy* coordinates and the pH values. (c) Standard curve of the smartphone reader response to different pH buffer solutions.

Cite this: *Lab Chip*, 2012, **12**, 4240–4243
www.rsc.org/loc

TECHNICAL INNOVATION

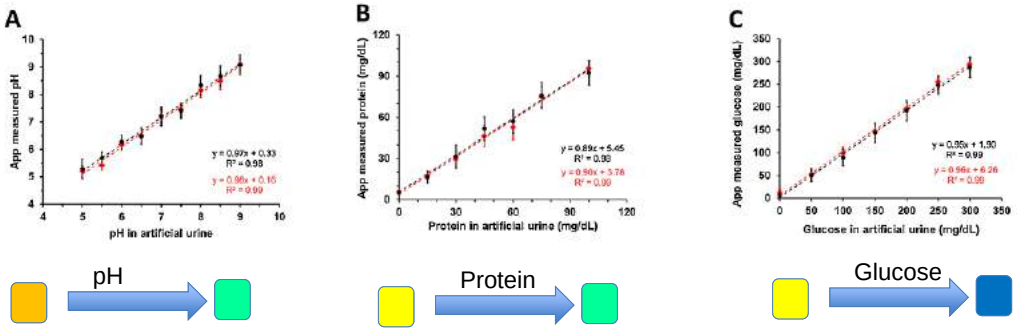
Point-of-care colorimetric detection with a smartphone†

Li Shen,[a] Joshua A. Hagen[b] and Ian Papautsky*[a]

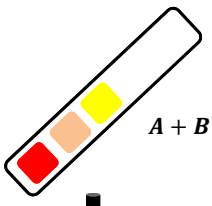
Radboud University

Prediction of these parameters from phone assays

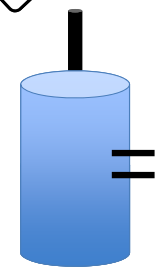
R^2 is a qualitative readout of the analysis

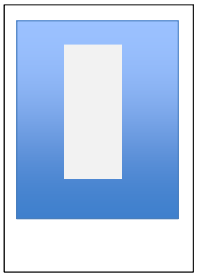


R^2 is determined by all steps in the analysis



$A + B \rightarrow C \rightarrow D + E$





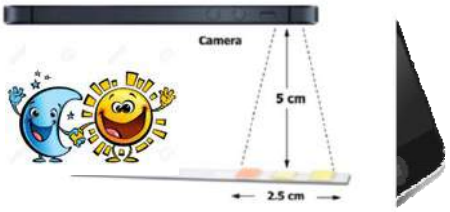
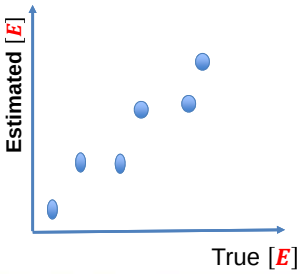
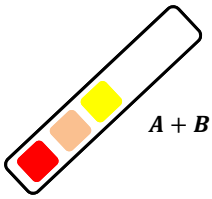


Fig. 1. Quantifying colorimetric tests through a smartphone reader. The smartphone captures and processes the image of the test zones, reducing time and errors related to visual inspection of the color reference chart.

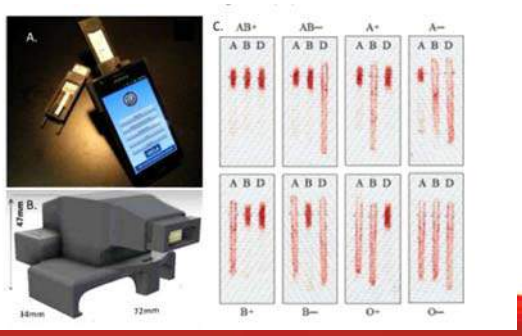


R^2 is determined by all steps in the analysis



$$A + B \rightarrow C \rightarrow D + E$$

Fig. 1. Quantifying colorimetric tests through a smartphone reader. The smartphone captures and processes the image of the test zones, reducing time and errors related to visual inspection of the color reference chart.



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How to make your phone setup as good as possible

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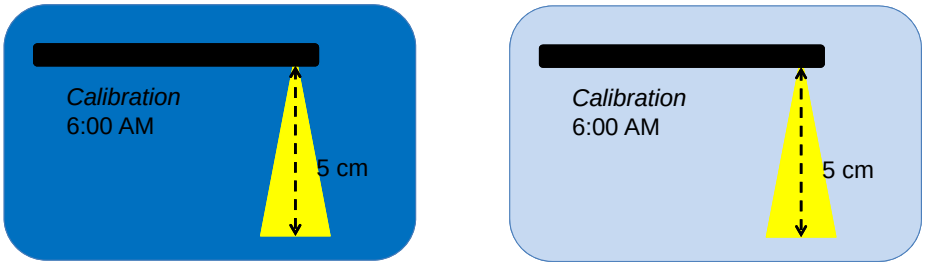
Measurement Quality is determined by all steps in the analysis

- Influences need to be identified to improve the measurement quality
  - Biases



  - variabilities

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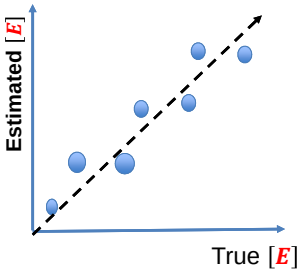
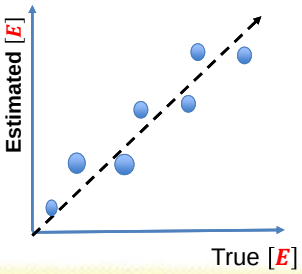
Measurement Quality is determined by all steps in the analysis

- Influences need to be identified to improve the measurement quality
  - Biases





  - variabilities

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Analysis of Variance

$$measured\ pH = true\ pH + height\ of\ measurement + CMOS\ chip\ type + indicator\ paper\ brand + time\ of\ day + analyst + paper + phone + \text{measurement error}$$

$$true\ pH = \text{result of your experiment} + \text{experimental error}$$

experimental design

random errors

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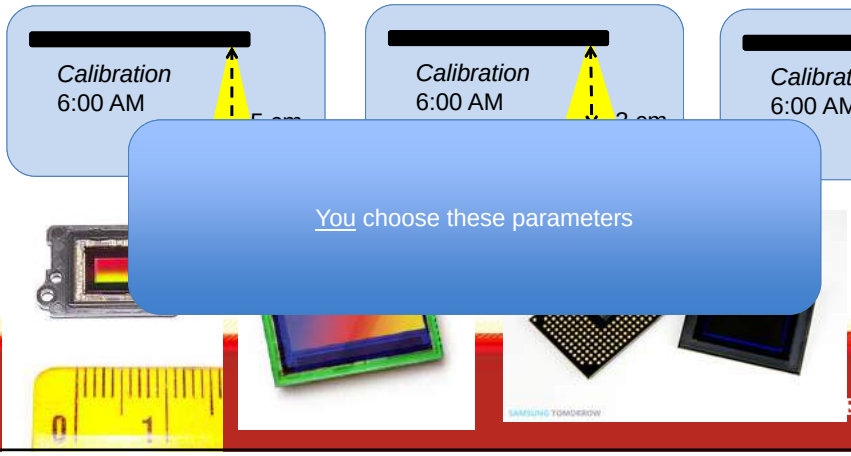
Experimental design

- The route from analyte solution to app readoud is long
- Many choices need to be made, choices influence each other
- Good data can only come from good measurements



Ordered

Categorical

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Experimental design

- The route from analyte solution to app readout is long
- Many choices need to be made, choices influence each other
- Good data can only come from good measurements
- ‘Design of Experiments’ invented by Roland Fisher →

Random

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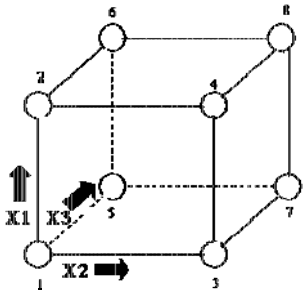
Design of Experiments

- A systematic way to design what experiments to do to find the best measurement
  - *E.g.* X1 = cmos chips Sony vs. Samsung
  - X2 = measurement height 5 cm vs 1 cm
  - X3 = measurement at 6:00 and at 18:00 (maybe also 12:00)
  - Repeat every experiment 1 … 8 several times, with different analysts
- But:
  - Maybe the optimum is not at an ‘experiment’
  - Should we do all experiments?



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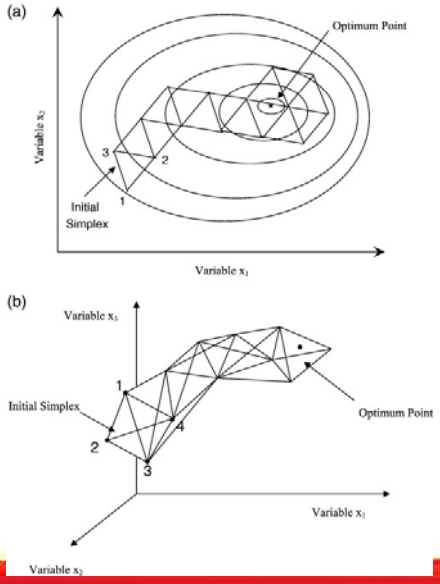
Optimization

You can do Design of Experiments iteratively, to obtain optimal settings for your setup

This is of course more difficult if you need
- To optimize many parameters at the same time
  - CMOS brand, paper brand, phone height, sampling protocol, photo protocol
- Have more criteria to optimize at the same time
  - Optimal color sensitivity & time window for measurement



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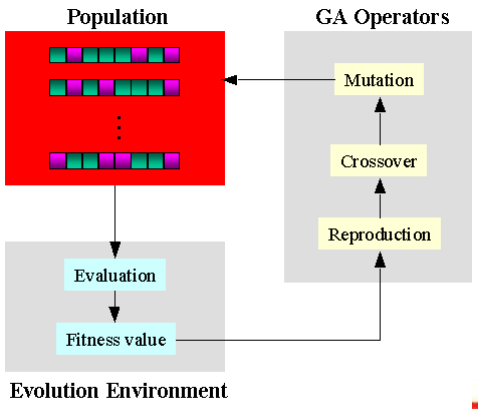
Genetic algorithms

- Use genetic principles to generate new experiments from old settings



Genetic Algorithm Evolution Flow

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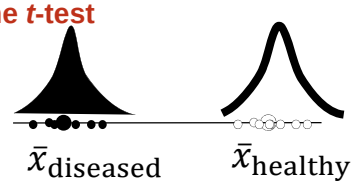
How to process your data as efficiently as possible

Univariate analysis: The *t*-test



A

$$t = \frac{\left|\bar{x}_{\text{diseased}} - \bar{x}_{\text{healthy}}\right|}{\sqrt{\frac{s^2_{\text{diseased}}}{n_{\text{diseased}}} + \frac{s^2_{\text{healthy}}}{n_{\text{healthy}}}}}$$

$\bar{x}$: average value
s^2 : variance
n : number of replicates

Use this formula for $n_{\text{diseased}} + n_{\text{healthy}}$>30

For more details, see 'college dictaat statistiek' or any statistics textbook

- Compare *t* with a tabulated value for a given confidence interval (95%)
- Or use the computer to calculate a *p*-value from this value of *t*

$p < 0.05$?

$p < 0.05$**?**

What does this mean?

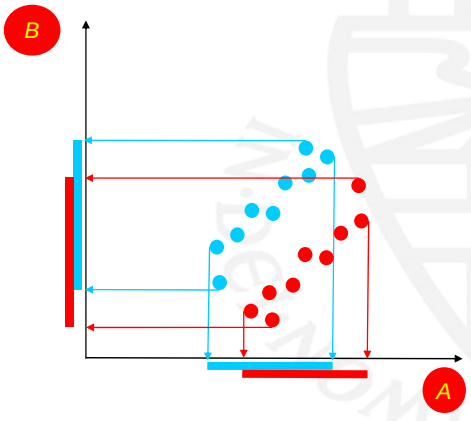
[redacted]

3. There is less than 5% chance that the data we have measured, results from a system where the null hypothesis is true

Multivariate advantage: correlations may be essential in finding differences between groups

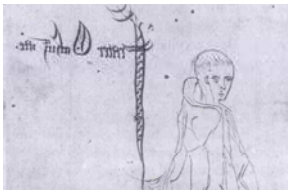
WHY IS THIS IMPORTANT?

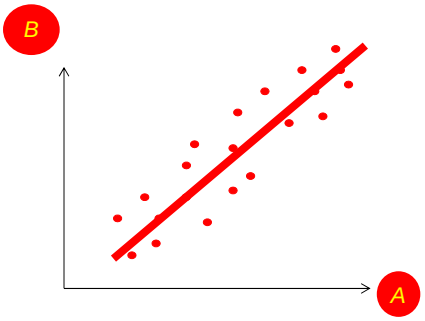
- *A* and *B* by themselves are not distinctive between both groups
- t-tests find two non-significant features
- Together they can describe the difference perfectly!
- The correlations of *A* and *B* in both groups are essential for this!



PCA on two variables

Draw <u>one</u> line to describe <u>two</u> variables

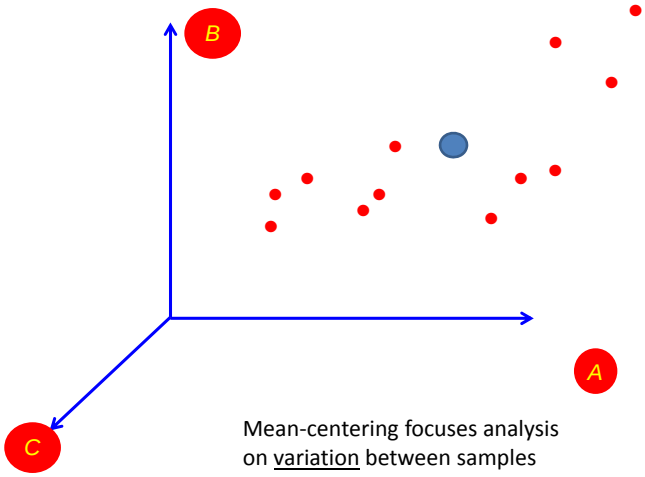




Mean Centering

3 variables

12 samples

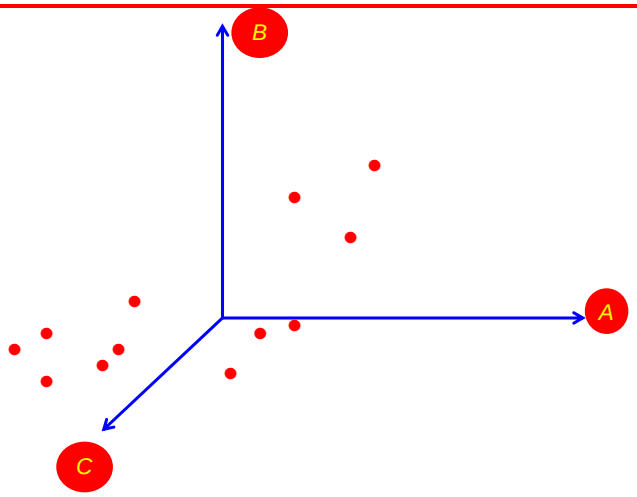


Mean-centering focuses analysis on <u>variation</u> between samples
Only then: correlation between features

Principal Component Analysis

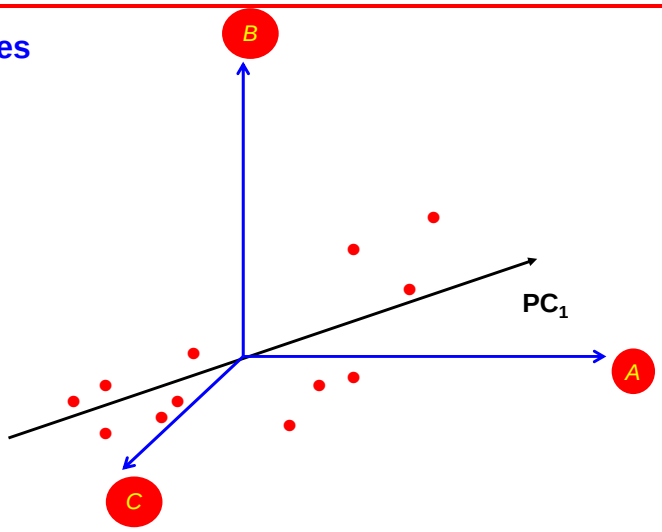
3 variables

12 samples

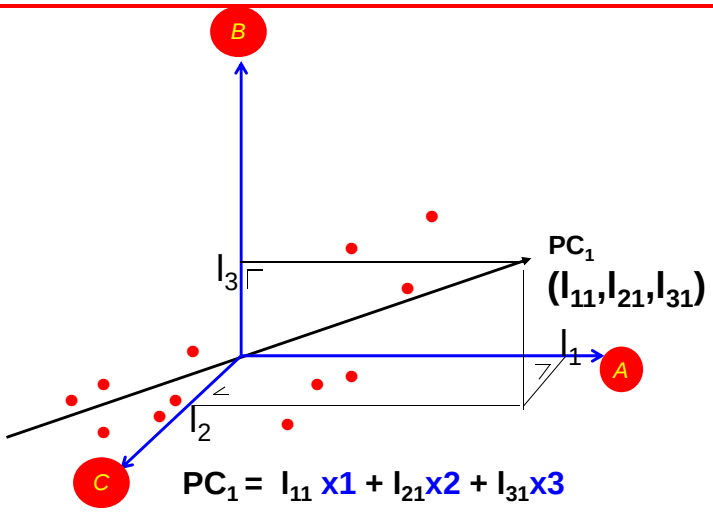


Principal Component Analysis

3 variables

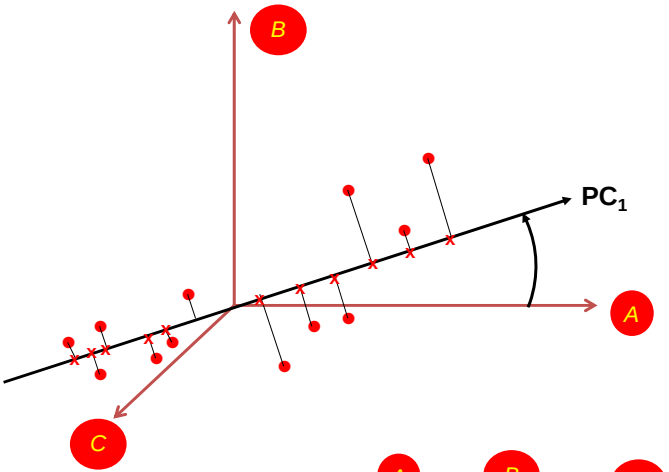


Principal Component Analysis



$PC_1 = l_{11}\,x1 + l_{21}x2 + l_{31}x3$

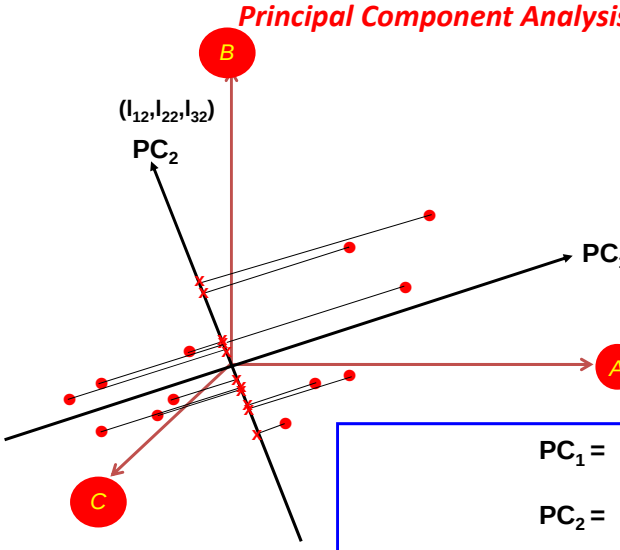
Principal Component Analysis



$PC_1 = l_{11}\,A + l_{21}\,B + l_{31}\,C$

Criterion: Maximum variation of projections (x)

Principal Component Analysis



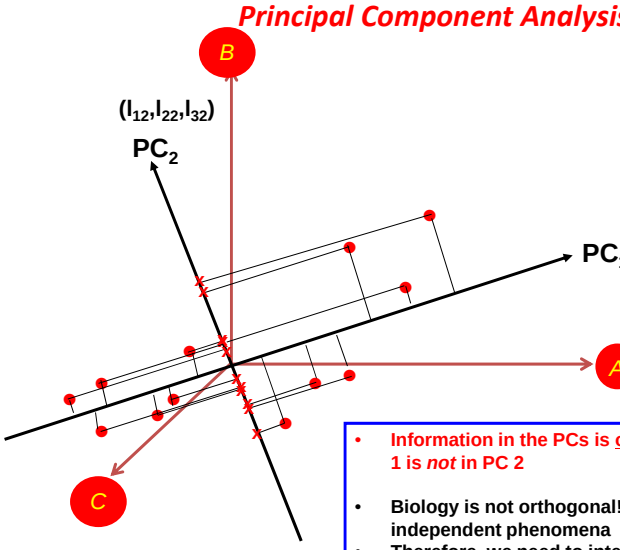
$PC_1 = l_{11}$ A $+ l_{21}$ B $+ l_{31}$ C

$PC_2 = l_{12}$ A $+ l_{22}$ B $+ l_{32}$ C

Criterion: Maximum variation of projections (x)

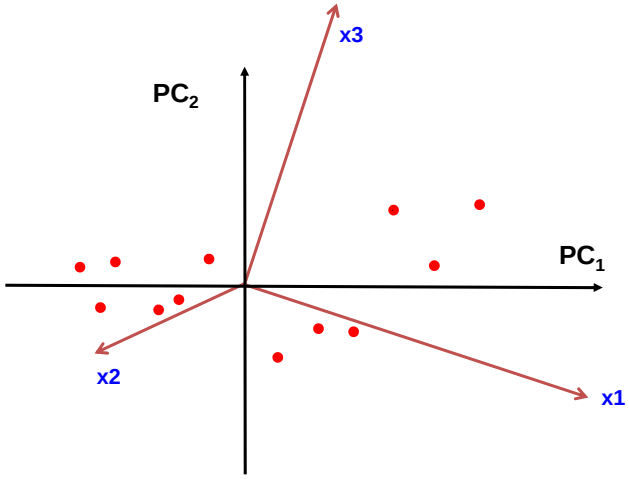
PC1 ⊥ PC2

Principal Component Analysis



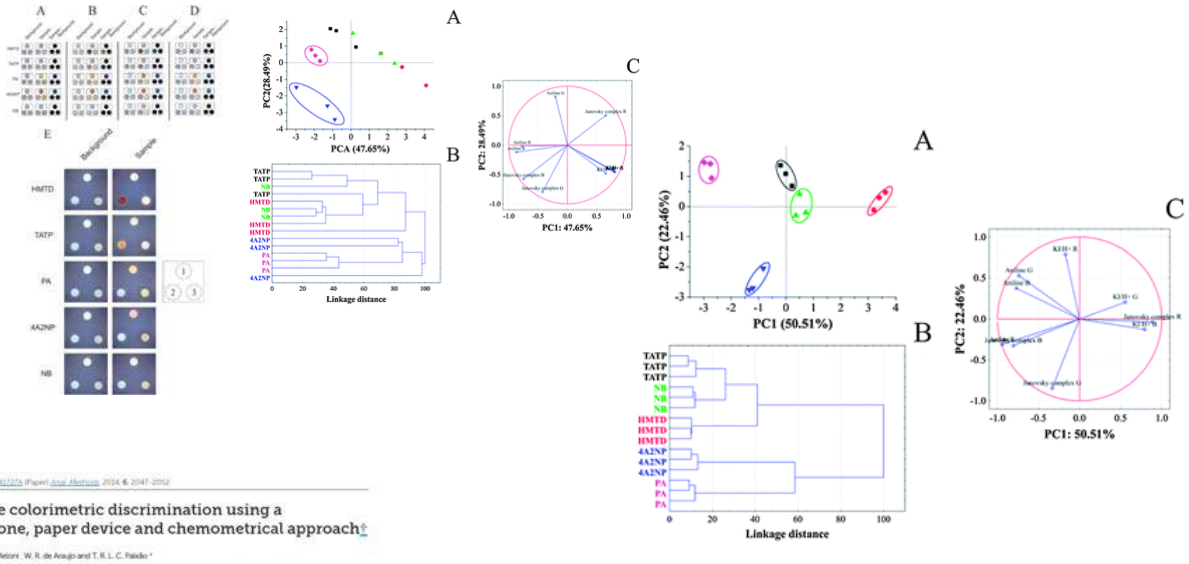
- **Information in the PCs is orthogonal: information in PC 1 is *not* in PC 2**
- **Biology is not orthogonal! PCs do not describe independent phenomena**
- **Therefore, we need to interpret all PCs at the same time!**

Principal Component Analysis



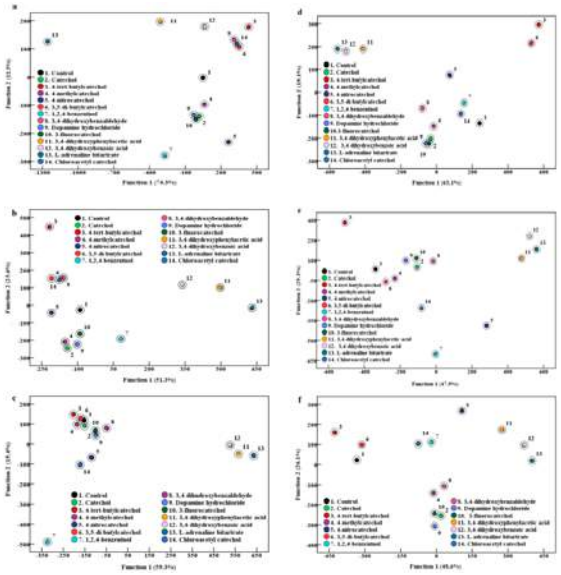
Each PC is a linear combination of all original variables; the coefficients are the loadings. (Each PC is a 'new' variable)

Principal Component Analysis

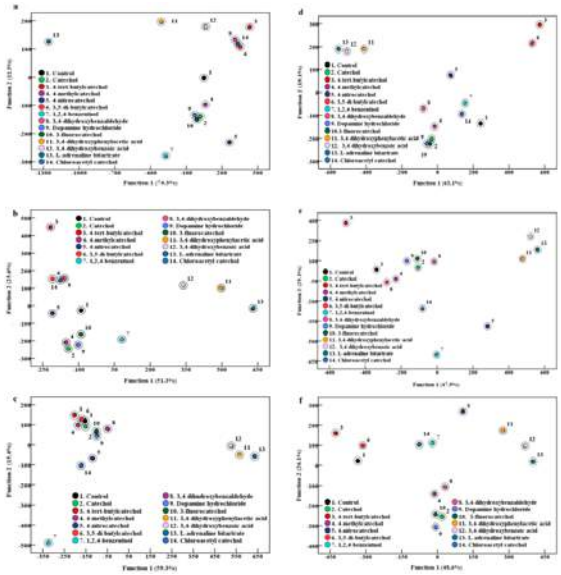


Explosive colorimetric discrimination using a smartphone, paper device and chemometrical approach†

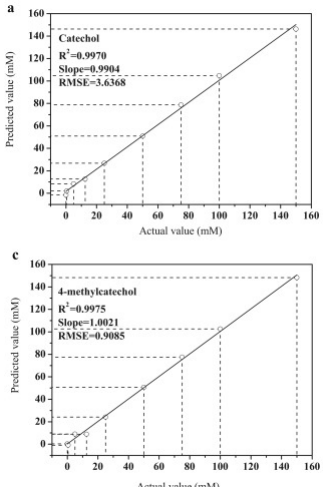
Principal Component Analysis for calibration



Principal Component Analysis for calibration



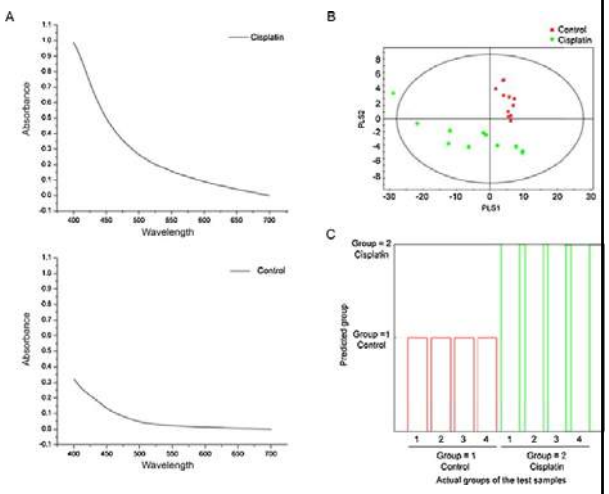
Calibration line



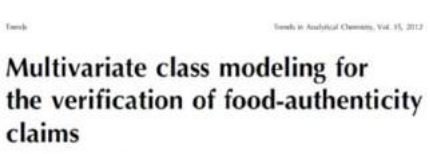
Principal Component Analysis for classification

Classifier

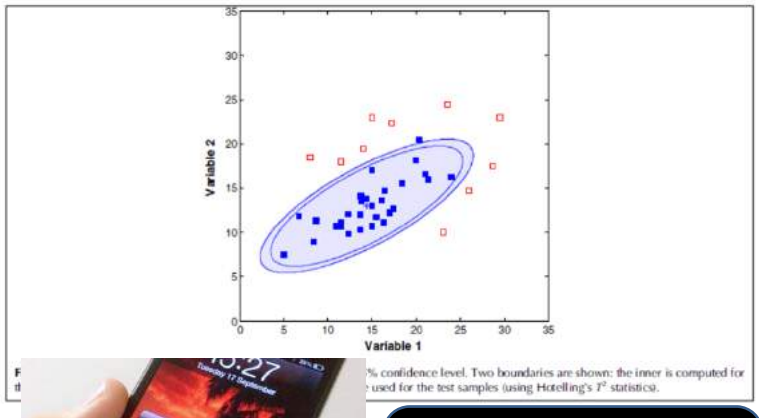
PCA-like model, where the green and red groups are as small as possible and the difference between both groups is as large as possible



Principal Component Analysis for class modeling



One-Class



PCA-like model, where the blue class is distinguished as much as possible from all red samples

This is completely New in smartphone colorimetry

Data handling in smartphone colorimetry

- Going from colorimetric measurement to readout is a multi-step process systematically different from ‘conventional’ spectroscopy
- You can use Design of Experiments and Optimization to improve calibration of your colorimetric setup
- The multivariate advantage provides a lot of relevant problems that you will meet in the application of smartphone colorimetry in real-life practice!
- Questions: jj.jansen@science.ru.nl

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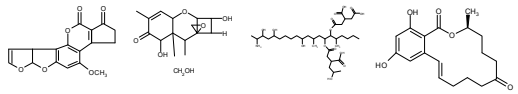
R-Biopharm AG • An der neuen Bergstraße 17 • 64297 Darmstadt, Germany • E-mail: info@r-biopharm.de • www.r-biopharm.com

What exactly are mycotoxins?

- Toxic chemical compounds produced by molds
- There are hundreds of compounds, only a few are relevant
- They are persistent compounds
- They are chemically a very heterogeneous group of compounds



- They may occur globally (with regional differences)
- Mycotoxins are natural contaminants that cannot 100% be controlled nor eliminated – but they can be "managed"!

Which agricultural commodities are of concern?

- Corn (AFB, FUM, DON, ZON)
- Wheat (Trichothecenes, ZON)
- Rice, Barley, Oat, Rye, Sorghum (AFB; OTA, Trichothecenes)
  - Corn, wheat, rice and barley cover > 90% of world cereal production
- Oilseeds (peanuts, soy) (AFB, OTA)
- Treenuts, fruits (raisins, figs, apples) (AFB,OTA, PAT)
- Coffee, liquorice, tea, spices, herbs (AFB, OTA)
- All products derived from these commodities (including feed)

Were do mycotoxins come from?

- Toxins can be formed during storage (insufficient drying) but also pre-harvest (in the field)
- Plants are more susceptible at certain stages of growth and to different weather conditions at those stages.
- Different conditions produce different molds-hence possibly different mycotoxins.
- Cereals may get infected by molds if the kernels are damaged (e.g. by insects or during transport)

Photos: courtesy Henry Njapau and Masja Straetemans

Why test for mycotoxins?

- Most countries have mycotoxin regulation(s)
- Mycotoxins have a significant economic and social impact
  - Direct crop revenue loss due to mycotoxins
  - Decrease of growth rate livestock
  - Trade flow losses
  - Public health costs & social costs
    - USA/Canada: Annually mycotoxin related losses ± $ 5 billion
    - Molds spoil app. 10% of the world's annual harvest (Science, 2010)

How are mycotoxins typically analyzed?

RIDA®SMART APP

Sometimes you need those analytical results rapidly..

And you may need those results on-site –
basically at any place at any time….

RIDA®SMART APP

Lateral flow based mycotoxin tests are the method of choice to make those rapid decisions……

Advantages:

- Fast and easy sample preparation and test execution
- No specialist skills required
- No handling with hazardous mycotoxin standards
- Quantitative results analysis with reader

But: Testing is done in a laboratory

RIDA®SMART APP

Now allow me to ask you a question:
What do these 2 have in common?

They are both lateral flow readers!

How does that work?

- Use a supported Google Inc. smartphone
- Buy your RIDA®SMART APP voucher at R-Biopharm
- Download the RIDA®SMART APP (QR code on voucher)
- Register and activate your voucher: http://app.r-biopharm.com

Test - Scan - Send - RIDA®SMART APP

How to generate results with RIDA®SMART APP

Testing (e.g. test for total aflatoxin with RIDA®QUICK Aflatoxin RQS ECO)

After testing

Place the RIDA®SMART APP cover on your test strip

Each box of the RIDA®QUICK mycotoxin test comes with it's own cover

Scan

Scan

Define your sample:
- Operator
- Sample ID
- Customer ID

Define the used lateral flow test:
- Scan QR-code on the SMART APP cover for test information

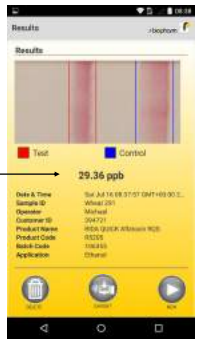
RIDA®SMART APP

Scan

RIDA®SMART APP

Scan

- The result is evaluated by the app and immediately available after measurement
- The result is automatically stored in the RIDA®SMART APP database or can be exported



RIDA®SMART APP

Send

Send your result directly after measurement…

…or after recalling from the RIDA® SMART APP database

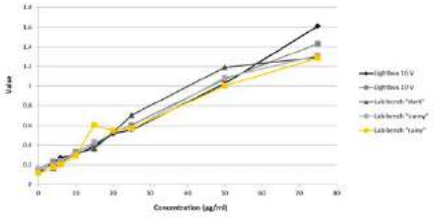
via email

or to any WiFi or bluetooth printer

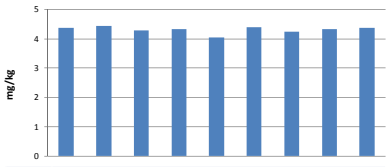
https://youtu.be/qjC1Q_4LCGI

Does it work?

- We have extensively validated the performance of the RIDA®SMART APP. For example the effect of light conditions:



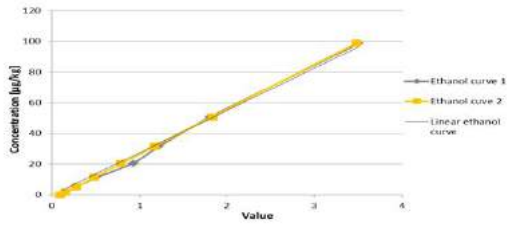
Does it work?
Inter reader variation



Reader	1	2	3	4	5	6	7	8	9
Mean	4,3766	4,4435	4,2868	4,3295	4,0461	4,3897	4,2357	4,3202	4,3809
SD	0,24	0,16	0,19	0,17	0,21	0,20	0,23	0,18	0,18
CV	5,43%	3,52%	4,51%	4,02%	5,27%	4,49%	5,36%	4,09%	4,12%

Fig 2: Inter reader variation. The same reference strip has been measured 20 times (n=20) with each smartphone. For this Nexus6 phones ordered in different countries all over the world were used.

Figure 3 : The figure shows the linearity and conformity of two individual recorded curves generated from real samples and analyzed under non controlled conditions.



Concentration [µg/kg]	5.3	11.3	20.2	31.6	50.8	98.7
mean	0.27	0.51	0.93	1.22	1.81	3.50
CV %	13.3	14.7	18.5	12.5	16.4	14.0

RIDA®SMART APP – on-site mycotoxin testing with smartphone-based test evaluation

Recovery rates

Fig 4: Recovery rates. Trilogy® reference materials were analyzed with all RIDA®SMART APP-compatible R-Biopharm lateral flow tests. Mycotoxin concentrations on the reference material certificates were set as target values.

RIDA®QUICK DON (Art. No. R3RQE)	Trilogy® reference material (wheat)							
Target value	ND	0.5	0.9	1.6	2.1	3.5	4.5	6.2
Recovery [%]	-	106	93	93	100	95	104	91
	Trilogy® reference material (corn)							
Target value	ND	0.5	1.1	1.9	2.7	3.6	4.8	6.2
Recovery [%]	-	113	104	102	105	100	104	97
RIDA®QUICK Aflatoxin RQS (Art. No. R5205)	Trilogy® reference material (corn)							
Target value	ND	1.7	5.9	14.3	20.2	31.6	50.8	98.7
Recovery [%]	-	-	97	110	104	106	96	82
RIDA®QUICK Aflatoxin RQS ECO (Art. No. R5206)	Trilogy® reference material (corn)							
Target value	ND	1.7	5.9	14.3	20.2	31.6	50.8	98.7
Recovery [%]	-	-	88	84	93	88	100	97
RIDA®QUICK Zearalenon RQS (Art. No. R3504)	Trilogy® reference material (corn)							
Target value	ND	59	88	121	163	267	472	1021
Recovery [%]	-	73	117	121	111	86	82	84
RIDA®QUICK Fumonisin RQS (Art. No. R5604)	Trilogy® reference material (corn)							
Target value	ND	0.6	1.0	2.2	3.2	6.8	9.2	12.5
Recovery [%]	-	92	111	98	94	101	84	-
RIDA®QUICK T-2 / HT-2 RQS (Art. No. R3304)	Oats sample (for spike level see target values)							
Target value	ND	50	100	200	400	600	800	1000
Recovery [%]	-	103	113	96	93	93	92	91

Does it work?

- And we validated the RIDA®SMART APP using Trilogy certified mycotoxin reference materials. Here are some data for aflatoxin (compared to the RIDA®QUICK SCAN reader):

Trilogy Reference Material	Reference value	Results	
		RIDA®QUICK SCAN	RIDA®SMART APP
AC-215	Blank	<4 µg/kg	<4 µg/kg
AC-285	5,9 µg/kg	4.5 µg/kg	5.2 µg/kg
AC-2203	11,1 µg/kg	13,2 µg/kg	10,8 µg/kg
AC-286	20,2 µg/kg	23,4 µg/kg	20,5 µg/kg
AC-290	32,2 µg/kg	26,7 µg/kg	26,1 µg/kg

- More data are of course available on request – to answer the question yes, it does work!

RIDA®SMART APP

Compatible RIDA®QUICK mycotoxin tests

Compatible with all quantitative RIDA®QUICK mycotoxin lateral flow tests

Aqueous extraction

Product	Article number	Test implementation	Detection range	
			RIDA®SMART APP	RIDA®QUICK SCAN
RIDA®QUICK Aflatoxin RQS ECO	R5206	5 min	4 - 100 µg/kg	4 - 100 µg/kg
RIDA®QUICK DON	R5904	5 min	500 - 5500 µg/kg	500 - 5500 µg/kg
RIDA®QUICK Fumonisin RQS	R5606	5 min	300 - 10000 µg/kg	300 - 10000 µg/kg
RIDA®QUICK T-2 / HT-2 RQS	R5304	5 min	50 - 10000 µg/kg	50 - 8000 µg/kg

Ethanol/methanol extraction

Product	Article number	Test implementation	Detection range	
			RIDA®SMART APP	RIDA®QUICK SCAN
RIDA®QUICK Aflatoxin RQS	R5205	5 min	4 - 100 µg/kg	4 - 100 µg/kg
RIDA®QUICK Zearalenon RQS	R5504	5 min	50 - 1000 µg/kg	75 - 500 µg/kg

RIDA®SMART APP

Features and compatible smartphones

- **Internal results database**
  Results are stored within the RIDA®SMART APP database and can be retrieved anytime
- **Serial mode and automatic sample ID assignment**
  Options for high sample throughput
- **Implemented service request function**
  Submit your service request via smartphone
- **Smartphone platform**
  Google Inc. Nexus 6, Nexus 6P and Motorola Moto X Pro, Google Inc. Pixel XL

RIDA®SMART APP

Why use smartphone technology?

First of all: Technology

- Camera quality in most smartphones is very good
- Smartphone software can do the evaluations and calculations

Smartphones are easily available (Just go online and order ☺) and affordable

They are globally certainly more easily available than "lateral flow readers"

RIDA®SMART APP

Why use smartphone technology?

Secondly: Mobility & Connectivity

- Results can be evaluated at any place, at any time –
  You can test at those places, where you actually need to take the decision


- Results can be shared by email or sent to a printer (Wi-Fi or Bluetooth)

RIDA®SMART APP

Why use smartphone technology?

But the best thing is: “Mycotoxin Big Data”

- As the central QC manager you can have real-time data “from the field”
  for mycotoxins and manage more effectively


RIDA®SMART APP

Why use smartphone technology?

Even bigger Mycotoxin Data?

- Combine those real-time data from the field with available online data from
  harvest predictive models and weather monitors – globally……

http://www.wheatscab.psu.edu/

RIDA®SMART APP

So to conclude: These are both lateral flow readers. But what is the difference between both?

With the RIDA® SMART APP you can:

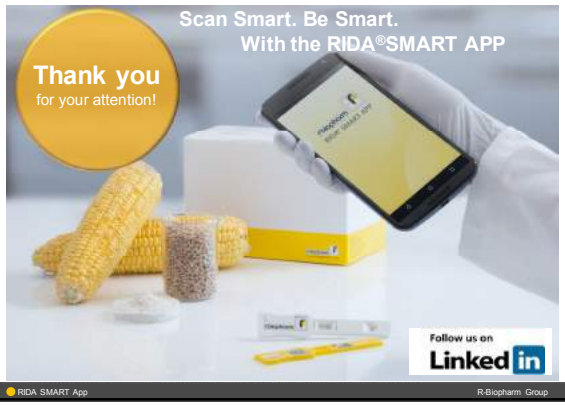
- Read and evaluate mycotoxin tests anytime, anywhere
- Send and print results - online from any place
- Get (online) access to technical support

- You could even call us ☺

RIDA®SMART APP

Scan Smart. Be Smart. With the RIDA®SMART APP

RIDA®SMART APP



Smartphone-based NIR scanners

Yannick Weesepoel [1]

During the past years, the field of vibrational spectroscopics is in the process of 'democratization'. The available spectroscopic hardware can nowadays be purchased for as little as USD 250, is miniaturized or build into a smartphone, and the software interface has been made dummy-proof. These recent developments pave the way for so-called 'food scanners' and can basically deal with applications which are complementary to the envisioned H2020 FoodSmartPhone applications. The commercial available food scanners are mainly based on near-infrared spectroscopy (NIR) and can therefore have applications in determination of the macro-composition of (unprocessed) foods, determination of food authenticity and various quality parameters like freshness. However, before a workable food scanner application can be deployed for citizens, a spectral database has to be constructed covering sufficiently the within-food product variation and the measuring practises variation by the end-user. In this lecture we will therefore touch upon the various aspects of building a smartphone-based NIR application for industry and consumers. This will cover the following topics:

- Sampling for a reliable database
- Protocols for reliable measuring
- Dealing with instrumental error and multivariate statistics
- Validation of your spectral database
- Communication of the results to the end-user
- Pilot-applications for NIR food scanners

Finally, we will touch upon the development of new type of scanners in the H2020 project PhasmaFOOD (www.phasmafood.eu). This project aims to develop a scanner for a wider array of food applications by combining different type of spectral sensors and imaging techniques.

Suggestions for further reading:

[1] Future trends in food authenticity: Pustjens, A. M.; Weesepoel, Y.; van Ruth, S. M., 1 - Food Fraud and Authenticity: Emerging Issues and Future Trends A2 - Leadley, C.E. In Innovation and Future Trends in Food Manufacturing and Supply Chain Technologies, Woodhead Publishing: 2016; pp 3-20.

[2] Spectral Error: Bazar, G.; Kovacs, Z.; Tsenkova, R., Evaluating Spectral Signals to Identify Spectral Error. PLoS One 2016, 11, 15.

[3] Validation: Alewijn, M.; van der Voet, H.; van Ruth, S., Validation of multivariate classification methods using analytical fingerprints - concept and case study on organic feed for laying hens. J. Food Compos. Anal. 2016, 51, 15-23.

[4] SCiO sensor case: Wilson, B. K.; Kaur, H.; Allan, E. L.; Lozama, A.; Bell, D., A New Handheld Device for the Detection of Falsified Medicines: Demonstration on Falsified Artemisinin-Based Therapies from the Field. Am. J. Trop. Med. Hyg. 2017, 96, 1117-1123.

[5] Data fusion: Borras, E.; Ferre, J.; Boque, R.; Mestres, M.; Acena, L.; Busto, O., Data fusion methodologies for food and beverage authentication and quality assessment - A review. Anal. Chim. Acta 2015, 891, 1-14.

[1] E-mail; yannick.weesepoel@wur.nl; Wageningen University & Research, RIKILT, Bu Authenticity and Bioassays, Wageningen, The Netherlands

[6] Case on hand-held NIR (MicroNIR): O'Brien, N.; Hulse, C. A.; Pfeifer, F.; Siesler, H. W., Technical Note Near infrared spectroscopic authentication of seafood. J. Near Infrared Spectrosc. 2013, 21, 299-305.

[7] Case on Oregano: Black, C.; Haughey, S. A.; Chevallier, O. P.; Galvin-King, P.; Elliott, C. T., A comprehensive strategy to detect the fraudulent adulteration of herbs: The oregano approach. Food Chem. 2016, 210, 551-557.

[8] PhasmaFOOD NIR sensor: Pugner, T.; Knobbe, J.; Gruger, H., Near-Infrared Grating Spectrometer for Mobile Phone Applications. Appl. Spectrosc. 2016, 70, 734-745.

Smartphone-based NIR scanners

Yannick Weesepoel – RIKILT, Wageningen University and Research

FoodSmartPhone course – June 30th 2017

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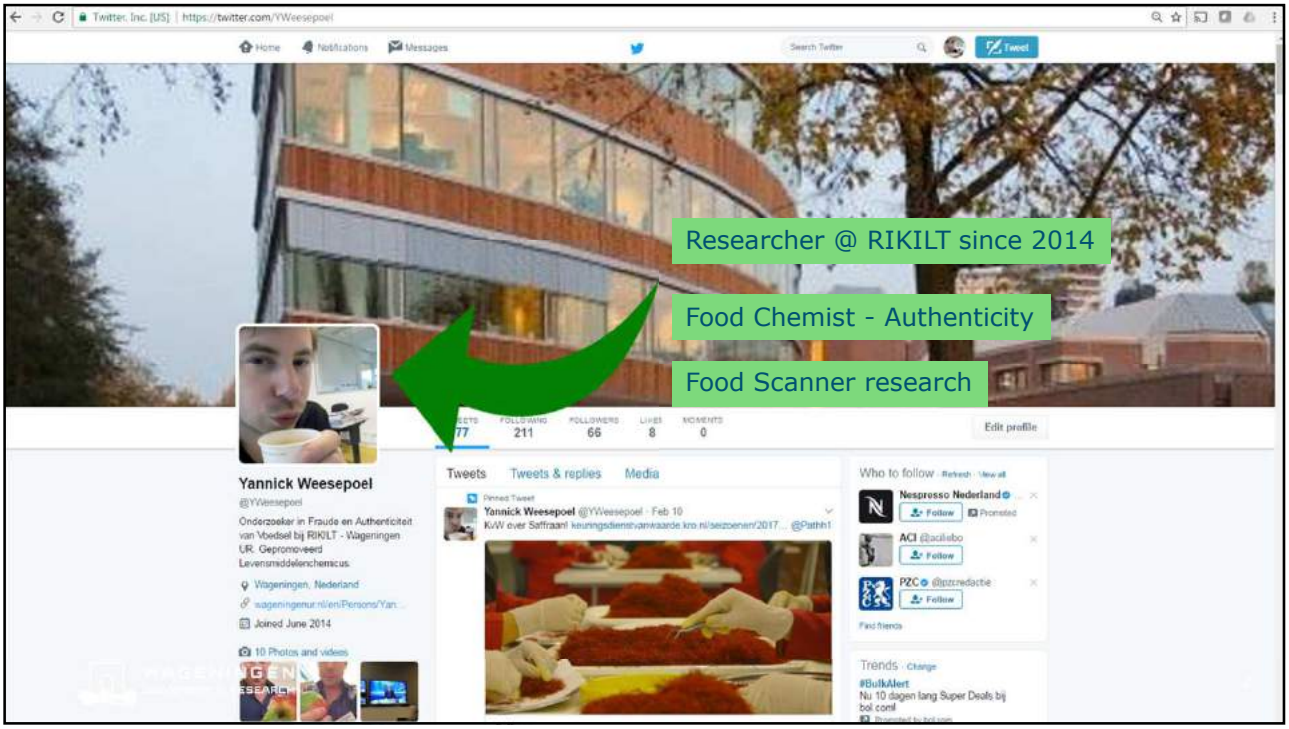
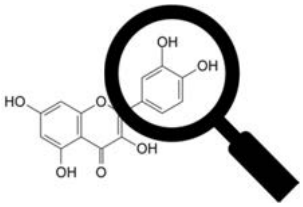


Food Authenticity & Analysis

Determining value of the product

Authentic or … ?



Targeted
vs.
non-targeted analysis

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3

Trends in Food Analysis

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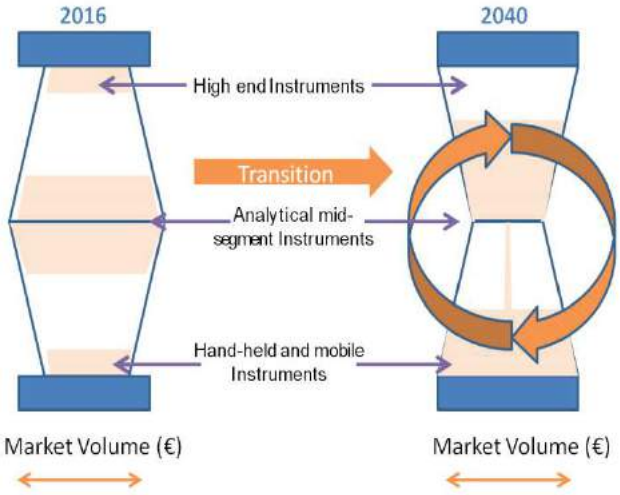
4

Market transition



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Source: Citizen Science Innovation Initiative

5

"Democratizing"* spectroscopy

Consumer
0.2-2k€

Portable
(5-20+k€)

Lab
(20-50k€)

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*Quote: D. Goldring - ConsumerPhysics

6

NIRsmartphones – Expected *very* soon

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In Today's Talk

- **Basics**
  - Chicken fillet - case
  - Building a conceptual authentication application by portable NIR
- **Present**
  - Current smartphone-based NIR scanners
  - Example applications
- **Future**
  - Next-Generation smartphone-based spectral scanners

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Vibrational spectroscopics in a nutshell

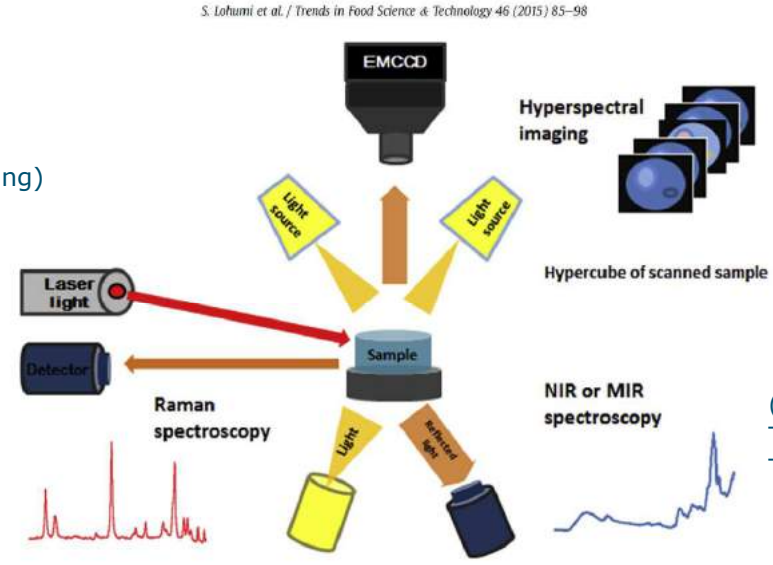

Fig. 1. Schematic diagram of typical vibrational spectroscopic techniques.

Raman scattering
(Rayleigh scattering)

Info rich
Lasers!

Multispectral
Hyperspectral

FT-NIR
FT-IR (MIR)

(Diffuse) reflectance
Transmission
Transflectance

NIR: Info poor
MIR: Info rich

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How to 'teach' your scanner

Representative sample set

Statistic/Chemometric transformation and algorithm

Database maintenance

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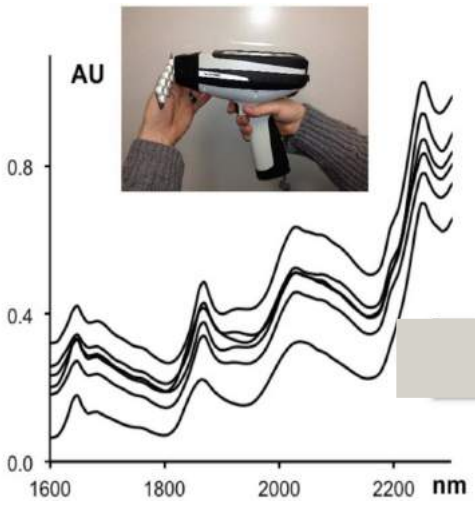
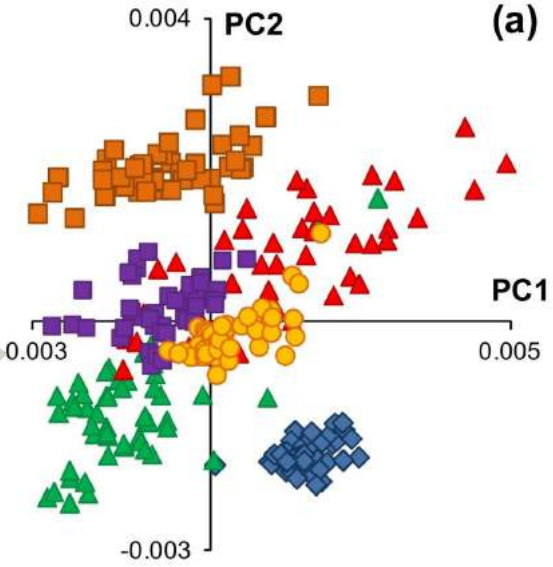
Chemometrics in a nutshell





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Zontov et al. J. Pharm. Biom. Analysis, 2016, 131, 87-93

Case Study: Chicken fillets

Macro component: Moisture/Protein

Micro component: Chilled vs. Thawed

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‘Off-line’ handhelds (examples)

MicroNIR 1700 ES (Viavi)
Range: 950 – 1650 nm
Operation: Power tablet /
Unscrambler X

IDRaman mini (Ocean Opt.)
Laser: 638 or 785 nm
Range: 400 – 2300 cm^{-1}
Operation: AA Batteries /
OceanView

FT-IR TruDefender (Thermo)
Range: 650 – 4000 cm^{-1}
Operation: Internal battery
Internal software

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Increase in animal welfare awareness

‘Basic’ chick
€ 5 – 7 / kg

‘New standard’ chick
€ 6 – 9 / kg

‘Free range’ chick
€ 11 – 13 / kg

‘Biological’ chick
€ 25 – 27 / kg

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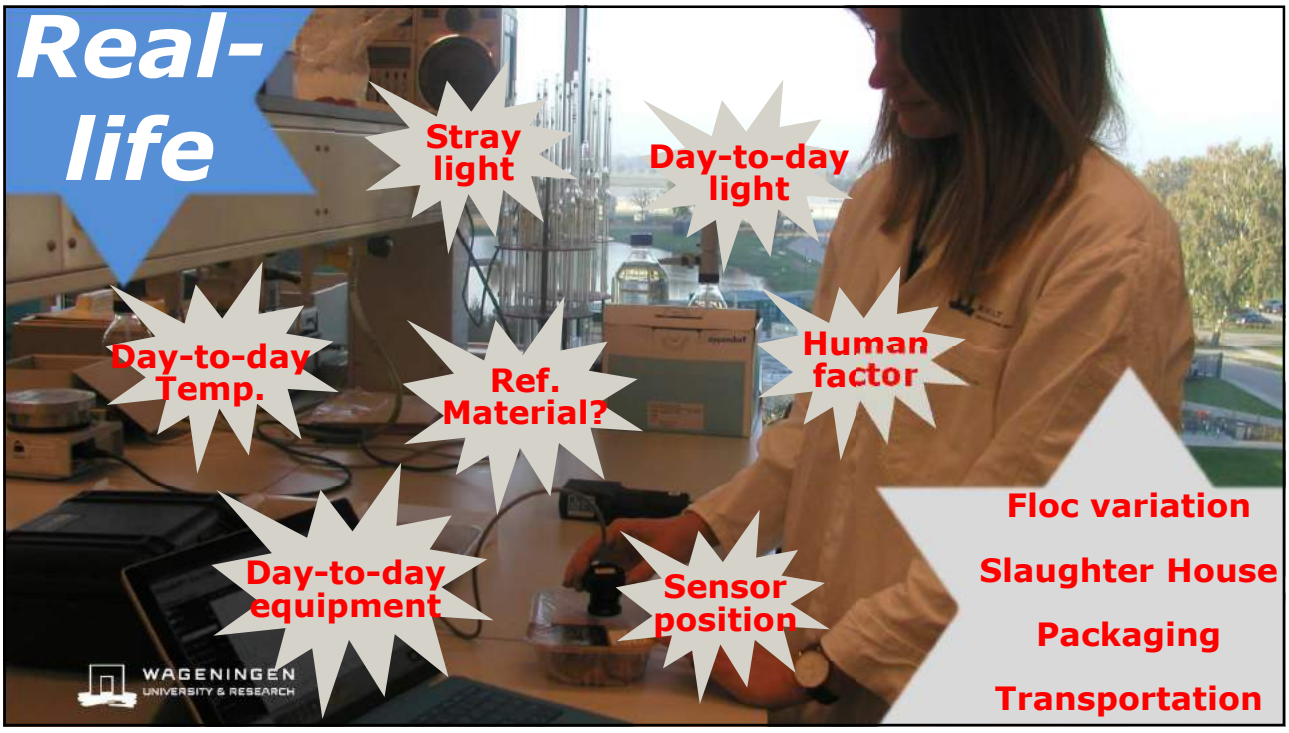

Sample plan

Through package and on meat

Ahold
70 samples
~650 scans
5 scans/sample

Musgrave
83 samples
~800 scans
5 scans/sample

4 Grow syst.
6 Lots
2 Weeks

3 Grow syst.
1 marinated
6 Lots
3 Weeks

Reference methods for:

- "Freshness" (in-house HADH method)
- Moisture content
- Protein content

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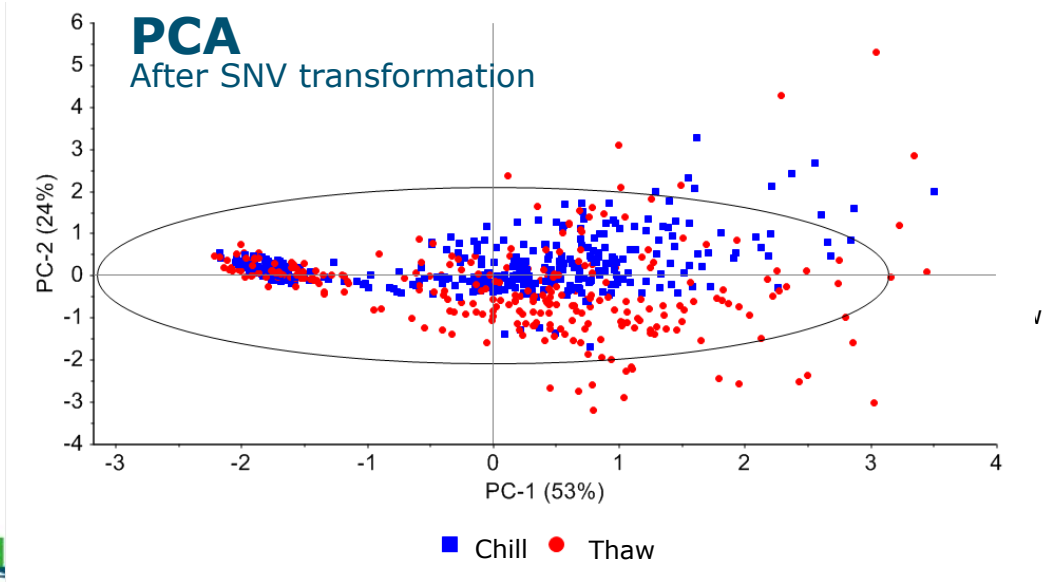
Spectroscopic data is relatively chaotic



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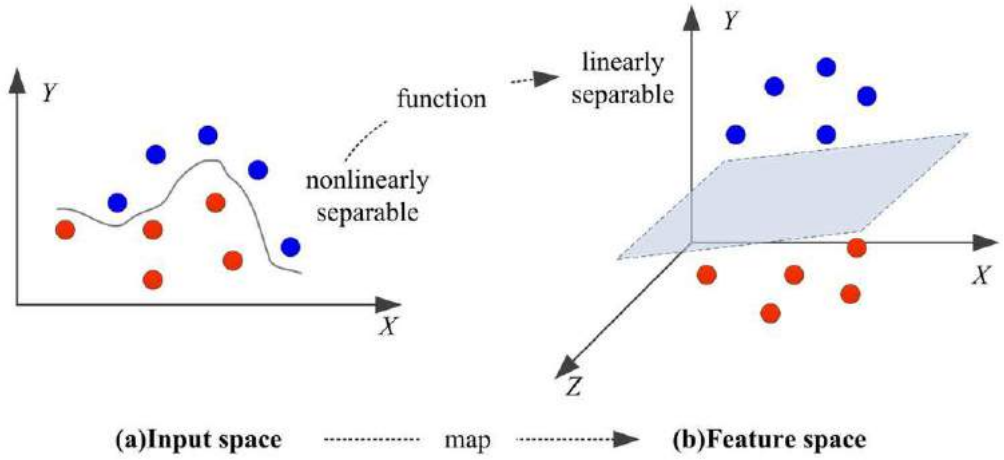
Machine learning: e.g. Support vector machine



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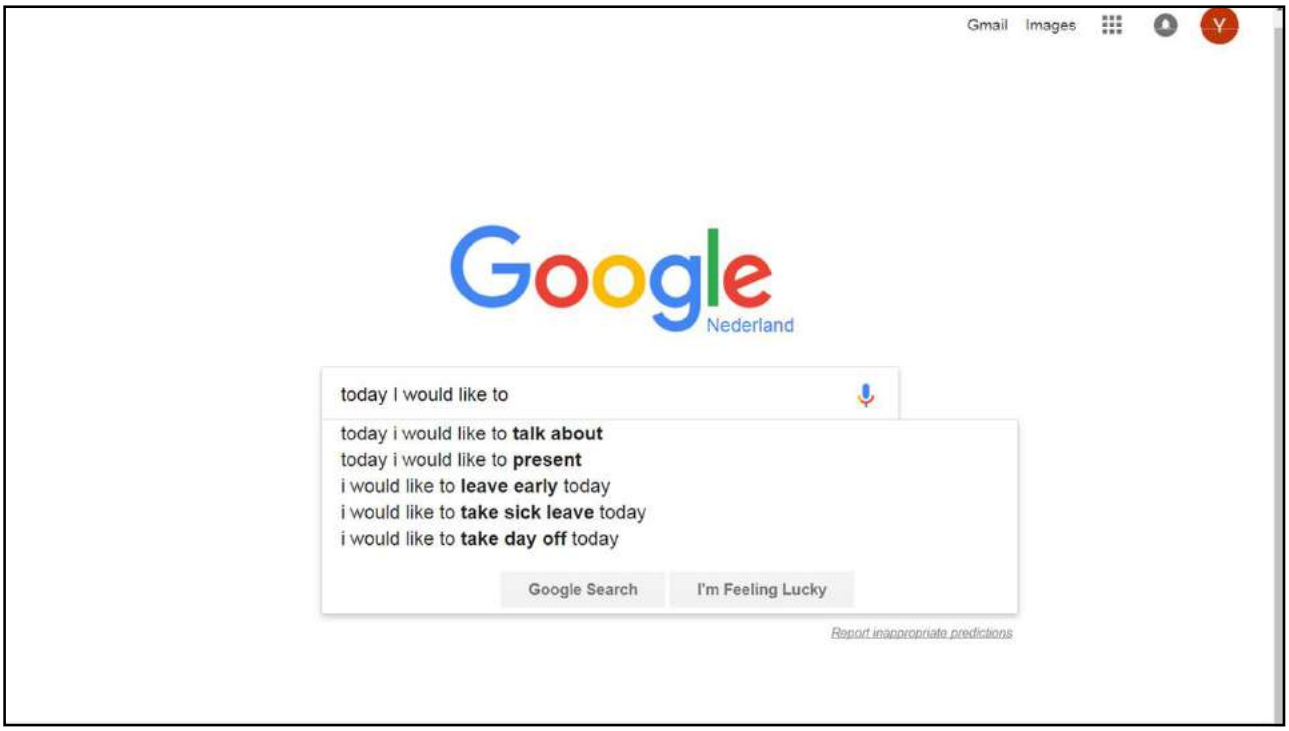
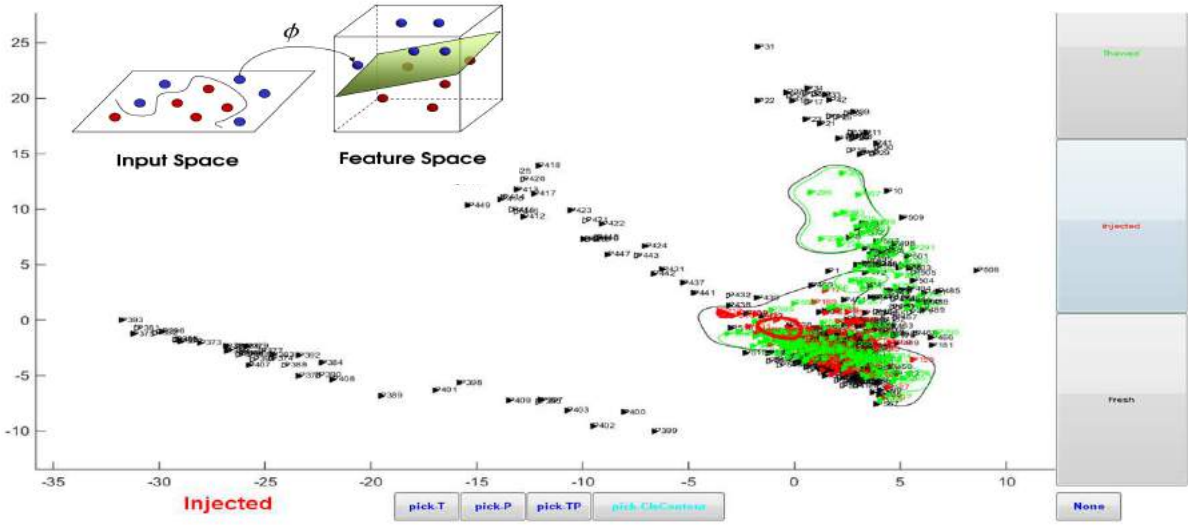


Visualization of SVM models



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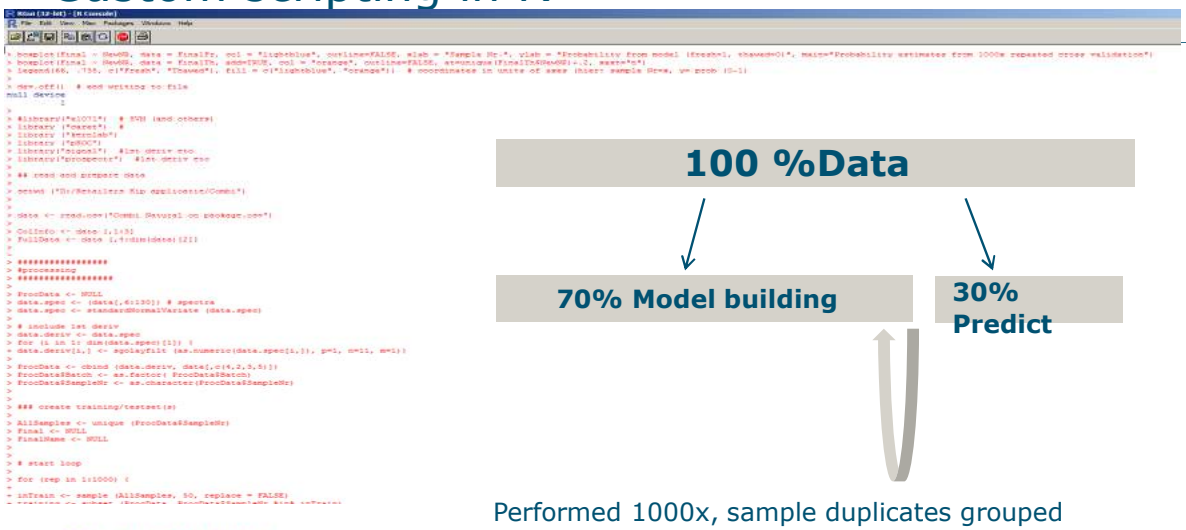
Chang Hsiung, Viavi Corp.

Validation (simulation of real situation) by Custom scripting in R



100 %Data

70% Model building

30% Predict

Performed 1000x, sample duplicates grouped

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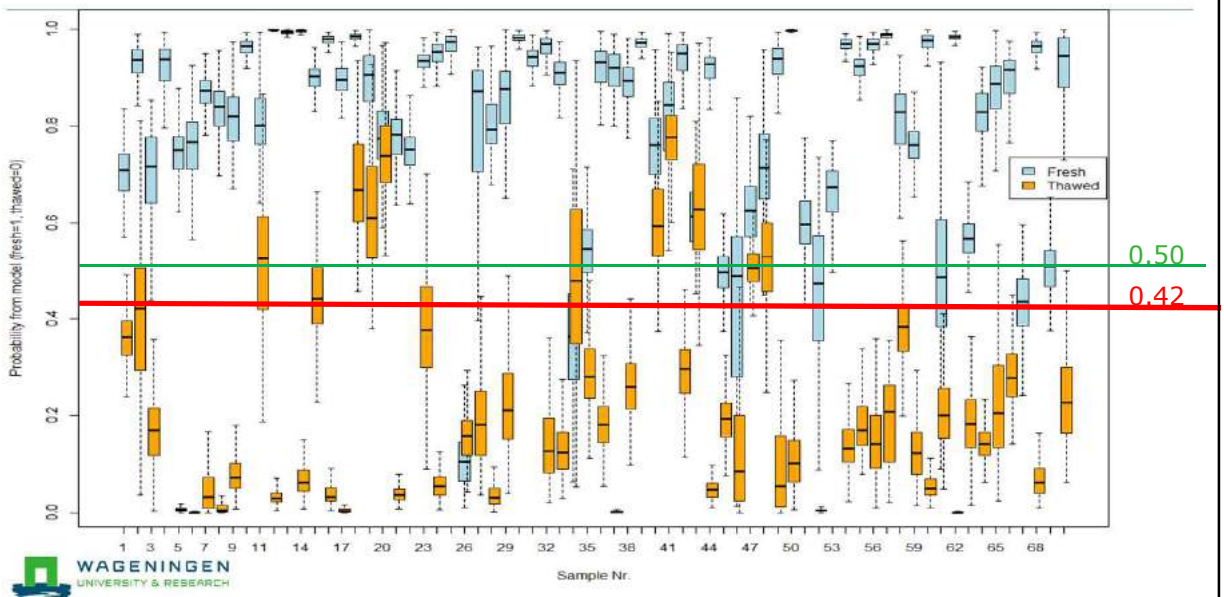
Support vector machine classification scaled on probability



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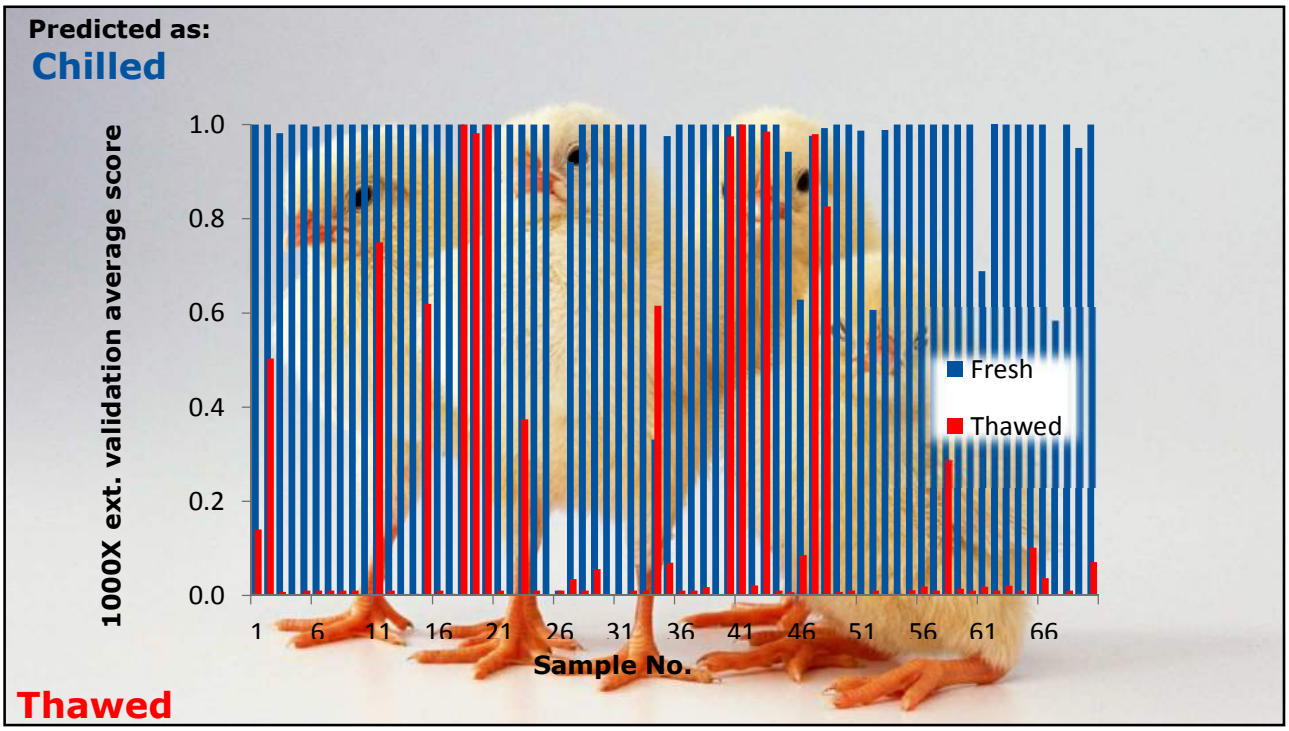
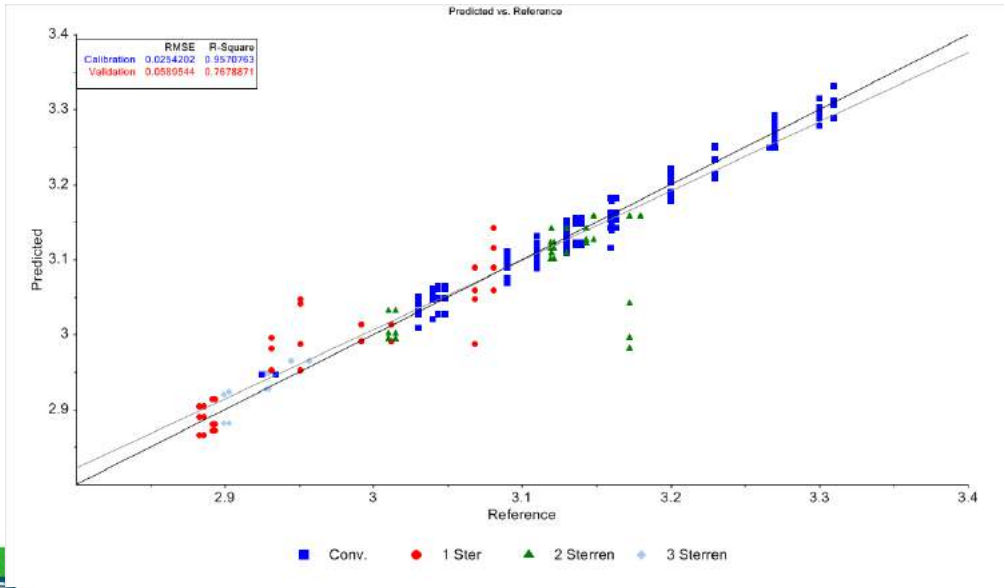


Moisture-Protein ratio by machine learning



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Lessons learned from the fillet case

- Moisture and protein regression promising
- 95% correct prediction of chilled products and 80% for thawed products → In the lab!

→Transferability outside lab?

→How do you cover all chicken meat in the world?

→Relatively expensive scanners for an authenticity problem

→NIR has limitations! Realistic expectations

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Present: Consumer spectroscopics

Spectroscopic analysis

NIR scanner

Smartphone

Easy to use

Time: ≈ 5 sec

Modelling

Classification

To which of the defined classes does the sample belong?

Estimation

What is the concentration of x in the sample?

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"On-line" handhelds - Available

SCiO (1.2) – ConsumerPhysics (NIR)
Range: 750 – 1059 nm
Operation: Apps/Cloud

Tellspec enterprise (NIR)
Range: ca. 900 – 1700 nm
Operation: Apps/Cloud

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And the winner is...

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Spectralengines.com

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Hard-ware to be released...

ChangHong H2(NIR)
Range: ca. 750 – 1059 nm
Operation: Apps/Cloud
Release: Q3 – 2017 (China)

Tellspec Gen. 1 (NIR)
Range: ca. ? nm
Operation: Apps/Cloud
Release: Q3 - 2017

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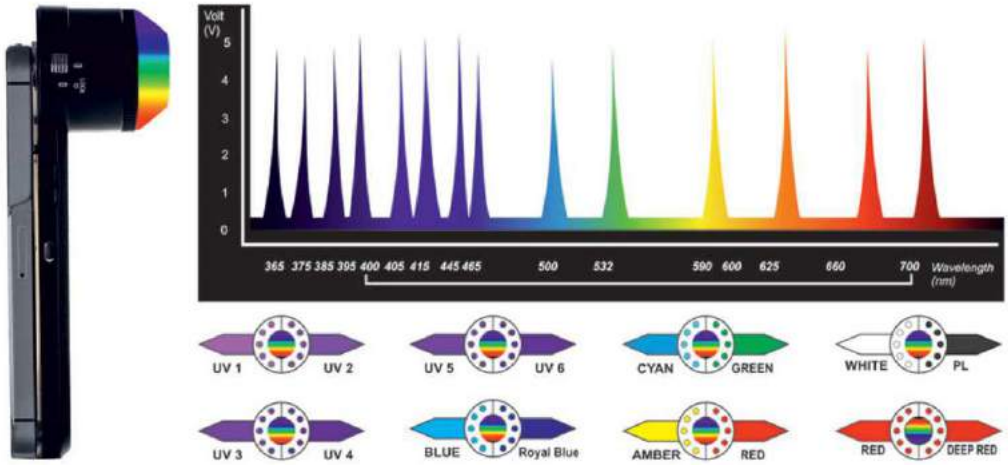
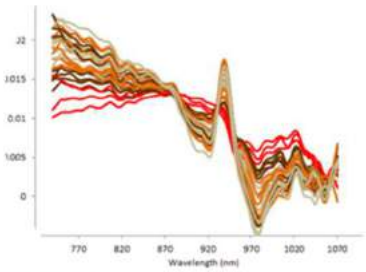
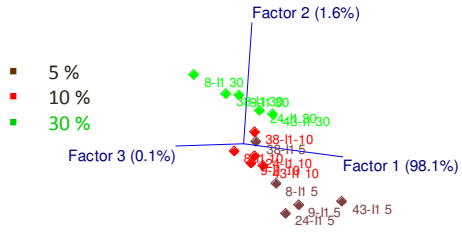
Smartphone-based MSI



Powders: Adulteration of ground nutmeg

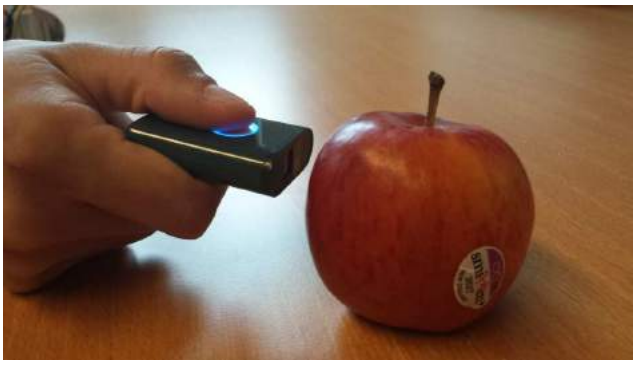


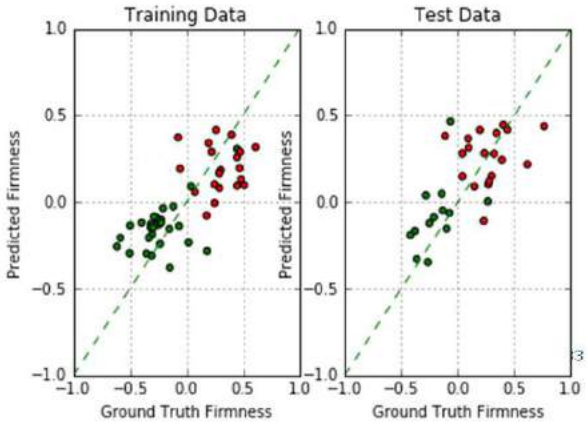





Pilot: Approximately 30% adulteration detectable

Fruits: Sensing of firmness





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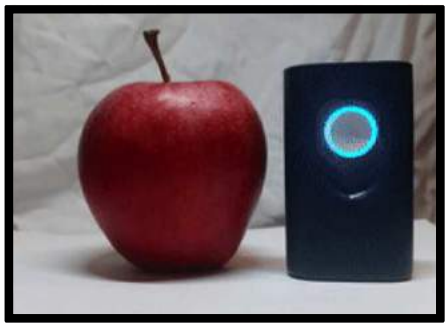
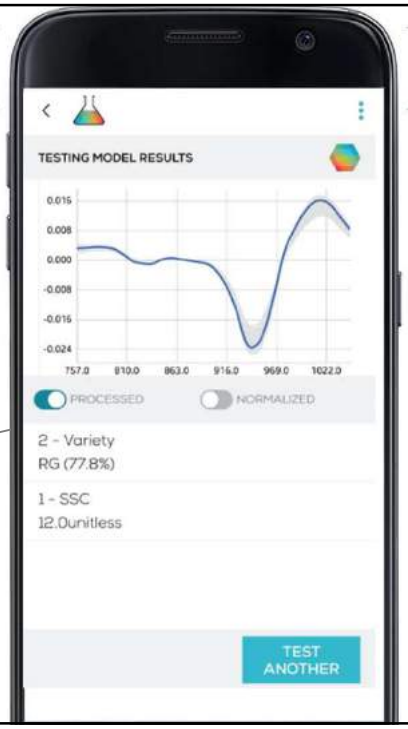
Sensing of Fruits



Variety: Variety X (78%)

Soluble Solids Content: 12%



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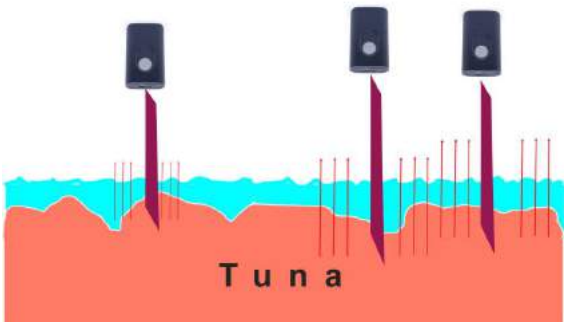
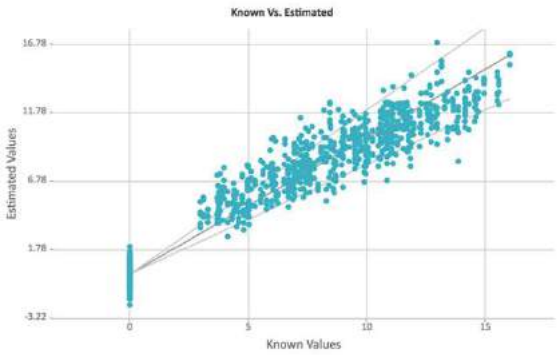
Frozen produce: Fish Glaze





Pilot: Accuracy approximately 3%

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pH7

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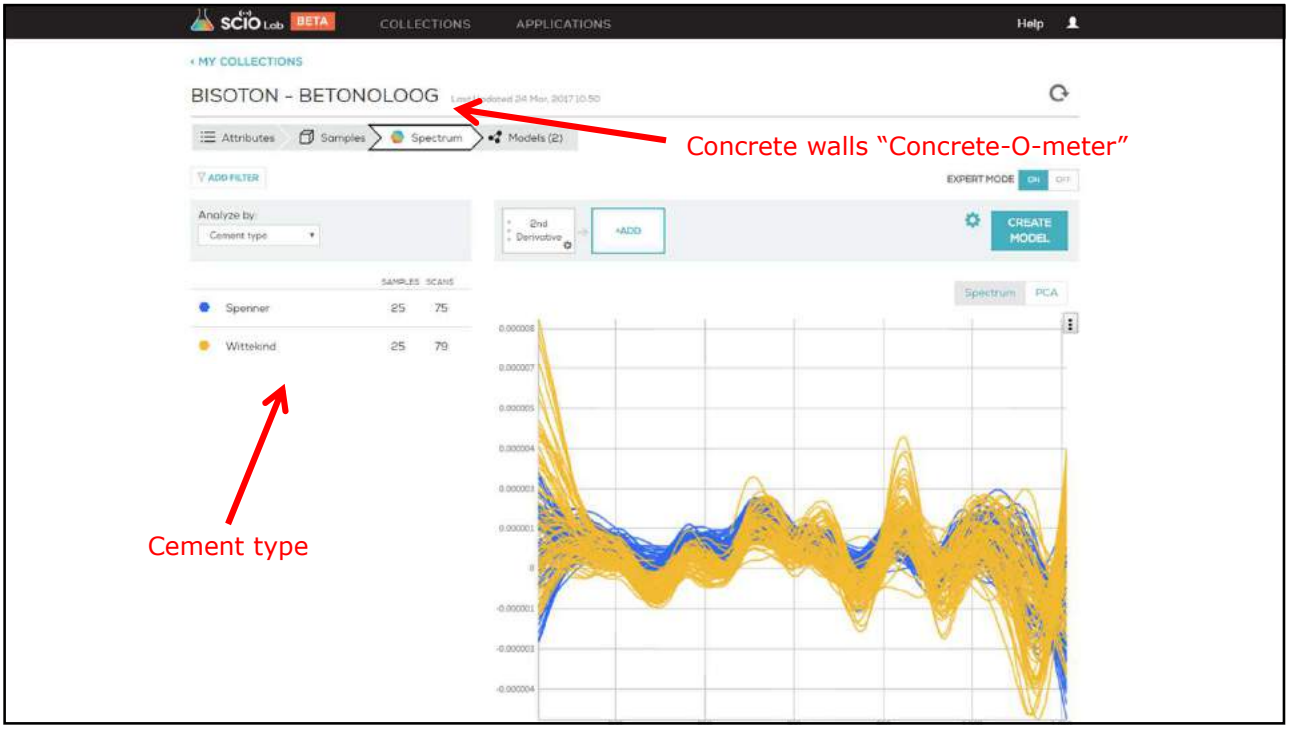

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Mechanisatie | Pompen | Mestbewerking | Gras | Beregening | Overige Werktuigen | Referenties

SIM MMA© SIM Module Mest Analyse

'Manure analysis'

SIM Holland introduceert een mestsensor volgens het "NIR" principe. Een handige tool om snel mest te analyseren.

De SIM MMA© werkt volgens NIR meettechniek en leest de mest. Met deze NIR meeting worden de gegevens verstuurd via "bluetooth" naar de app op je mobiele telefoon. De telefoon stuurt deze gegevens naar een platform met een ijk-lijn in de Cloud waar de gegevens omgerekend worden naar Droge stof (Ds.), Fosfaat (P), Kalium (K) en Stikstof (N) die in de mest aanwezig is. Binnen enkele seconden komen deze gegevens met een uniek monsternummer en datum terug op de app in de telefoon zodat men deze NIR mestgegevens direct kan aflezen. Dit gebeurd in enkele seconden afhankelijk van uw verbinding.

De telefoon legt ook de locatie vast waar het monster genomen wordt. De gegevens van elk monster kunnen later opgeslagen worden op een computer of verwijderd indien niet meer nodig.

De SIM MMA© werkt met een usb kabel of een Li-On accu en weegt inclusief de accu 136 gram. Om gebruik te maken van het platform met de ijk-lijn in de Cloud is een abonnement nodig wat tegen een laag tarief aangeboden wordt op jaarbasis. Met de SIM MMA© is het snel en eenvoudig meten van varkens- en rundveemest, en op korte termijn ook vaste mest. De oplossing van deze tijd. De methode is een non-destructieve meting d.w.z. dat de kwaliteit van het product behouden blijft. De ijk-lijnen van andere mestsoorten zal in korte tijd uitgebreid worden.

Dry matter, Phosphate, Potassium, Nitrogen

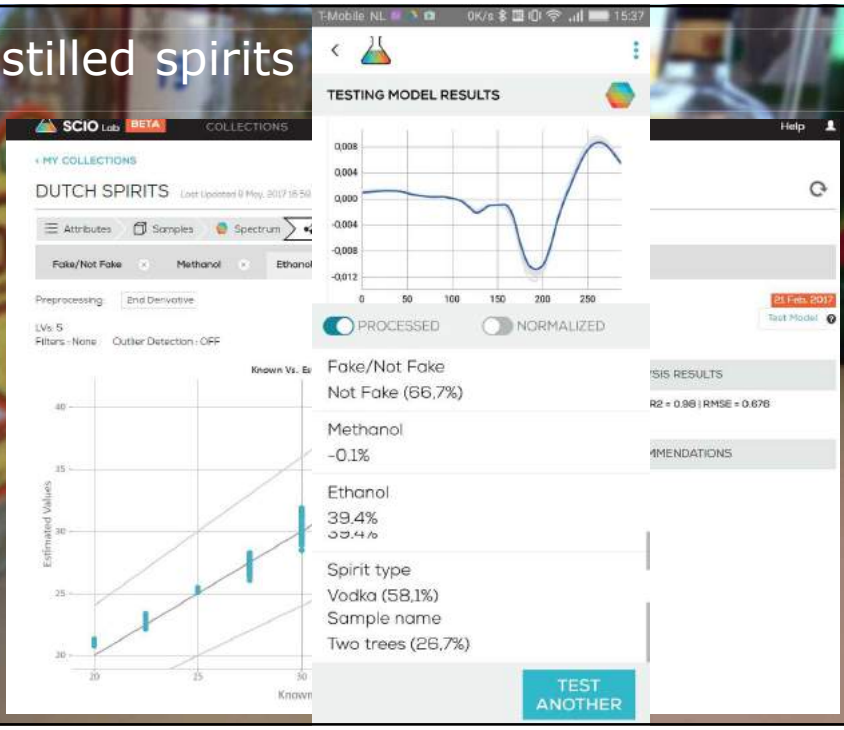
Voordelen van de SIM MMA® op een rijtje:
- Controle bij aan- en verkoop
- Gestuurd bemesten
- Snel en kostenefficiënt werken

Er wordt gewerkt aan een SIM MMA in-line NIR meetsysteem wat zeer binnenkort op de markt gebracht gaat worden. De bedoeling is om dit NIR systeem te koppelen aan een GPS module zodat uitrijden van meststoffen met GPS en hoeveelheid voedingstoffen zoals Stikstof, Kali en Fosfaat per hectare gestuurd kan worden. Efficiënt uitrijden en weten wat men doet is in de toekomst een belangrijk stuk gereedschap voor de boer en handelaar.

Download onze MMA leaflet in pdf formaat

???

Liquids: Distilled spirits



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Still...

- What happens when consumers start using the applications?


PhD


Mom


Student


Gf(!) - Dad


Phd


Student


Me

- What about...
  - Toxins, allergens, pesticides, ...
  - Shelf life of products
- 'Universal device'?

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What about the end-users? Some journalists last year ....

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Start at: 1.43

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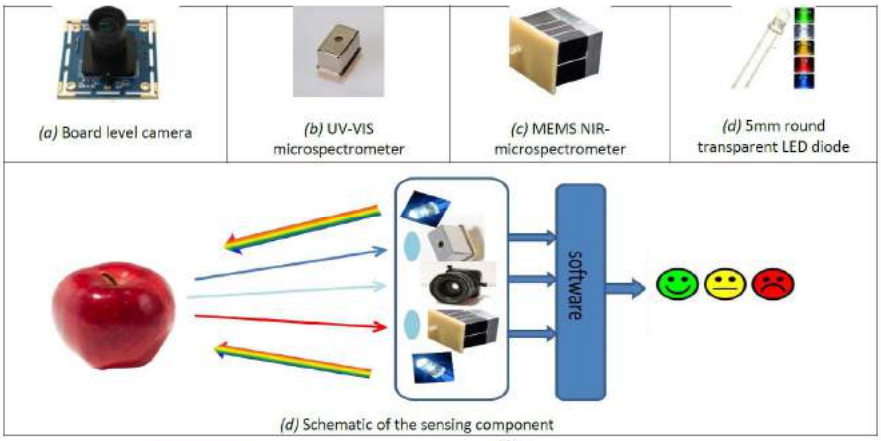
Future: Sensor fusion – FoodSmartPhone's sister

"Portable photonic miniaturised smart system for on-the-spot food quality sensing"



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 732541

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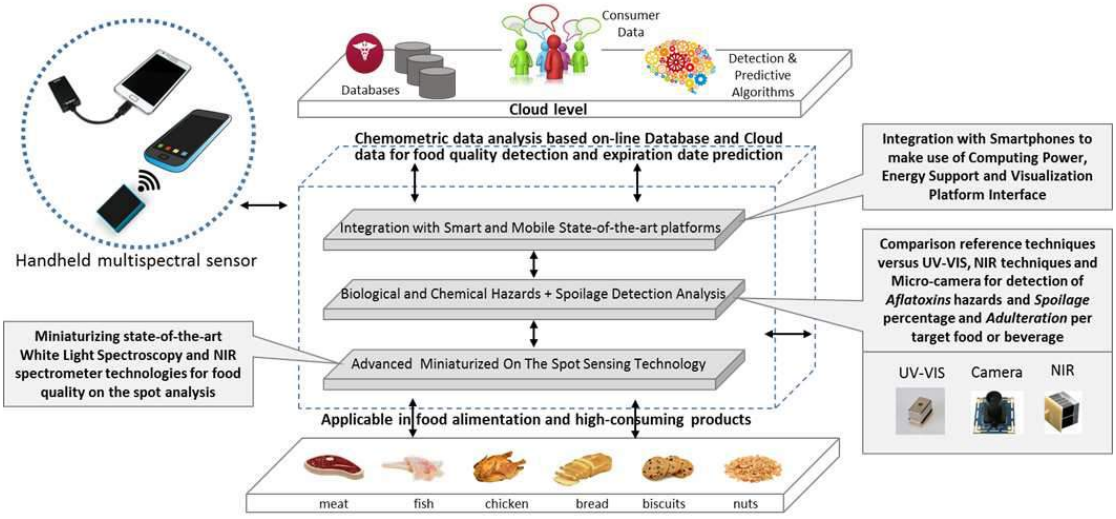
Sensor combination & data fusion for a more universal food scanner



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 732541

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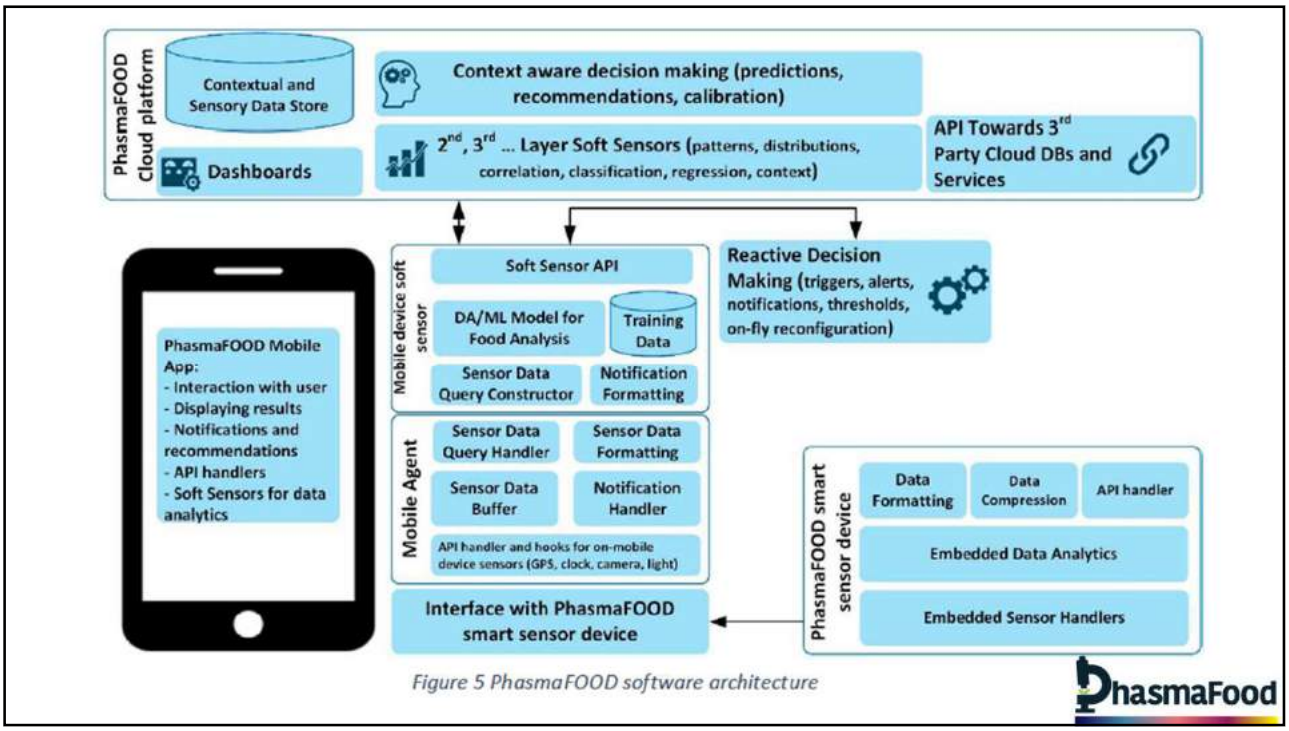
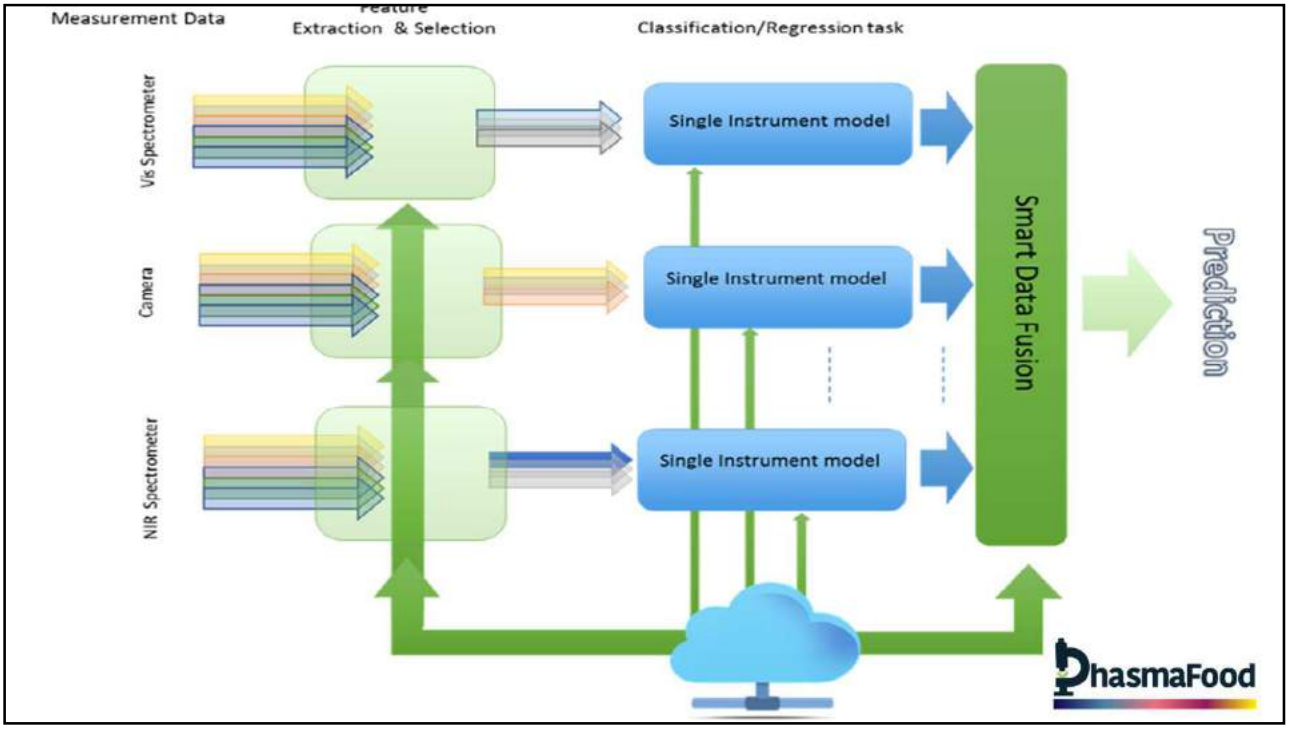
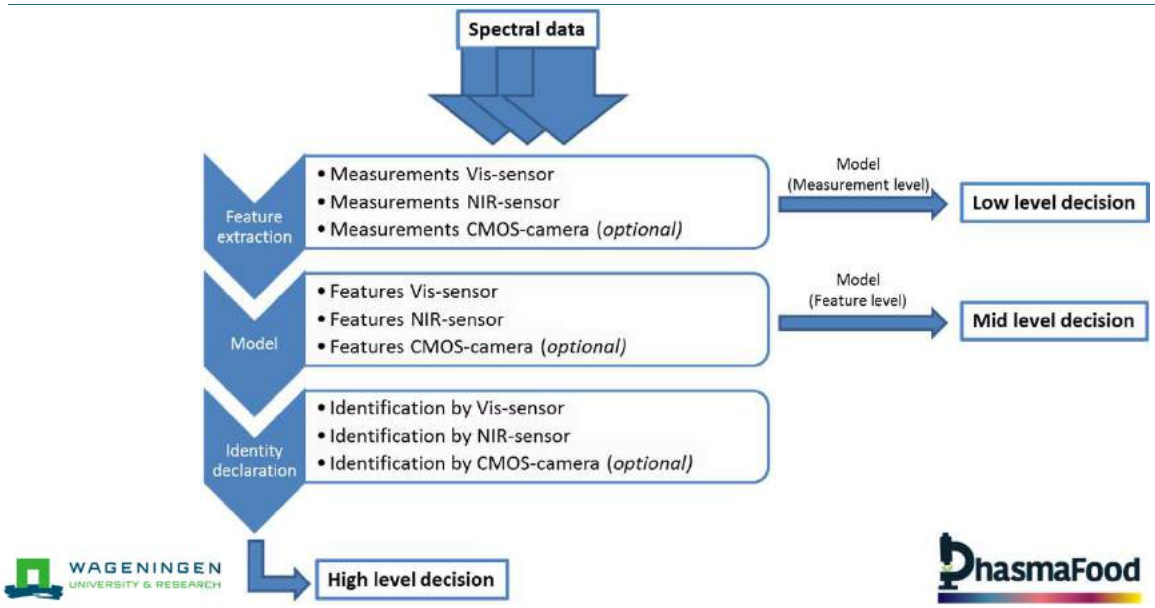


Figure 5 PhasmaFOOD software architecture



Data-fusion strategies



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PhasmaFood

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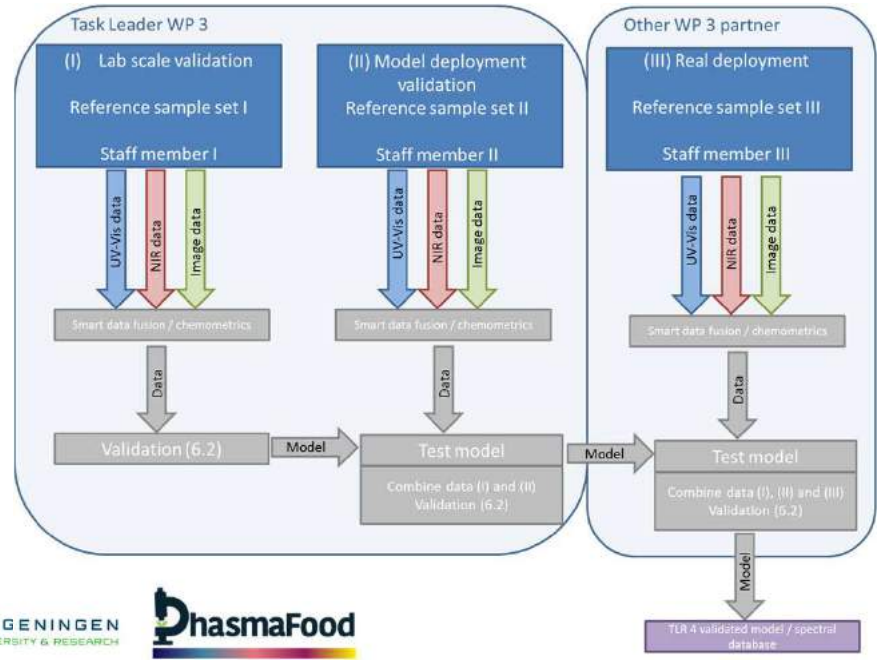
Validation strategies



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On-going initiatives on enabling citizen science

nationale wetenschaps agenda

world food center

COAST Community of Innovation

Topsector Agri& Food

Topsector Chemie

HORIZON 2020

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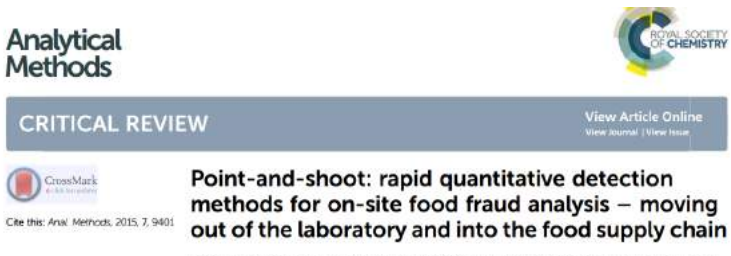
Further reading...

- Popular Science (Dutch) – Quest 05/2017

- Scientific literature (English)



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Thank you! – Q & A - Credits

Chicken fillet case:

RIKILT – Authenticity:
Saskia van Ruth
Saskia.vanruth@wur.nl

Consumer spectroscopics:

- Nutmeg case (RIKILT): Laura Lanseros de las Heras & Isabelle Silvis (isabelle.silvis@wur.nl)
- Fish Glaze: Paul Hiscoe (paul@ph-7.co.uk) – PH Seven London – www.ph-7.co.uk
- Distilled spirits (RIKILT): Stevan van der Hoek, Yannick Weesepoel
- Concrete: Casper Heijkoop www.bisoton.nl
- Fruits:
  - Wageningen Food & Biobased Research, Computer Vision: Lydia Meesters (lydia.meesters@wur.nl) & Hendrik de Villiers (hendrik.devilliers@wur.nl)
  - Pieter Dekker (RIKILT): (pieter.dekker@wur.nl)

Future:

Michel Nielen & Wim Beek
Michel.nielen@wur.nl
Wim.beek@wur.nl

http://phasmafood.eu/
Yannick Weesepoel
Yannick.weesepoel@wur.nl

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Mobile Microscopy, Sensing and Diagnostics through Computational Photonics

Aydogan Ozcan, Ph.D.
Electrical Engineering Department, Bioengineering Department, California NanoSystems Institute
University of California, Los Angeles, CA
ozcan@ucla.edu ; http://innovate.ee.ucla.edu/ ; http://org.ee.ucla.edu/

My research focuses on the use of computation/algorithms to create new optical microscopy, sensing, and diagnostic techniques, significantly improving existing tools for probing micro- and nano-objects while also simplifying the designs of these analysis tools. In this presentation, I will introduce a new set of computational microscopes which use lens-free on-chip imaging to replace traditional lenses with holographic reconstruction algorithms. Basically, 3D images of specimens are reconstructed from their "shadows" providing considerably improved field-of-view (FOV) and depth-of-field, thus enabling large sample volumes to be rapidly imaged, even at nanoscale. These new computational microscopes routinely generate >1–2 billion pixels (giga-pixels), where even single viruses can be detected with a FOV that is >100 fold wider than other techniques. At the heart of this leapfrog performance lie self-assembled liquid nano-lenses that are computationally imaged on a chip. These self-assembled nano-lenses are stable for >1 hour at room temperature, and are composed of a biocompatible buffer that prevents nano-particle aggregation while also acting as a spatial "phase mask." The field-of-view of these computational microscopes is equal to the active-area of the sensor-array, easily reaching, for example, >20 mm^2 or >10 cm^2 by employing state-of-the-art CMOS or CCD imaging chips, respectively.

In addition to this remarkable increase in throughput, another major benefit of this technology is that it lends itself to field-portable and cost-effective designs which easily integrate with smartphones to conduct giga-pixel tele-pathology and microscopy even in resource-poor and remote settings where traditional techniques are difficult to implement and sustain, thus opening the door to various telemedicine applications in global health. Some other examples of these smartphone-based biomedical tools that I will describe include imaging flow cytometers, immunochromatographic diagnostic test readers, bacteria/pathogen sensors, blood analyzers for complete blood count, and allergen detectors. Through the development of similar computational imagers, I will also report the discovery of new 3D swimming patterns observed in human and animal sperm. One of this newly discovered and extremely rare motion is in the form of "chiral ribbons" where the planar swings of the sperm head occur on an osculating plane creating in some cases a helical ribbon and in some others a twisted ribbon. Shedding light onto the statistics and biophysics of various micro-swimmers' 3D motion, these results provide an important example of how biomedical imaging significantly benefits from emerging computational algorithms/theories, revolutionizing existing tools for observing various micro- and nano-scale phenomena in innovative, high-throughput, and yet cost-effective ways.

Biography: Dr. Aydogan Ozcan received his Ph.D. degree at Stanford University Electrical Engineering Department. After a short post-doctoral fellowship at Stanford University, he was appointed as a research faculty at Harvard Medical School, Wellman Center for Photomedicine in 2006. Dr. Ozcan joined UCLA in 2007 and he is currently the Chancellor's Professor at UCLA and an HHMI Professor with the Howard Hughes Medical Institute, leading the Bio- and Nano-Photonics Laboratory at UCLA Electrical Engineering and Bioengineering Departments, and is also the Associate Director of the California NanoSystems Institute at UCLA.

Dr. Ozcan holds 34 issued patents and 20+ pending patent applications for his inventions in telemedicine, mobile health, nanoscopy, wide-field imaging, lensless imaging, nonlinear optics, fiber optics, and optical coherence tomography. Dr. Ozcan gave more than 35 plenary/keynote talks and 300+ invited talks and is also the author of one book, the co-author of more than 450 peer reviewed publications in major scientific journals and conferences. In addition, Dr. Ozcan is the founder and a member of the Board of Directors of Holomic/Cellmic LLC, which was named a Technology Pioneer by The World Economic Forum in 2015.

Prof. Ozcan received several major awards including the 2011 Presidential Early Career Award for Scientists and Engineers (PECASE), which is the highest honor bestowed by the United States government on science and engineering professionals in the early stages of their independent research careers. Dr. Ozcan received this prestigious award for developing innovative optical technologies and signal processing approaches that have the potential to make a significant impact in biological science and medicine; addressing public health needs in less developed countries; and service to the optical science community including mentoring and support for underserved minority undergraduate and graduate students. Dr. Ozcan also received the 2015 UCLA Postdoctoral Scholars Mentoring Award for his commitment to training and mentoring of postdoctoral researchers. In addition, Dr. Ozcan received the inaugural Rahmi M. Koc Science Medal, the International Commission for Optics (ICO) Prize, the inaugural SPIE BioPhotonics Technology Innovator Award, the Army Research Office (ARO) Young Investigator Award, SPIE Early Career Achievement Award, NSF CAREER Award, NIH Director's New Innovator Award, the Office of Naval Research (ONR) Young Investigator Award, the IEEE Photonics Society Young Investigator Award and the MIT's Technology Review TR35 Award for his seminal contributions to near-field and on-chip imaging, and telemedicine based diagnostics.

Prof. Ozcan is also the recipient of the 2016 IEEE Photonics Society Distinguished Lecturer Award, 2013 and 2015 Microscopy Today Innovation Awards, 2012 Popular Science Brilliant 10 Award, 2012 National Academy of Engineering (NAE) The Grainger Foundation Frontiers of Engineering Award, 2011 Innovators Challenge Award presented by the Rockefeller Foundation and mHealth Alliance, the 2010 National Geographic Emerging Explorer Award, the 2010 Bill & Melinda Gates Foundation Grand Challenges Award, the 2010 Popular Mechanics Breakthrough Award, the 2010 Netexplorateur Award given by the Netexplorateur Observatory & Forum in France, the 2009 and 2016 Wireless Innovation Award organized by the Vodafone Americas Foundation as well as the 2008 Okawa Foundation Award, given by the Okawa Foundation in Japan.

Prof. Ozcan was selected as one of the top 10 innovators by the U.S. Department of State, USAID, NASA, and NIKE as part of the LAUNCH: Health Forum organized in 2010. He also received the 2012 World Technology Award on Health and Medicine, which is presented by the World Technology Network in association with TIME, CNN and AAAS.

Dr. Ozcan is a Fellow of SPIE, OSA, IEEE, AIMBE, RSC and the Guggenheim Foundation, and is a Lifetime Member of AAAS, SPIE and OSA.

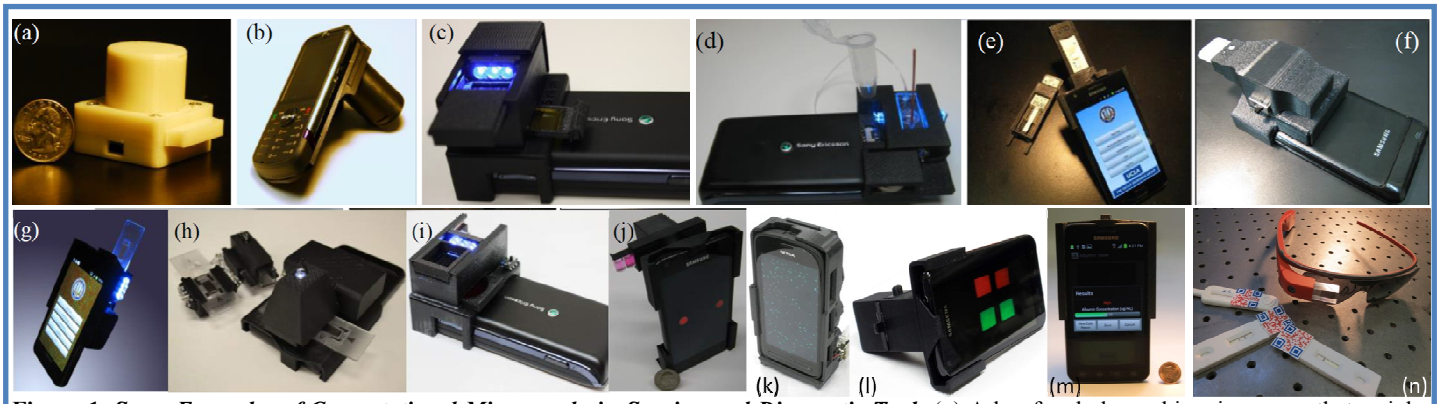

Figure 1. ***Some Examples of Computational Micro-analysis, Sensing and Diagnostic Tools*** (***a***) A lensfree holographic microscope that weighs ~45 grams. (***b***) A cellphone that is modified based on the same lensless holographic microscopy technology. (***c***) A wide-field fluorescent microscope that is installed on a cellphone using a compact and cost-effective optical interface. (***d***) An imaging fluorescent *flow-cytometer* installed on a cellphone. (***e-f***) A cellphone attachment for automated reading and quantification of immunochromatographic rapid diagnostic tests (RDTs). (***g-h***) A compact and cost-effective blood analysis platform installed on a cellphone for the measurement of the density of red and white blood cells as well as hemoglobin concentration in blood samples. (***i***) An optical attachment for *E. coli* detection on a cellphone using quantum dot based sandwich assay in glass capillary tubes, with a detection sensitivity of ~5-10 CFU/mL. (***j***) A personalized allergen testing platform running on a cellphone that images and automatically analyzes colorimetric assays toward sensitive (~1 ppm) and specific detection of allergens in food samples. (***k***) Cellphone based fluorescent microscope that is capable of imaging single nanoparticles. (***l***) Detection and spatial mapping of mercury contamination in water samples using a smart-phone (sensitivity: ~3-4 ppb). (***m***) Smartphone-based urinary albumin tester. (***n***) Immunochromatographic diagnostic test analysis using Google Glass.

Related References: http://goo.gl/uYeiKn

Hands-on practicals: strip test analysis

Analysis of the mycotoxin DON in barley and beer

Jeroen Peters, Laura Bartlett, Michel Nielen

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Sponsored by: CHARM SCIENCES INC

Program

- 13:45 – 15:45 Introduction and Hands on labwork
- 15:45 – 16:15 Break
- 16:15 – 17:00 5 min presentations and closing
- 17:00 – 17:15 Return to RIKILT

RIKILT WAGENINGEN UR

The challenge of your team:
Estimate the DON content in unknown samples by a strip test immunoassay.

1. Design an experiment how to do this
2. Perform the analysis according to your protocol and the information available from us
3. Read the strip tests results (a) visually, (b) by the incubator/reader and (c) by smartphone camera/app
4. Estimate the DON content in the unknown sample(s) based on the results of 3a, 3b and 3c.
5. Compare with the EU legal limits (find them)
6. Explain the working principle of this ligand binding strip test.
7. Prepare max 3 slides for a max 3 minutes pptx presentation for plenary discussion about your **experiment design, all the results and explanation of your findings**!

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Four sub-groups (day 1)
Within each sub-group share the tasks 1-7

- Unknown barley samples, 2 g each:
  - Group 1 Barley sample blank, 4, 9
  - Group 2 Barley sample blank, 5, 10
  - Group 3 Beer sample B1 and B2 (blanks), S1 and S5
  - Group 4 Beer sample B1 and B3 (blanks), S2 and S7
- Strip tests available:
  - DON Q2 tests (see Charm protocol)
- Extraction/sample preparation:
  - Barley: extract 2 g with 10 mL water
  - Negative control = blank sample
  - Positive DON control = simulates 1 ppm in barley

RIKILT WAGENINGEN UR

Four sub-groups (day 2)
Within each sub-group share the tasks 1-7

- Unknown barley samples, 2 g each:
  - Group 1 Barley sample blank, 2, 7
  - Group 2 Barley sample blank, 3, 8
  - Group 3 Beer sample B1 and B2 (blanks), S3 and S6
  - Group 4 Beer sample B1 and B3 (blanks), S4 and S8
- Strip tests available:
  - DON Q2 tests (see Charm protocol)
- Extraction/sample preparation:
  - Barley: extract 2 g with 10 mL water
  - Negative control = blank sample
  - Positive DON control = simulates 1 ppm in barley

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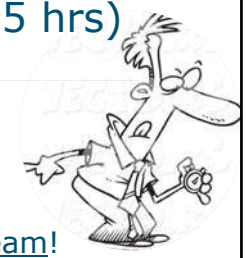
You have limited time (until 15:45 hrs)



Critical issues.....

- Team work and too many tasks: start to make a division of tasks in your team!
- DON (Deoxynivalenol) is toxic, apply health & safety lab rules at all times!
- There is one **DON Q2** incubator (4 strips) and one **DON Q2** reader (1 strip). Incubation time is 2 min per strip: **planning issue**!
- Use any information source present (incl. the internet, the lab assistant, your lecturer, etc.)

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Coffee break 15:45 – 16:15

Results of samples and standards

Some calculations following the Charm protocol, *assuming that 1 ppm DON is present* in barley

1. 1 ppm = 1000 ppb DON in barley = 1000 ng/g = 2000 ng/2 g barley.
2. 2000 ng DON ends in 10 mL water: = 200 ng/mL water.
3. From 2., 50 µL is mixed with 1 mL buffer: ≈ 10 ng/mL buffer.
4. From 3., 300 µL is pipetted onto strip: ≈ 3 nanogram/strip test.

Legal limit DON according to 1881/2006/EC:
DON 1250 µg/kg = 1250 ng/g "for unprocessed cereals for food".

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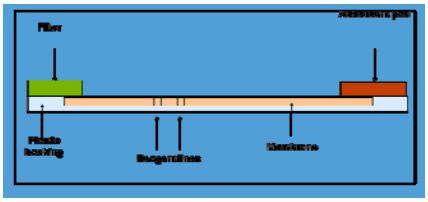
		children (16) and labelled and sold as such (4)	
	2.3.5	Baby foods other than processed cereal-based foods for infants and young children (3) (4)	10,0
▼M1	2.4	**Deoxynivalenol** (17)	
	2.4.1	Unprocessed cereals (18) (19) other than durum wheat, oats and maize	1 250
	2.4.2	Unprocessed durum wheat and oats (18) (19)	1 750
	2.4.3	Unprocessed maize (18), with the exception of unprocessed maize intended to be processed by wet milling (37)	1 750 (20)

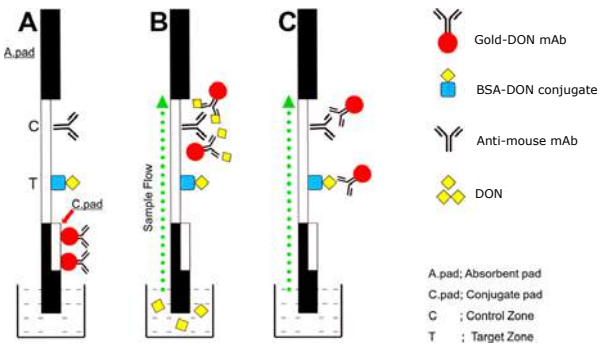
▼M1

	Foodstuffs (1)	Maximum levels (µg/kg)
2.4.4	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption, with the exception of foodstuffs listed in 2.4.7, 2.4.8 and 2.4.9	750
2.4.5	Pasta (dry) (22)	750
2.4.6	Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals	500
2.4.7	Processed cereal-based foods and baby foods for infants and young children (3) (7)	200
2.4.8	Milling fractions of maize with particle size > 500 micron falling within CN code 1103 13 or 1103 20 40 and other maize milling products with particle size > 500 micron not used for direct human consumption falling within CN code 1904 10 10	750 (20)
2.4.9	Milling fractions of maize with particle size ≤ 500 micron falling within CN code 1102 20 and other maize milling products with particle size ≤ 500 micron not used for direct human consumption falling within CN code 1904 10 10	1 250 (20)
2.5	**Zearalenone** (17)	
2.5.1	Unprocessed cereals (18) (19) other than maize	100
2.5.2	Unprocessed maize (18) with the exception of unprocessed	350 (20)

How does the strip test work?



Possibly:



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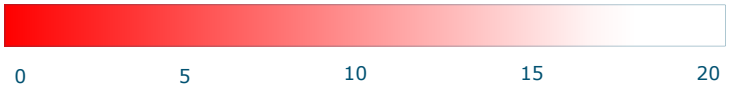
Visual and Smartphone application

- Calibration/Dose response curve
- For example: 20 ppm → 20 mg/kg → 40 µg/2 g
- in 10 ml MQ or blank barley extract
- 40 µg in 10 ml ≈ 4 µg/ml
- Serial dilutions in MQ or blank barley extract



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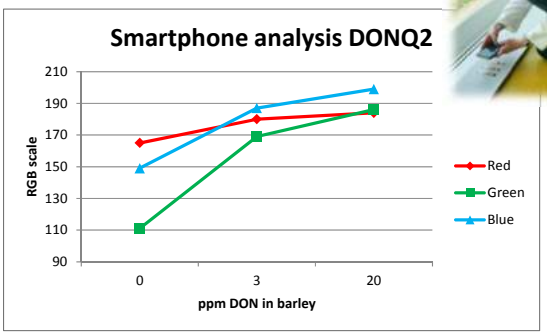
Visual and Smartphone application

Visual

Color Grab App



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...but what was the real content??

Day 1: The real content?

sample	DON added (ppm)	DON measured (ppm)
Barley 2, 3	3	4.8
Barley 7, 8	20	21
Beer S1,S2	1	1.3
Beer S5	1	1.7
Beer S7	1	1.8

Blanks?





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...has been determined by Laura!

Day 2: The real content?

sample	DON added (ppm)	DON measured (ppm)
Barley 4, 5	3	4.8
Barley 9, 10	20	21
Beer S3	1	1.3
Beer S6	1	1.7
Beer S8	1	1.8

Blanks?





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...has been determined by Laura!

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

2nd FoodSmartphone Summer School

Food Applications, QA/QC and Validation

18 - 22 June 2018

Prague, Czech Republic

FoodSmartphone.eu

University of Chemistry and Technology Prague
Technická 3, 166 28 Prague 6, Czech Republic

UCT PRAGUE

Certificate of Participation

Awarded to

for successfully completion of the

2nd FoodSmartphone Summer School
Food Applications, QA/QC and Validation

held in Prague from 18 – 22 June 2018

and organised by the H2020 Marie-Curie project FoodSmartphone in co-operation with the Department of Food Analysis and Nutrition, University of Chemistry and Technology, Prague

FoodSmartphone.eu

Prof. Jana Hajslova, PhD.
Course Director

Prof. Jana Pulkrabova, PhD.
Head of Department of Food Analysis and Nutrition

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Department of Food Analysis and Nutrition

FoodSmartphone.eu

2nd FoodSmartphone Summer School: Food Applications, QA/QC and Validation

18 - 22 June 2018, Prague, Czech Republic

Organised by the H2020 Marie-Curie project FoodSmartphone in co-operation with the Department of Food Analysis and Nutrition, University of Chemistry and Technology, Prague.

Course Director: Prof. Jana Hajslova
Course organizer: Martina Vlckova
Co-organizer: Mr. Wim Beek (H2020 FoodSmartphone project)

Course venue: Department of Food Analysis and Nutrition, University of Chemistry and Technology, Prague, Technicka 3, Prague 6, Lecture room B32 (3. Floor, building B)

Monday, 18 June 2018

10:30	Registration with coffee / tea	
11:15-11:30	Welcome, short introduction to the programme	Prof. Jana Hajslova (UCT Prague)
11:30-12:30	**Introduction to QA/QC and ISO 17025 accreditation in testing laboratories**	Prof. Vladimír Kocourek (UCT Prague)
12:30-13:30	*Lunch (Carbon club)*	
13:30-13:45	*Group photo outside*	
13:45-15:00	**Analytical validation of MS based methods according to EU requirements**	Prof. Jana Hajslova (UCT Prague)
15:00-15:30	*Coffee, tea & refreshments*	
15:30-17:00	**Specific food quality and safety application requirements**	Petr Cuhra (CAFIA)
17:00-18:00	Questions, Discussion	Prof. Jana Hajslova, Prof. Vladimir Kocourek (UCT Prague), Petr Cuhra (CAFIA)
19:00-21:00	*Course Dinner (Kulatak restaurant)*	

Tuesday, 19 June 2018

08:30-09:00	*Coffee & tea*	
09:00-10:00	**Barilla Quality &Food Safety management system**	Antonio Nespoli (Barilla)
10:00-11:00	**Validation: how to make your data as informative as possible and how to Ensure that your predictions are real**	Dr. Jeroen Jansen (Radboud University)
11:00-11:30	*Coffee & tea*	
11:30-12:00	Discussion	Antonio Nespoli (Barilla), Dr. Jeroen Jansen (Radboud University), Prof. Hajslova (UCT Prague)
12:00-13:00	*Lunch (Carbon club)*	
13:00-14:30	**Hands-on labwork**	3 groups (Dr. Lucie Drabova, Dr. Vojtech Hrbek, Kamila Hurkova)
14:30-15:00	*Coffee, tea & refreshments*	
15:00-17:00	**Concepts and Guidelines for Validation of Screening Methods for Residue Analysis: EU Requirements**	Dr. Roger Galve, Dr. Pablo Salvador (CSIC)
17:00-18:00	**Happy hour presentations**	Prof. Jana Hajslova (UCT Prague)

Wednesday, 20 June 2018

10:00-12:00	Czech Agriculture and Food Inspection Authority (CAFIA)	Dr. Martin Kubik, Dr. Radim Stepan (CAFIA)

Thursday, 21 June 2018

08:30-09:00	*Coffee & tea*	
09:00-11:00	**Workshop: development of fit-for-purpose validation protocols for smartphone-based assays**	Dr. Cuong Cao, Dr. Katrina Campbell (QUB)
11:00-11:30	*Coffee & tea*	
11:30-13:00	**Hands-on labwork**	3 groups (Dr. Lucie Drabova, Dr. Vojtech Hrbek, Kamila Hurkova)
13:00-14:00	*Lunch (Carbon club)*	
14:00-15:30	**Hands-on labwork (continued)**	3 groups (Dr. Lucie Drabova, Dr. Vojtech Hrbek, Kamila Hurkova)
15:30-16:00	*Coffee, tea & refreshments*	
16:00-17:00	**Critical comparison and benchmarking of technologies**	Prof. Jana Hajslova (UCT Prague)
17:00-18:00	**Happy hour presentations**	Prof. Jana Hajslova (UCT Prague)

Friday, 22 June 2018

08:30-09:00	*Coffee & tea*	
09:00-10:30	**Collaborative validation studies**	Dr. Katerina Mastovska (Covance)
10:30-11:00	*Coffee & tea, meet the expert*	
11.00-11.15	Course certificates	Prof. Jana Hajslova (UCT Prague
11:15-12:00	*Lunch (Carbon club)*	

Participants' Evaluation of FoodSmartphone Summer School

Term of FoodSmartphone Summer School: 18 – 22 June 2018

_____

Name:

_____

1. Summer School was organized in accordance with my expectations.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

2. All topics of my interest were relevant to me.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

3. The content / programme was well organized and easy to follow.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

4. This training experience will be useful in my work.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

5. The documents distributed were helpful.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

6. The trainers were knowledgeable about the training topics.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

7. The time allocated for the training was sufficient.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

8. Can you suggest any changes / improvements / other topics for future FoodSmartphone Summer School?

_____

_____

_____

9. What did you missed in programme?

_____

_____

_____

Thank You for Your Feedback !

UNIVERSITY OF
CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology

Introduction to QA/QC
and ISO 17025 accreditation in testing laboratories

Department of Food Analysis and Nutrition, UCT Prague

Vladimír Kocourek

Prague, June 2018

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Accredited entity according to ČSN EN ISO/IEC 17025:2005:

University of Chemistry and Technology Prague

Metrological and Testing laboratory

Technická 5, 166 28 Prague 6, Czech Republic

The Laboratory is qualified to update standards identifying the test procedures.

The laboratory has a flexible scope of accreditation permitted as detailed in the Annex.

Updated list of activities provided within the flexible scope of accreditation is available at the laboratory (from the Head of Laboratory).

The laboratory is qualified to provide expert opinions and to interpret test results.

Tests:

Ordinal number	Test procedure/method name	Test procedure/method identification	Tested object
1.	Determination of pesticide residues and their metabolites by GC-MS method and calculation of sums from the measured values (multi-residue method) [1]	KM 01	Food, beverages and water*, food raw materials, fats, oils, honey, food supplements, baby and infant food, novel food***, human and animal tissues and body fluids, plant materials, crops, feedstuffs and preparations
2.	Determination of pesticide residues and their metabolites by LC-MS method and calculation of sums from the measured values (multi-residue method 2) [2]	KM 02 (ČSN EN 15662)	Food, beverages and water**, food raw materials, plant materials, food supplements, baby and infant food, novel food***, crops, feedstuffs and preparations
3.	Determination of dithiocarbamate fungicides by SPME/GC-MS method	KM03	Food of plant origin, baby and infant food, crops, feedstuffs and preparations
4.	Determination of growth regulators, desiccants and herbicides by LC-MS method [3]	KM 04	Food, beverages and water*, food raw materials, human and animal tissues and body fluids, food supplements, baby and infant food, novel food***, crops, feedstuffs and

"Accredo - delivering confidence"

ilac global trust
Testing – Calibration – Inspection

The ILAC Mutual Recognition Arrangement (MRA) supports international trade by promoting international confidence and acceptance of accredited laboratory and inspection results.

NÁRODNÍ AKREDITAČNÍ ORGÁN

Czech Accreditation Institute (CAI) is EC notified as a national accreditation body for accreditation of the Testing Laboratories according to EN ISO/IEC 17025:2005

European Co-operation for Accreditation (EA): organisation associating national accreditation bodies; EA MLA signatory

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Flexible scope

Flexibility

Ordinal number of test methods
1-25

The Laboratory is allowed to modify the test methods listed in the Annex within the specified scope of accreditation provided the measuring principle is observed.

The flexible approach to the scope of accreditation cannot be applied to the tests not included in the Annex.

EA-2/15 M (2008) „Requirements for the Accreditation of Flexible Scopes“

ILAC-G18:04/2010 „Guideline for the Formulation of Scopes of Accreditation“

When a laboratory is granted a flexible scope, it is allowed to include additional activities in its scope of accreditation on the basis of its own validations without evaluation by the accreditation body prior to operation of the activity.

The possibility of introducing new, modified or developed methods under flexible scope does not include introduction of new measurement principles of testing.

UCT PRAGUE

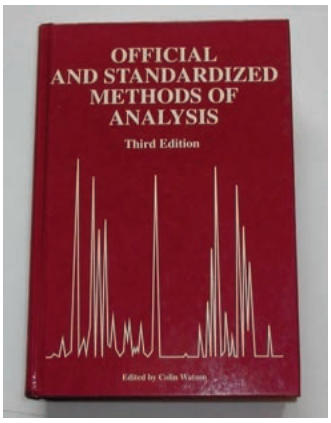
Flexible scope of accreditation

- ✓ **Flexibility concerning object/matrix/sample**
  changes with respect to various matrices within a product area (e.g. LC-MS method which is extended from determination of mycotoxins in cereals and bakery products for the determination of mycotoxins in herbal food supplements).
- ✓ **Flexibility concerning parameters/components/analytes**
  changes with respect to parameters (e.g. the extension of DON determination in cereals to other mycotoxins in cereals by LC-MS method) .
- ✓ **Flexibility concerning the performance of the method**
  changes in the performance of the method for a given matrix type and a given analyte (e.g. the modification of measuring range and uncertainty).
- ✓ **Flexibility concerning the method**
  This means flexibility which allows adoption of methods that are equivalent to methods already covered by accreditation (e.g. new method based on the same measuring principle).

If a laboratory develops new testing methods or modifies them, it requires a sound technical understanding of the techniques used. This competence can be acquired, e.g. by participation in suitable research projects or developing projects, in projects for the development or standardisation of test method etc.

UCT PRAGUE

Choice of Method



It is important to appreciate the difference between an 'analytical method' (combination of steps illustrated by the 'analytical process') and an 'analytical technique' (chemical or instrumental procedure by which analytical data is eventually obtained). In selecting a method we shall consider the following parameters:

- **sample type (matrix) and size (lot or a little);**
- **data required (qualitative/quantitative);**
- **expected level(s) of analyte(s);**
- **precision & accuracy expected;**
- **likely interferences;**
- **number & frequency of samples for analysis.**

Consider a standard method if available - as this will save on development time. However the method must be checked to prove that it suitable for your laboratory/situation. Modification may well be required.

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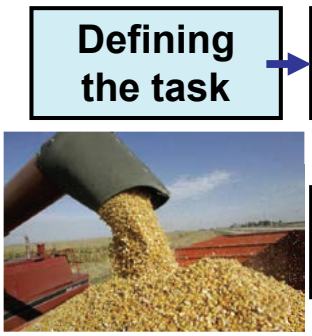
Analytical method for decision making



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Analytical (measurement) method performance

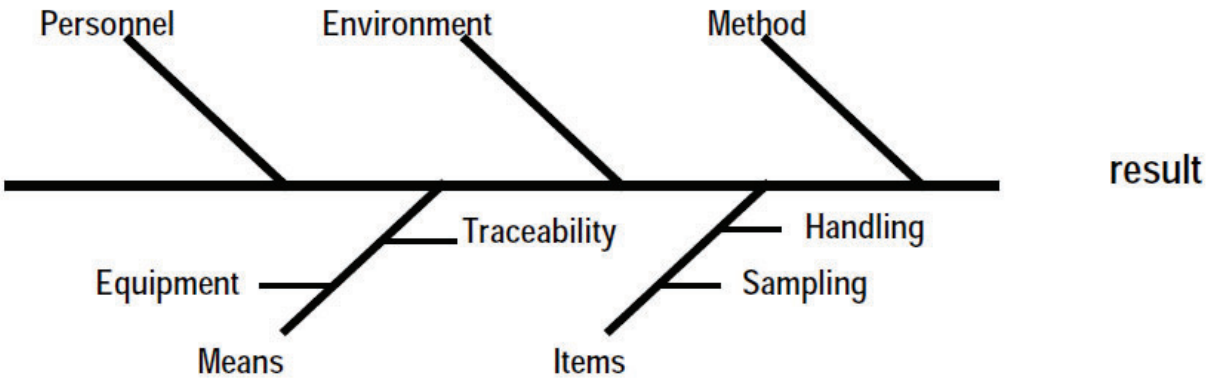

Figure 2 — Ishikawa diagram

ISO/WD 15725-1 Accuracy (trueness and precision) of measurement methods and results Part 1: Introduction and basic principles

VŠCHT PRAHA

QUALITY ASSURANCE (QA)

- QMS documented and reviewed
- laboratory environment and facilities are suitable
- educated and trained personnel
- training procedures and records
- specifications for reagents, and reference materials (RMs)
- equipment maintained and calibrated
- procedures for sample handling
- documented and validated methods
- metrological traceability of results
- evaluation of measurement uncertainty
- internal quality control procedures - QC
- participation in proficiency testing (PT)
- procedures for checking and reporting results
- procedures for implementing preventive and corrective actions
- internal quality audit and review procedures

VŠCHT PRAHA

QUALITY CONTROL (QC)

QC procedures relate to ensuring the quality of results obtained for specific samples or sets of samples and include:

- analysis of **QC samples**
- analysis of **measurement standards (including RMs)**
- analysis of **blind samples**
- analysis of **sample blanks and reagent blanks**
- analysis of **spiked samples**
- analysis in **duplicate / replicate**
- **use of QC charts to monitor trends**
- ***participation in proficiency testing (PT) and EQC programes***

VŠCHT PRAHA

WHAT IS VALIDATION?

► **Validation is a process, within which the method is demonstrated to be suitable for its purpose. It documents methods performance !**

► **During validation process, methods Performance characteristics are estimated.**

► **Validation documents, that the methods performance characteristics are capable of producing results in line with the needs of the analytical problem.**

Is it possible to detect pesticide residues at regulation levels using the method ?
Is it possible to correctly quantify the amount of residues in apple/orange/… ?
…

► **Validation procedure (protocol) is related to a particular analyte and matrix**

Applicability

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There are six validation principles:

Analytical measurements should be made to satisfy an agreed requirement

Analytical measurements should be made using methods and equipment which have been tested to ensure they are fit for their purpose

Analytical measurements made in one location should be consistent with those elsewhere

Staff making analytical measurements should be both qualified and competent to undertake the task

Laboratories should have well defined quality control and quality assurance procedures

There should be an independent assessment of the technical performance of the laboratory

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VALIDATION

VALIDATION PARAMETERS

- PRECISION
- TRUENESS

ACCURACY

Where possible, the validation of in-house validated methods shall include a certified reference material.

- RANGE & LINEARITY
- LIMIT OF DETECTION & LIMIT OF QUANTIFICATION
- SPECIFITY & SELECTIVITY
- RUGGEDNESS

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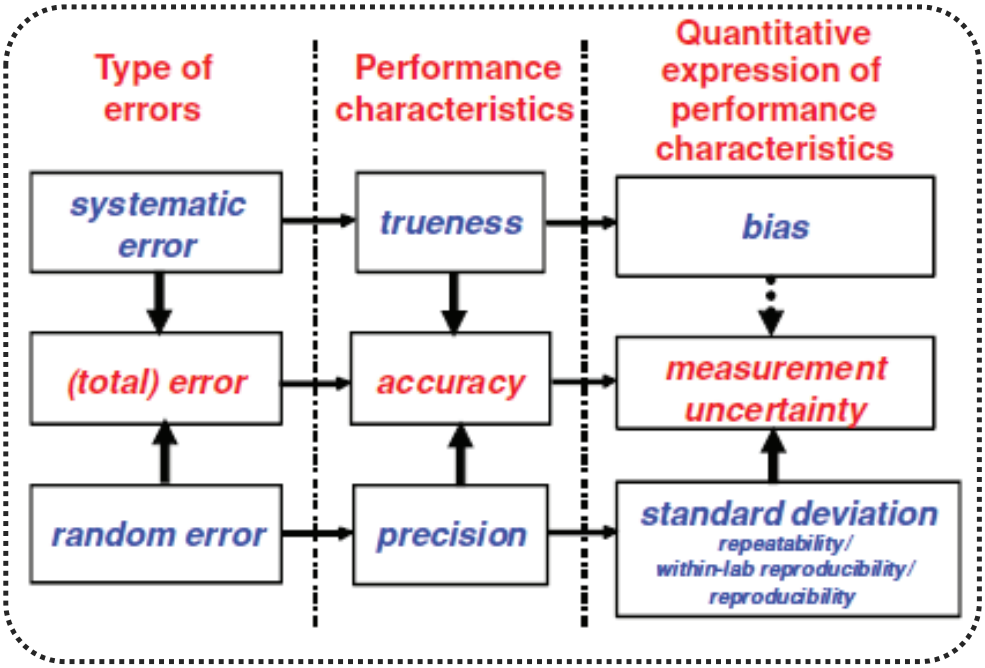
TRUENESS AND PRECISION = ACCURACY

RELATIONSHIPS BETWEEN TYPE OF ERROR, RELATED CHARACTERISTICS AND THEIR QUANTITATIVE EXPRESSION



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TRUENESS AND PRECISION = ACCURACY

ERRORS OF MEASUREMENT

What is included in result value (X)?

$$X = \mu + \varepsilon + \Sigma\,\delta$$

True value is an idealized concept and „true value" cannot be known exactly!

*Hence the **REFERENCE VALUE** represents a true value in routine practice*

Reference value usually provided with reference to:

- Certified reference material
- Reference measurement procedure
- Known amount of analyte added into the sample (spike)

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TRUENESS

TRUENESS is closeness of agreement between the mean of of replicates (measured values) and a **REFERENCE (TRUE)** value.

Trueness is inversely related to systematic error:
The lower the systematic error, the higher the trueness…

Estimate of a systematic error: bias

In analytical chemistry: RECOVERY

Correction of the result can be carried out using the recovery

=> compensation for an estimated systematic effect

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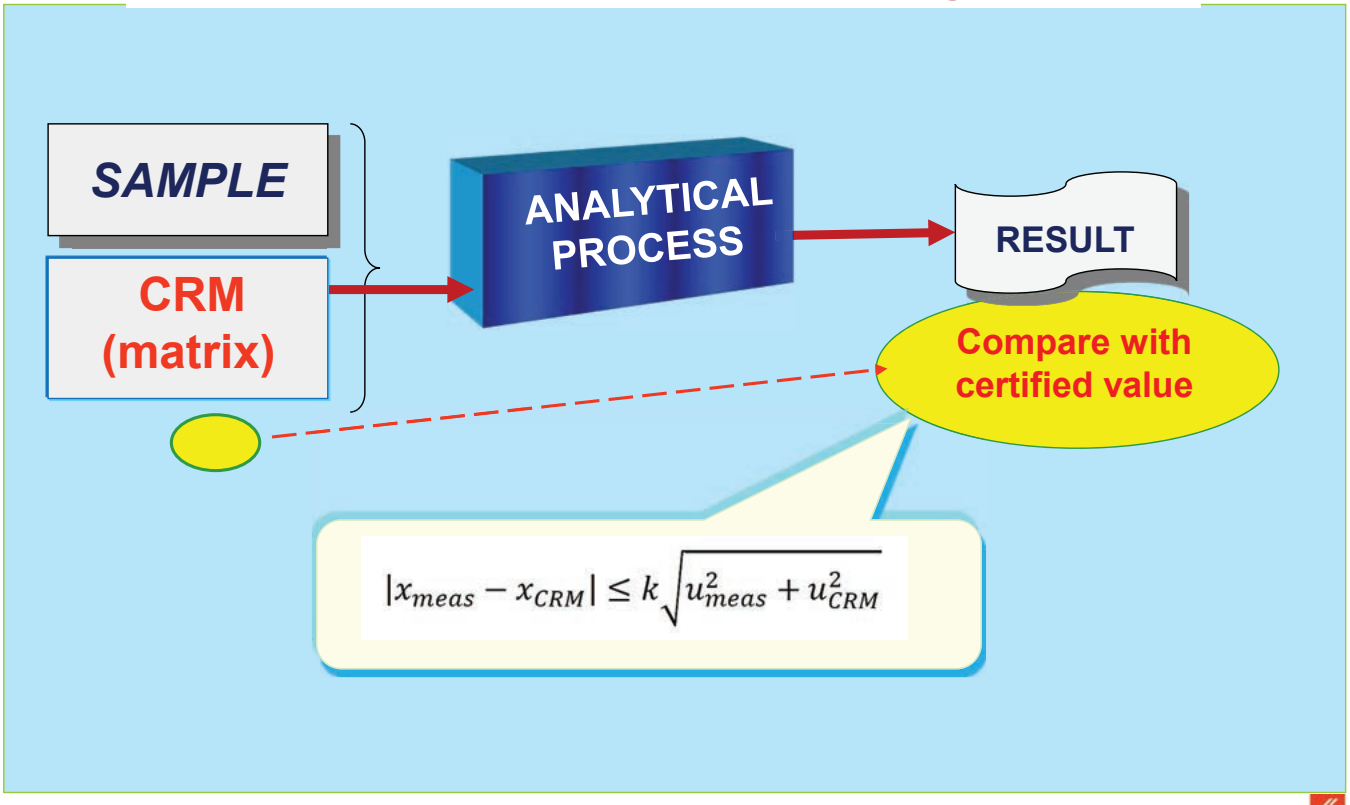
Traceability to reference material: trueness

Assessment of trueness using CRM



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Certified Reference Material (CRM, SRM)

CERTIFICATE OF ANALYSIS

ERM®- BC717

MAIZE		
Compound	Mass fraction	
	Certified value [1] [µg/kg]	Uncertainty [2] [µg/kg]
Zearalenone	83	9

1) Unweighted mean of accepted mean values, independently obtained by 18 laboratories and traceable to the SI.

2) Estimated expanded uncertainty *U* with a coverage factor *k* = 2, corresponding to a level of confidence of about 95 %, as defined in the Guide to the Expression of Uncertainty in Measurement (GUM), ISO, 1995. Uncertainty contributions arising from characterisation as well as from homogeneity and stability assessment were taken into consideration.

This certificate is valid for one year after purchase.

http://www.erm-crm.org/html/homepage.htm

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PRECISION

REPEATABILITY AND REPRODUCIBILITY

Repeatability: a set of conditions that includes

- the same measurement, procedure, operators, same measuring system, operating conditions and location, and replicate measurements on the same or similar objects over a short period of time

Reproducibility: a set of conditions that includes

- different locations, operators, measuring systems, or even methods on the same or similar objects.

Intermediate precision (*intra-laboratory reproducibility*):

- the same laboratory, method, procedure but within an extended period of time - may include new calibrations, calibrants, operators, measuring systems, etc.

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PRECISION

INCREASING NUMBER OF CONSIDERED RANDOM ERROR SOURCES

REPEATABILITY		INTRA-LABORATORY REPEATABILITY		REPRODUCIBILITY	
SAMPLE:	SAME	SAMPLE:	SAME	SAMPLE:	SAME
OPERATOR:	SAME	OPERATOR:	DIFFERENT	OPERATOR:	DIFFERENT
INSTRUMENT:	SAME	INSTRUMENT:	SAME / DIFF.	INSTRUMENT:	DIFFERENT
TIME PERIOD:	SHORT	TIME PERIOD:	LONG	TIME PERIOD:	LONG
CALIBRATION:	SAME	CALIBRATION:	DIFFERENT	CALIBRATION:	DIFFERENT
LAB:	SAME	LAB:	SAME	LAB:	DIFFERENT

- Precision value is related to a certain analyte and concentration level

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PRECISION

INCREASING NUMBER OF CONSIDERED RANDOM ERROR SOURCES

REPEATABILITY | INTRA-LABORATORY REPEATABILITY | REPRODUCIBILITY

- Repeated analyses of a sample containing analyte(s) at:
  - level close to expected concentration in analyzed matrix
  - level close to regulatory limit
  - low level close to limit of quantification of the method
- Appropriate number of repeats: 8 – 15 (at least 5)
- Calculated as standard deviation or relative standard deviation (RSD)

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QC in routine testing: repeability limit (r)

Calculated from standard deviation of results under repeatability conditions (replicate analyses):

$$r = f \cdot \sqrt{2} \cdot \sigma_r$$

$$r = 2{,}8 \cdot s_r$$

Practical use:

…value less than or equal to which the absolute difference between two test results obtained under repeatability conditions may be expected to be with a probability of 95 %.

$$|x_1 - x_2| \leq r$$

VŠCHT PRAHA

Reproducibility limit (R)

Reproducibility - expected to give the largest variation in results - is a measure of the variability in results between laboratories[1-2)]

$$R = 2{,}8 \cdot s_R$$

Practical use:

$$|X_1 - X_2| \leq R$$

[1)] **Reproducibility may also refer to the variation observed between laboratories using different methods (intending to measure the same quantity).**

[2)] **Intermediate precision is sometimes (improperly) referred to as 'within-laboratory reproducibility'**

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PRECISION

INCREASING NUMBER OF CONSIDERED RANDOM ERROR SOURCES

REPEATABILITY → INTRA-LABORATORY REPEATABILITY → REPRODUCIBILITY

- **Can be estimated within an inter-laboratory study…**

Two components:

σ^2_r – **intra-laboratory variance (typical)**

σ^2_L – **between laboratories variance**

$$\sigma^2_R = \sigma^2_r + \sigma^2_L$$

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PRECISION

INCREASING NUMBER OF CONSIDERED RANDOM ERROR SOURCES

REPEATABILITY → INTRA-LABORATORY REPEATABILITY → REPRODUCIBILITY

- **Can be estimated within an inter-laboratory study…**

…however…

- **inter-laboratory study is time-demanding and costly**
- **it is problematic to find sufficient number of competent laboratories**
- **in multi residue analysis it is almost impossible to perform this kind of study for all analytes / matrices / concentration levels**

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PRECISION

REPRODUCIBILITY - HORWITZ

- Reproducibility can be alternatively estimated from an empirical model developed based on numerous inter-laboratory studies…

Horwitz empirical model of precision:

…the RSD_R can be expressed as a function of the concentration …

William Horwitz (1918-2006): Anal. Chem. 1982, 54, 67A

…..„one of the most intriguing relationships in modern analytical chemistry“

PRECISION

REPRODUCIBILITY - HORWITZ

Relative standard deviation – variation coefficient:

- **lower concentration of analyte → increasing RSD**
- **nature of analyte, matrix, analytical method etc.: less important – even can be ignored !**

$$RSD = 2^{(1-\,0.5*\log X)}$$

X is an analyte concentration expressed as a mass ratio

Method performance criteria: Reg. 401/2006/EC

(a) Performance criteria for aflatoxins

Criterion	Concentration Range	Recommended Value	Maximum permitted Value
Blanks	All	Negligible	—
Recovery — Aflatoxin M1	0,01-0,05 mg/kg	60 to 120 %	
	> 0,05 mg/kg	70 to 110 %	
Recovery-Aflatoxins B_1, B_2, G_1, G_2	< 1,0 mg/kg	50 to 120 %	
	1-10 mg/kg	70 to 110 %	
	> 10 mg/kg	80 to 110 %	
Reproducibility RSD_R	All	As derived from Horwitz Equation (*) (**)	2 × value derived from Horwitz Equation (*) (**)
Repeatability RSD_r may be calculated as 0,66 times Reproducibility RSD_R at the concentration of interest.			

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Method performance criteria: Reg. 401/2006/EC

Performance criteria for deoxynivalenol

Level µg/kg	Deoxynivalenol		
	RSD_r %	RSD_R %	Recovery %
> 100-≤ 500	≤ 20	≤ 40	60 to 110
> 500	≤ 20	≤ 40	70 to 120

(b) Performance criteria for ochratoxin A

Level µg/kg	Ochratoxin A		
	RSD_r %	RSD_R %	Recovery %
< 1	≤ 40	≤ 60	50 to 120
≥ 1	≤ 20	≤ 30	70 to 110

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Method performance criteria: Reg. 401/2006/EC

Performance criteria for zearalenone

Level µg/kg	Zearalenone		
	RSD_r %	RSD_R %	Recovery %
≤ 50	≤ 40	≤ 50	60 to 120
> 50	≤ 25	≤ 40	70 to 120

Performance criteria for Fumonisin B_1 and B_2 individually

Level µg/kg	Fumonisin B_1 and B_2 individually		
	RSD_r %	RSD_R %	Recovery %
≤ 500	≤ 30	≤ 60	60 to 120
> 500	≤ 20	≤ 30	70 to 110

Performance criteria for T-2 and HT-2 toxin individually

Level µg/kg	T-2 and HT-2 toxin individually		
	RSD_r %	RSD_R %	Recovery %
15-250	≤ 30	≤ 50	60 to 130
> 250	≤ 25	≤ 40	60 to 130

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Proficiency testing (PT)

Regular participation in proficiency testing (also known as external quality assessment, EQA) is a recognised way for a laboratory to monitor its performance against both its own requirements and the norm of peer laboratories.

PT helps to highlight variation between laboratories (reproducibility), and systematic errors (bias).

Accreditation bodies strongly encourage laboratories to participate in PT as an integral part of their quality management.
In certain instances, accreditation bodies may specify participation in a particular PT scheme as a requirement for accreditation.

It is important to monitor PT results as part of the QC procedures and take action as necessary.

- ✓ Requirements for the competence of PT providers are described in ISO/IEC 17043,
- ✓ Selection, use and interpretation of PT schemes: see Eurachem Guide on www.eurachem.org

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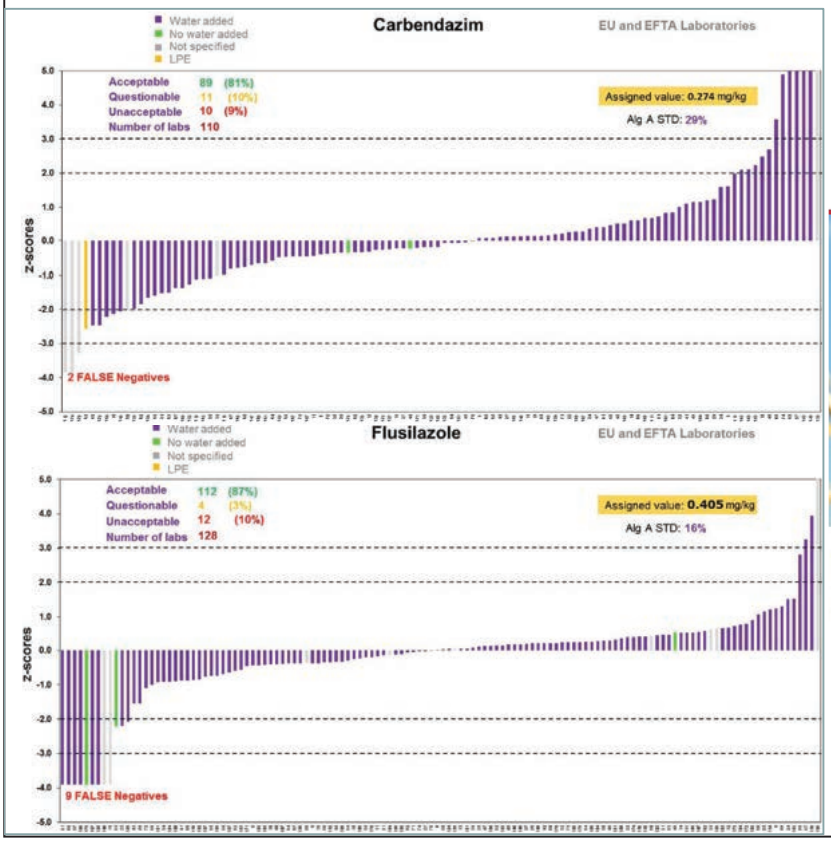
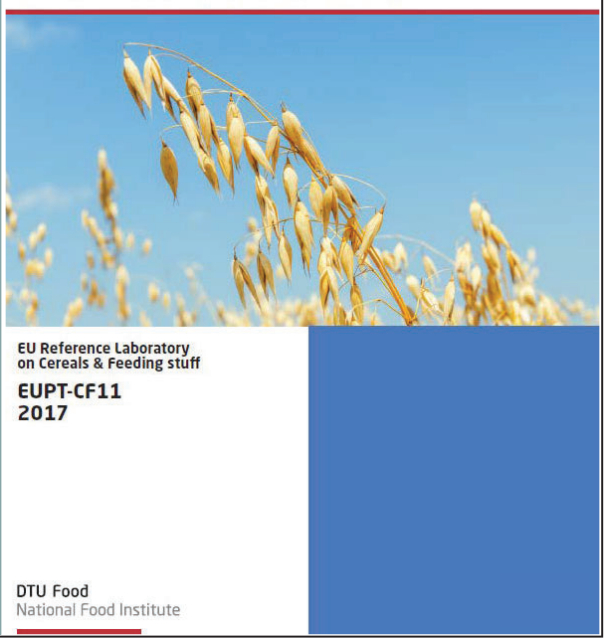
Example of EU PT results

149 EU and EFTA laboratories, from 30 different countries
82 labs classified as „GOOD“ (53 in category „A“)
UCT: 18 pesticides quantified, weighted z scores = 0.1





FAPAS®: Food Analysis Performance Assessment Scheme

CERTIFICATE OF PARTICIPATION

This certificate confirms that:

University of Chemistry and Technology (Food Analysis)

took part in:
Food Chemistry Proficiency Test 04335
Multi-mycotoxins in Maize

and were allocated laboratory number 91.

The performance of the laboratory is shown in the relevant report, which is available from the secure pages at fapas.com

FAPAS®, FEPAS®, GeMMA, LEAP®
Fera Science Ltd (Fera)
Sand Hutton
York YO41 1LZ

tel: +44 1904 462100
fax: +44 1904 500440
info@fapas.com
fapas.com

FAPAS®, FEPAS®, GeMMA and LEAP® are UKAS accredited, giving independent confirmation that we comply with the requirements of International Standard ISO/IEC 17043:2010. Additionally, Fera Science Ltd (Fera) is an ISO 9001 certified organisation.

The Food and Environment Research Agency

CERTIFICATE OF PARTICIPATION

This certificate confirms that:

University of Chemistry and Technology (Food Analysis)

took part in:
Food Chemistry Proficiency Test 14177
Total Fat , Saturates , Mono-unsaturates , Poly-unsaturates , Total Trans Fatty Acids , Omega-3 fatty acids , Omega-6 fatty acids , Omega-9 fatty acids , alpha Linolenic Acid (C18:3 n-3) (ALA) , Linoleic Acid (C18:2 n-6) , Oleic Acid (C18:1(n9)cis) in Mixed Fat Spread

and were allocated laboratory number 12.

The performance of the laboratory is shown in the relevant report, which is available from the secure pages at fapas.com

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UCT PRAGUE

LINEARITY, CALIBRATION AND RANGE

GENERAL RECOMENDATIONS FOR LINEAR CALIBRATION

- **There should be five or more calibration points (standards)**
- **Even spacing over the concentration range of interest**
- **The calibration range should encompass 0–150% or 50–150% of the concentration likely to be encountered in samples**
- **Calibration standards should be run at least in duplicate in random order**

- **VALIDATION RANGE is the interval of analyte concentration within which the method can be regarded as validated**
- **Typically narrower than linear range**
- **In practice, most methods will be validated at only one or two levels of concentration. The validated range may be taken as a reasonable extrapolation from these points at concentration scale**

UCT PRAGUE

LIMIT OF DETECTION / QUANTIFICATION

Limit of Detection (LOD): the smallest concentration of analyte in the test sample which can be reliably distinguished from zero.

►LOD is concentration of analyte which induce **signal (S)** that is 3 times higher than the background **noise level (N)**. **S/N=3**

Limit of Quantification (LOQ): the smallest concentration of analyte in the test sample which can be reliably quantified.

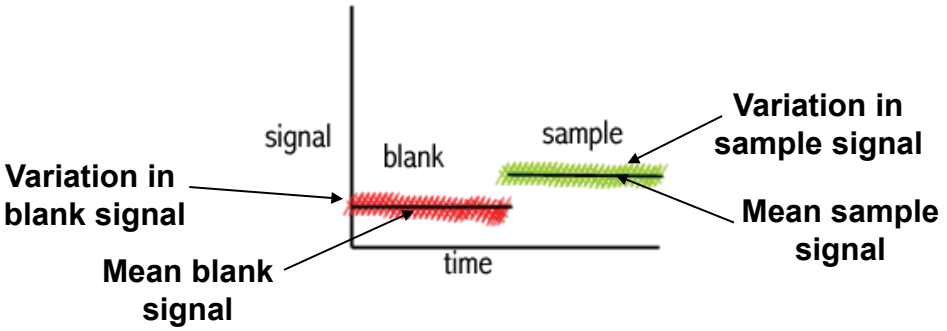
►LOQ is concentration of analyte which induce **signal (S)** that is 10 times higher than the background **noise level (N)**. **S/N=10**, LOQ usually corresponds to lowest calibration point.

S/N can be usually calculated in processing software

UCT PRAGUE

Method validation -
detection & quantitation limits

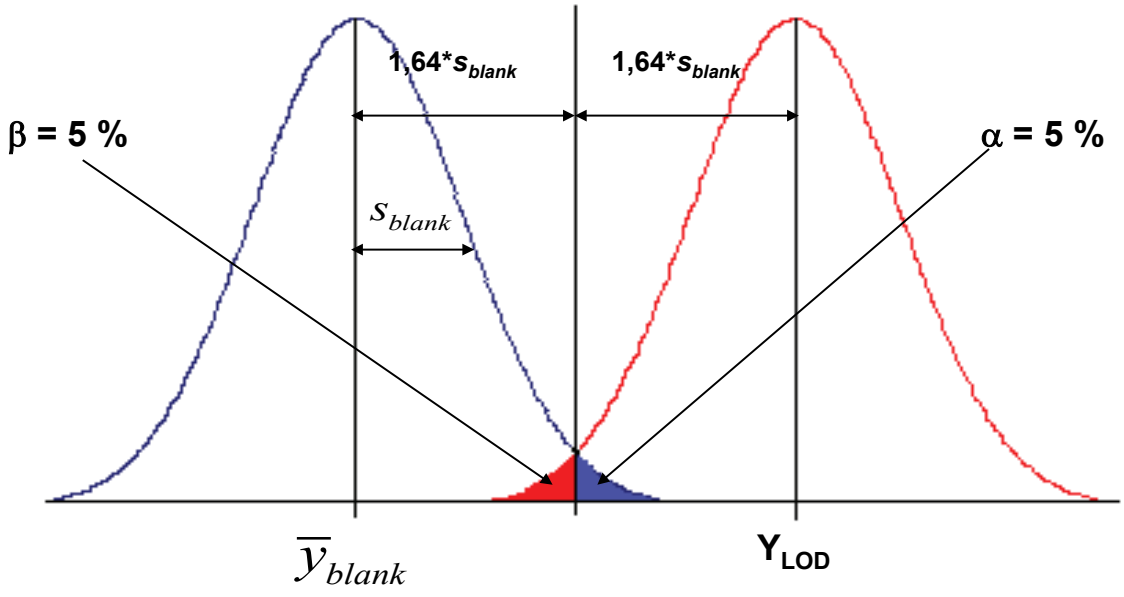
A method is not acceptable for reliable detection or quantitation if the analyte level is likely be fall beneath the limit(s) calculated based upon the blank signal and its standard deviation. Analyte pre-concentration then becomes necessary.



Mean sample signal must be sufficiently larger than the blank so that positive detection or accurate quantitation is possible

UCT PRAGUE

Signal at LOD vs. background noise



Response to blank **Analyte signal**

UCT PRAGUE

LIMIT OF DETECTION / QUANTIFICATION

HOW TO ESTIMATE LOD & LOQ

SPIKING OF BLANK MATRIX WITH DECREASING AMOUNT OF ANALYTES

SAMPLE PREPARATION & MEASUREMENTS

CALCULATION OF S/N VALUES

Method detection limit: LOD

STANDARD SOLUTION WITH DECREASING AMOUNT OF ANALYTES

MEASUREMENTS

CALCULATION OF S/N VALUES

Instrument detection limit: IDL

UCT PRAGUE

Codex Alimentarius: numerical values for the criteria

Minimum Applicability:

The method has to be applicable for the specified provision, specified commodity and the maximum level (ML).

The minimum applicability (ma) of the method depends on the maximum limit (ML) to be assessed, and can either be expressed in terms of the reproducibility standard deviation (s_R) or in terms of LOD and LOQ.

CL 2008/7-MAS, March 2008

Based on the reproducibility:

ML ≥ 0.1 mg/kg → ma = ML - 3 s_R

ML < 0.1 mg/kg → ma = ML - 2 s_R

Based on the Limit of Detection (LOD):

ML ≥ 0.1 mg/kg → ma = LOD ≤ 0.1 ML

ML < 0.1 mg/kg → ma = LOD ≤ 0.2 ML

Based on the Limit of Quantitation (LOQ):

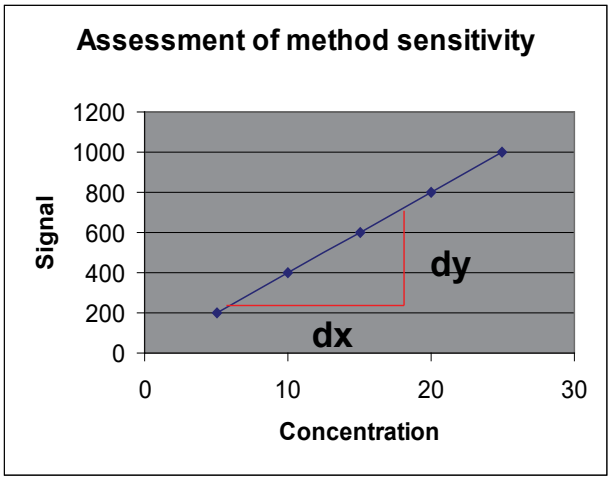
ML ≥ 0.1 mg/kg → ma = LOQ ≤ 0.2 ML

ML < 0.1 mg/kg → ma = LOQ ≤ 0.4 ML

UCT PRAGUE

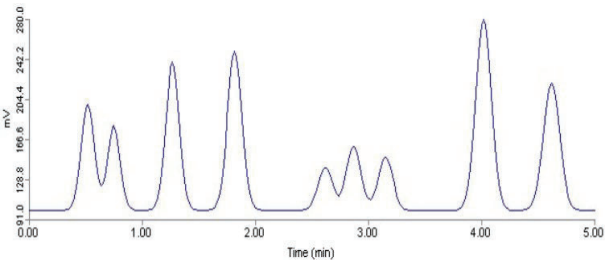
Sensitivity and selectivity

Sensitivity is the change in measured signal for unit change in concentration and can be obtained from the calibration graph



Sensitivity = dy/dx

Selectivity is the ability of a method to discriminate between the target analyte and other constituents of the sample. In many instances selectivity is achieved by high performance separation using chromatographic or electrophoretic techniques.



HPLC chromatogram

UCT PRAGUE

SELECTIVITY AND SPECIFITY

Selectivity: the degree to which a method can quantify the analyte accurately in the presence of interferents.

- **Selective method** – the results are influenced by the sample matrix (interferents, cross-reactivity, matrix effects,…)
- **Specific methods** – the results are not influenced by the presence of sample matrix

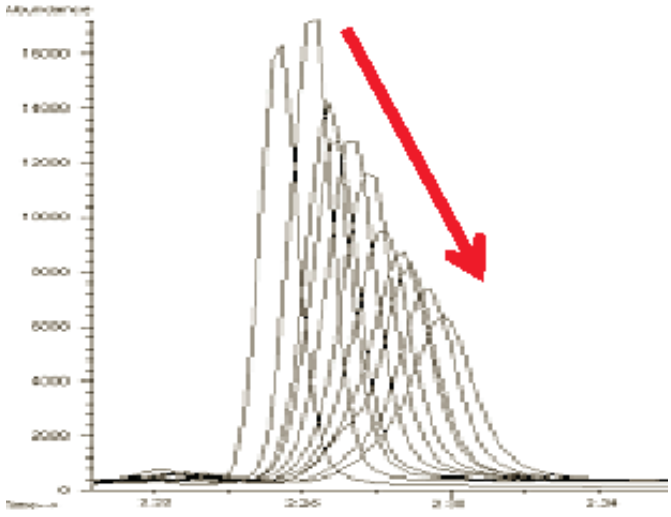
The presence and influence of any sample matrix interference on method results should be tested and described

UCT PRAGUE

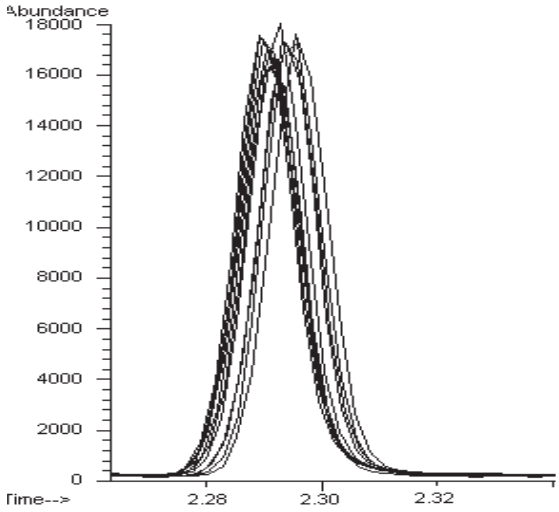
SELECTIVITY AND SPECIFITY

MATRIX EFFECTS:

DECREASE (INCERASE) AND PEAK SHAPE DETERIORATION



High matrix content



Low matrix content

UCT PRAGUE

RUGGEDNESS

Ruggedness of an analytical method is the resistance to change in results when minor deviations are made from the experimental conditions described in the SOP.

► The aspects of the method that are likely to affect results should be identified and described in SOP

Examples of factors relevant to ruggedness:

- ► pH of a solution
- ► stability of the instrumental system
- ► extraction time
- ► concentration of (derivatization) reagents
- ► temperature/time of (derivatization) reaction
- ► time allowed for completation of whole analytical process

UCT PRAGUE

WHAT IS UNCERTAINTY ?

Theory:

"Uncertainty is a parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand"

(International Vocabulary of Basic and General Terms in Metrology, ISO, Geneva, Switzerland, 1993, ISBN 92-67-10175-1)

Practice:

The uncertainty on the result may arise from many sources, such as sampling, incomplete extraction of the measurand, matrix effects, purity of chemicals, instruments and operator bias, random effects etc.

ANALYTICAL RESULT CANNOT BE VIEWED ONLY AS A SEPARATE VALUE!

COMBINED UNCERTAINTY - TWO APPROACHES FOR QUANTIFICATION

"BOTTOM-UP" /"ERROR BUDGET"/

Estimation of individual contribution of each step of the analytical process to the final result (combination of all individual components)

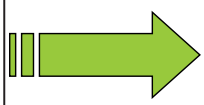
(Guide to the expression of uncertainty in measurement (GUM). ISO, Geneva 1995)
(Quantifying Uncertainty in Analytical Measurement. Ellison S.L.R., Rösslein M., Williams A. (Eds.), EURACHEM/CITAC Guide 4, 2000)

"TOP-DOWN"

Repeatibility of determination (expressed as standard deviation) and uncertainty of recovery (expressed as standard deviation obtained from rectangular distribution) are used.

Proficiency testing, CRM, recovery tests

(ISO/TS 21748:2004 Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation)



WHAT IS EFFECTIVE SOLUTION IN "REAL-LIFE" SITUATIONS?

USEFUL DOCUMENTS

Pure Appl. Chem., Vol. 74, No. 5, pp. 835–855, 2002.

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

ANALYTICAL, APPLIED, CLINICAL, INORGANIC, AND
PHYSICAL CHEMISTRY DIVISIONS
INTERDIVISIONAL WORKING PARTY FOR HARMONIZATION OF
QUALITY ASSURANCE SCHEMES FOR ANALYTICAL LABORATORIES*

HARMONIZED GUIDELINES FOR SINGLE-LABORATORY VALIDATION OF METHODS OF ANALYSIS

(IUPAC Technical Report)

Prepared for publication by
MICHAEL THOMPSON[1], STEPHEN L. R. ELLISON[2], AND ROGER WOOD[3,‡]

UCT PRAGUE

USEFUL DOCUMENTS

Criteria for validation of methods used in official control of contaminants and residues in food and feed:

COMMISSION DECISION

of 14 August 2002

implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

(notified under document number C(2002) 3044)

(Text with EEA relevance)

2002/657/EC

UCT PRAGUE

Conformity assessment – General reguirements for the competence of testing and calibration laboratories (ISO/IEC 17025:2005)

The laboratory shall use test methods, including methods for sampling, which meet the needs of the customer and which are appropriate for the tests it undertakes.
Methods published in international, regional or national standards shall preferably be used.

Appropriate methods have been also published by reputable technical organizations, or in relevant scientific texts or journals, or specified by the manufacturer of the equipment.
Laboratory-developed methods or methods adopted by the laboratory may also be used if they are appropriate for the intended use and if they are validated.

The laboratory shall validate nonstandard methods, laboratory developed methods, standard methods used outside their intended scope, and modifications of standard methods to confirm that the methods are fit for the intended use.
Testing laboratories shall apply procedures for estimating uncertainty of measurement based on the method validation data.

UCT PRAGUE

Conformity assessment – General reguirements for the competence of testing and calibration laboratories (ISO/IEC 17025:2005)

All documents and methods issued to personnel in the laboratory shall be reviewed and approved for use by authorized personnel prior to issue.

Documents are periodically reviewed and, where necessary, revised to ensure continuing suitability and compliance with applicable requirements;
Invalid or obsolete documents are promptly removed from all points of issue or use, or otherwise assured against unintended use;

Procedures shall be established to describe how changes in documents maintained in computerized systems are made and controlled.

All records and data shall be stored and retained in such a way that they are readily retrievable in facilities that provide a suitable environment to prevent damage or deterioration and to prevent loss.

The laboratory shall have procedures to protect and back-up records stored electronically and to prevent unauthorized access to or amendment of these records.

UCT PRAGUE

ISO IEC FDIS 17025:2017

- Scope
- Normative references
- Terms and definitions
- General requirements
- Structural requirements
- Resource requirements
- Process requirements
- Management system requirements
- Annexes

VŠCHT PRAHA

ISO IEC FDIS 17025:2017

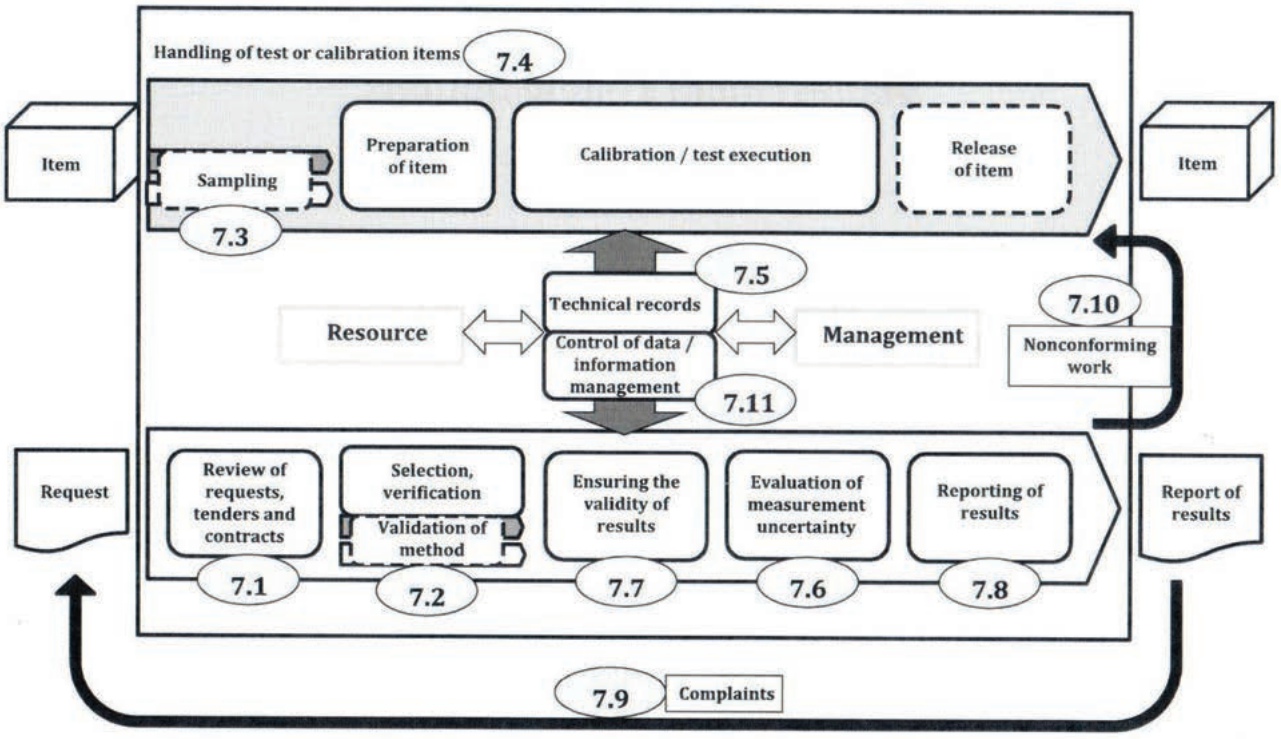


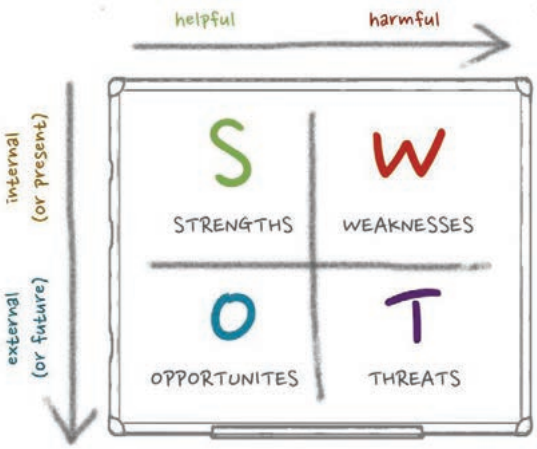
Figure B.1 — Possible schematic representation of the operational processes of a laboratory

VŠCHT PRAHA

Decision rules: conforming / not conforming

- Laboratories often make statements on conformity of the results with certain specifications (compliance with limits, test passed (yes/no), etc.)
- Clear decision rules are to be communicated, documented and to be applied
- Decision rules have to relate to associated risks

Duck or rabbit?



Your SWOT

Strengths
- What do you do better than others?
- What's your talent?
- What competences do you have that are relevant and valuable for the job?
- Which specific and transferable skills do you have?

Weaknesses
- Which tasks and responsibilities your don't like?
- What are the development opportunities your manager and your peers flagged?
- What can you improve?

Opportunities
- In which industry/field/position could you easily move?
- What development / training would be value adding for you?
- Which industry/company is in particular in need of your type of profile?

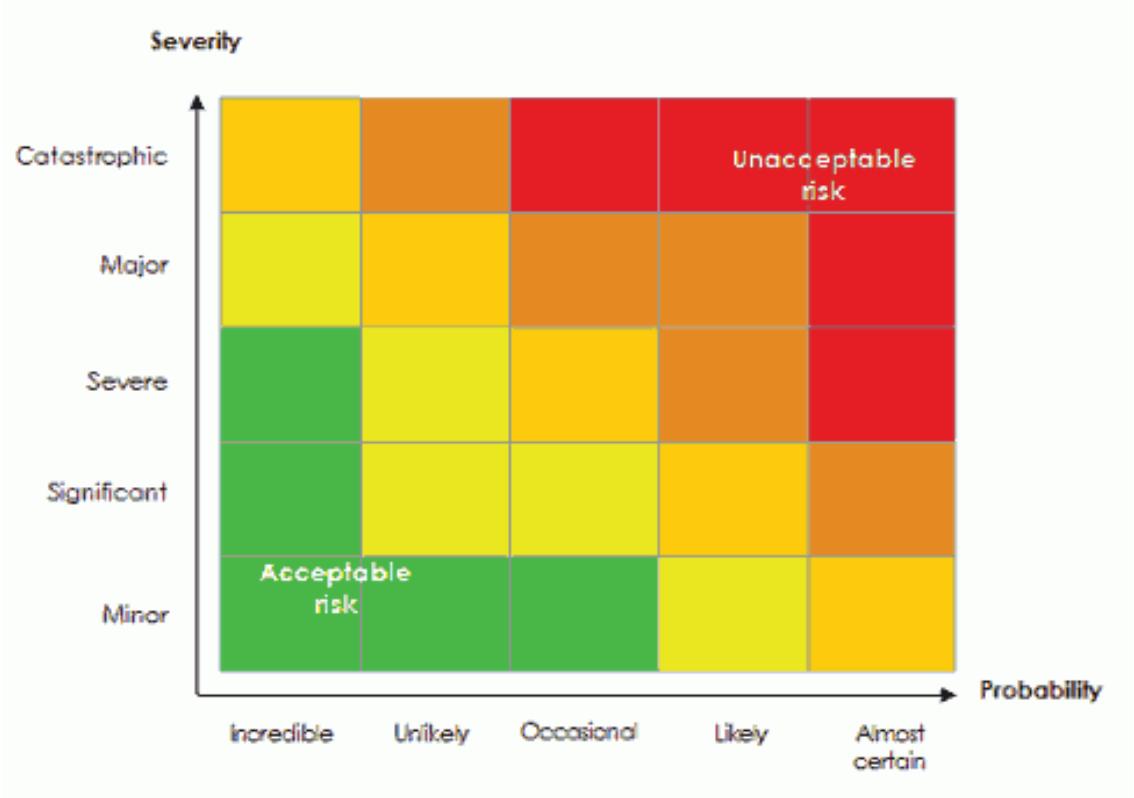
Threats
- What do other candidates have more/better than you?
- What are the obstacles in your way to the job?

Chromatographic-based methods		Immunochemical-based methods
• Validation (in compliance to regulations) • Allows compound identification and structural elucidation of unknown • Multi-target	Strength	• Limited sample treatment • Simple, cheap, portable • Managing of large number of samples
• Expensive • Sophisticated (skilled personnel is required for operating and interpreting results) • Operated in laboratory	Weakness	• Excessively selective • Long time needed for the development (to obtain bioreagents, mainly antibodies)
• Simplified (QuEChERS) sample preparation for high-throughput and multiresidue analysis • Biomarkers in biological fluids	Opportunities	• Provide up-to date information on occurrence • Provide epidemiologic data
• Emerging mycotoxins • Masked mycotoxins	Threats	• New matrices • Multiplex analysis

Current Opinion in Biotechnology

FMEA

Risk-based thinking and acting



(Failure Mode and Effects Analysis)

ANALYTICAL QUALITY CONTROL AND METHOD VALIDATION PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS IN FOOD AND FEED

Document No. DG SANTE/11813/2017, *implemented by 01/01/18*

Document is intended for laboratories involved in official control of pesticide residues in food and feed in the European Union. The document supports the validity of data reported within official controls on pesticide residues and used for checking compliance with maximum residue levels (MRLs) or assessment of consumer exposure to pesticides.

The key objectives are:

- ✓to provide a harmonized, cost-effective quality assurance and quality control system in the EU
- ✓to ensure the quality and comparability of analytical results
- ✓to ensure that acceptable accuracy is achieved
- ✓to ensure that false positives or false negatives are avoided
- ✓to support compliance with, and specific implementation of ISO/IEC 17025 (accreditation standard)

This document is complementary and integral to the requirements in ISO/IEC 17025.

UCT PRAGUE

Pesticides standards

"Pure" standards should be of known purity and each must be uniquely identified and the date of receipt recorded. They should be stored at low temperature, preferably in a freezer, with light and moisture excluded, i.e. under conditions that minimise degradation. The identity of freshly acquired "pure" standards should be checked if the analytes are new to the laboratory.

When preparing stock standards of "pure" standards of analytes and internal standards, the identity and mass of the "pure" standard and the identity and amount of the solvent must be recorded. The solvent(s) must be appropriate to the analyte (solubility, no reaction) and method of analysis. Moisture must be excluded during equilibration of the "pure" standard to room temperature before use and concentrations must be corrected for the purity of the "pure" standard.

Not less than 10 mg of the "pure" standard should be weighed using a 5 decimal place balance. The ambient temperature should be that at which the glassware is calibrated, otherwise preparation of the standard should be based on solvent-mass measurement.

Existing stock and working solutions may be tested against newly prepared solutions by comparing the detector responses obtained from appropriate dilutions of individual standards or mixtures of standards.
The means from at least 5 replicate measurements for each of two solutions (old and new) should not normally differ by more than ±10 %. The mean from the new solution is taken to be 100%. Differences in apparent concentration between old and new standards must be investigated.

UCT PRAGUE

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

Analytical validation of MS based methods according to EU requirements

Jana Hajslova, Jana Pulkrabova,
Josep Rubert

FoodSmartphone.eu

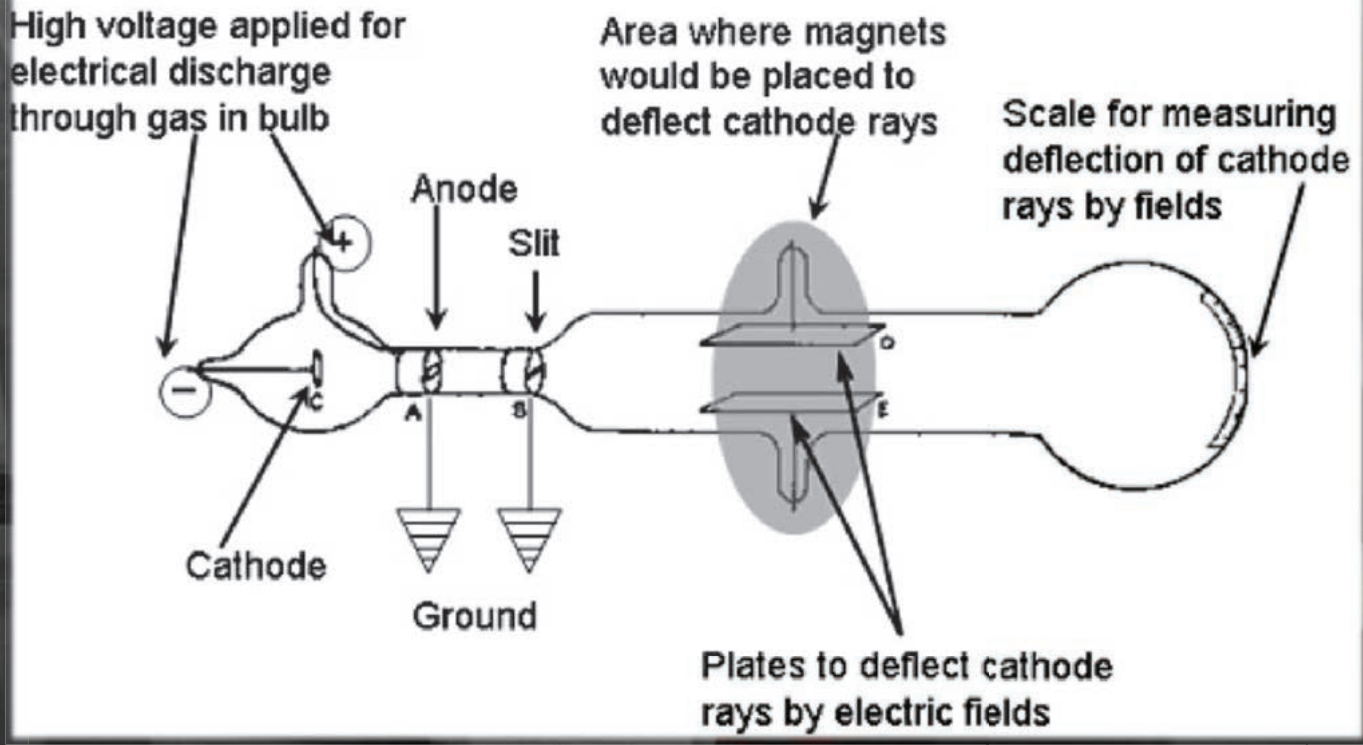
Prague, June 18 – 22, 2018

The performance of mass analyzers

Identification of Unknowns

Identification points

Thomson's Apparatus for Research on Cathode Rays



Mass Spectrometry

The history of mass spectrometry

Nobel prize pioneers

Joseph John Thomson
1906 Nobel Prize for Physics
(*theoretical and experimental investigations on the conduction of electricity by gases*)

Francis William Aston
1922 Nobel Prize for Chemistry
(*mass spectrograph, of isotopes, in a large number of non-radioactive elements*)

Wolfgang Paul
1989 Nobel Prize for Physics
(for the development of the ion trap technique)

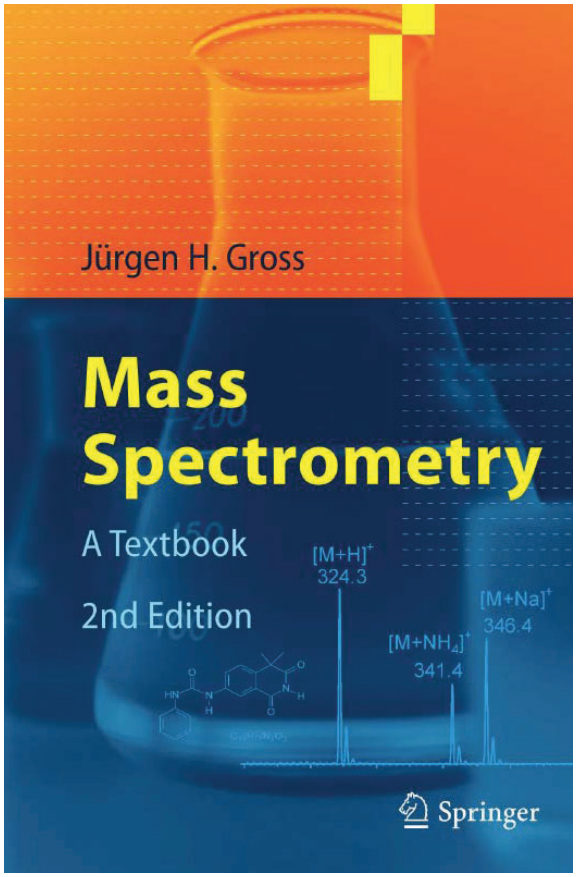
John Bennet Fenn
2002 Nobel Prize for Chemistry
(for the development of Soft Desorption ionization Method)

Koichi Tanaka
2002 Nobel Prize for Chemistry
(mass spectrometric analyses of biological macromolecules)

MS

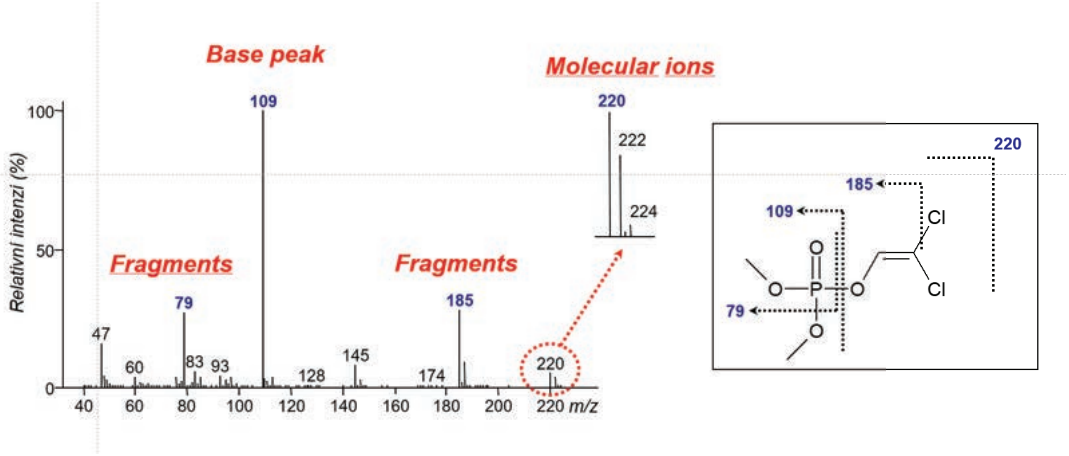
The principle of MS ***is measurement of the mass of a molecule***, more precisely, the mass-to-charge ratio (***m/z***) of the ion, being created by losing or gaining a charge from a neutral species in the ion source.

Once formed, ions are electrostatically directed into the mass analyser where they are separated according to their m/z, and finally detected (Gross 2011).



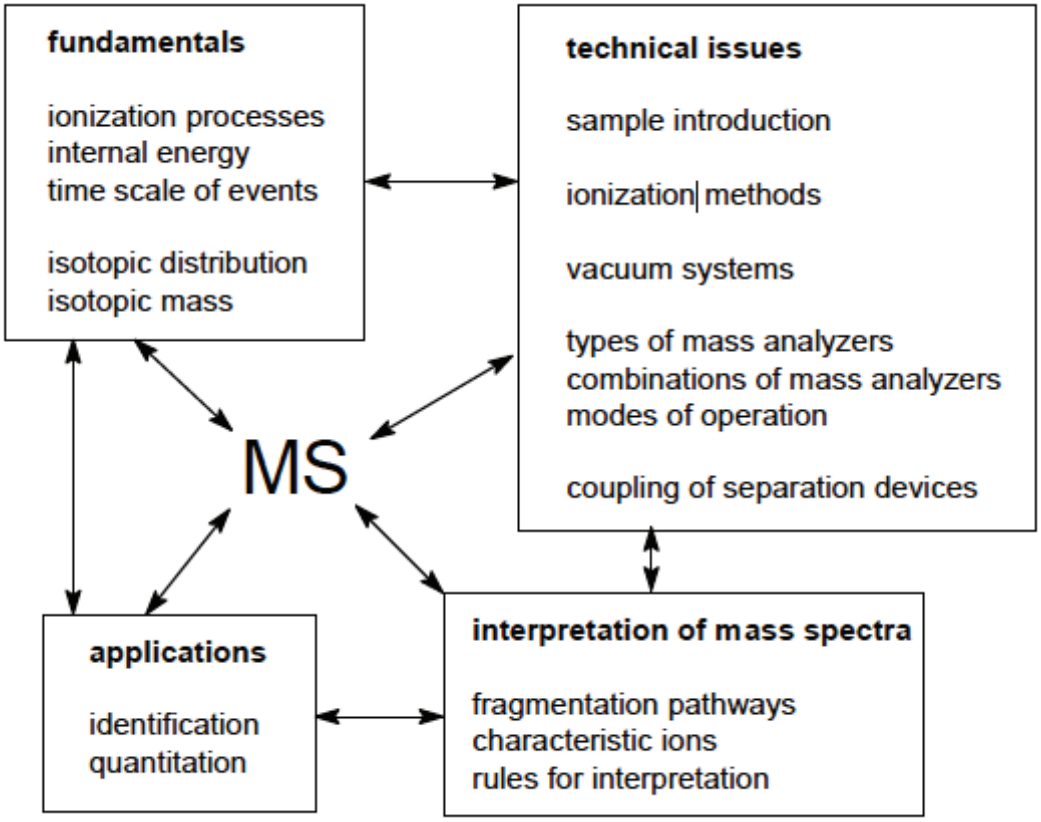
MS Tutorial

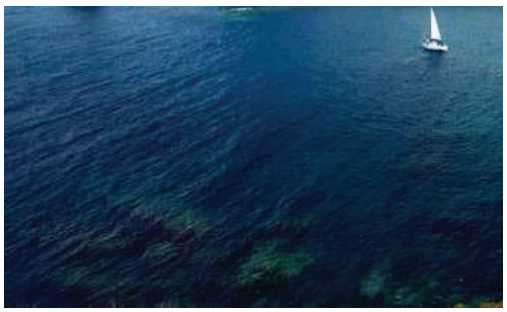
https://www.youtube.com/watch?v=NuIH9-6Fm6U



Key application and field of application	Explanation
Elemental and isotopic analysis Physics Radiochemistry Geochemistry	Elemental identification and isotopic abundance measurement of both short-lived and stable species in physics and radiochemistry (nuclear waste), in geochemistry and more recently in the life sciences.
Organic and bio-organic analysis Organic chemistry Polymer chemistry Biochemistry and medicine	Identification and structural characterization of molecules from small to very large as provided either by chemistry, physiological processes, or polymer chemistry.
Structure elucidation Organic chemistry Polymer chemistry Biochemistry and medicine	Mass spectrometric experiments can be arranged consecutively to study mass-selected ions in tandem mass spectrometry (MS/MS or MS^2). Eventually products are subjected to a third level (MS^3) and so forth (MS^n).
Coupling to separation techniques Quality control Environmental analysis Complex mixture analysis Petroleum chemistry Food chemistry	MS can be coupled to separation methods such as gas chromatography (GC) and liquid chromatography (LC). 'Hyphenation', i.e., as GC-MS or LC-MS, delivers high selectivity and low detection limits for the analysis of trace compounds in complicated matrices or the deconvolution of complex mixtures.
Material sciences	eral distribution of compounds on surfaces (microelectronics, slices of tissue) into images, which in turn can be correlated to optical images.
Miniaturization Field portable MS Space missions Military applications	Mass spectrometers can be very small. Portable instruments allow for environmental on-site analysis, detection systems for explosives and warfare chemicals, and last but not least for many space missions.

Interconnected MS workflow





Mass spectrometry is multifaceded rather than to be viewed from a single perspective.

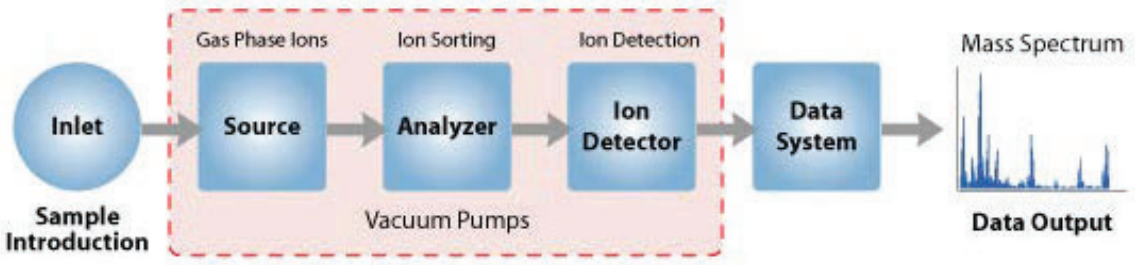
Black Box

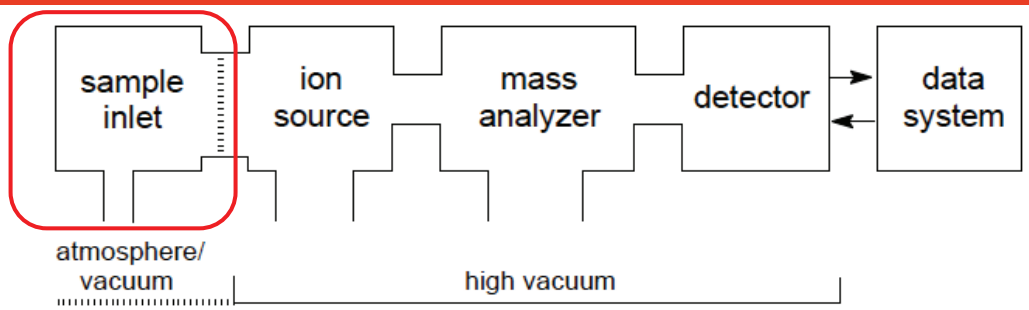
Which parts of MS do you know?

All these boxes have the same scheme, are you able to define this scheme?

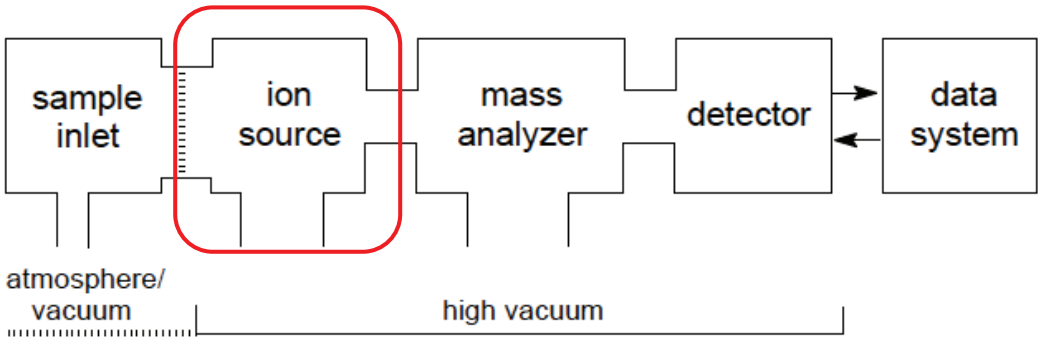
A simple basic scheme

- There is a simple basic scheme that all mass spectrometers follow.





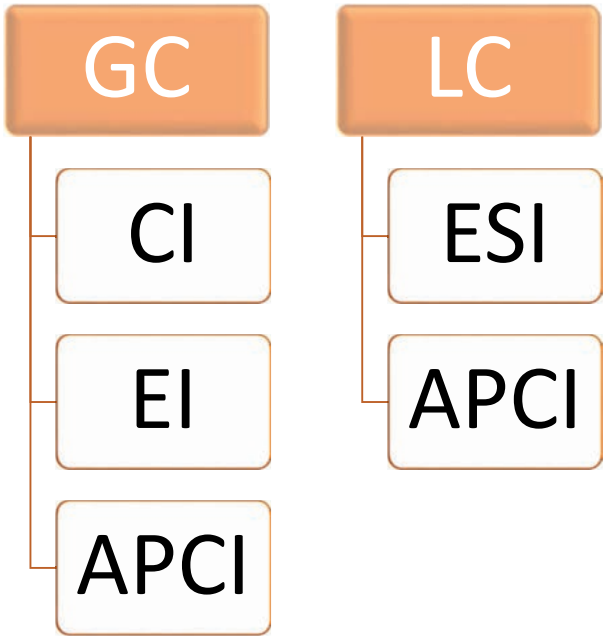
Inlet system	Principle	Analytes
Reservoir/reference inlet	heated reservoir with sample vapor	low to medium boiling liquids
Direct insertion probe, DIP	sample in heated/cooled glass/metal vial as particles or film of analyte	solids, waxes or high-boiling liquids
Direct exposure probe, DEP	sample particles or film of analyte on resistively heated metal filament	solids of extremely low volatility, especially if thermally labile
Gas chromatograph, GC	elutes directly into ion source	volatile components of mixtures
Liquid chromatograph, LC	connected via particle beam interface	analytes suitable for EI that cannot be separated by GC due to high polarity



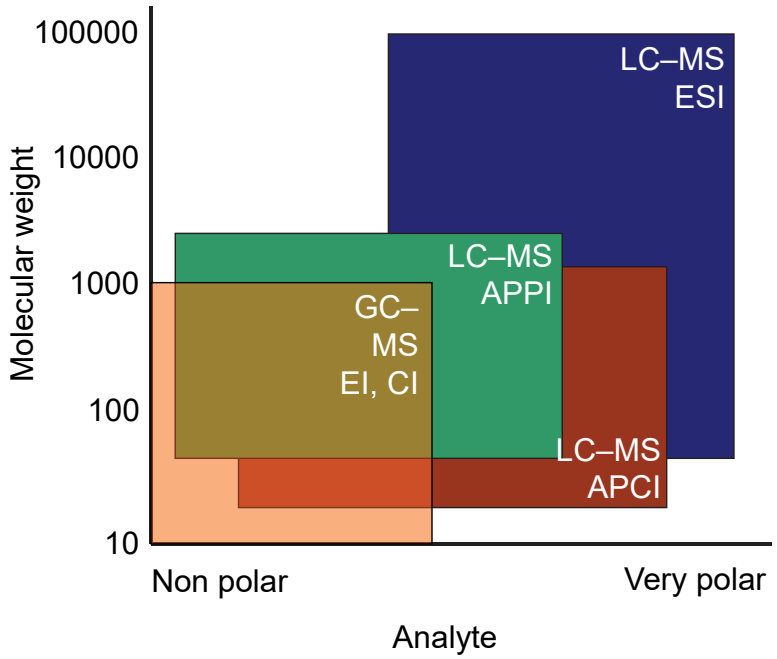
EI: fragment ions are less specific, or fragmentation is too extensive. (70 eV)

APCI:

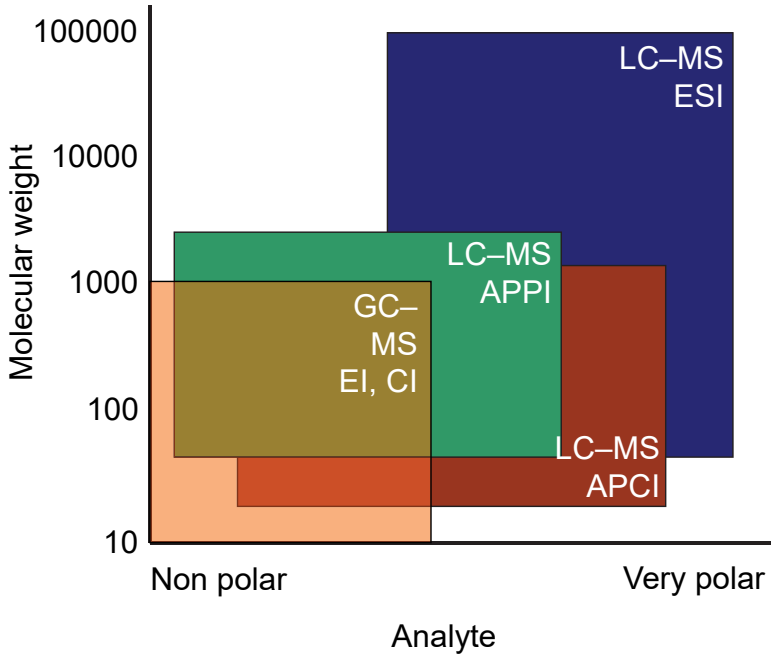
- Protonated molecular ions reduced fragmentation.
- Less fragmentation.
- Greater universality.
- Flexibility to determine volatile and semi-volatile compounds of low and intermediate polarity

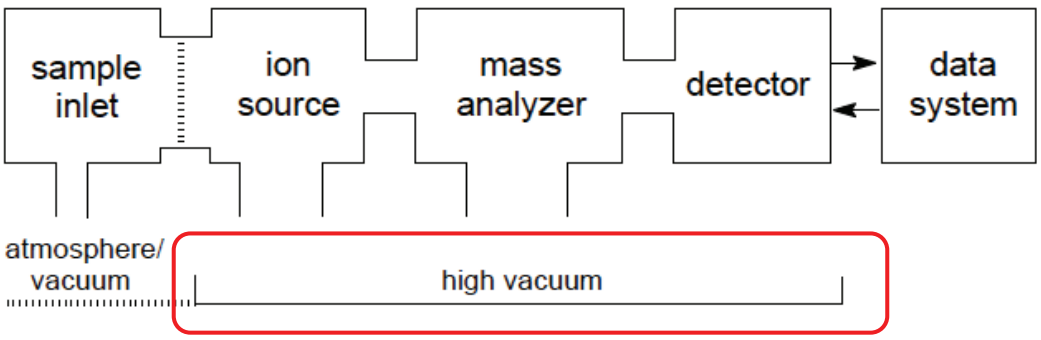


Application potential of MS techniques

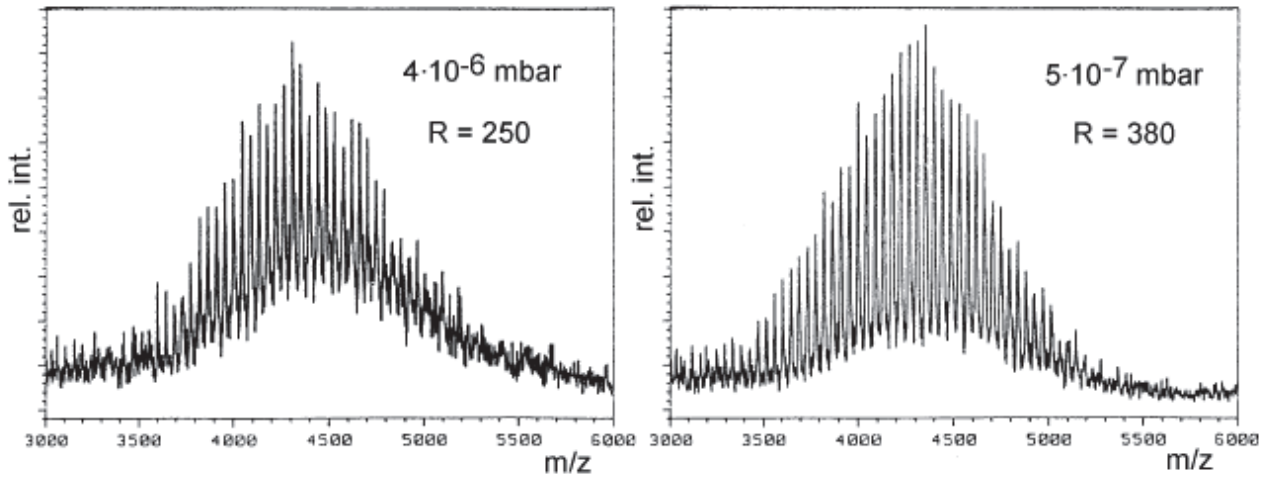


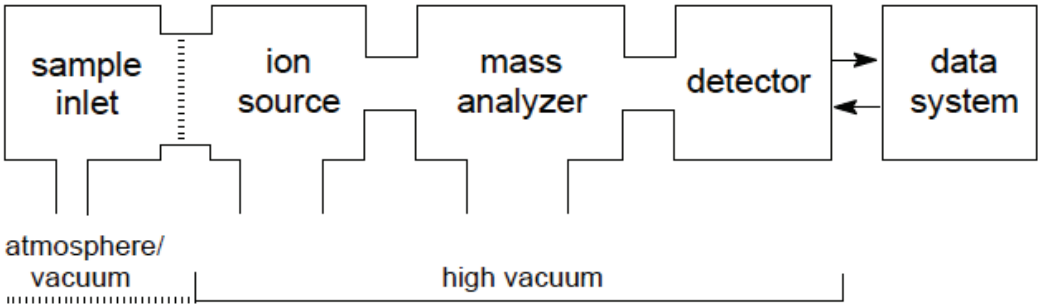
Application potential of MS techniques





Improved vacuum conditions result in **an elongated** mean **free path for the ions** and thus **reduce the risk of collisions in transit through the analyzer**.

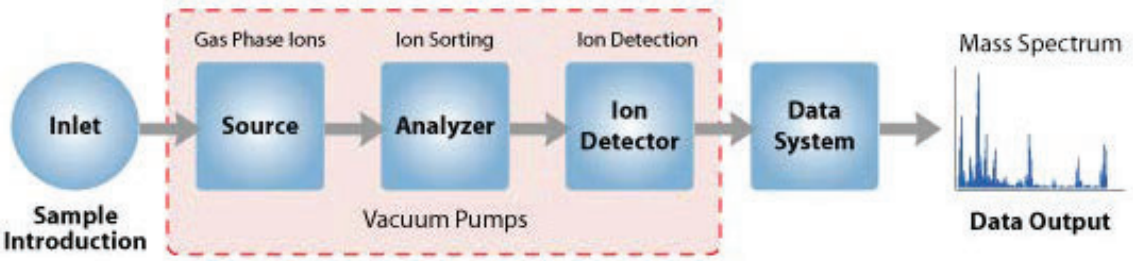




Pressure range [Pa]	Pressure range [mbar]	Pressure range [mtorr]	Vacuum	Gas flow
$10^5 - 10^2$	1 bar – 1 mbar	750 torr – 750 mtorr	Rough vacuum (RV)	Viscous flow
$10^2 - 10^{-1}$	$1 - 10^{-3}$	750 – 0.75	Medium vacuum (MV)	Knudsen flow
$10^{-1} - 10^{-5}$	$10^{-3} - 10^{-7}$	$0.75 - 7.5\times10^{-5}$	High vacuum (HV)	Molecular flow
$< 10^{-5}$	$< 10^{-7}$	$< 7.5 \times 10^{-5}$	Ultrahigh vacuum (UHV)	Molecular flow

The performance of mass analyzers is typically quantified in terms of:

- Mass resolving power and mass resolution
- Mass accuracy
- Acquisition speed
- Dynamic range
- Tandem analysis capabilities



Mass resolution vs. mass resolving power

Who-is-Who?

Mass resolution

- The separation observed in a mass spectrum is termed mass resolution, R, or simply resolution. **Mass resolution is given as the smallest difference in m/z (Δm/z) that can be separated for a given signal.**

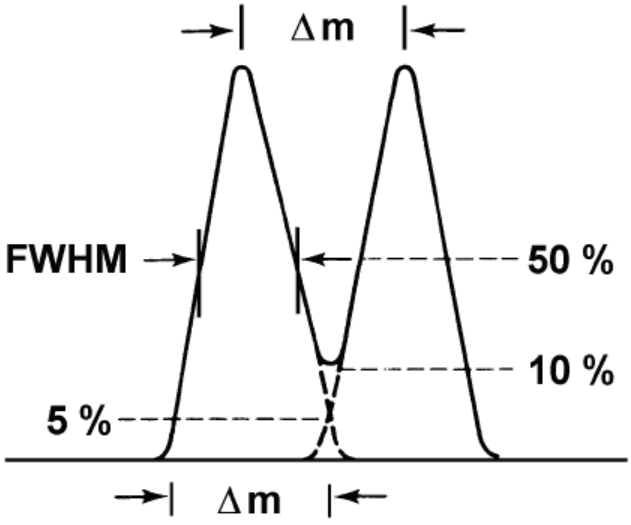
Mass resolving power

According to Commission Decision 2002/657/EC, HRMS is defined as the resolving power of 10.000 for the entire mass range at 10% valley. Moreover, Commission Decision 2002/657/EC does not specify a criterion for mass accuracy.

Nowadays, this value is roughly expressed as 20.000 FWHM (full width at half maximum).

Mass resolving power

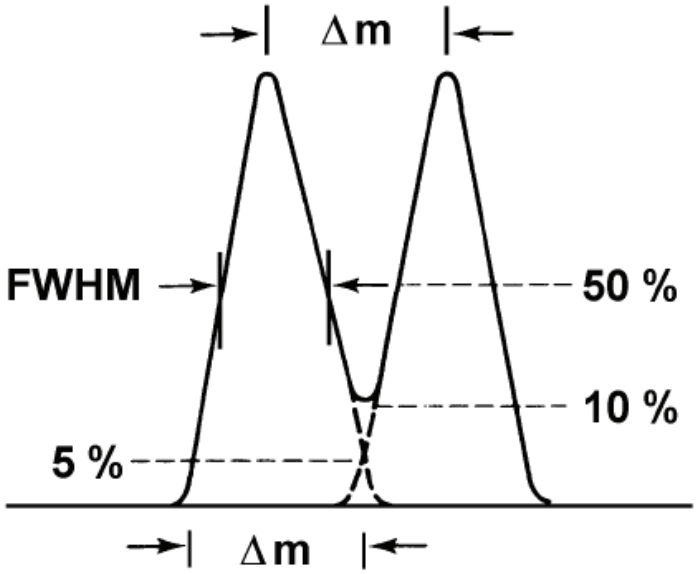
- The ability to distinguish between ions differing in the quotient mass/charge by a small increment.
- 2 definitions:
  - 10% Valley
  - The full width at half maximum (FWHM)



10% valley

Two neighboring peaks are assumed to be sufficiently separated when the valley separating their maxima has decreased to 10% of their intensity.

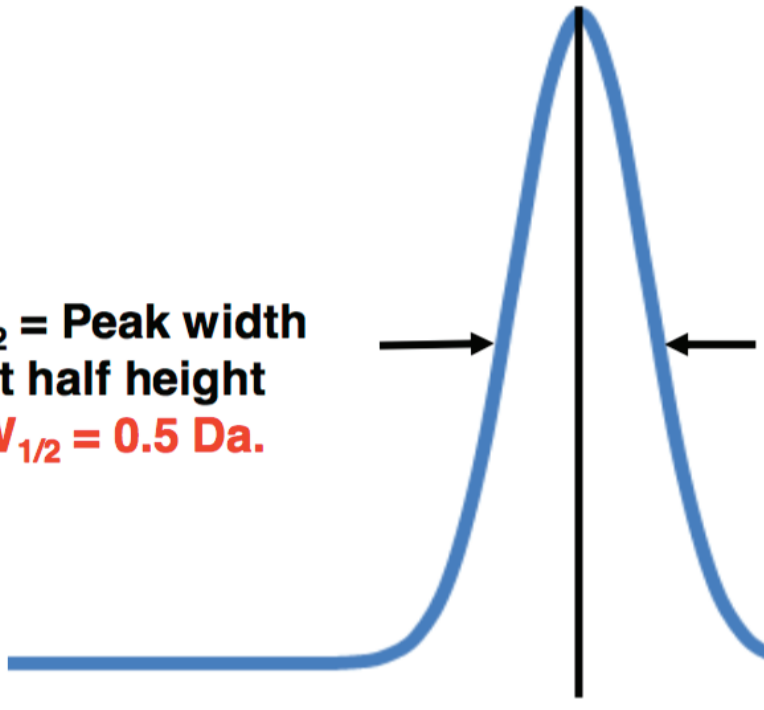
Two peaks of equal height, masses m1 and m2, when there is overlap between the two peaks to a stated percentage of either peak height (**10% is recommended**), then the resolving power is defined as m1/(m1 – m2). **The percentage overlap (or 'valley') concerned must always be stated.**



The full width at half maximum (FWHM)

Mass resolution = (ion mass)/(mass peak width)

$R = (m/z) / W_{1/2}$



$W_{1/2}$ = Peak width at half height

$W_{1/2}$ = 0.5 Da.

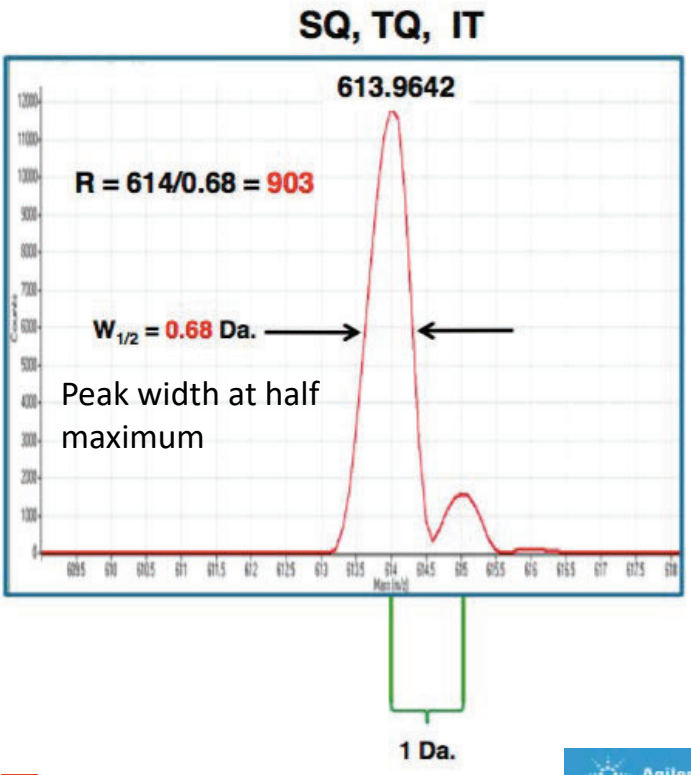
Example:

R = 200 Da./0.5 Da.

R = 400

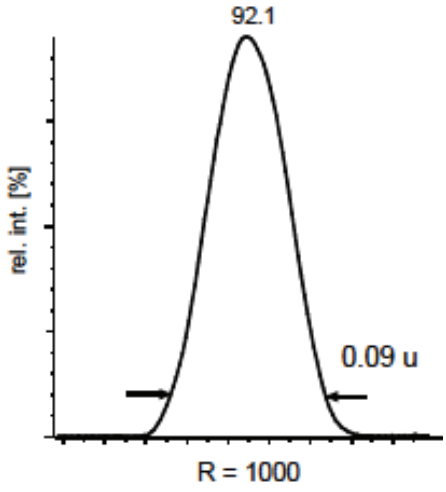
m/z = 200 Da.

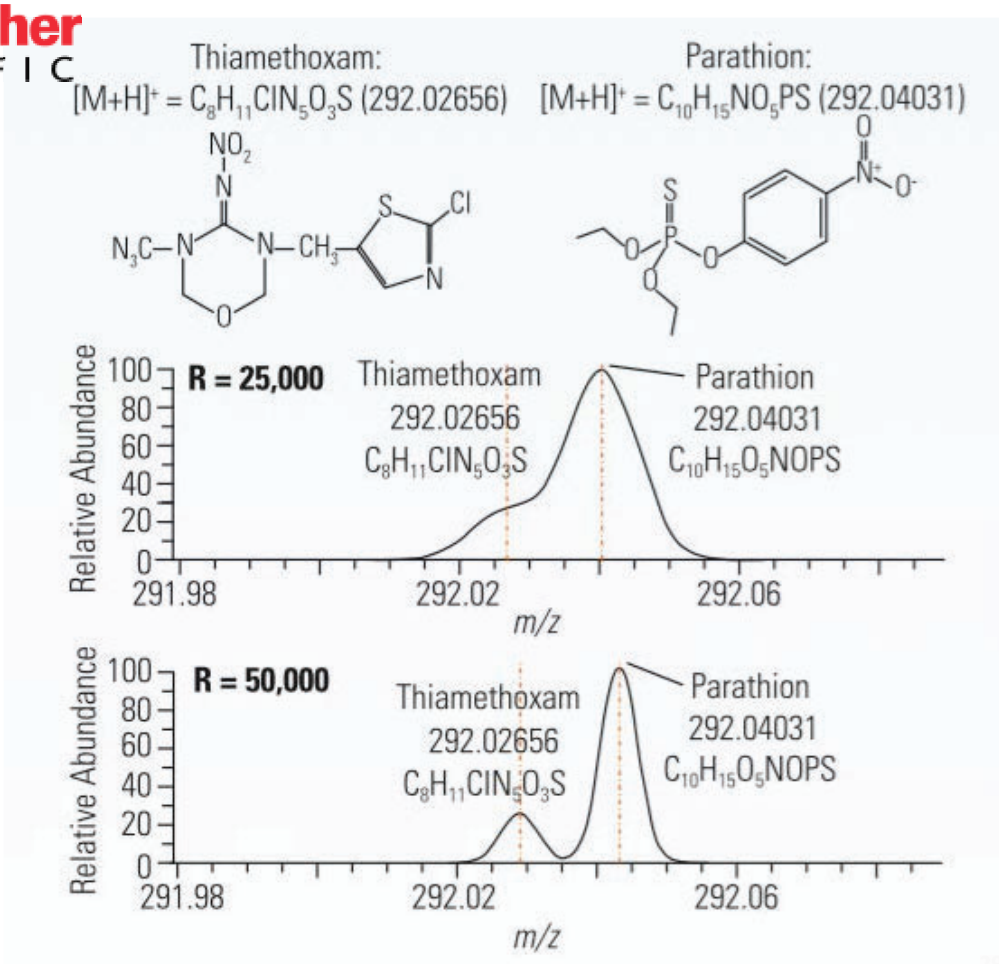
m/z = 613.964203



How can resolving power help us?

- A mixture of xylene (*m/z* 92.0581) and toluene (*m/z* 92.0626) at different settings of resolution





The performance of mass analysers is typically quantified in terms of:

- Mass resolving power and mass resolution
- **Mass accuracy**
- Acquisition speed
- Dynamic range
- Tandem analysis capabilities

What is mass accuracy?

The absolute mass accuracy, Δm/z, is defined as the difference between measured accurate mass and calculated exact mass:

$$\Delta m/z = m/z_{experimental} - m/z_{calculated}$$

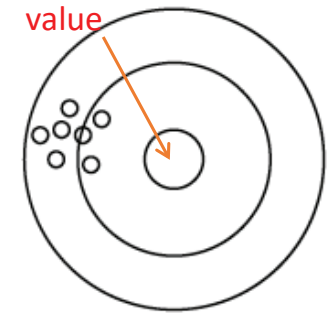
Mass error = (exact mass) – (accurate mass)

Mass error in parts per million (ppm) =

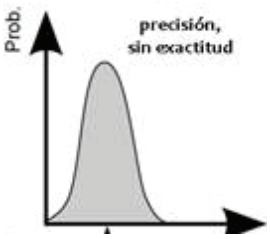
$$\frac{\text{(mass error)}}{\text{(exact mass)}} \times 10^6$$

Why do we need accuracy and precision?

The concepts of accuracy and precision can best be illustrated using the analogy to a target



P+ A-

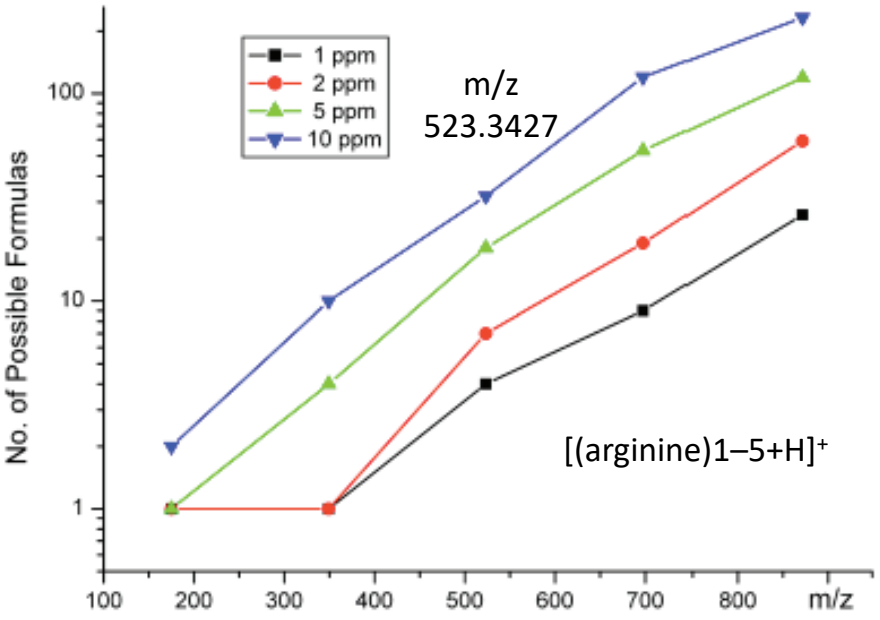


THE ANALOGY

High resolution separates adjacent signals (Precision)

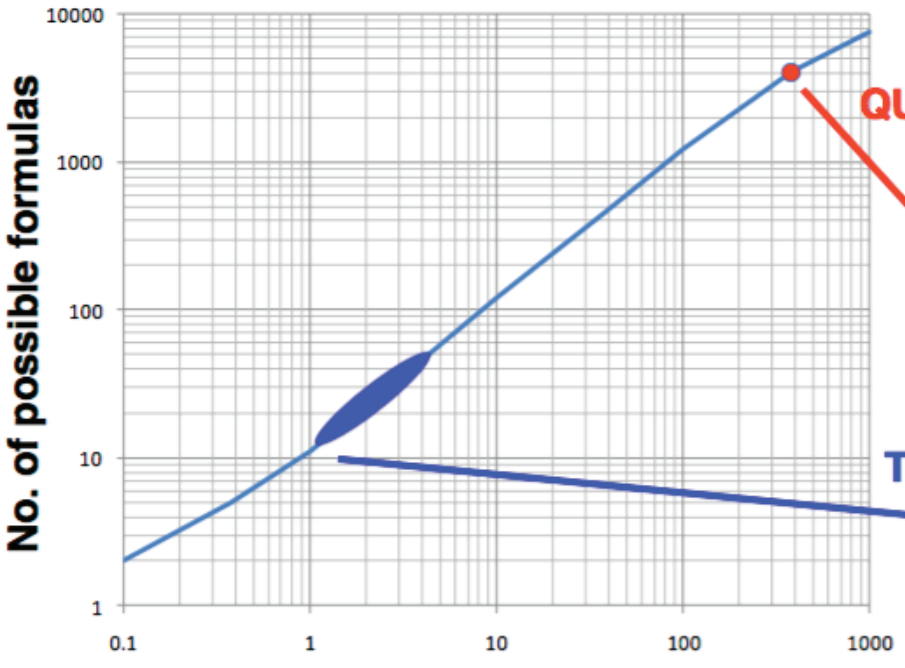
Accurate mass can deliver molecular formulas (Accuracy)

Molecular formulas based on a free selection among the elements C, H, N, O as a function of relative mass error vs. m/z.



The higher the mass error the larger number of candidates

Possible chemical formulas for m/z = $C_{10}F_8$ = 271.98667



mass uncertainty		# of Possible Formulas
ppm	amu	
1000	0.3	7657
368	0.1	4050
100	0.03	1223
37	0.01	466
10	0.003	120
4	0.001	43
1	0.0003	11
0.4	0.0001	5
0.1	0.00003	2

Formulas made of:
C.H.N.O.F. & Cl

Agilent Technologies

The performance of mass analysers is typically quantified in terms of:

- Mass resolving power and mass resolution
- Mass accuracy
- **Acquisition speed**
- Dynamic range
- Tandem analysis capabilities

Scan speed

- Scan Speed refers to the scans per second which can be performed per second. For LC-MS and GC-MS a high number of scans per peak is required to perform correct peak picking and mass spectral deconvolution of full scan mass spectra.
- The time taken to complete this series of events (MS experiments) is referred to as the analytical cycle time.

Have you calculated The Scan speed ☺?

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Multi-analyte high performance liquid chromatography coupled to high resolution tandem mass spectrometry method for control of pesticide residues, mycotoxins, and pyrrolizidine alkaloids

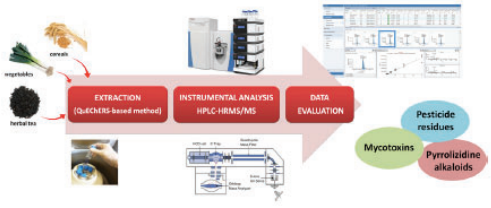
Zbynek Dzuman [a], Milena Zachariasova [a,*], Zdenka Veprikova [a], Michal Godula [b], Jana Hajslova [a]

[a] University of Chemistry and Technology, Prague, Technicka 3, Prague 6, 16628, Czech Republic
[b] Thermo Scientific, Slunecna 27, Prague 10, 10000, Czech Republic

HIGHLIGHTS

- HPLC–HRMS/MS method for analysis of 389 multi-class food contaminants was developed.
- The employed core–shell analytical column showed very good separation efficiency.
- Validation for matrices wheat, leek, and tea was performed.
- Recoveries and limits of quantification complied with the EU legislation.
- The mass spectral library of fragment ions in high resolution was created.

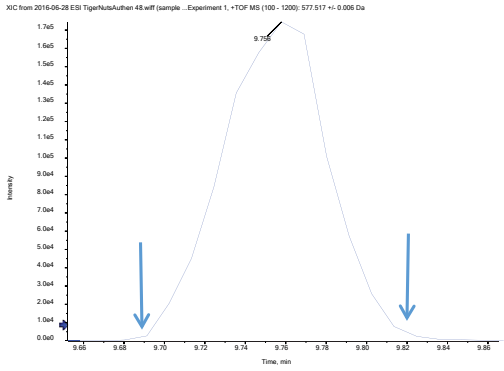
GRAPHICAL ABSTRACT



Q-Orbitrap vs. QTOF system

MS: 3 spectra s^{-1} for 70,000 FWHM (333 ms)
MS/MS: 12 spectra s^{-1} for 17,500 FWHM (83 ms)
The time demands for one full MS–dd-MS/MS cycle would be approx.:

- 1.2 s (333ms for MS plus 10x83ms for MS/MS).
- approx. 10 data points for 12 s chromatographic peak are earned



MS: 100 ms
MS/MS: 50 ms (10 spectra)
The accumulation time took 0.65 s, under these conditions:

- 15 data points (10 s peak)
- 18 data points (12 s peak)
- 23 data points (15 s peak)

What do you need for your MS methods?
- Target method requirements
- Omics strategies

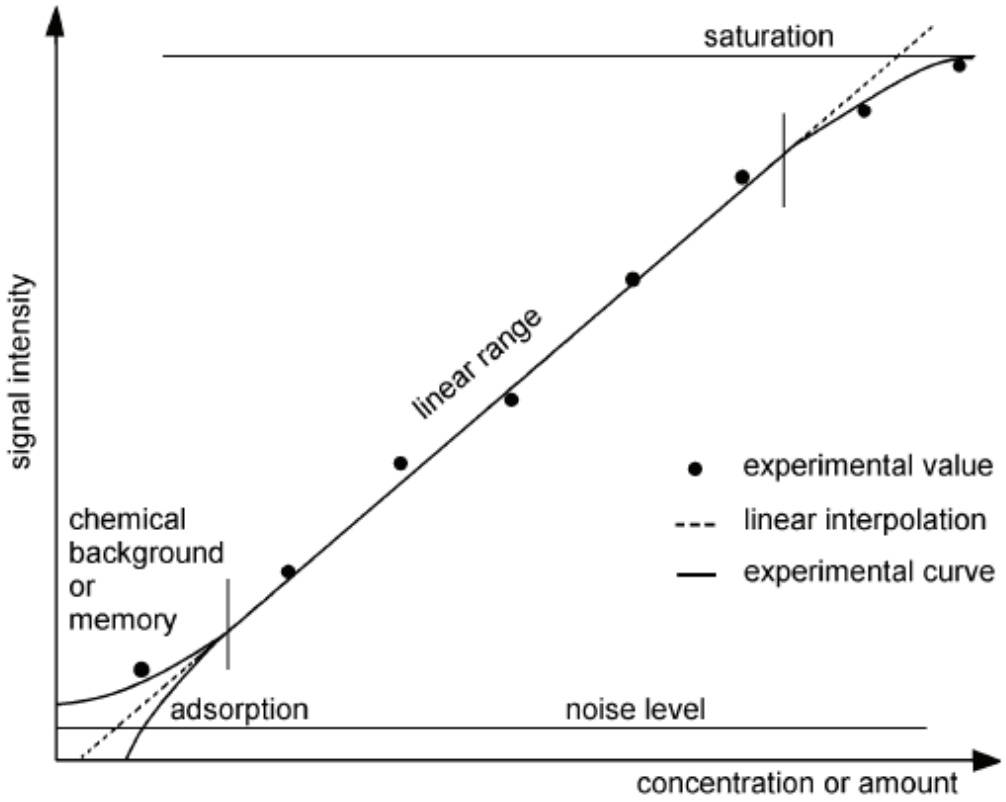
The performance of mass analysers is typically quantified in terms of:

- Mass resolving power and mass resolution
- Mass accuracy
- Acquisition speed
- **Dynamic range**
- Tandem analysis capabilities

Dynamic range

The dynamic range is the ratio obtained by dividing the intensity of the most intense signal by that of the weakest while both are correctly detected in the same spectrum.

Dynamic range



Dynamic range

- LRMS: > 5 orders of linear dynamic range
- HRMS: A minimum linear dynamic range from a few ppb to ppm.
  - A wide linear dynamic range offers substantial advantages: time saved due to lack of re-assays and avoiding complications that are usually associated with dilution.

The performance of mass analysers is typically quantified in terms of:

- Mass resolving power and mass resolution
- Mass accuracy
- Acquisition speed
- Dynamic range
- **Tandem analysis capabilities**

Tandem analysis capabilities

- Tandem mass spectrometry comprises the acquisition and study of the spectra of ionic products or precursors of m/z-selected ions, or of precursor ions of a selected neutral mass loss.
- Tandem MS is also denoted as mass spectrometry/mass spectrometry from which the common acronym MS/MS is derived.

LR vs HR

- Mass resolving power and mass resolution
- Mass accuracy
- Acquisition speed
- Dynamic range
- Tandem analysis capabilities

Focusing on mass resolving power and mass accuracy, low- (LRMS) and high-resolution mass spectrometry (HRMS) can be differentiated

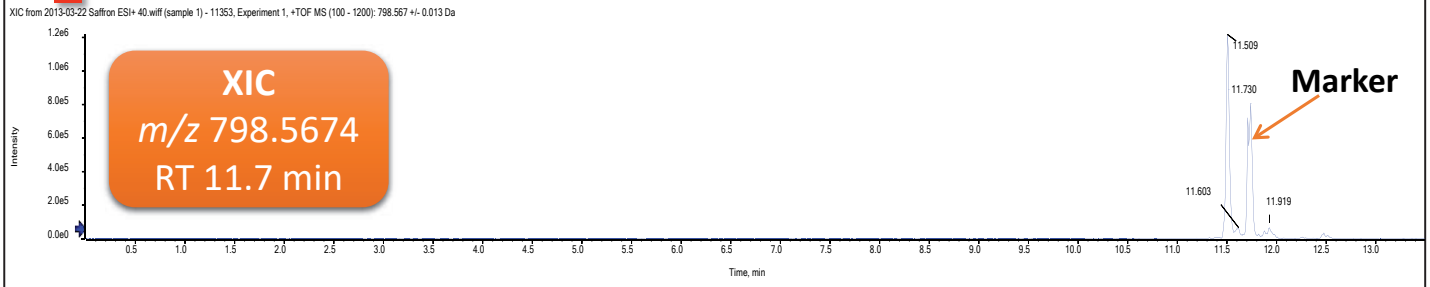
LR vs HR

The attributive low resolution (LR) is generally used to describe spectra obtained at R = 500–2000. High resolution (HR) is appropriate for R > 5000. However, there is no exact definition of these terms.

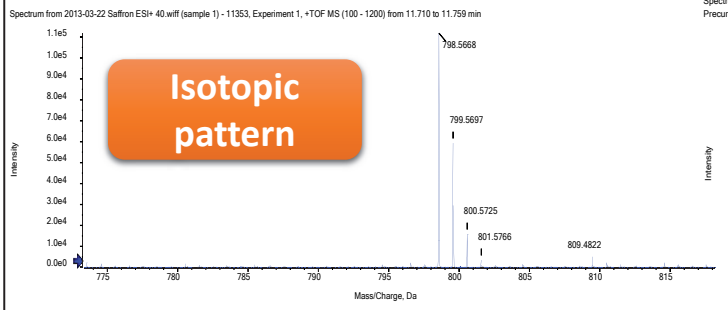
Identification of compounds: Workflow

- PeakView® (Qualitative softwares):
  - Its mass (m/z), isotopic pattern, retention time (RT) and MS/MS
  - Molecular formula
  - MS/MS Pathway
- Libraries: MassBank, METLIN, MMCD, CSFMetabolome, DrugBank, LMSD, PubChem, KEGG, BioCyc, MetaCyc, HumanCyc, Reactome

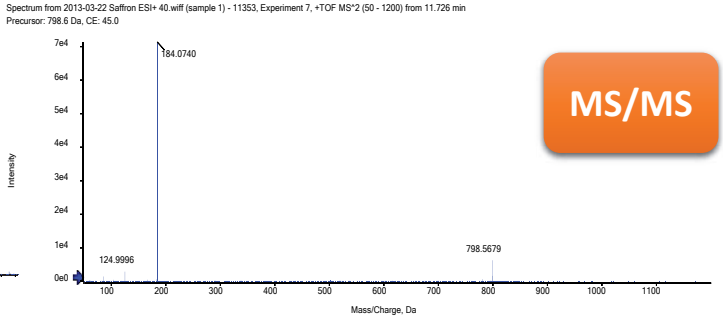
Marker identification: Saffron (PDO)



XIC
m/z 798.5674
RT 11.7 min



Isotopic pattern

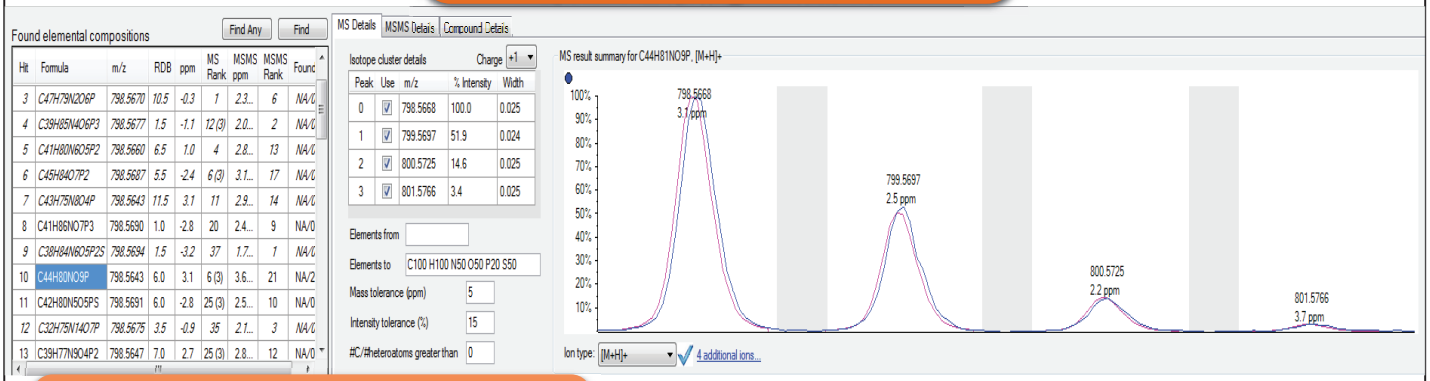


MS/MS

Formula Finder:
1. MS (accurate mass)
2. Isotopic pattern (Theoretical vs. Experimental)
3. MS/MS data, fragment ions

Marker identification: Saffron (PDO)

Formula Finder:
1. **MS (accurate mass)**
2. **Isotopic pattern (Theoretical vs. Experimental)**
3. **MS/MS data, fragment ions**



47 Candidates→$C_{44}H_{80}NO_9P$

ChemSpider
The free chemical database

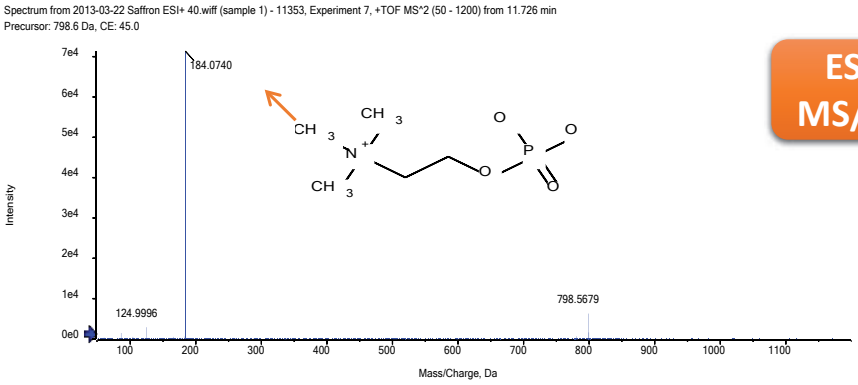
Oxidized glycerophospholipids

Marker identification: Saffron (PDO)

$C_{44}H_{80}NO_9P$

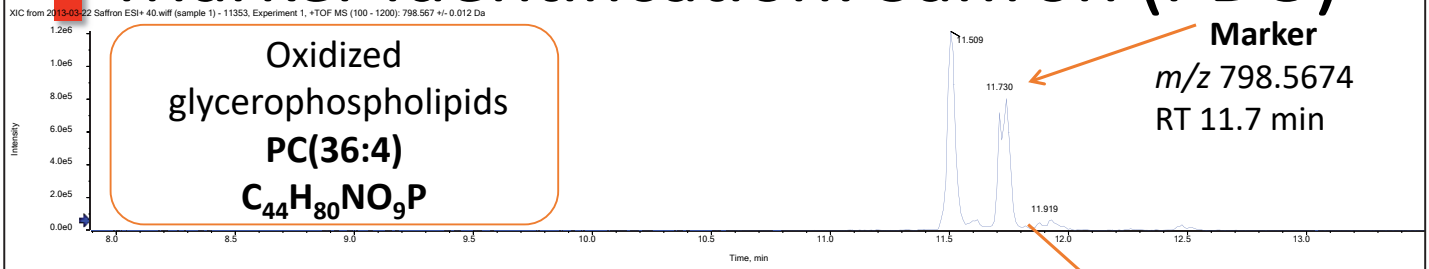
ChemSpider
The free chemical database

Oxidized glycerophospholipids
PC(36:4)



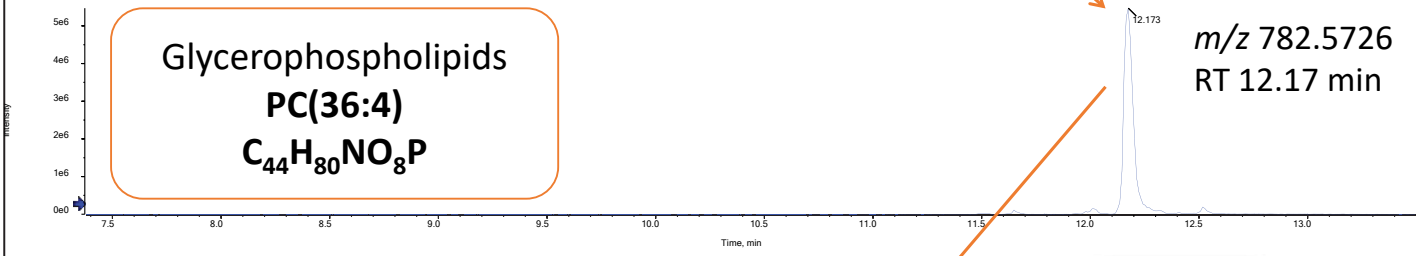
ESI+ MS/MS

Marker identification: Saffron (PDO)



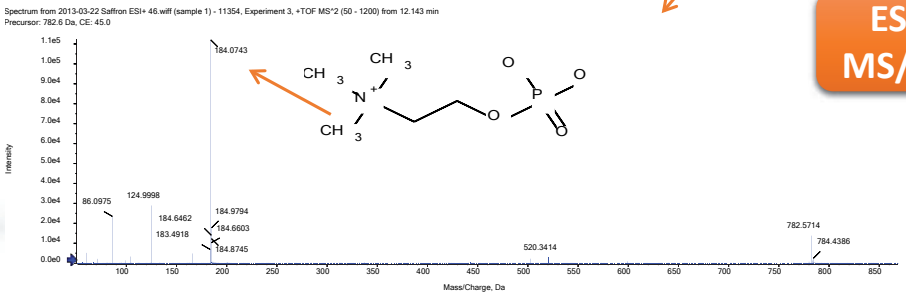
Oxidized glycerophospholipids
PC(36:4)
$C_{44}H_{80}NO_9P$

Marker
m/z 798.5674
RT 11.7 min



Glycerophospholipids
PC(36:4)
$C_{44}H_{80}NO_8P$

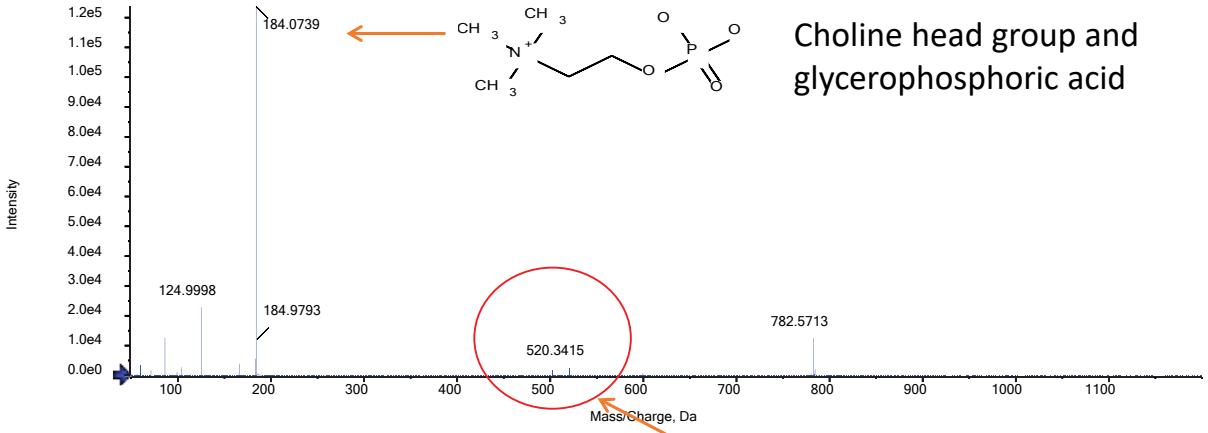
m/z 782.5726
RT 12.17 min



ESI+ MS/MS

Marker identification: Saffron (PDO)

Spectrum from 2013-03-22 Saffron ESI+ 40.wiff (sample 1) - 11353, Experiment 2, +TOF MS^2 (50 - 1200) from 12.188 min
Precursor: 782.6 Da, CE: 45.0



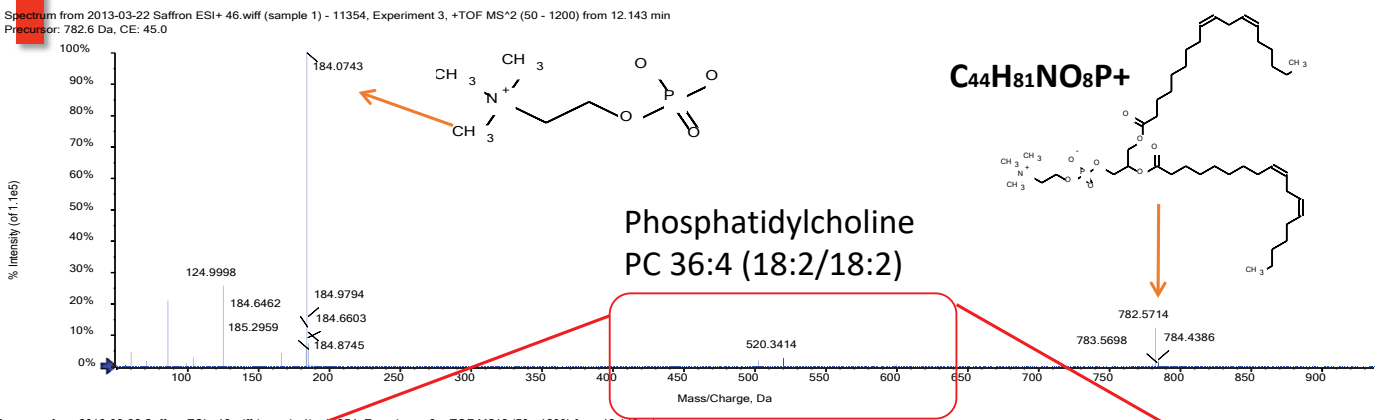
Choline head group and glycerophosphoric acid

ESI+

These product ions could be useful in order to identify hydrophobic chains, and subsequently this marker.

Marker identification: Saffron (PDO)

Spectrum from 2013-03-22 Saffron ESI+ 46.wiff (sample 1) - 11354, Experiment 3, +TOF MS^2 (50 - 1200) from 12.143 min
Precursor: 782.6 Da, CE: 45.0

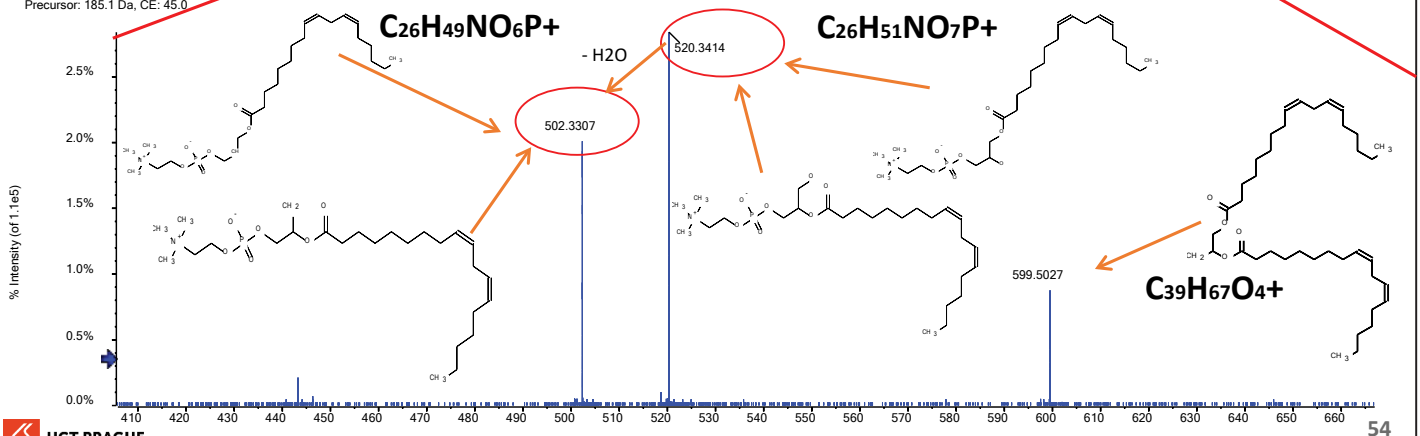


$C_{44}H_{81}NO_8P+$

Phosphatidylcholine
PC 36:4 (18:2/18:2)

Spectrum from 2013-03-22 Saffron ESI+ 46.wiff (sample 1) - 11354, Experiment 3, +TOF MS^2 (50 - 1200) from 12.143 min
Precursor: 782.6 Da, CE: 45.0

Spectrum from 2013-03-22 Saffron ESI+ 46.wiff (sample 1) - 11354, Experiment 2, +TOF MS^2 (50 - 1200) from 0.060 min
Precursor: 185.1 Da, CE: 45.0



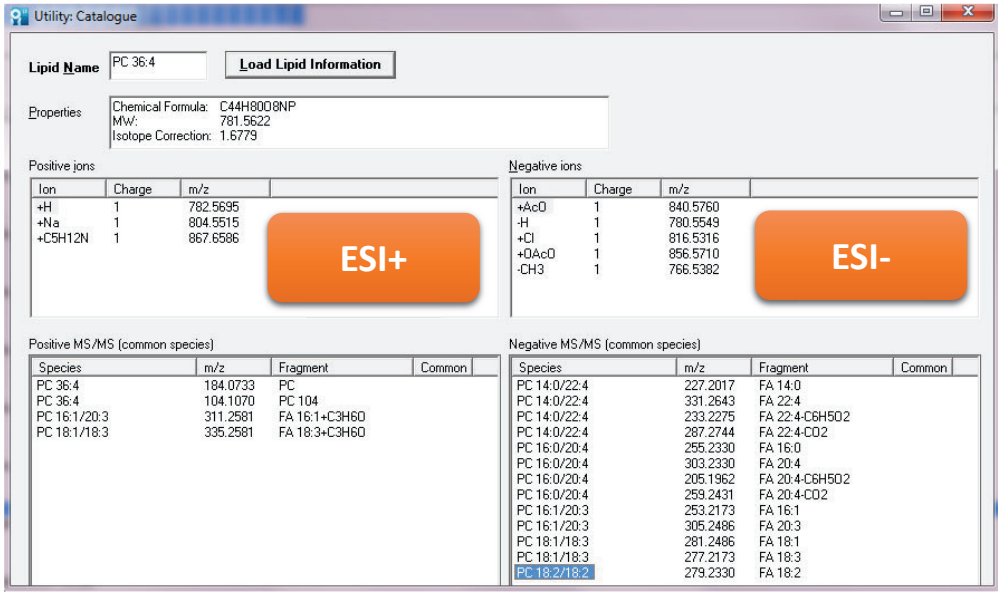
$C_{26}H_{49}NO_6P+$

- H_2O

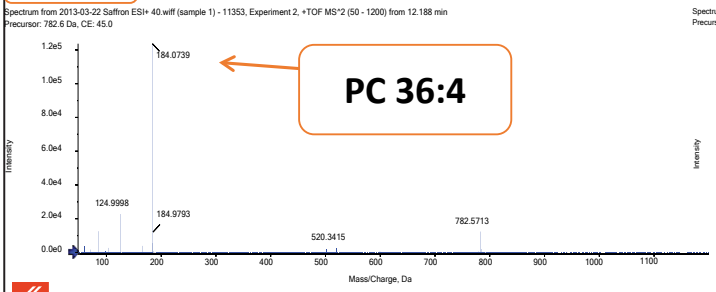
$C_{26}H_{51}NO_7P+$

$C_{39}H_{67}O_4+$

Marker identification: Saffron (PDO)

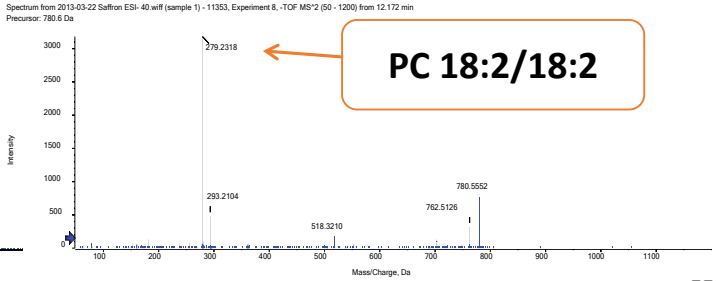


ESI+

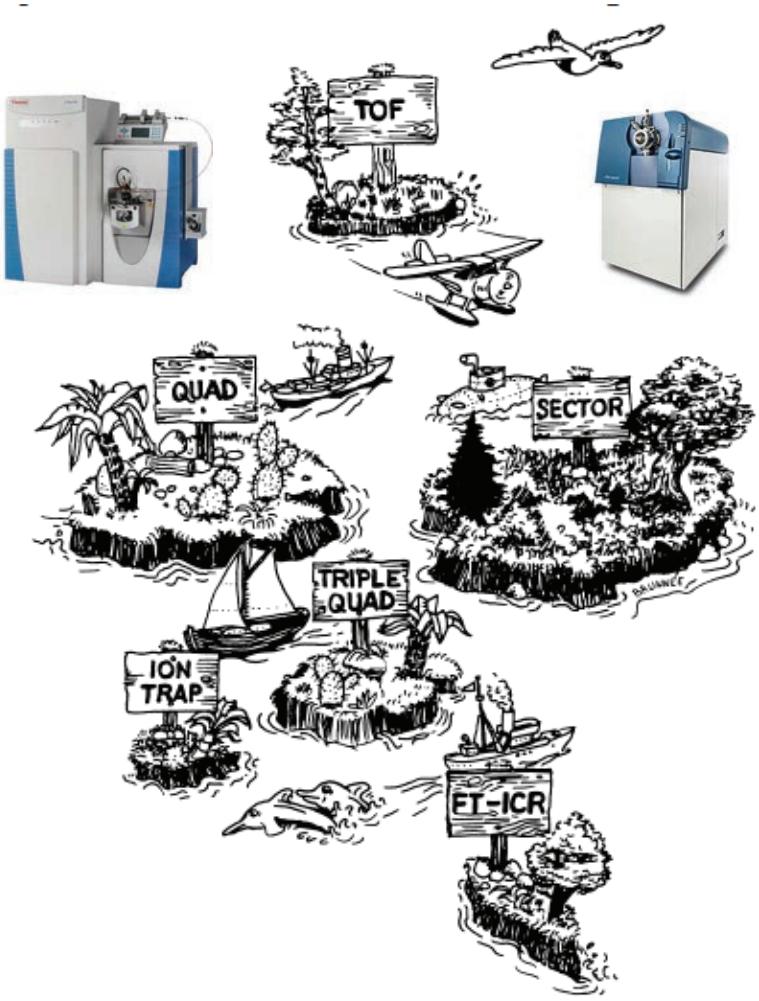


PC 36:4

ESI-



PC 18:2/18:2



Quality assurance in MS

Requirements related to separation

- The minimum acceptable retention time (RT) - at least twice the retention time corresponding to the void volume of the column.
- RT of the analyte in the extract should correspond to that of the calibration standard with a tolerance of ±0.1min, for both GC and LC .
- Larger retention time deviations are acceptable where both retention time and peak shape of the analyte match with those of a suitable IL-IS, or evidence from validation studies is available.
- Isotopically –labelled internal standrds (IL-IS) can be particularly useful where the chromatographic procedure exhibits matrix induced retention time shifts or peak shape distortions.
- Overspiking with the analyte suspected to be present in the sample will also help to increase confidence in the identification.

The use of isotopically labelled std.

IL-ISs can be used to:

- **accurately compensate for both analyte losses and volumetric variations during the procedure**
- **To compensate for matrix effects and response drift in the chromatography-detection system.**
- **Losses during extract storage (e.g. due to degradation)will also be corrected for by the IL-IS.**
- **Use of IL-ISs will not compensate for incomplete extraction of incurred residues**

Requirements for mass spectrometry

MS detection can provide:

- **mass spectra**
- **isotope patterns**
- **signals for selected ions**

identification based on MS spectra – fairly depends on SW

Identification based on selected ions – can be defined

Recommendations regarding identification using MS spectra

- For **reference spectra**, the same instruments and conditions used for analysis of the samples shoul be used
- The reference spectrum in the instrument software can originate from a previous injection (without matrix present), but is preferably obtained from the same analytical batch
- To avoid **distortion of ion ratios** the concentration of the analyte ions must not overload the detector.

In case of full scan measurement, careful **subtraction of background** spectra, either manual or automatic, by deconvolution or other algorithms, may be required to ensure that the resultant spectrum from the chromatographic peak is representative.

Requirements for identification using selected ions

- **Selected ions** must be sufficiently selective for the analyte in the matrix being analysed and in the relevant concentration range.
- **Molecular ions, (de)protonated molecules or adduct ions** are highly characteristic for the analyte and should be included in the measurement and identification procedure whenever possible

Requirements for identification using selected ions, *cont.*

- In general, and especially in single-stage **MS, high m/z ions are more selective** than low m/z ions (e.g. m/z < 100). However, high mass m/z ions arising from loss of water or loss of common moieties may be of little use.
- Although characteristic isotopic ions, especially Cl or Br clusters, may be particularly useful, the **selected ions should not exclusively originate from the same part of the analyte molecule**. The choice of ions for identification may change depending on background interferences.
- In high resolution MS, the **selectivity of an ion of the analyte is determined by the narrowness of the mass extraction window** (MEW) that is used to obtain the extracted ion chromatogram. The narrower the MEW, the higher the selectivity.

Requirements for identification using selected ions, *cont.*

Extracted ion chromatograms of sample extracts

- Peaks should have similar retention time
- Peak shape and response ratio to those obtained from calibration standards analysed at comparable concentrations in the same batch.
- Chromatographic peaks from different selective ions for the analyte must fully overlap.
- Where an ion chromatogram shows evidence of significant chromatographic interference, it must not be relied upon for identification

Identification requirements for different MS techniques

Unit resolution MS

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		minimum number of ions	other
Unit mass resolution	Single MS quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3[d] Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap. Ion ratio from sample extracts should be within **±30% (relative)** of average of calibration standards from same sequence
	MS/MS triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	

[a] preferably including the molecular ion, (de)protonated molecule or adduct ion
[b] including at least one fragment ion
[c] < 1 mDa for m/z < 200
[d] in case noise is absent, a signal should be present in at least 5 subsequent scans

Identification requirements for different MS techniques

High resolution MS

Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a, b, c]	S/N ≥ 3[d] Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap. Ion ratio: see D12

a) preferably including the molecular ion, (de)protonated molecule or adduct ion
b) including at least one fragment ion
c) < 1 mDa for m/z < 200
d) in case noise is absent, a signal should be present in at least 5 subsequent scans

Confirmation

- For a higher degree of confidence in identification, further evidence may be gained from additional mass spectrometric information. For example, evaluation of full scan spectra, isotope pattern, adduct ions, additional accurate mass fragment ions, additional product ions (in MS/MS), or accurate mass product ions.
- The chromatographic profile of the isomers of an analyte may also provide evidence. Additional evidence may be sought using a different chromatographic separation system and/or a different MS-ionisation technique.

THANK YOU FOR YOUR ATTENTION!

Specific Food Quality and Safety Application Requirements

Petr Cuhra

Czech Agriculture and Food Inspection Authority (CAFIA)

Official Food and Feed Control in the CR

Ministry of Agriculture

Ministry of Health

CAFIA:
Food of non-animal origin
Catering service

SVA:
Food of animal origin

CISTA:
Feedingstuffs

PHPB:
Catering service

CAFIA Competencies

Official control of

- production, import, distribution, storage and retail of foods od non-animal origin
- retail of foods of animal origin
- tobacco products

Requirements on Food

Based on Legislation....

Ratio of non-compliance samples in 2017 / 2018 (totally 12329 samples analysed)

↓

Food Safety – related to health (microbiology, pesticides, contaminants, food additives, allergens, toxins etc.)

1,5%

Food Quality– related to composition (nutritional composition, adulteration, labelling, GMO, irradiation etc.)

4,8%

Food Safety Examples

results from official control
period 01/2017 – 06/2018

Food Safety

Contaminants

- **COMMISSION REGULATION (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs**
  1. Nitrates
  2. Mycotoxins
  3. Metals
  4. 3-monochloropropanediol (3-MCPD) and glycidyl fatty acid esters
  5. Dioxins and PCBs
  6. Polycyclic aromatic hydrocarbons
  7. Melamine and its structural analogues
  8. Inherent plant toxins

1. Nitrates

Maximum limits (ML) for

- spinach, lettuce, rucola, processed cereal-based foods and baby foods - contaminant
- however $NaNO_3$ and KNO_3 are also food additves (cheese, meat and fish products)

Findings (01/2017 – 06/2018)

- 135 samples analysed, **2 samples exeeded ML (1,48%)**
- Rucola (conatminant), Bacon (food additive)

2. Mycotoxins

Maximum limits (ML) for

- Aflatoxins – peanuts, nuts, dried fruits, cereals, milk, spices, baby foods
- Ochratoxin A – cereals and cereal products, raisins, coffee, wine, grape juice, spices, liquorice, baby foods
- Patulin – fruit drinks and baby foods
- Deoxynivalenol, Zearalenole – cereals and cereal products
- Fumonisin – maize and maize products,
- T-2 and HT-2 toxin – cereals (not yet adopted),
- Citrinin - food supplements based on rice fermented with red yeast *Monascus purpureus*
- Ergot sclerotia and ergot alkaloids – cereals and cereal products

2. Mycotoxins

Aflatoxins

Specifics requirments for official control (sampling, import control)

Findings (01/2017 – 06/2018)

- 262 samples analysed
- **5 samples exeeded ML (1,91 %)**
- 2x Hazelnuts (Azerbaijan), 3x Figs (Turkey, Greece)

2. Mycotoxins

Ochratoxin A

Specifics requirments for official control (sampling)

Findings (01/2017 – 06/2018)

- 196 samples analysed
- **4 samples exeeded ML (2,04 %)**
- 3x Raisins (Iran), 1x Herbal tea (Slovakia)

2. Mycotoxins

Deoxynivalenol

Findings (01/2017 – 06/2018)

- 99 samples analysed
- **0 samples exeeded ML**

Zearalenone

Findings (01/2017 – 06/2018)

- 106 samples analysed
- **0 samples exeeded ML**

Fumonisins

Findings (01/2017 – 06/2018)

- 106 samples analysed
- **0 samples exeeded ML**

2. Mycotoxins

Patulin

Findings (01/2017 – 06/2018)

- 50 samples analysed
- **0 samples exeeded ML**

T-2 and HT-2 toxins

Findings (01/2017 – 06/2018)

- 58 samples analysed
- **only 1 sample pozitive finding, no ML adopted yet**

3. Metals

Maximum limits (ML) for

- Lead – milk, baby foods, meat, offal, fish, cereals, pulses, sea foods, vegetables, fruits, fats, wine, food supplements, honey
- Cadmium - cereals, vegetables, fruits, funghi, cocoa, , sea foods fats, baby foods, meat, offal, fish, food supplements,
- Mercury – fish and fishery products, food supplements
- Tin (inorganic) – canned food and beverages,
- Arsenic (inorganic) - rice

3. Metals

Lead

Findings (01/2017 – 06/2018)

- 221 samples analysed
- **1 samples exeeded ML (0,45 %)**
- Food Supplement

Cadmium

Findings (01/2017 – 06/2018)

- 213 samples analysed
- **1 samples exeeded ML (0,47 %)**
- Parsnip

3. Metals

Mercury

Findings (01/2017 – 06/2018)

- 61 samples analysed
- **0 samples exeeded ML, 18 positive samples (30%)**

Tin

Findings (01/2017 – 06/2018)

- 7 samples analysed
- **0 samples exeeded ML, 3 positive samples (43%)**

Arsenic

Findings (01/2017 – 06/2018)

- 43 samples analysed
- **0 samples exeeded ML, 23 positive samples (53%)**

4. MCPD and glycidol

Maximum limits (ML) for

- 3-monochloropropanediol (3-MCPD) – Hydrolysed vegetable protein, soy sauce
- Glycidyl fatty acid esters expressed as glycidol – vegetable oils and fats, baby foods

4. MCPD and glycidol

3-MCPD

Findings (01/2017 – 06/2018)

- 24 samples analysed
- **0 samples exeeded ML, 1 positive sample (4%) – techlology for production of HVP and soy sauce was changed / improved**

Glycidyl fatty acid esters expressed as glycidol

Findings (01/2017 – 06/2018)

- 28 samples analysed (just monitoring – ML since February 2018)
- **0 samples exeeded ML, 15 positive samples (54%)**

5. Dioxins and PCBs

Maximum limits (ML) for

- Sum of dioxins (WHO-PCDD/ F-TEQ)
- Sum of dioxins and dioxin-like PCBS (WHO- PCDD/F-PCB-TEQ)
- Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6)
  - – Meat and meat products, liver, fish and fishery products, marine oil, milk and diary products, eggs, animal fat, vegetable oils and fats, baby foods

5. Dioxins and PCBs

Sum of dioxins (WHO-PCDD/ F-TEQ)

Sum of dioxins and dioxin-like PCBS (WHO- PCDD/F-PCB-TEQ)

Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6)

Findings (01/2017 – 06/2018)

- 13 samples analysed
- **0 samples exeeded ML, 9 positive samples (69%)**
- **exceedances in case of accident and fatal fails....**

6. Polycyclic aromatic hydrocarbons

Maximum limits (ML) for

- Benzo(a)pyrene
- Sum of benzo(a)- pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene
  - Oils and fats, Cocoa beans and derived products, Cocoa fibre, Coconut oil, Smoked meat and smoked meat products, smoked fish and smoked fishery products, Smoked sprats and canned smoked sprats, baby foods, banana chips,
  - food supplements, dried herbs and spices

6. Polycyclic aromatic hydrocarbons

Benzo(a)pyrene

Sum of benzo(a)- pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene

Findings (01/2017 – 06/2018)

- 82 samples analysed
- **1 samples exeeded ML, 77 positive samples (97%)**
- **Food Supplement (herbal tea)**

Contaminants - summary

Analyte	Number of samples	Positive	% Positive	Non-compliance	% Non-compliance
Ochratoxin A	196	63	32	4	**2,04**
Aflatoxins	262	21	7	5	**1,91**
Nitrates	135	127	94	4	**1,48**
Sum of PAH	79	77	97	1	**1,27**
Benzo[a]pyren	82	65	79	1	**1,22**
Cadmium	213	104	49	1	**0,47**
Lead	221	27	12	1	**0,45**

Food Safety

Pesticides

- **REGULATION (EC) No 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on maximum residue levels of pesticides in or on food and feed of plant and animal origin**
  - 1375 active substances covered by pesticide legislation
    - 478 of them approved as PPP (MRLs set)
    - 788 of them not approved as PPP (MLR = 0,01 mg/kg)
  - big challenge from point of analytical methods
  - use of multiresidual methods (MRM) and „single“ methods – based on mass spectrometry (LC and GC/MS)

Samples for pesticide residues

Year	2010	2012	2013	2014	2015
Total No. of Samples	1148	1251	1038	1567	1262
- positive samples	336	758	603	1048	836
- non - compliance	29	27	34	43	22
Number of analysed pesticides	355	427	427	440	453

Ratio of positive and non-compliance samples

Year	2010	2012	2013	2014	2015
Total No. of Samples	1148	1251	1038	1567	1262
- positive (%)	**29**	**61**	**58**	**67**	**66**
- non- compliant (%)	**2,5**	**2,2**	**3,3**	**2,7**	**1,7**
Number of analysed pesticides	355	427	427	440	453

Pesticides

Most frequently exceeded pesticides

9x chlorpyrifos

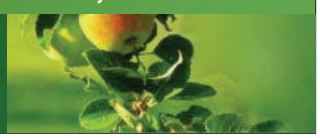
6x carbofuran, tolfenpyrad

5x dinotefuran

3x propargite, folpet

Findings (01/2017 – 06/2018)

- 1690 samples analysed
- **41 samples exeeded MRL (2,4%),**
  - **11x Tea (China, Japan),**
  - **7x Apples (Poland, Czech Republic),**
  - **5x Goji (China),**
  - **1x Apricots, Grapes, Strawberries, Spinach, Cauliflower, Cabbage, Carrot, Mango...**



Contaminants and Pesticides - summary

Analyte	Number of samples	Positive	% Positive	Non-compliance	% Non-compliance
Pesticide residues	1690	1115	66	41	2,43
Ochratoxin A	196	63	32	4	2,04
Aflatoxins	262	21	7	5	1,91
Nitrates	135	127	94	4	1,48
Sum of PAH	79	77	97	1	1,27
Benzo[a]pyren	82	65	79	1	1,22
Cadmium	213	104	49	1	0,47
Lead	221	27	12	1	0,45

Food Safety

Food Additives

REGULATION (EC) No 1333/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on food additives

- food additives
  - intentionally added to the foods for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage ,
  - are not normally consumed as a food,
- approx. 390 food additives covered by legislation
  - only part of them with maximum limits – priority for official control
  - combinations commodity x additive – positive list
  - aversion against food additives

Hazard Food?

E101
E161
E300
E309
E375
E621

E160a
E251
E307
E325
E440

E160d
E262
E308
E330
E 554

Contains 16 food additives – is it too much?

Hazard Food?

E101 vit. B_2
E161 lutein
E300 vit. C
E309 δ-tocopherol
E375 niacin
E621 glutamate Na

E160a carotenes
E251 nitrates
E307 α-tocopherol
E325 lactate Na
E440 pectins

E160d lycopen
E262 acetate Na
E308 γ-tocopherol
E330 citric acid
E 554 phosphates

They seem to be natural compounds – not so bad as it looked...

It is just Tomato.....

To consider food with „E“ automaticaly as hazard food is not correct

- number of food additives is natural part of food
- use of food additives is exactly specified and restricted
- most of food additives are considered as not toxic

Food Additives

Most frequent non-compliances (N/C)

- colours (E110, E120, E129, E131, E133...) – 32 out of 710 samples
- sweeteners (sucralose, acesulfam K) – 7 out of 201 samples
- preservatives (SO2, sorbic acid, benzoic acid) – 52 out of 1178 samp.
- other – polyphosphates etc.

Findings (01/2017 – 06/2018)

- 2760 samples analysed
- **81 samples non-compliance (2,93%)**
  - **wine (SO2, colours)**
  - **honey (caramel)**
  - **soft drinks (sucralose)**
  - **confectionery (colours)**
- often „only“ missing labelling of FA – not related to the food safety

Reasons for N/C
1. approved FA for commodity x ML exceeded
2. approved FA for commodity x not in labelling
3. not approved FA for particular commodity

Contaminants, Pesticides and Food Additives- summary

Analyte	Number of samples	Positive	% Positive	Non-compliance	% Non-compliance
Food additives	2760			81	2,93
Pesticide residues	1690	1115	66	41	2,43
Ochratoxin A	196	63	32	4	2,04
Aflatoxins	262	21	7	5	1,91
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Sum of PAH	79	77	97	1	1,27
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Cadmium	213	104	49	1	0,47
Lead	221	27	12	1	0,45

Food Safety

Microbiology

COMMISSION REGULATION (EC) No 2073/2005 on microbiological criteria for foodstuffs

- microorganisms and related products (toxins - Staphylococcal enterotoxins, metabolites – Histamine)
- Limits for selected microorganisms
  - Listeria monocytogenes
  - Salmonella spp.
  - Escherichia coli
  - Campylobacter spp.
  - Enterobacteriaceae
  - Cronobacter spp.

Microbiology

Most frequent non-compliances (N/C)

Salmonella spp. – 8 out of 2338 samples (0,34%)

L. monocytogenes – 11 out of 3579 samples (0,31%)

Enterobacteriaceae – 34 out of 102 samples (33%)

E. coli – 7 out of 305 samples (2,3%)

Findings (01/2017 – 06/2018)

- 3579 samples analysed
- **103 samples non-compliance (2,88%),**
  - L. monocytogenes – salads, humus spread, delicatessen
  - Salmonella – meat product (raw), salads, spinach, confectionery
  - Enterobacteriaceae - ice creams, ice (for soft drinks)

Contaminants, Pesticides, Food Additives, Microbiology - summary

Analyte	Number of samples	Positive	% Positive	Non-compliance	% Non-compliance
Food additives	2760	-	-	81	2,93
Microbiology	3579	-	-	103	2,88
Pesticide residues	1690	1115	66	41	2,43
Ochratoxin A	196	63	32	4	2,04
Aflatoxins	262	21	7	5	1,91
Nitrates	135	127	94	4	1,48
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Requirements on Food

Based on Legislation....

Ratio of non-compliance samples in 2017 / 2018 (totally 12329 samples analysed)

↓

Food Safety – related to health (microbiology, pesticides, contaminants, food additives, allergens, toxins etc.)

1,5%

Food Quality– related to composition (nutritional composition, adulteration, labelling, GMO, irradiation etc.)

4,8%

Food Quality Examples

results from official control
period 2002 - 2018

Food Quality

Quality requirements

- **EU REGULATIONs and DIRECTIVEs**
  1. Milk and milk products
  2. Olive oils
  3. Chocolate
  4. Spirit drinks
  5. Fruit and vegetables
  6. Juices and nectars
  7. Wine
  8. Tobacco
  9. .....

Food Quality

Nutritional labelling

- **REGULATION (EU) No 1169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the provision of food information to consumers**
  1. Proteins – 21 out of 833 samples (2,52%)
  2. Fat – 15 out of 937 samples (2,04%)
  3. Carbohydrates/sugars – 14 out of 880 samples (1,59%)
  4. Vitamins – 20 out of 420 samples (4,76%)
     - vitamin C - 12 out of 143 samples (8,39%)
  5. Energy – 1 out of 23 samples (4,35%)
  6. Fiber .....

Food Quality

Adulteration

- **REGULATION (EU) No 1169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the provision of food information to consumers**
- Food information shall not be misleading
  - as to the characteristics of the food and, in particular, as to its nature, identity, properties, composition, quantity, durability, country of origin or place of provenance, method of manufacture or production;
  - by attributing to the food effects or properties which it does not possess;
  - by suggesting that the food possesses special characteristics when in fact all similar foods possess...
- Food information shall be accurate, clear and easy to understand for the consumers;

Adulteration

Analytical challenge

- analytical strategy depends on food commodity a type of adulteration
- adulteration could be very „simple“ (addition of water into meat), but also very sophisticated (addition of enzymaticaly hydrolysed sugars)
- to reveal adulteration needs skilled staff and different techniques and methods including isotopic method (NMR, IRMS), chromatography, mass spektrometry, PCR, sensory analysis etc.

Some examples....

Wine – data from 2015

- more than 450 non-compliance samples
  - 64 water addition
  - 53 addition of synthetic glycerol
  - 44 ethanol from other origin than grapes
  - 29 addition of sugars (maltose and saccharose)
  - 24 addition of synthetic colours (tartrazine, ponceau, azorubine)
  - 14 geographical origin
  - 12 addition of sweeteners (saccharin, aspartam…)
  - 4 addition of citric acid

Honey

- 106 non-compliance samples
  - 63 activity of diastase
  - 21 addition of caramel (E 150a)
  - 21 sugar addition (delta d^{13}C
  - 2 β-fructofuranosidase
  - geographical and botanical origin

Jams and Marmalades

Determination of fruit content – calculation from characteristic markers (organic acids, sugars, flavoniods, mineraks, aminoacids atc...)

Since 2003 approx. 120 samples, out of them 44 non-compliance:

- lower fruit content
- presence of undeclared fruit (apples, pumpkin...)

Extrem finding: declaration: 40% blueberries
reality <15% blueberries, 30% apples + aroma

Coffee

Coffee authentification – based on sugar determination of possible adulterants

- other plants (chicory, cereals, figs, malt….)
- carbohydrates (starch, maltodextrin, sugars ....)
- coffee husks

Analysis of sugars (mono- a disacharides)

- glucose after hydrolysis – starch, maltodextrins, cereals
- xylose and manitol – coffee husks,
- fruktose - chicory

Coffee

Since 2000 have bee analysed approx. 100 coffee samples, (mostly soluble), out of them 21 non-compliance - amounts of replacements – between 5% and 82% (!!!)

Coffee content: approx. 20% (rest ~ 80% starch)

Cocoa

S BUDGET LOW-FAT COCOA POWDER

- declaration: 100 % cocoa content
- findings:
  - 80 % cocoa content (theobromine and caffein content)
  - 16 % of carbohydrates (starch, maltodextrine)
  - 4 % cocoa husks (LAT lignoceric acid triptamid)

Cocoa and cocoa products

- Baron Chocolate – milk chocolate requirements:
  - cocoa matter content: min 25%
  - other fat than cocoa butter (CBE): max. 5%
- Findings
  - cocoa matter content: 20 %
  - other fat than cocoa butter (CBE): 14 %

CAFIA corrective tools

- **Measures**
  - ban of the production or placing on the market
  - ban of the use of packaging, instruments and equipment
  - ban of the use of manufacturing premises
  - destruction of unsafe foodstuffs
  - imposition of corrective measures
- **Fines in law**
  - **more than 100 mil CZK (approx. 4 mil EUR) per year**
- **Publication**
  - **media, internet, CAFIA web**

CAFIA website
www.szpi.gov.cz

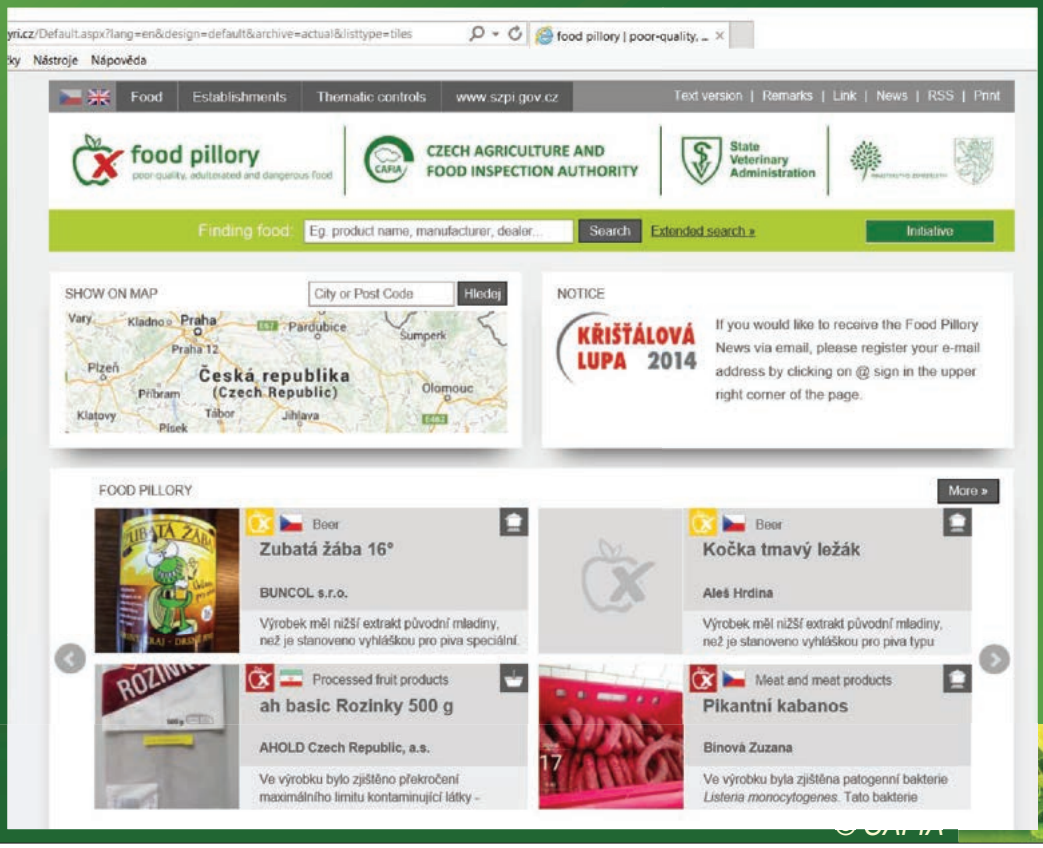
„Foods On Pillory“

Since 2012

- transparent publication of all non-compliant samples
- three categories of infringement
  - food safety
  - food quality
  - food adulteration
- all details regarding producer, retail, lot number, findings etc.
- popular by consumers….

Since 2012….

„Foods On Pillory“
www.potravinynapranyri.cz

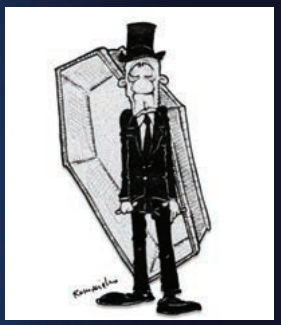


Summary

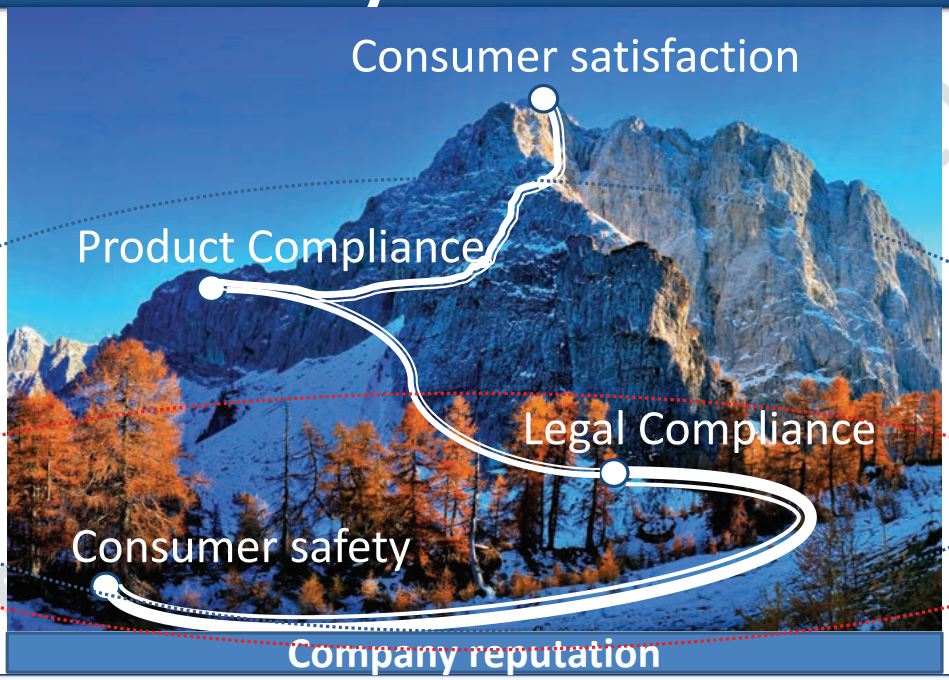
- Requirements on Food Safety and Quality
  - have to reflect food law and legislation
  - have to cover wide range of methods
  - should be based on risk analysis
  - should be flexible and should be changed according to the actual information and alerts (RASFF, AACS)
- To set Food Quality and Safety Application Requirements is not easy job...

Thank you for your attention

Q&FS: The way we are seen ….and why we are here!



Q&FS Team Journey



Culture of quality

People

Barilla
The Italian Food Company. Since 1877.

Quality of our products

Designed Quality

PRODUCT STANDARDS

Ensure consistency:
QUALITY CONTROL PLANS
- Raw Material
- Packaging
- Finished product

Includes Tasting

Feedback Path

Feedback Path

Listen to People:
- Consumer tests
- Point of Sales Surveys
- Consumer contact

Quality Pyramid

Conformity Index

Manufactured Quality

PROCESS STANDARDS

Complaints

Perceived Quality

Barilla
The Italian Food Company. Since 1877.

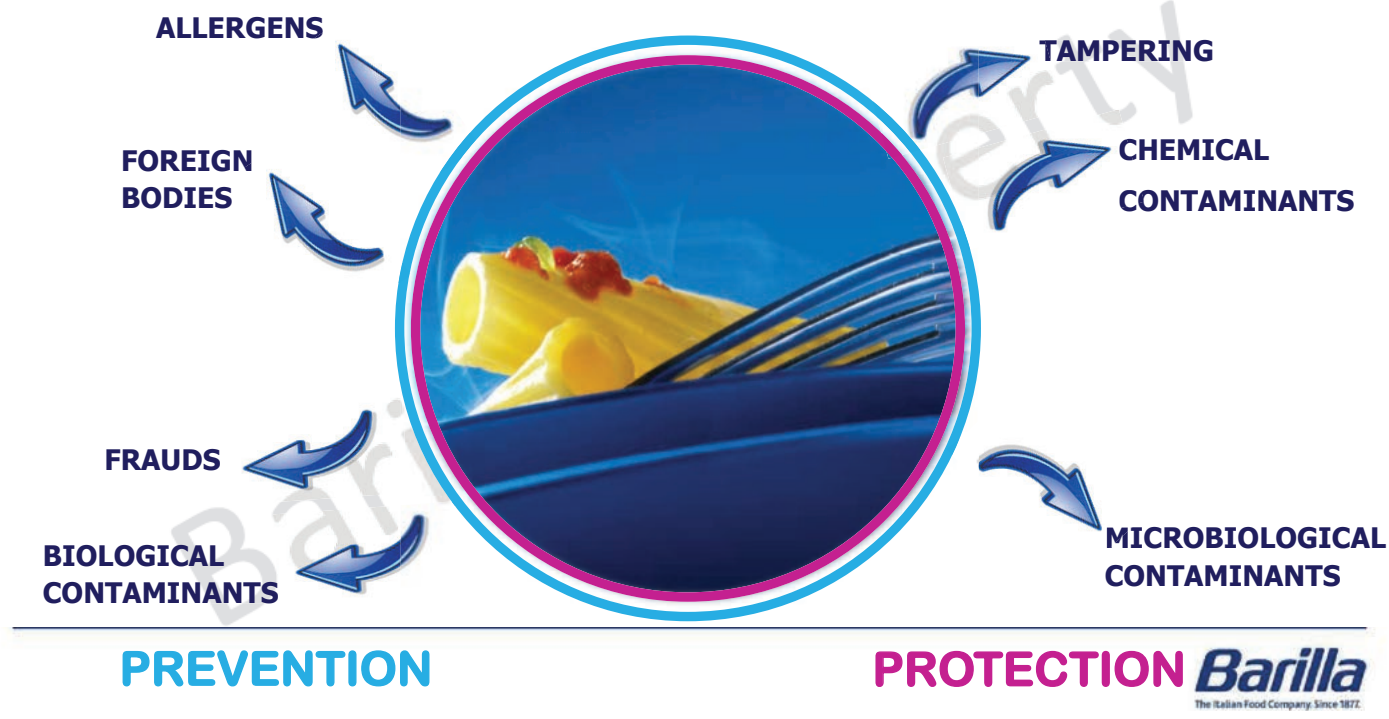
QUALITY AT BARILLA

How do we Measure Quality?

MEASURABLE CHARACTERISTICS
-Color, Dimensions, Moisture, Instrumental Texture
-Attributes have clear target and range
-Found in PRODUCT SPECIFICATION

SENSORIAL CHARACTERISTICS
-Flavor, Texture (MOST IMPORTANT TO CONSUMERS)
-Attributes do not have clear target- need reference
-NOT EASILY MEASURED

FOOD SAFETY: What we need to defend from



Food Safety	Food Defence	Food Fraud
HACCP Hazards	TACCP Threats	VACCP Vulnerabilities
Prevention Of Unintentional/ Accidental Adulteration Science based Food borne illness	Prevention Of Intentional Adulteration Behaviourally and ideologically motivated	Prevention Of Intentional Adulteration Economically motivated

Food related Risks

BARILLA FOOD SAFETY FROM FARM TO FORK

RAW MATERIAL	FINISHED PRODUCT TRANSFORMATION	DISTRIBUTION	POINT OF SALES	CONSUMER HOUSE
Raw materials Risk Assessment Suppliers' selection and approval with on site audit: As avg. each day one expert entrusted by Barilla audits a supplier Preventive mitigation actions applied to supply chain (ex: DON management; grano duro and eggs traceability) Raw materials control plan at acceptance stage	Production sites support by the definition of the "1200 rules of the know how" (GMP) Definition of the Critical Control Point of the process (HACCP) Analytical control plans, specific for each phase of the production process	Good Distribution Practices (GDP): 143 requisites to be respected Continuous training of distributors to spread food safety culture Detailed audits on pest control practices and on cleaning plans in the distribution platforms	Monitoring of our products on points of sales, to guarantee the fully compliance with the food safety standards	Prompt actions whenever a critical problem may rise. Key information given for consumers' safeguard Direct support to the consumers regarding food safety topic

KEY FACTS

- 22 mln — € invested in prevention and quality control activities
- 3 mln — test carried out to monitor quality and food safety
- 100 % — From 2018 Barilla product volumes complying with the most advanced international Q&FS standards.
- 8500 — items collected and checked in points of sales
- 445000 — Checks on packs from the shelves
- 2200 — Analysis completed on Emerging risks

HACCP: the cornerstone of Food Safety

HAZARD ANALYSIS

CRITICAL CONTROL POINT

- The HACCP system was developed by NASA to ensure that astronauts would not be plagued by diarrhoea, vomiting, food poisoning or other food-borne hazards during their stay in outer space.
- It has been adopted by the Codex Alimentarius Commission as a system suitable to ensure food safety worldwide.
- Regulation 852/2004 defined the key points of Hygiene including HACCP
- Starting from 1 January 2006, the entire system applies to all food businesses in the EU

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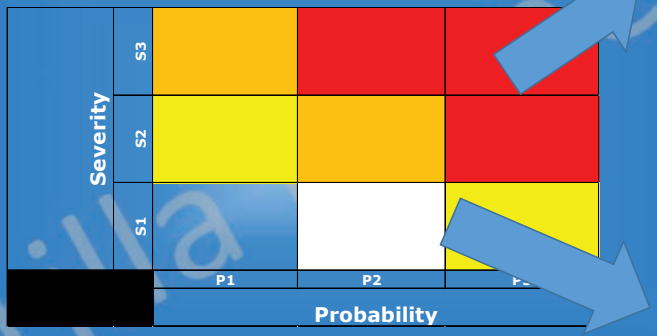
Hazard Analysis: identify and rank hazards

A: IDENTIFY relevant Hazards for a given product/process: chemical, phyisical, biological, allergens
B: RANK according to Severity vs. Probability
C: CONTROL the hazard

	Impact on consumers' health
Very high (S3)	When the hazard **can threaten consumers' life** or cause immediate irreversible consequences.
High (S2)	When the hazard **does not cause immediate risk to consumers' life**, but it can cause damage with immediate and reversible or chronic effects.
Low (S1)	When the hazard **does not cause damage to consumers' health** but customers'/consumers' expectations are not met.



Management via CCPs

Management via Pre requisites programs

	Observed frequency
High (P3)	When the hazard occurs frequently (**more than once a year**) or periodically (**at least once every year**)
Medium (P2)	When the hazard occurs sporadically (**once every 2 or more years**)
Low (P1)	When the hazard **has never occurred in the last 10 years**, but it may occur.

Pre-requisite programs and CCPs: examples

Famous PRPs:

1. GMPs
2. IPM (Integrated pest management)
3. Cleaning and Sanitization
4. Supplier program (selection, validation, surveillance)

Famous CCPs:

- Metal Detector / X-RAY (all)
- Alcohol addition in shelf stable bread / minicakes
- Time/temperature treatment (sauces, filled pasta)

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PRPs: GMPs AND IPM

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PRP: Vendor assurance, in 5 points

Ensure compliance and performance via:

1. **Approval:** all suppliers before starting supply barilla are audited and approved by barilla representative or a delegate from accredited auditors
2. **Setting up specification and quality requirements** (SQR) attached to contracts
3. **Conformity of each delivery (certified by supplier)**
4. **Surveillance (base on risk assessment)**
   - Analytical surveillance plan
   - Audit (even unannounced)
5. **Non conformities management**
   - Fixing issues and supporting continous improvement
   - In case of Major or recurring Non Conformities Suppliers can be audited

Pre-requisite programs and CCPs: examples

Famous PRPs:

1. GMPs
2. IPM (Integrated pest management)
3. Cleaning and Sanitization
4. Supplier program (selection, validation, surveillance)

Famous CCPs:

- Metal Detector / X-RAY (all)
- Alcohol addition in shelf stable bread / minicakes
- Time/temperature treatment (sauces, filled pasta)

CCPs: Detection systems for FB

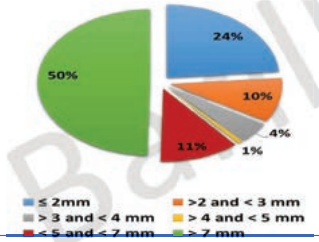
Biggest issues: hard materials

Ingestible dimension

No official rules
FDA= potential risk >7mm,
possible risk <7mm
Holland <2mm safe threshold

Case by case evaluation
Type of FB and dimension

Dimensions



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CCP: Thermal treatment and TPA

Barilla **TPA** is **Accountable** to certify **new** and **existing thermal processes,**
every time that it **represents a Critical Control Point (CCP).**
In all other cases, TPA might be consulted or simply informed regarding thermal process applied.

CCP

NOT CCP

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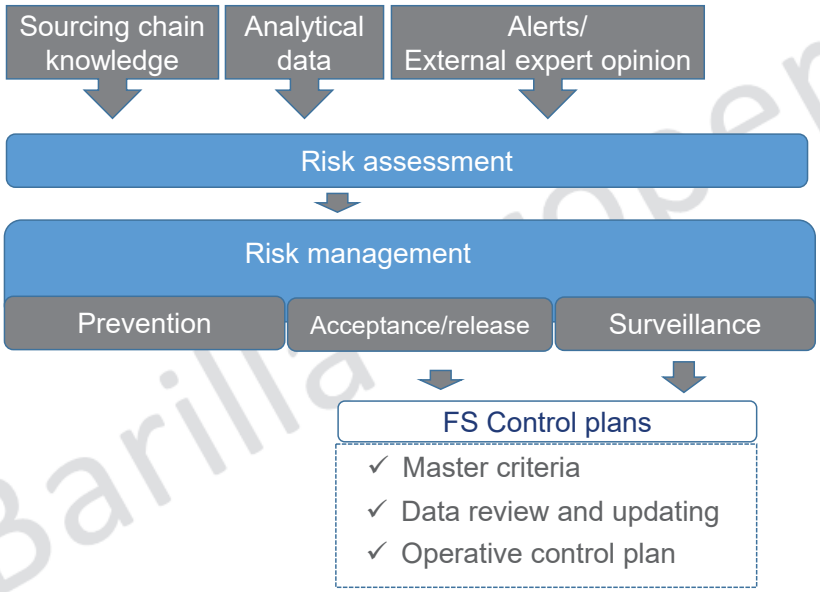
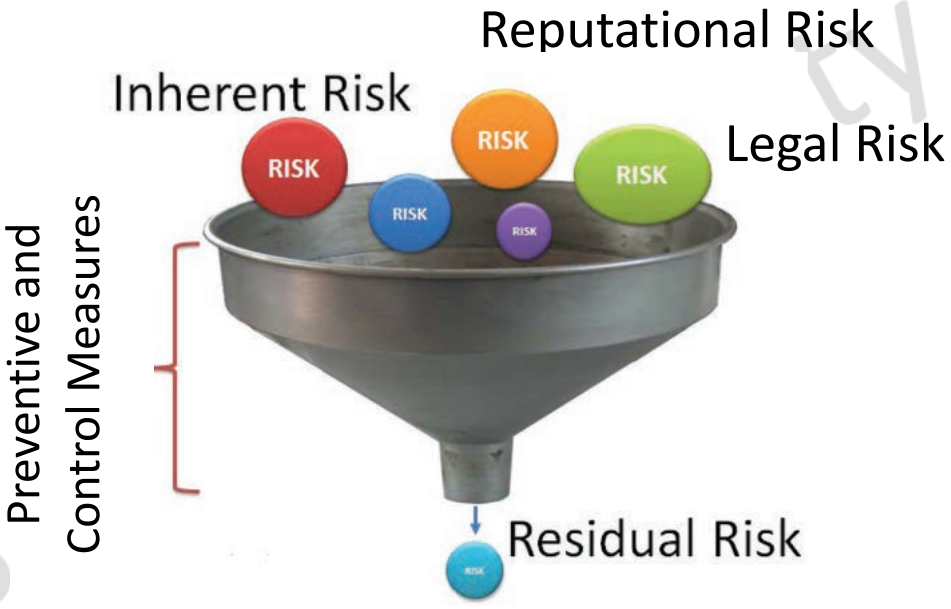
Food Safety Control plans



But after all…Residual Risk remains



When crisis arise..

ESCALATION OF AN INCIDENT

Event → Incident → Crisis

Main causes

- Harmful raw material
- Product non conformities (failure of CCP, ineffective quarantine, etc.)
- Irrational consumer (and authority…)

Detected

- By ourselves (self check)
- Notified by suppliers
- Reported by authorities (RASSF)
- Consumer/customer complaints

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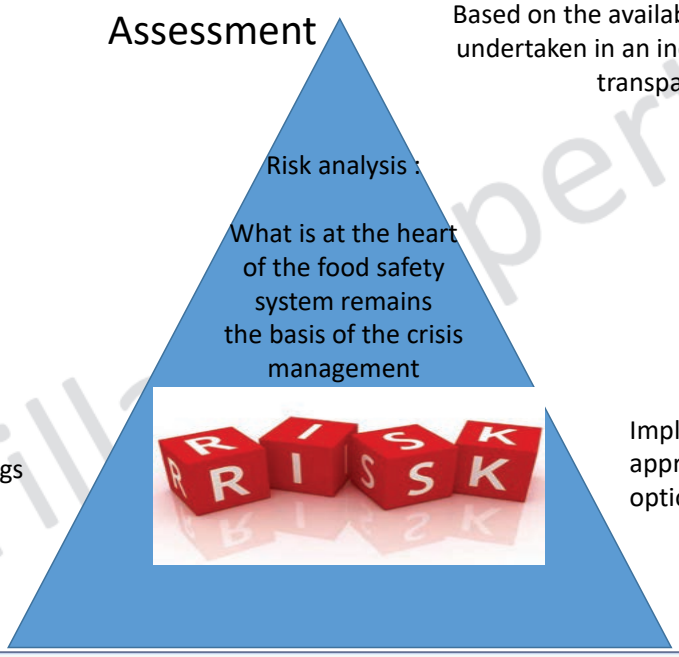
…we should stay rationale

Assessment

Based on the available scientific evidence and undertaken in an independent, objective and transparent manner.



Among all stakeholders including the explanation of risk assessment findings and the basis of risk management decisions

Communication

Implement policies and select appropriate prevention and control options

Management

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Risk assessment: Emergency Response service

ITALY & EUROPE: TRISKELION

AMERICAS: RQA GROUP

AAA: Evaluating the best providers

HOW DOES IT WORK IN PRACTICE?

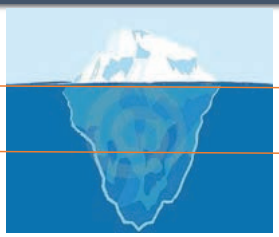
Example of crisis management

TIME FRAME

FRIDAY 18/11/16 4:30 pm	SATURDAY 19/11/16 8:30 am	SUNDAY 20/11/16 11:00 am	MONDAY 21/11/16 2:30 pm
Issue outbreak in Barilla French plant	Samples shipped from the plant to Triskelion	Preliminary risk assessment paper ready and sent to crisis team	Final risk assessment paper

24h OVER THE WEEKEND (AFTER SAMPLE RECEIPT) TO GET THE PRELIMINARY RISK ASSESSMENT

HAZARDS: BEYOND EMERGED



Known Emerged HAZARDS

Known Emerging HAZARDS

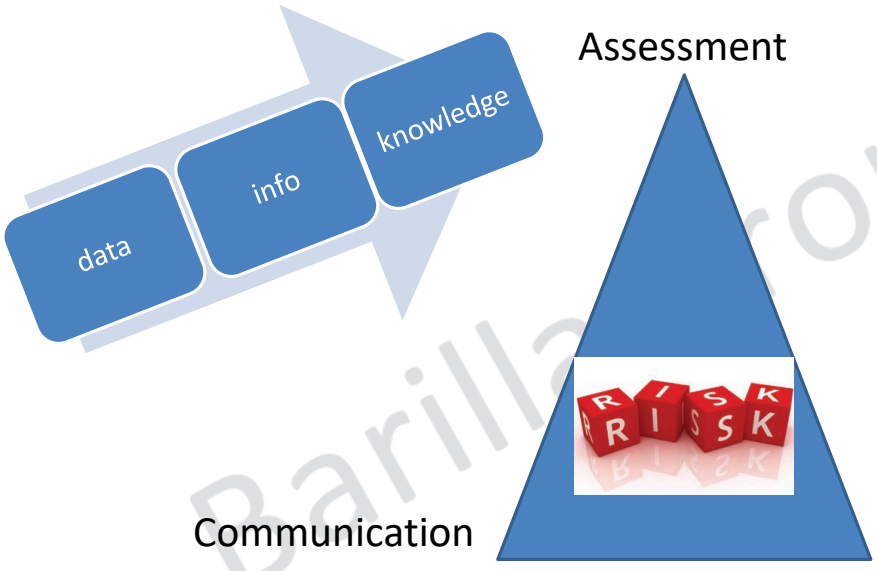
UnKnown HAZARDS

Emerging RISKs are related to a Food Safety hazard that is:

- **not yet regulated** by law *or*
- regulated by law, but for which a scientific-legislative **modification** is **on going** *or*
- characterized by a **change in** the consumers or stakeholders **perception**

Among examples of **Unknown RISKs** we have Food Frauds:

RISK PREVENTION MODEL



DOES A STANDARD RISK ASSESSMENT MODEL WORKS FOR FOOD FRAUDS?

WHY DOESN'T IT WORK?

$$X = XI + I$$

OR

FRAUD RISK PREVENTION MODEL

Assessment

- m Motivation
- o Opportunity
- m Means

Communication

Management

Food frauds: an emerged global risk

pwc

PwC › Assurance & Audit › Business risk › Third party trust › Fight food fraud

The war on $40bn food fraud

While food fraud is not new, the motivation to adulterate or counterfeit food for financial gain is growing and a new solution is needed. Current food safety management systems are not always designed for fraud detection or mitigation, but new food safety guidelines require it.

Companies are losing money and customers are losing faith. Food fraud is estimated to cost the global food industry US$30 to $40 billion every year[1]. But that's just the economic cost.

Beyond the financial impact food fraud can lead to serious public health risks and damage brands. Trust in food is being challenged as your fraud vulnerabilities increase.

What you don't know can hurt you. Take action.

BARILLA RMs CATEGORIES VULNERABILITY RANKING

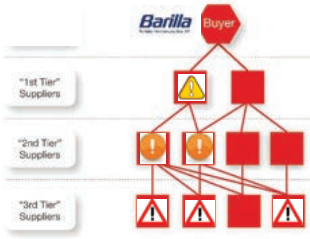
RAW MATERIAL CATEGORY	STRATEGICITY			PRICE VOLATILITY	PRICE	EXTERNAL RISK	GUT feel	Vulnerability scores
	Purchasing budget	Communication	score					
OLIVE OIL								
EGG AND PRODUCTS THEREOF								
ORGANIC (BIO) RMs								

KEY LEARNINGS AFTER MODEL APPLICATION

Work with INSIDERS

Expert in the specific sector

To:
- collect INSIGHTS
- Identify key vulnerable points
- Develop unconventional ways to reveal frauds



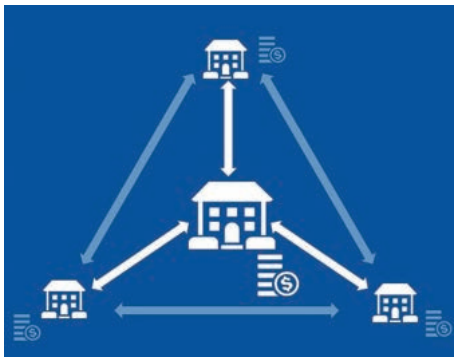
Focus on SUPPLY CHAIN COMPLEXITY

Often the most important source of frauds risks

Structure ORGANIZATION

To prevent frauds you need:
- Specific competences/ role in the team
- Auditing program on sub suppliers
- Specific control plans with conventional/ unconventional analysis
- To question even your good, long and trusted relationship with suppliers

Traceability with Blockchain



Main Advantages:

Safety
- Reduced risks for food frauds and food safety

Trust
- Certified and validated Storytelling
- Higher trustability due to supply chain visibility for consumers

Efficiency
- Quick data uploading and unloading

An application of Blockchain to prevent food frauds using Smartphone

An application of Blockchain to prevent food frauds using Smartphone

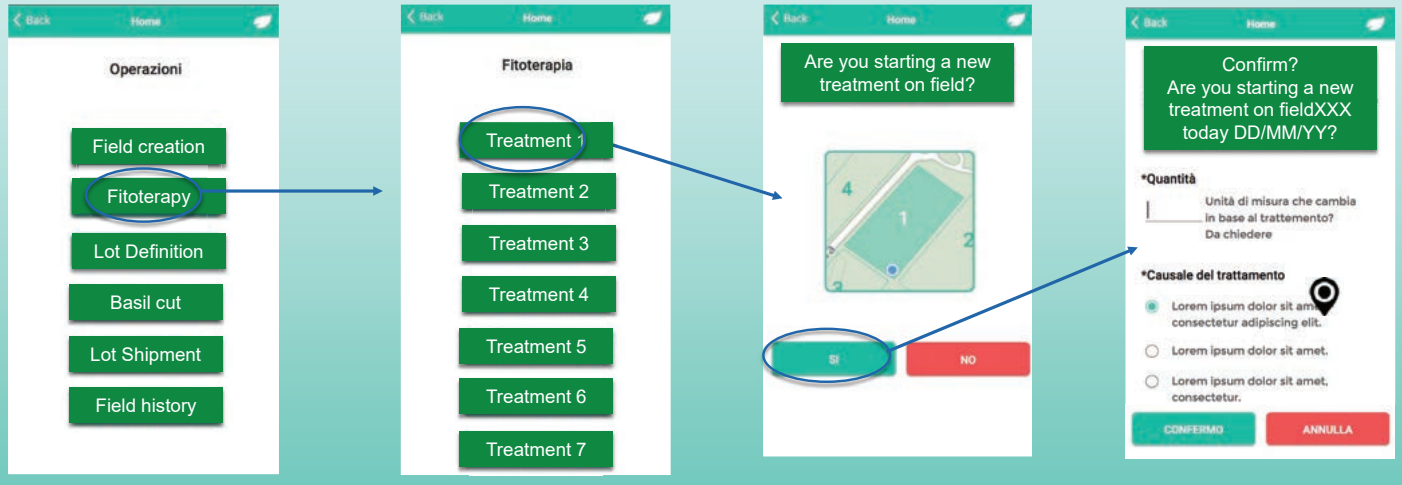
Farmer LOG IN

Home Page log-in and visualization

An application of Blockchain to prevent food frauds using Smartphone

Select a treatment

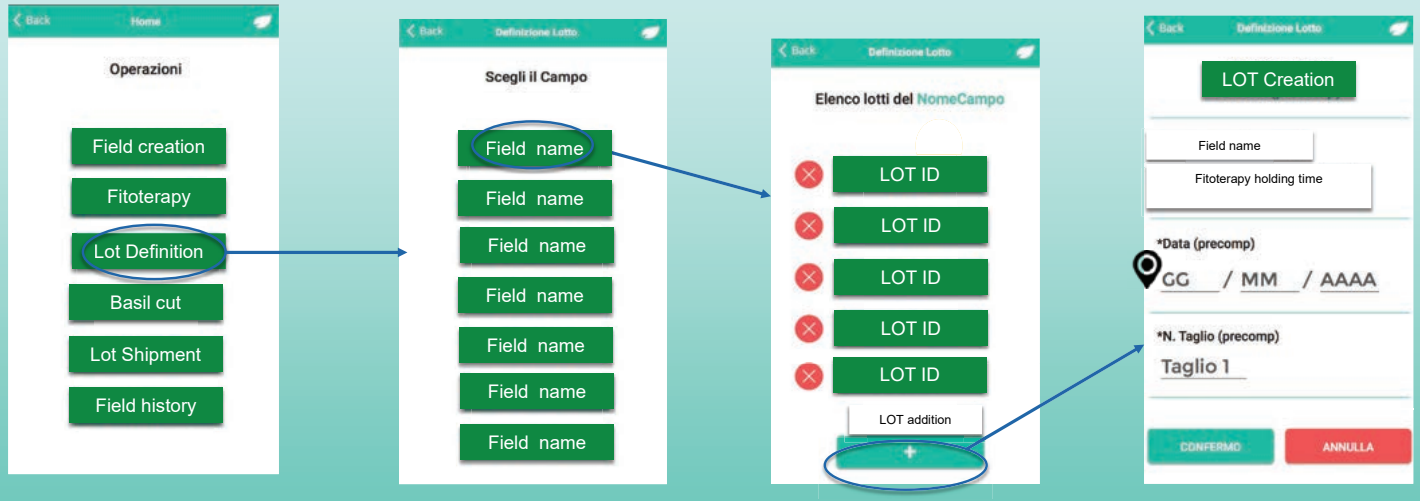
Fitoterapy



An application of Blockchain to prevent food frauds using Smartphone

Lot definition

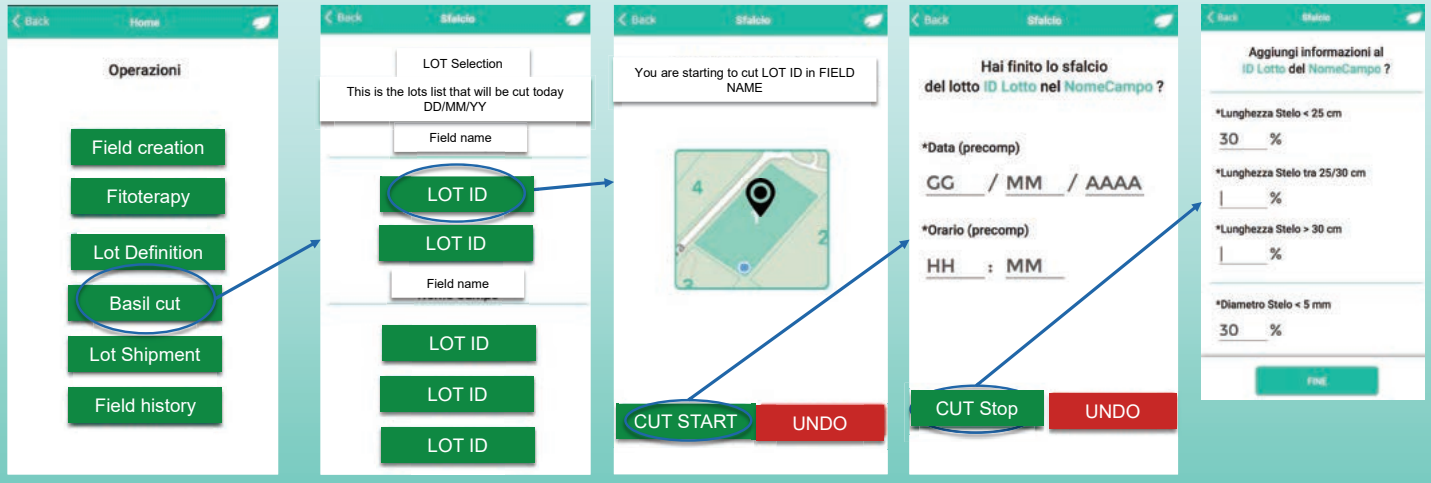
Lot Definition



An application of Blockchain to prevent food frauds using Smartphone

Basil Cut

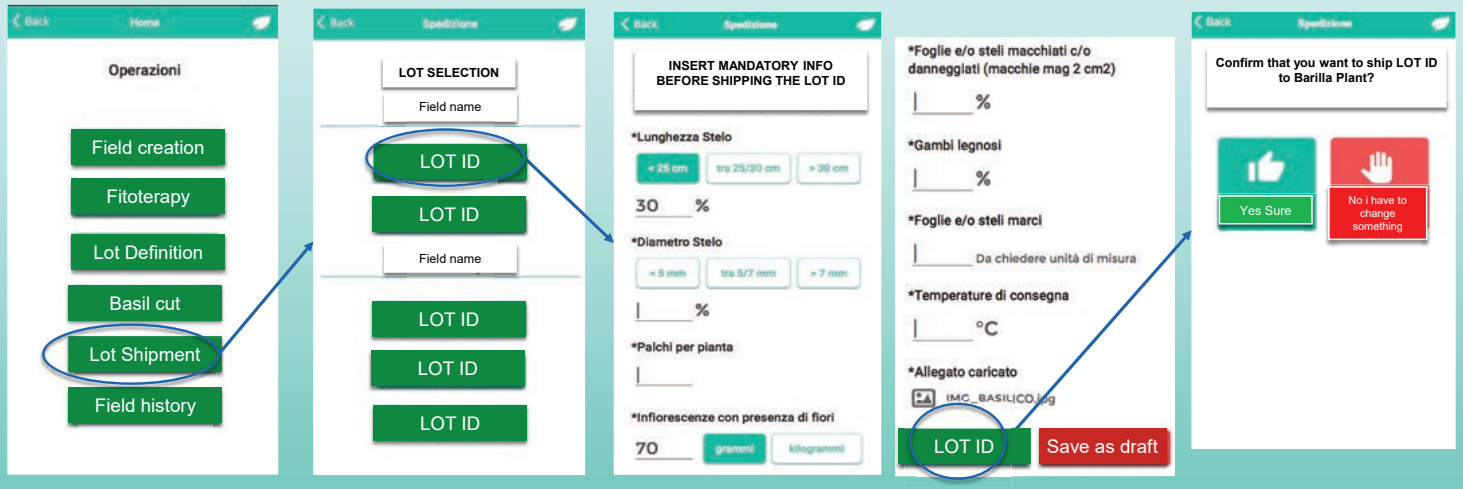
Basil Cut



An application of Blockchain to prevent food frauds using Smartphone

Lot Shipment

Spedizione: Flusso operativo

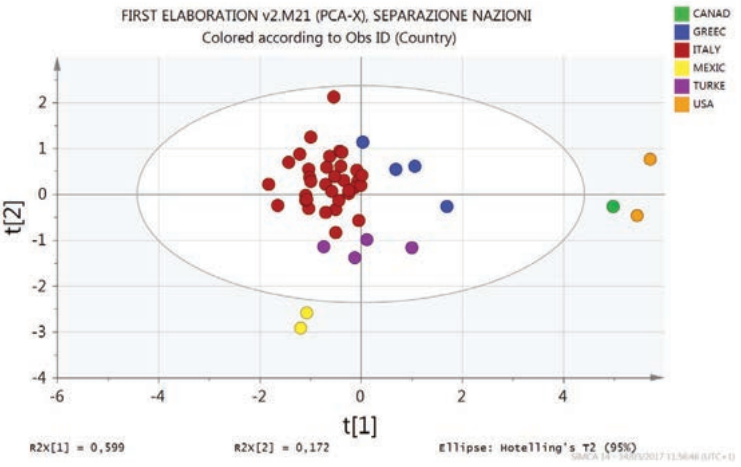


ANOTHER POSSIBLE APPLICATION

It is claiming:
- 100% Aureo Wheat type
- 100% Italian

Untargeted techniques – DURUM WHEAT case

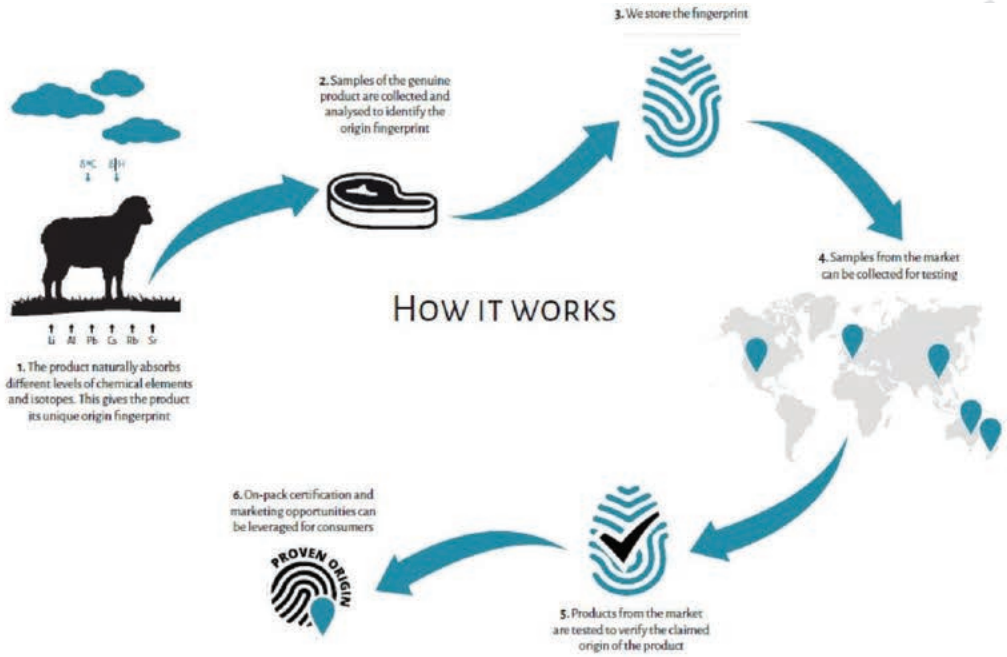
TRACEABILITY AND AUTHENTICITY BY INORGANIC APPROACH (Isotopes)



Untargeted techniques

NGS
(New Generation Sequencing)

Fingerprint



ANOTHER POSSIBLE APPLICATION

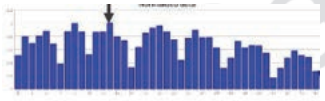


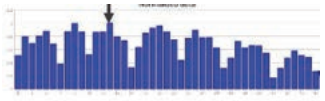


+



+



IS IT SAME WHEAT??

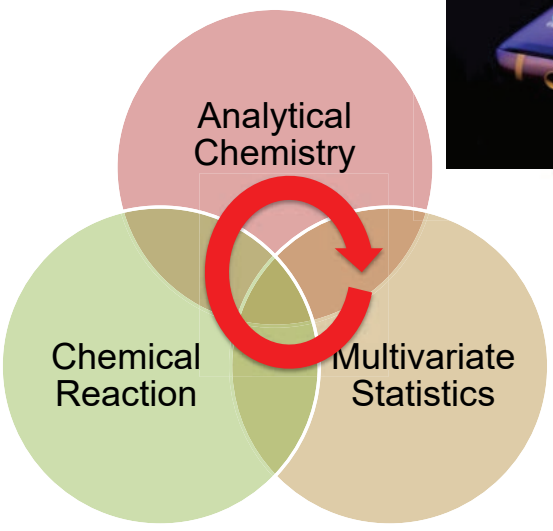
Validation of your sensor results

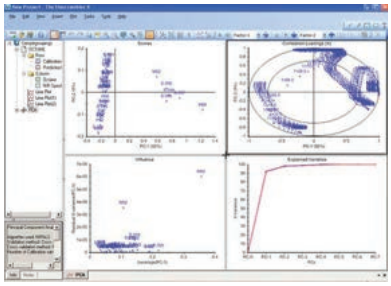
Jeroen Jansen
Assistant Professor / acting department head

Analytical Chemistry and Chemometrics

Radboud University

Chemometrics: bringing together three worlds

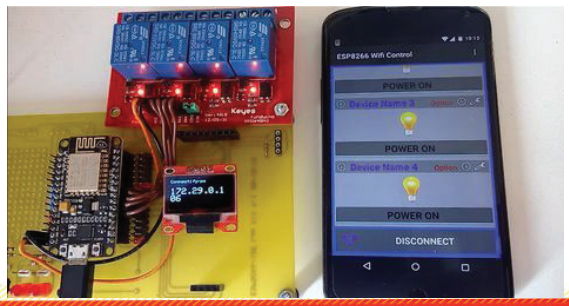




Radboud University

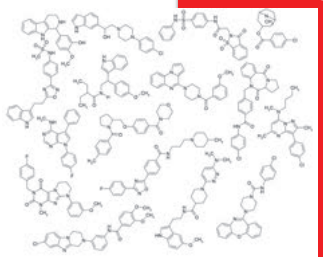
Sequence of analytical development

Lead Discovery → Sensor Development → Sensor Validation



Sequence of analytical development

Lead Discovery → Sensor Development → Sensor Validation



Sequence of analytical development

Lead Discovery → Sensor Development → Sensor Validation

Official Regulations

Sequence of chemometric model development

Model Fitting → Model Calibration → Model Validation

Official Regulations

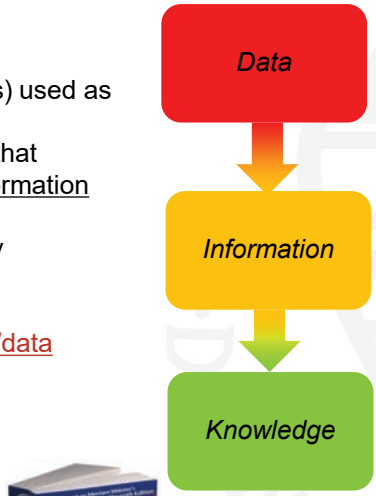
Data, Information and Knowledge

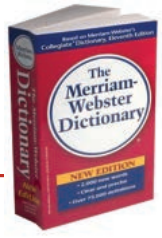
Data

1: factual information (as measurements or statistics) used as a basis for reasoning, discussion, or calculation
2 : information output by a sensing device or organ that includes both useful and irrelevant or redundant information and must be processed to be meaningful
3 : information in numerical form that can be digitally transmitted or processed

Source: http://www.merriam-webster.com/dictionary/data

'givens' is a synonym
'gegevens', 'donnees' are translations
This means you can only obtain information, by keeping the data intact





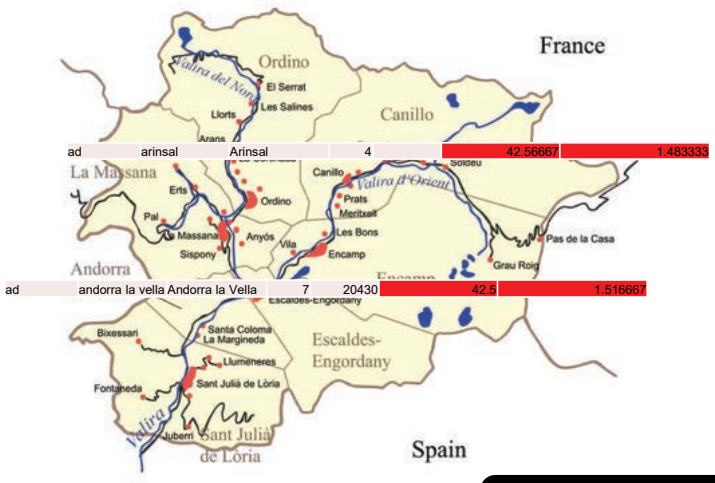
Example: from coordinates to a route

Country	City	AccentCity	Region	Population	Latitude	Longitude
ad	aixas	Aixàs	6		42.48333	1.466667
ad	aixirivali	Aixirivali	6		42.46667	1.5
ad	aixirivall	Aixirivall	6		42.46667	1.5
ad	aixirvall	Aixirvall	6		42.46667	1.5
ad	aixovall	Aixovall	6		42.46667	1.483333
ad	andorra	Andorra	7		42.5	1.516667
ad	andorra la vella	Andorra la Vella	7	20430	42.5	1.516667
ad	andorra-vieille	Andorra-Vieille	7		42.5	1.516667
ad	andorre	Andorre	7		42.5	1.516667
ad	andorre-la-vieille	Andorre-la-Vieille	7		42.5	1.516667
ad	andorre-vieille	Andorre-Vieille	7		42.5	1.516667
ad	ansalonga	Ansalonga	4		42.56667	1.516667
ad	anyos	Anyós	5		42.53333	1.533333
ad	arans	Arans	4		42.58333	1.516667
ad	arinsal	Arinsal	4		42.56667	1.483333
ad	aubinya	Aubinyà	6		42.45	1.5
ad	auvinya	Auvinya	6		42.45	1.5
ad	bicisarri	Biçisarri	6		42.48333	1.466667
ad	bixessarri	Bixessarri	6		42.48333	1.466667
ad	bixisarri	Bixisarri	6		42.48333	1.466667
ad	canillo	Canillo	2	3292	42.56667	1.6
ad	casas vila	Casas Vila	3		42.53333	1.566667
ad	certers	Certers	6		42.46667	1.5
ad	certes	Certés	6		42.46667	1.5
ad	eixirivall	Eixirivall	6		42.46667	1.5
ad	el pui	El Pui	4		42.55	1.516667
ad	els bons	Els Bons	3		42.53333	1.583333

Data → Information → Knowledge

http://www.maxmind.com/en/worldcities

Example: from coordinates to a route

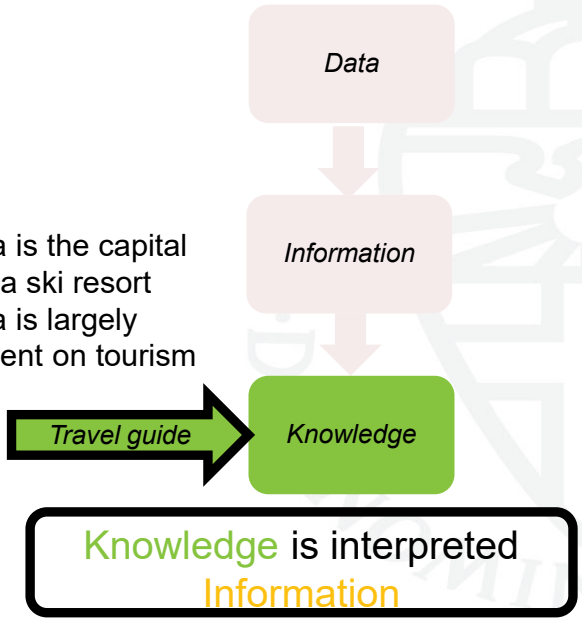


Data → Information → Knowledge

Information is organised data

https://en.wikipedia.org/wiki/File:Andorramap.png

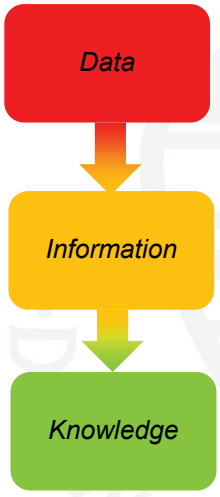
Example: from coordinates to a route



- Andorra is the capital
- Arinsal a ski resort
- Andorra is largely dependent on tourism

https://en.wikipedia.org/wiki/File:Andorramap.png

From data to knowledge



- Data needs to be organised, before it can be interpreted
- In the example:
  - The data were 'locations'
  - The information were 'their mutual distances' on a map
  - The knowledge was the likelihood of a road existing between both cities
- The models we will make, are always focused on information
- Information needs interpretation for new knowledge.
- **Knowledge is the field of specialists**
- We always have to start with 'locations' → Analytical Chemical Data

Data tables in analytical chemistry

WHAT IS INSIDE THE MATRIX?

Analytical chemical data : the data matrix

Variables
'coordinates'

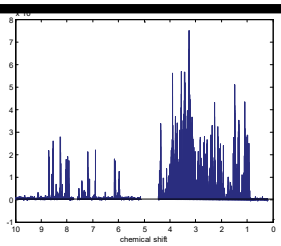
Samples, 'cities'

- In most chemometric data, the number of measured (spectral) variables is considerably larger than the number of samples ('fat' data)
- Spectral variables are often mutually dependent
- Dependence = redundancy!

Analytical chemical data : the data matrix

Variables (chemical shifts)

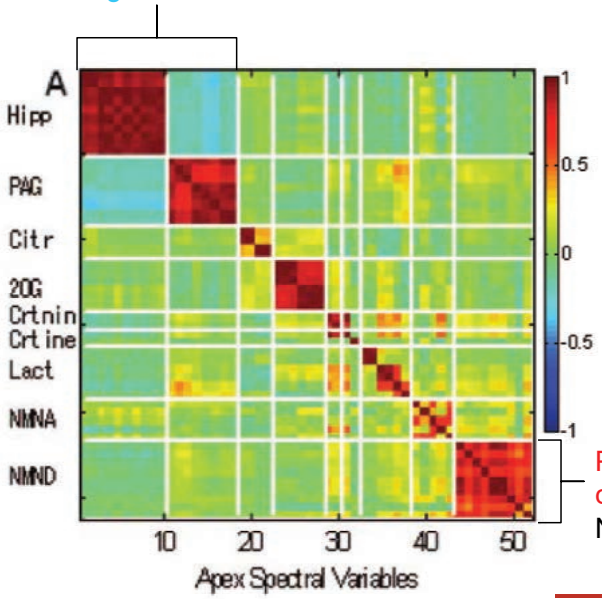
Samples



- # spectral variables (>250) >> #samples (<10)
- NMR chemical shifts are often mutually dependent
  - Spin-spin coupling
  - Protons from the same compound
  - Chemically related compounds from the same reaction
- A model does not need to describe >250 variables, it can describe the underlying chemical compounds
- Dependence = redundancy!

Biochemical and Physical Correlations in NMR spectroscopy

Negative, biochemical correlation between hippurate and PAG



$$r = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2}\sqrt{\sum_{i=1}^{n}(Y_i - \bar{Y})^2}}$$

The correlation between feature X and Y can be quantified by the 'correlation coefficient' r that lies between -1 (negative) and 1 (positive) correlation

Positive, physical correlation between NMND peaks

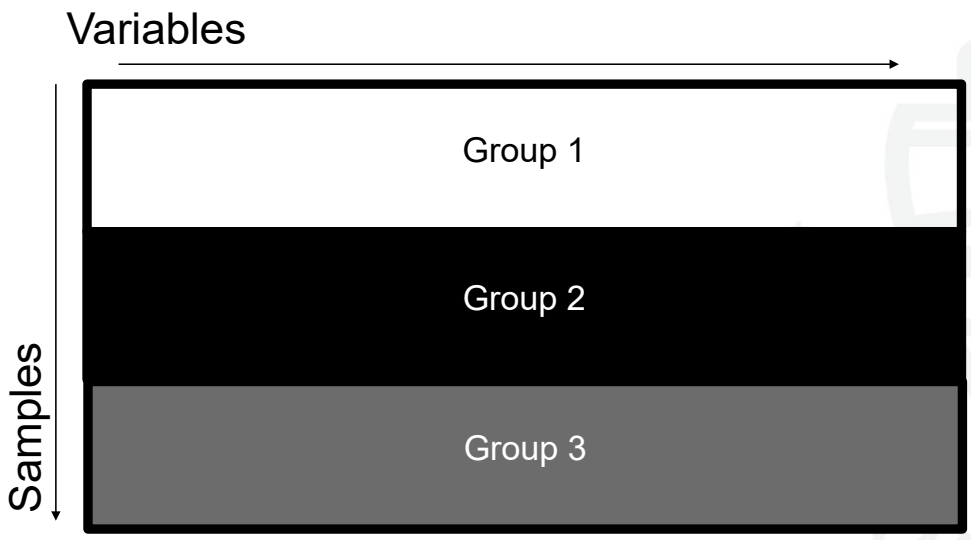
Analytic Properties of Statistical Total Correlation Spectroscopy Based Information Recovery in ^{1}H NMR Metabolic Data Sets

Alexessander Couto Alves, Mattias Rantalainen, Elaine Holmes, Jeremy K. Nicholson,* and Timothy M. D. Ebbels*

Analytical chemical data: Replication

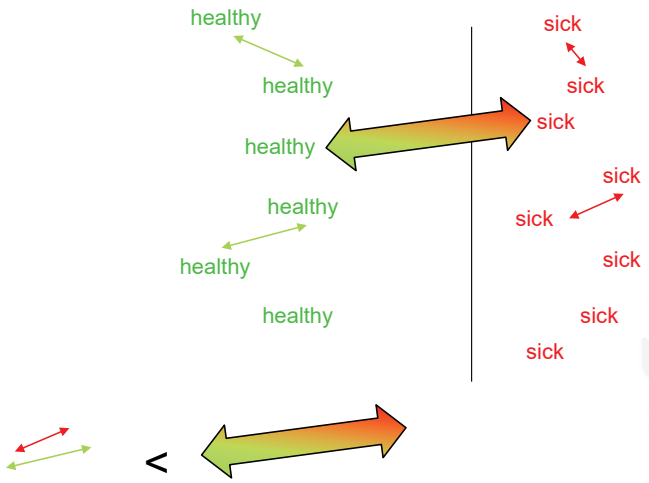
replication = redundancy!

Analytical chemical data: multiple groups



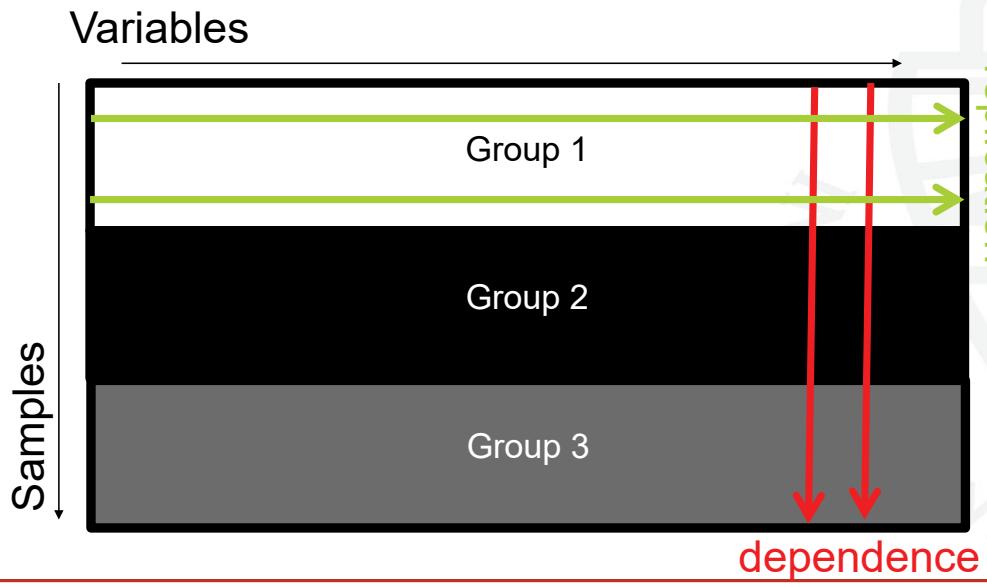
Differences : Technical<Biological<Between groups
This follows from the replication

Analytical chemical data: multiple groups



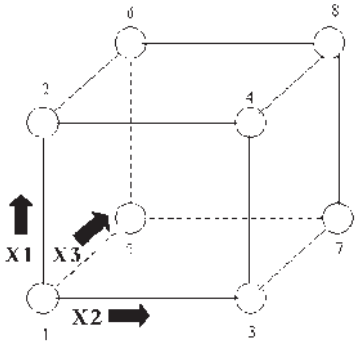
Differences : Technical/Biological < Between groups
This follows from the replication

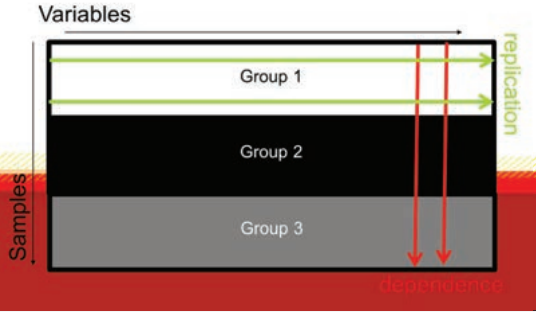
Analytical chemical data: multiple groups of multivariate data in a matrix



Design of Experiments → groups

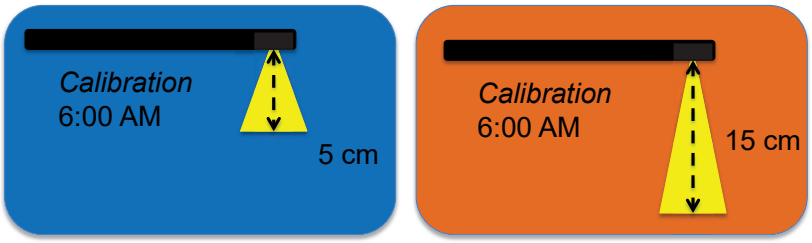
- A systematic way to design what experiments to do to find the best measurement
  - *E.g.* X1 = cmos chips Sony vs. Samsung
  - X2 = measurement height 5 cm vs 1 cm
  - X3 = measurement at 6:00 and at 18:00 (maybe also 12:00)
  - Repeat every experiment 1 … 8 several times, with different analysts
- But:
  - Maybe the optimum is not at an 'experiment'
  - Should we do all experiments?

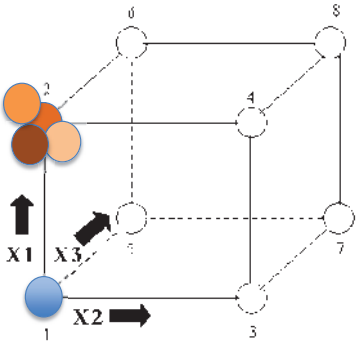




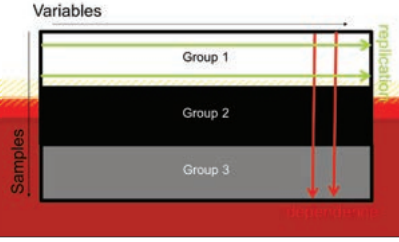
Measurement Quality is determined by all steps in the analysis

- Influences need to be identified to improve the measurement quality
  - Biases





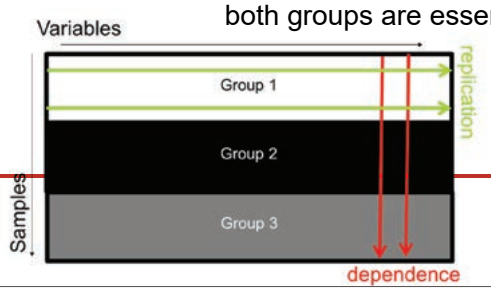
  - Variabilities → replication

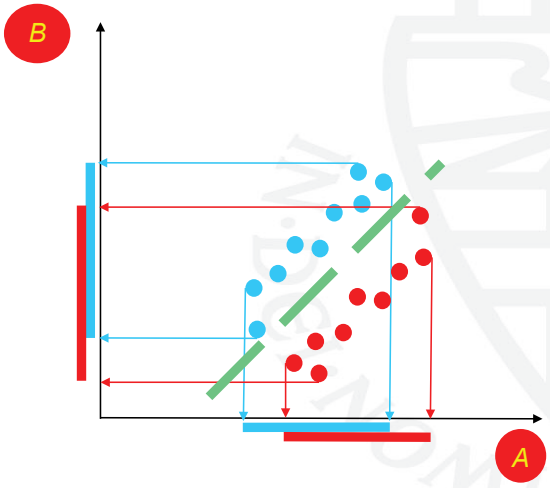


Multivariate advantage: The importance of variable dependence

WHY IS THIS IMPORTANT?

- *A* and *B* by themselves are not distinctive between both groups
- t-tests find two non-significant features
- Together they can describe the difference perfectly!
- The correlations of *A* and *B* in both groups are essential for this!





Analytical chemical data

- Information is replicated across sample groups
- Different variables are mutually dependent
- This is redundant information:
  - That is useful to understand the system
  - And to simplify its description

- This holds for every analytical chemical measurement
- But different platforms may have specific aspects to keep in mind

Geometric interpretation of analytical chemistry data tables

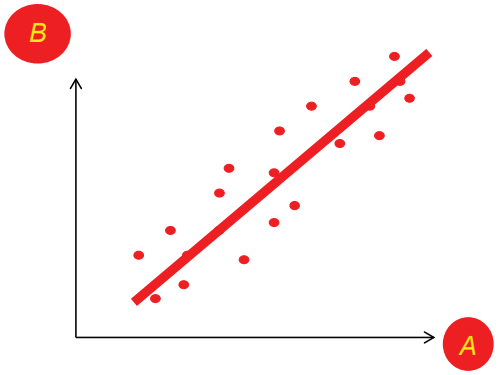
WHAT IS INSIDE THE MATRIX? (PART 2)

Institute for Molecules and Materials

Radboud University Nijmegen

PCA on two variables

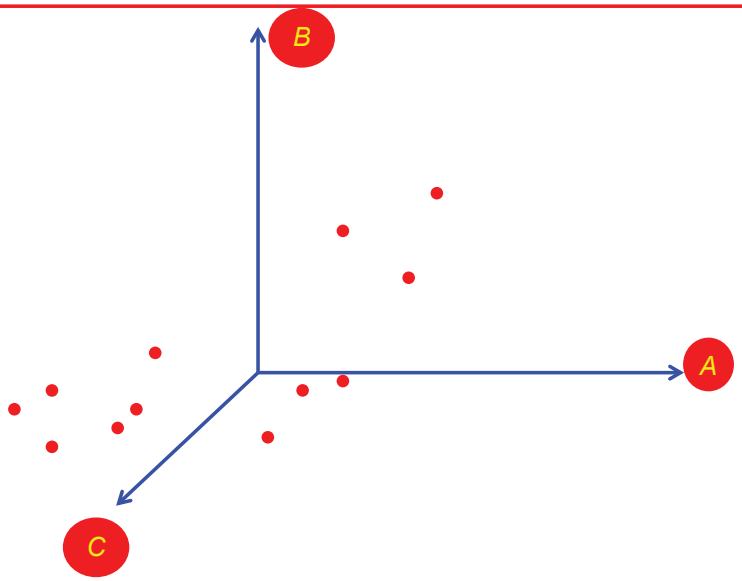
Draw <u>one</u> line to describe <u>two</u> variables



Principal Component Analysis

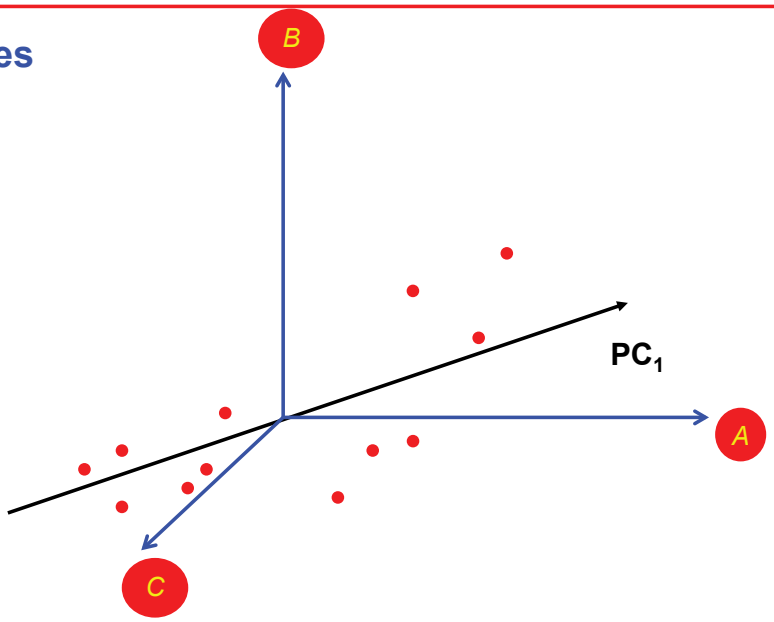
3 variables

12 samples

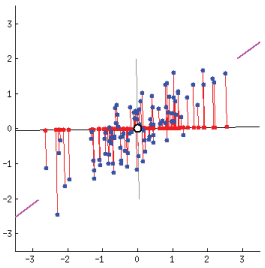


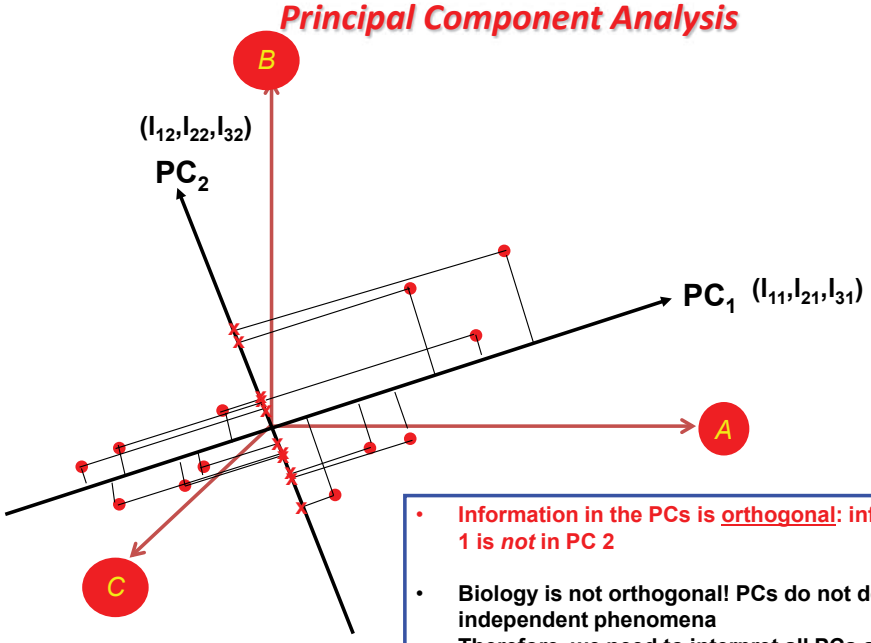
Principal Component Analysis

3 variables



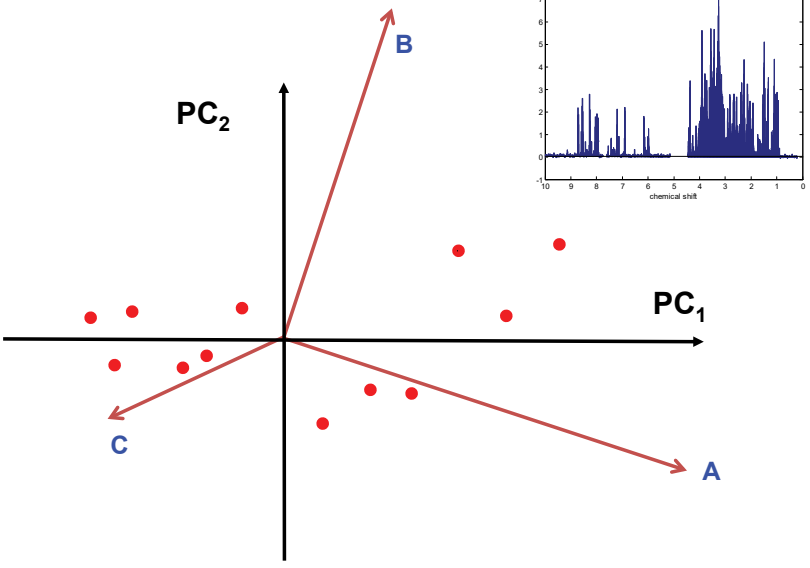
Principal Component Analysis



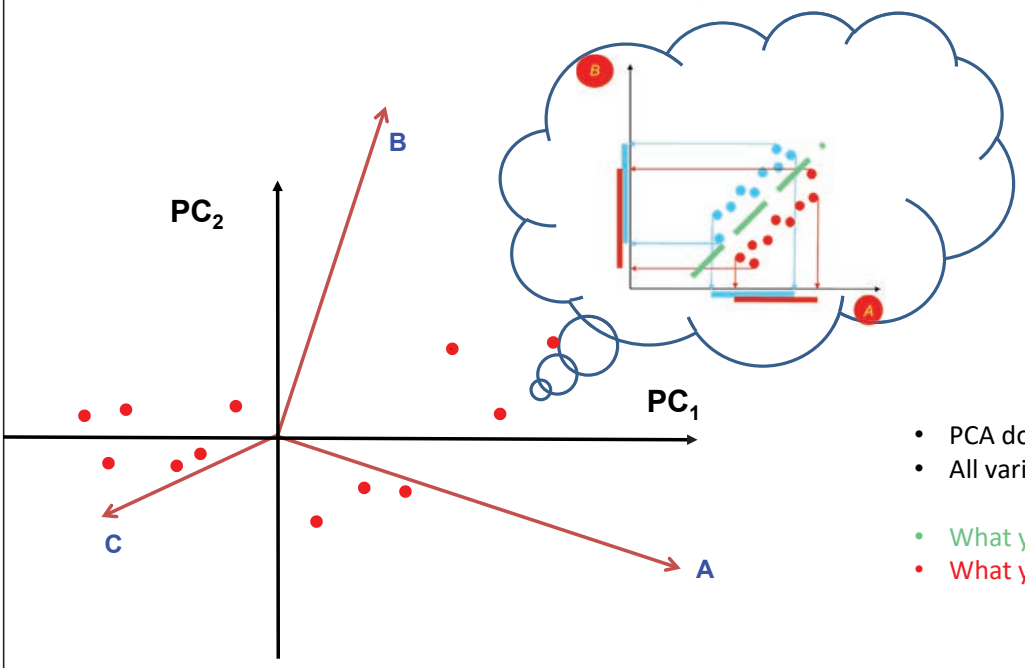


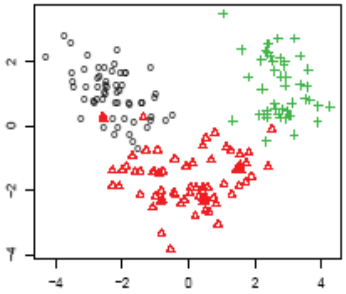
- Information in the PCs is orthogonal: information in PC 1 is *not* in PC 2
- Biology is not orthogonal! PCs do not describe independent phenomena
- Therefore, we need to interpret all PCs at the same time!

Principal Component Analysis



Principal Component Analysis

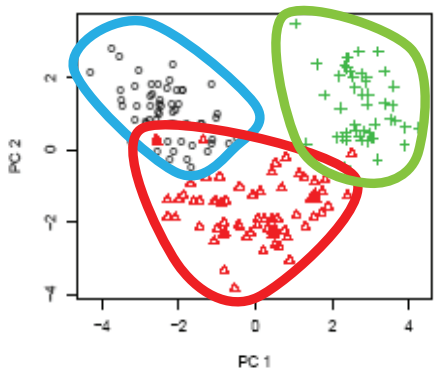


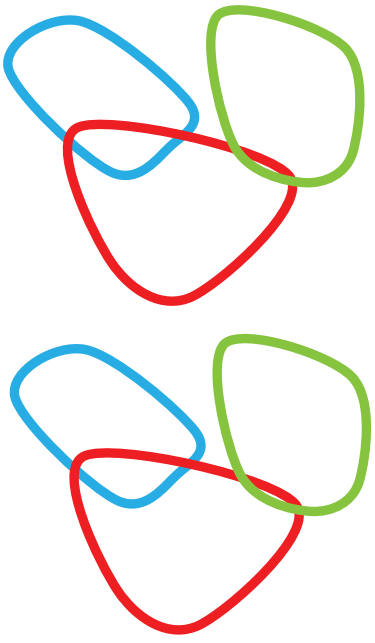


- PCA does not use the grouping in the data
- All variables and samples are considered equal

- What you see is what you get!
- What you do not see might still be there!

Discrimination ‘pulls apart’ and ‘squeezes’





Partial Least Squares = PCA for classification/regression

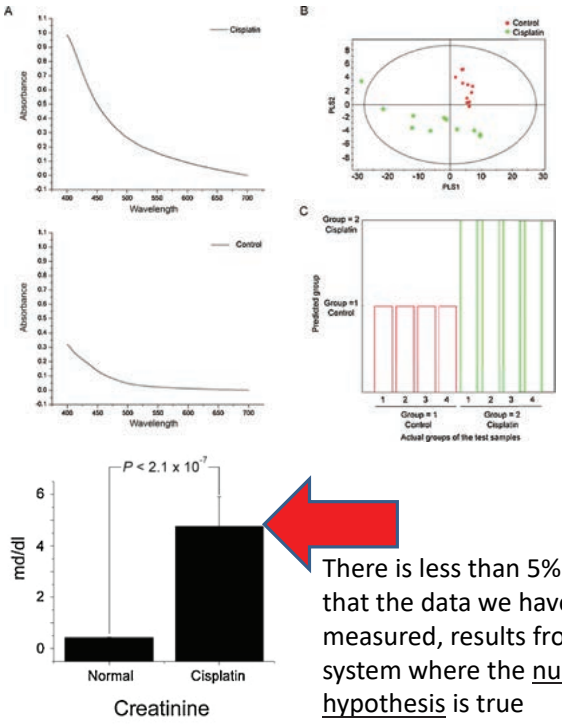
Analytica Chimica Acta
Volume 845, 3 October 2014, Pages 15-22

A smartphone metabolomics platform and its application to the assessment of cisplatin-induced kidney toxicity

Hyuknam Kwon[a], Jooeun Park[a], Yongjin An[a], Jaeho Sim[b], Sunghyouk Park[a,*]

Classifier

PCA-like model, where the green and red groups are as small as possible and the difference between both groups is as large as possible



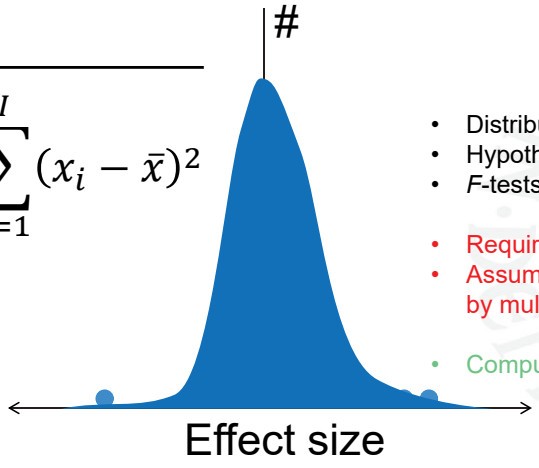
There is less than 5% chance that the data we have measured, results from a system where the null hypothesis is true

Turning multivariate data into probabilities

VALIDATION OF MULTIVARIATE CLASSIFIERS AND REGRESSION

Statistics: Full of theory

$$s_x = \sqrt{\frac{1}{I-1}\sum_{i=1}^{I}(x_i - \bar{x})^2}$$



- Distributions
- Hypotheses
- *F*-tests

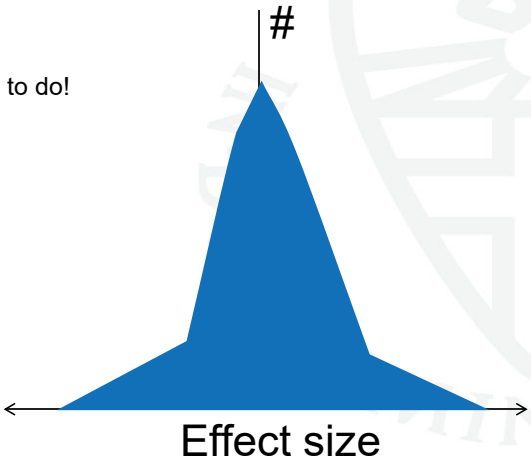
- Requires many assumptions
- Assumptions are (even more) rarely met by multivariate data

- Computer power may save us

Chemometrics: sparse in data

Resampling methods: data-driven validation
Using the data itself to assess whether a difference is significant
Advantageous:
- Not dependent on distribution of data
- Possible for low number of samples
- Easy to understand
- Already what Fisher (applied statistics) proposed to do!

- "A $p < 0.05$ should be OK"



http://nl.wikipedia.org/wiki/Ronald_Aylmer_Fisher

Principle of data-driven validation

1. By applying a chemometric method you obtain one result from all available data.

2. This is a scaled-down version of a population result, with associated uncertainty. ← "classical" statistics

3. We try to simulate this uncertainty by scaling down the result of point 1.

→ Resampling on your data.

Validation

=Estimating prediction error.

Basic Principle: test how well your model works with new data, it has not seen yet!

Split data in **training** and **test** set.

Several ways:

- One large test set
- Leave one out and repeat: LOO
- Leave *n* objects out and repeat: LNO
- . . .

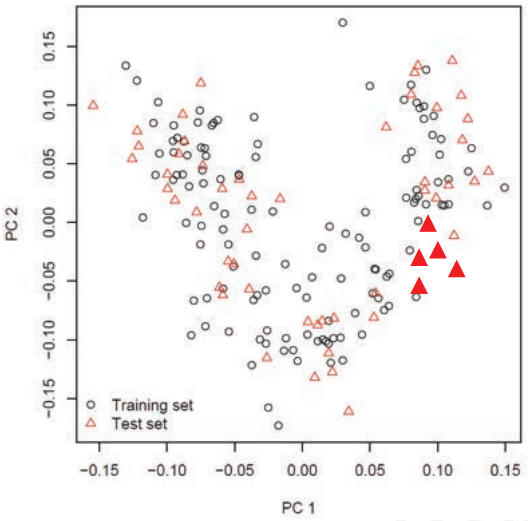
Apply entire modeling procedure on the test set

Training and test sets

Split in **training** and **test** set.

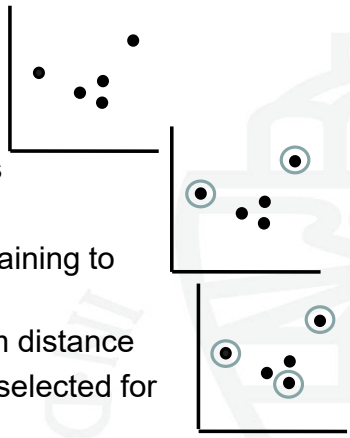
- Test set should be representative of training set
- OK in case of numerous data (100-500)
- Selection using Duplex method or **SOMs** (better than Random choice)*
- In case of Random: Check for extremely unlucky divisions + **repeat**
- Training set may be further split up into:
  - Validation Set
  - Training (sub)set
- Apply whole procedure on the test and validation sets

Random selection example



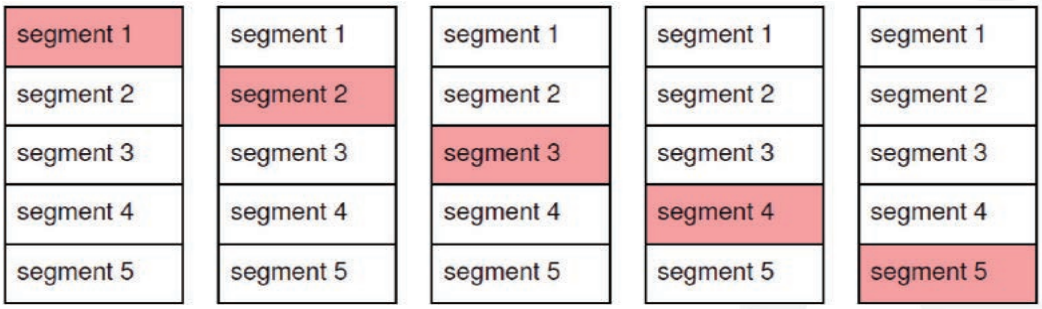
*: Westad, Anal. Chim. Acta, 2015

Training and test sets (2)



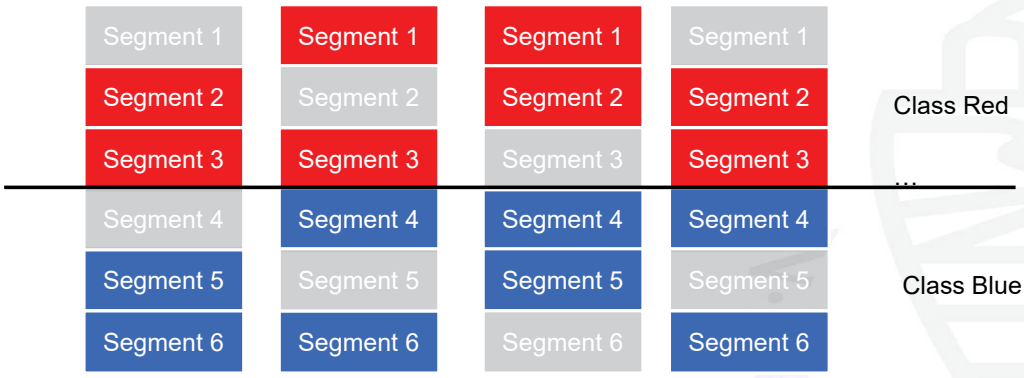
- Kennard Stone:
  1. calc distance between all sample pairs
  2. Most distant ones in training set (◯)
  3. Calculate minimum distance of all remaining to those already selected
  4. Select sample with maximum minimum distance
  5. Repeat 3 and 4 until enough samples selected for test set
- Duplex:
  - Step 1 and 2 of KS
  - Second most distant pair in test set
  - Repeat step 3 and 4 of KS, alternating for sample in training set and test set.

Cross-validation (CV): multiple test sets



- Most simple case: Leave-One-Out (=LOO, segment=1 sample). Normally 10-20% out (=L*n*O). (Stratified.)
  - Evaluate:
    - larger test set → higher accuracy of predictability
    - Smaller test set → more robust model
- To estimate prediction error OR model parameter (*e.g.* #LVs).
- Remark: for final model use whole data set.

Cross-validation, resampling for designed/grouped data

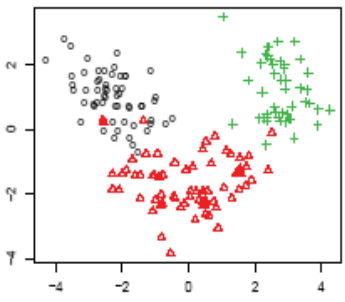


- Red and Blue group
- Representative test set (gray sections of both classes)
- Stratified resampling

Compare results without CV and with CV

Percentage of misclassifications:

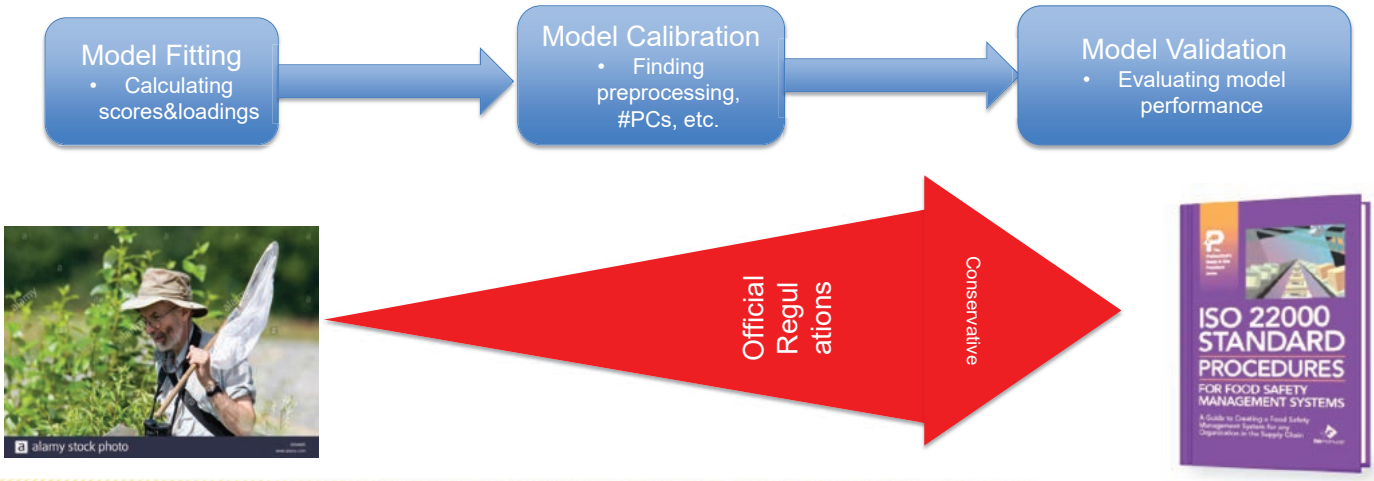
	Full set (177)		Test set (50)	
number of variables	2	13	2	13
LDA	11	0	16	2
LDA (Bayes)	10	0	14	2
Fisher LDA	15	6	20	10
QDA	8	-	16	-
QDA (Bayes)	8	-	12	-



Optimizing & reporting

VALIDATION OF MULTIVARIATE CLASSIFIERS AND REGRESSION

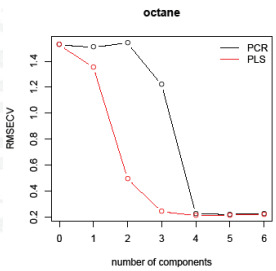
Sequence of chemometric model development



In case of parameter optimization

In case of e.g. PLS:

- Number of latent variables need to be optimized
- This can <u>also</u> be done with CV
  - (for each # LVs make model using training set and test performance with validation set, then choose optimal # LVs)

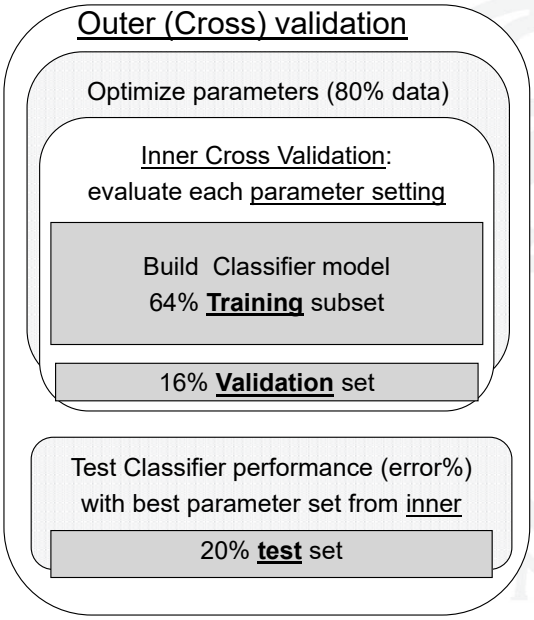


- Also indication of model performance is required
- Cannot be based on previous data: new independent data required: double (nested) CV

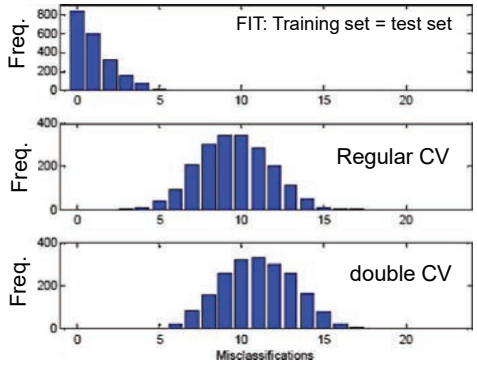
Double Cross-validation
Cross Model Validation

If parameters need to be optimized (e.g. *k* of *k*NN, #latent variables)

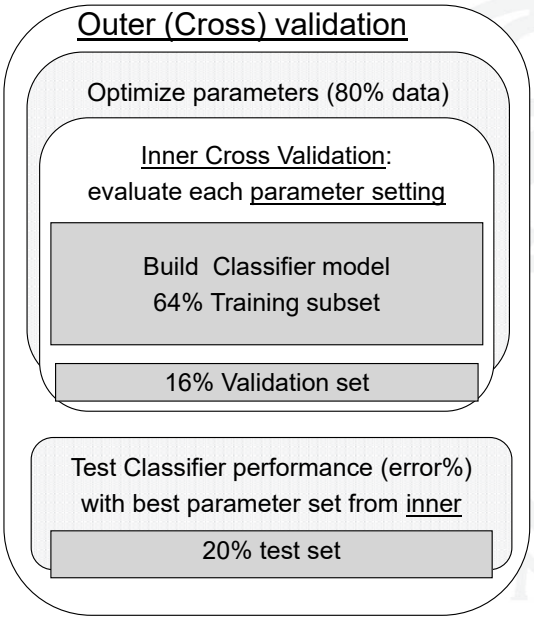
- Apply inner validation on the training set = (validation+training subset to find optimal parameter value.
- Then assess performance with remaining test data in outer loop.



Double Cross-validation
or Cross Model Validation



Westerhuis *et al.,* Assessment of PLSDA cross validation; Metabolomics (2008) 4:81–89

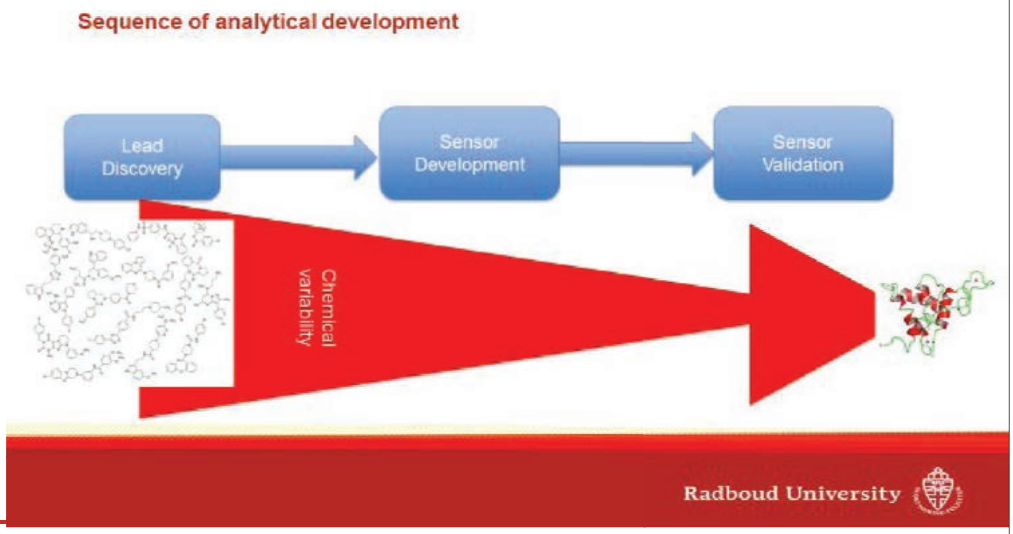


Extensions

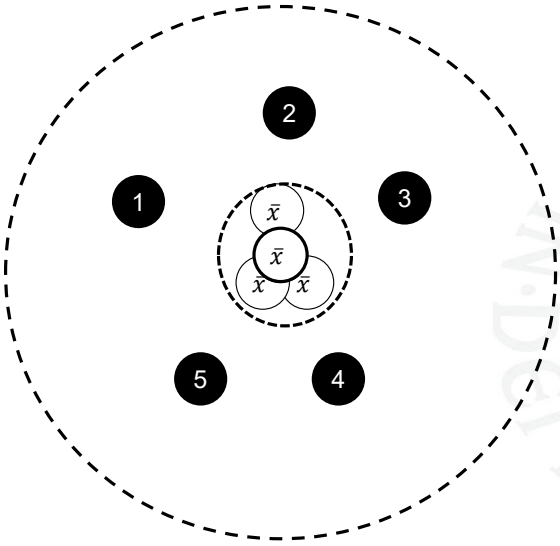
- The *p*-value is relevant, but mainly at the sensor validation

- Jackknife
- Bootstrap

Essential for Evidence-based lead discovery and Sensor development

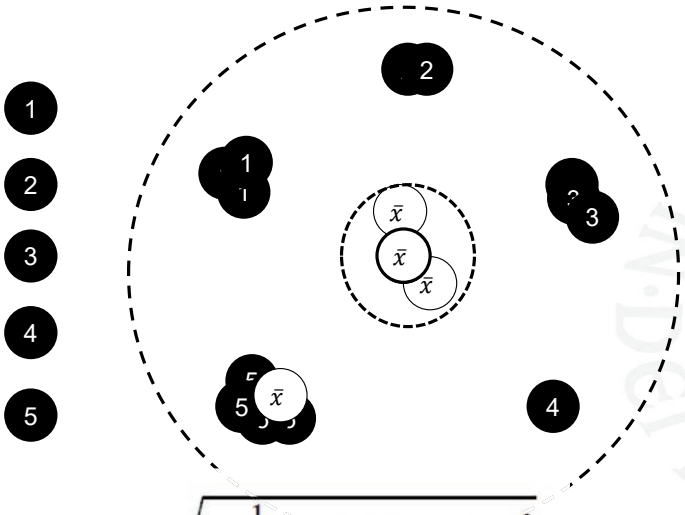


Schematic Jackknife



Data are taken out to produce data subsets (samples) with which models are constructed -> e.g. b of PLS (θ_i)

Schematic Bootstrap



$$\widehat{\mathrm{se}}_B = \sqrt{\frac{1}{B-1}\sum_i \left(\hat{\theta}_i^* - \hat{\theta}_\cdot^*\right)^2},$$

Bootstrap samples of objects 1-5 are taken with replacement, i.e. bootstrap samples are of same size as original but can contain replicates.

$\hat{\theta}_i^*$ = calculated measure based on bootstrap sample of data

$\hat{\theta}_\cdot^*$ = mean of B $\hat{\theta}_i^*$

Bootstrap *vs.* Jacknife

$$\widehat{\mathrm{se}}_B = \sqrt{\frac{1}{B-1}\sum_i \left(\hat{\theta}_i^* - \hat{\theta}_\cdot^*\right)^2},$$

$$\widehat{\mathrm{se}}_{\mathrm{jack}} = \sqrt{\frac{n-1}{n}\Sigma\left(\hat{\theta}_{(i)} - \hat{\theta}_{(\cdot)}\right)^2},$$

$\frac{n-1}{n} > \frac{1}{B-1}$, to compensate for larger variation in bootstrap

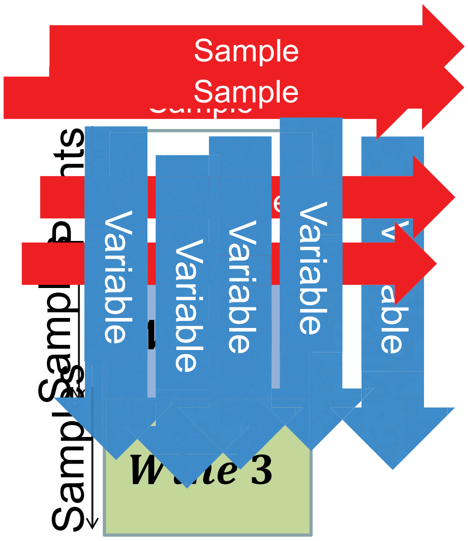
Usage: i.e.

$$\hat{\Theta} - t_{df;\alpha/2}\hat{se} \le \Theta \le \hat{\Theta} + t_{df;\alpha/2}\hat{se}$$

or better non-parametric interval based on quantiles

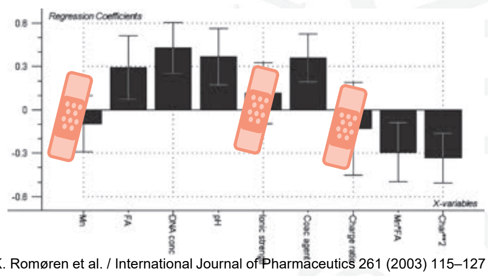
From LOO Cross-validation to Jackknife

Itteratively remove sample (and/) or variable and estimate parameter and use resulting distribution



$$\widehat{\mathrm{se}}_{\mathrm{jack}} = \sqrt{\frac{n-1}{n}\Sigma\left(\hat{\theta}_{(i)} - \hat{\theta}_{(\cdot)}\right)^2},$$

e.g. θ_i = regression coefficient



K. Romøren et al. / International Journal of Pharmaceutics 261 (2003) 115–127

Lit. example: by LOO both LV optimization and resulting (Jackknife) sets used for construction of regression models; resulting in variance on Regr. Coeff.'s. This way unimportant Variables are removed.

Remarks

Bootstrap

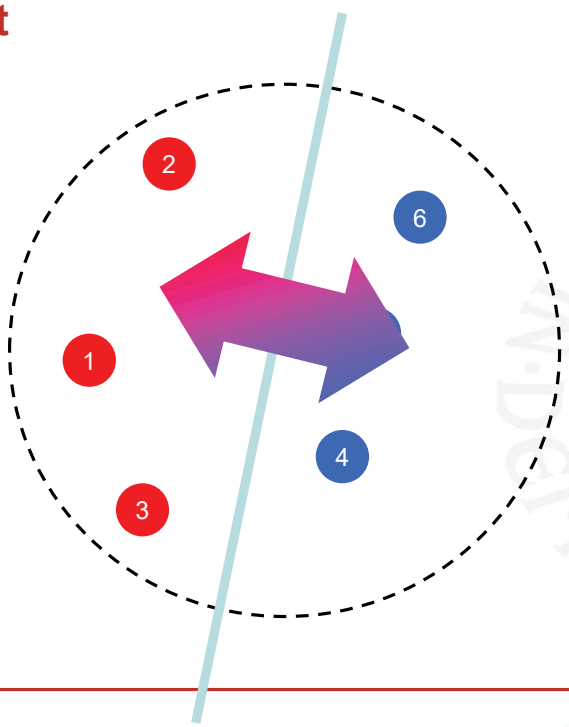
- More computer-intensive
- More widely-used → 'real statistics'

Jackknife

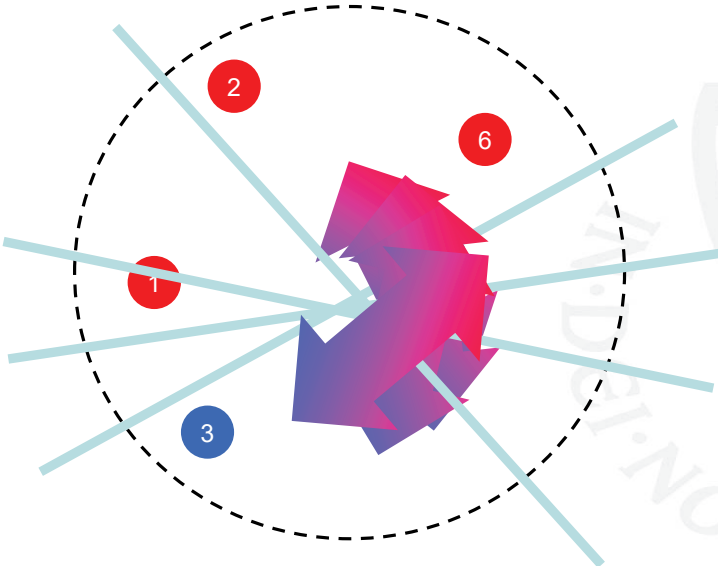
- Cross validation: in general fewer calculations than using bootstrap

- What value of probability is a 'good' one?
  - P<0.05 is a 'choice'
  - What is a realistic (data-driven) value?

Permutation Test

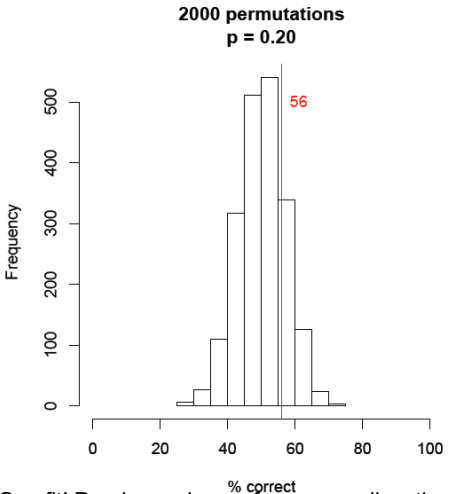


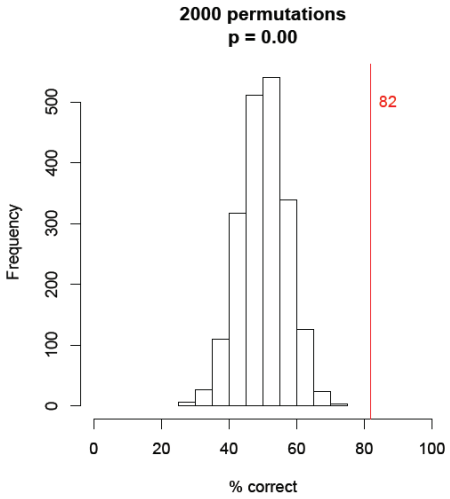
Permutation Test



Permutation tests

Shuffle class labels: then you know the outcome should be rubbish...





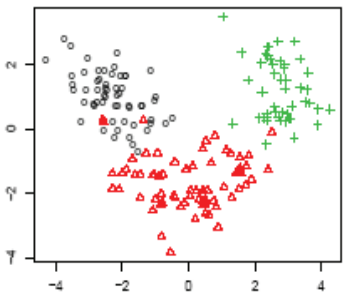
Overfit! Random noise performs as well as the data!

Misclassification

Wine data

Percentage of misclassifications:

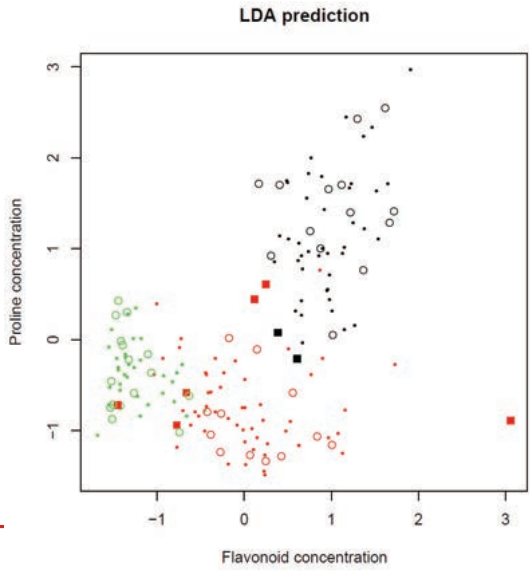
	2 var	13 var
LDA	16	2
LDA (Bayes)	14	2
Fisher LDA	20	10
QDA	16	-
QDA (Bayes)	12	-



- Grignolino NOT Barbera
- Barbera NOT Grignolino
- Bardolo NOT Grignolino
- Grignolino NOT Bardolo
- …

Wine data: test set result

Test set of 50 samples



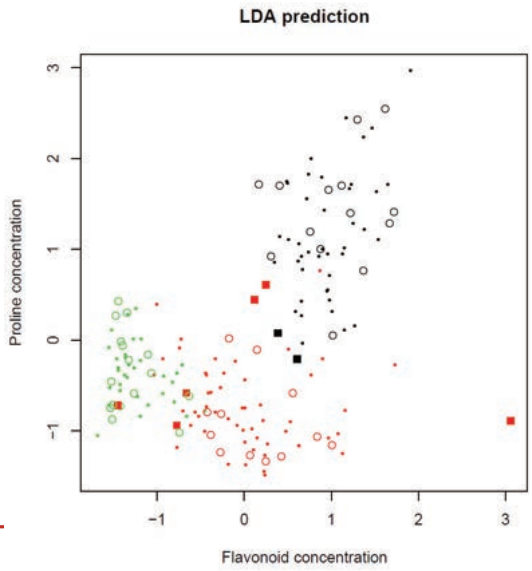
onfusion matrix

		Predicted class		
		Grignolino	Barbera	Bardolo
True class	Grignolino	14	2	0
	Barbera	3	12	3
	Bardolo	0	0	16

- Grignolino NOT Barbera
- Barbera NOT Grignolino
- Bardolo NOT Grignolino
- Grignolino NOT Bardolo
- …

Wine data: test set result

Test set of 50 samples



onfusion matrix

		Predicted class		
		Grignolino	Barbera	
True class	Grignolino	14	2	Sens
	Barbera	3	12	Spec
		PPV	NPV	

If 2 classes:

- Sensitivity: % of correct classifications of target class ('diseased'; now Grinolino)
- Specificity: % of correct classifications of the other class
- Positive Predictive Value: % of correct predictions
- Negative Predictive Value: % ….
- Unbalanced data: Matthews Correlation Coefficient

Some other measure of accuracy

Regression (e.g. PLS, OPLS):

- RMSEP Root Mean Square Error of Prediction → absolute value!

$$RMSEP = \sqrt{\frac{1}{m}\sum_i (\hat{y}_i - y_i)^2}$$

- Q^2: (normalized 'version' of RMSEP)

$$Q^2 = 1 - \frac{\sum_{i=1}^{m} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{m} (y_i - \bar{y})^2}$$

Also 1- Q^2 version exists (i.e. perfect prediction -> Q^2=0)

$\hat{y}_i$ is predicted y, y_i is true y of data object i, $\bar{y}$ is mean y

Conclusions

- Every step of analytical development should be evidence-based
- 'classical' statistics do not work for multivariate data
- Computer power allows 'resampling'

- Structured way of downsampling and testing with existing data
- Cross-validation, Jacknife/bootstrap, permutations
- Double Cross-validation is essential!

- Reporting the results is an important aspect !

Literature

See also (and more discussion on the associated statistics):

- R. Wehrens, H. Putter, and L.M.C Buydens; The bootstrap: a tutorial; *Chemom. Intell. Lab. Syst.*, 54(1), (2000) 35-52
- F. Westad, F. Marini, Validation of chemometric models - A tutorial, *Analytica Chimica Acta* 893 (2015) 14-24

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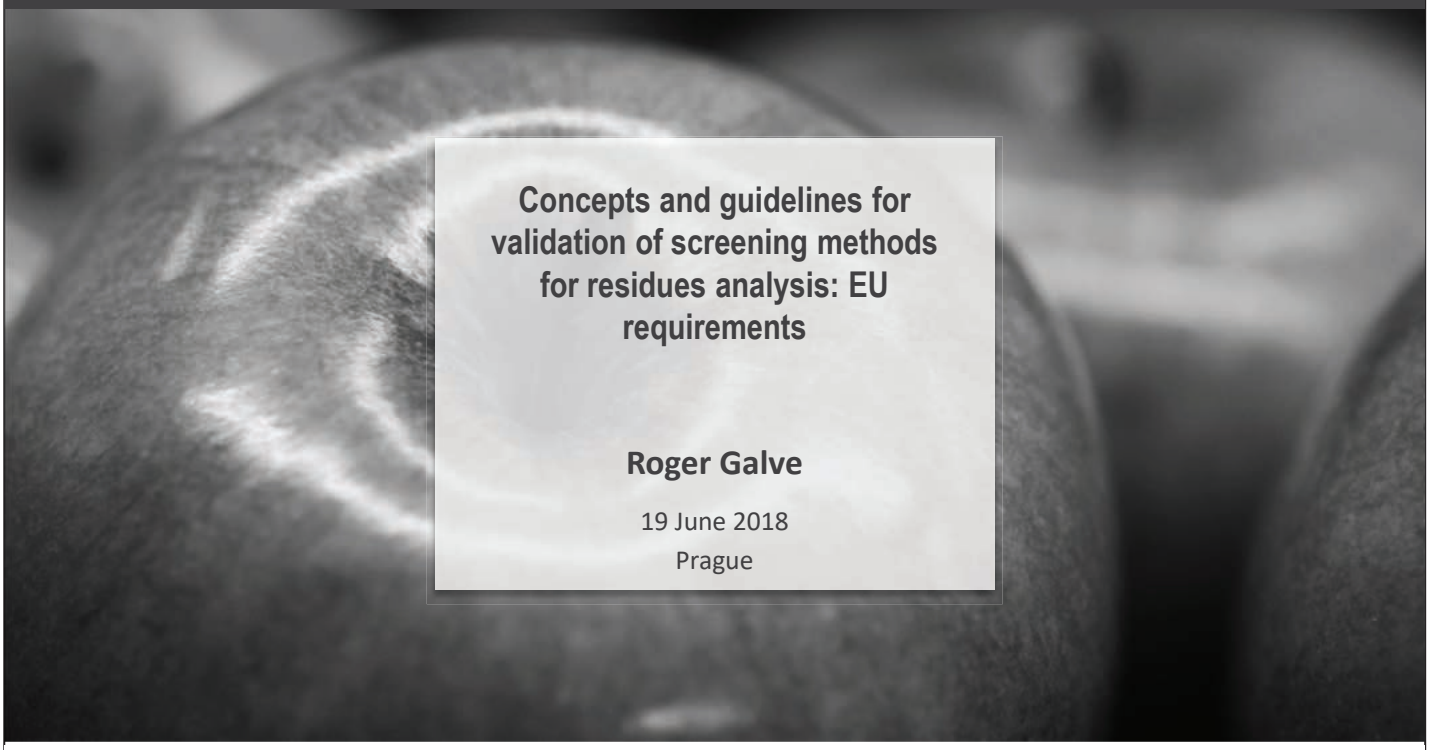
FoodSmartphone Project



Concepts and guidelines for validation of screening methods for residues analysis: EU requirements

Roger Galve

19 June 2018

Prague

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Regulations EC

- **Commission Decision 2002/657/EC**

  Guideline document regarding validation of screening methods

- **Regulation (EC) No 1107/2009**
- **Council Directive 91/414/EEC**
- **Regulation (EC) N0 396/2005**

Initial validation of screening methods in the originating laboratory

Validation of screening methods in the receiving laboratory following their transfer to the lab

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The process of **method validation** is intended to demonstrate that a method is **fit-for-purpose**. This means that when a test is performed by a properly trained analyst using the specified equipment and materials and exactly following the method protocol, accurate, reliable and consistent results can be obtained within specified statistical limits for sample analysis.

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Method validation

- The **validation** should demonstrate,
  - The identity and concentration of the **analyte**.
  - Taking into account for **matrix effects**,
  - Provide a statistical characterization of **recovery** results,
  - Indicate if the frequency of **false positives and negatives** are acceptable
- When the method is followed using suitable analytical standards, **results** within the established performance criteria should be obtained on the same or **equivalent sample material** by a trained analyst in any experienced residue testing laboratory.

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Performance parameters for analytical methods

- Selectivity
- Calibration
- Linearity
- Matrix Effects
- Trueness and Recovery
- Precision
- Limit of Quantification (LOQ)
- Analytical Range
- Ruggedness
- Method Documentation

Selectivity

➢ **Selectivity** should be evaluated to demonstrate that **no interferences** occur which significantly affect the analysis.

- It is required that common interferences are checked by **analysing a blank** for every batch of reagents.
- When **reagents and/or solvents are changed** between batches of samples, **additional reagent blank evaluations** should be performed.
- **Background levels** of plasticizers, septa bleed, cleaning agents, reagent impurities, laboratory contamination, carry-over, etc. tend to show up in reagent blanks and **must be recognized** by the analyst when they occur.
- Also, analyte-to-analyte **interferences must be known** by checking individual analytes in mixed standard solutions.
- The ultimate test of selectivity involves the **rates of false positives and negatives** in the analyses. To estimate rates of false positives and negatives during method validation, an adequate number of blanks per matrix should be analysed along with spiked matrices at the analyte reporting level.

Calibration

- **Calibration errors** are usually a minor component of the total **uncertainty**, but they affect **optimization** of the final protocol.
  - It must be known in advance whether the **calibration curve is linear or quadratic**, passes through the **origin**, and is affected by the **sample matrix** or not.
- The following calibration procedures are recommended for the initial method validation:
  - Determinations at five or more concentrations.
  - The reference standards should be spaced over the concentration range.
  - The fit of the calibration function must be plotted and inspected by calculation of the residuals (differences between the actual and calculated concentrations of the standards). Residuals of the calibration curve should not deviate by more than ± 20 – 30 % (30% for calibration concentrations near the instrument LOQ).

Linearity

- Linearity can be tested by examination of a **plot of residuals produced by linear or nonlinear (quadratic) calibration function** on the concentrations, using at least five concentration levels.
- Quality of fit usually is calculated by the **coefficient of determination (R^2)**, but may be misleading because it places greater significance on standards with **higher concentrations**.
- Ideally, the value of the intercept should be close to zero to reduce errors in calculating residue concentrations at low levels, although **the calibration curve should not be forced through the origin** without justification

Matrix Effects

- **Extracts of blank matrix**, preferably of the same or similar type as the sample, should be used for calibration.

  To compensate for matrix effects,

  - Standard addition
  - Isotopically labeled internal standards (IS)
  - Chemical analogues.

- However, these approaches are often **difficult in MRMs** because there are too many residues in different matrices at different levels to devise routine procedures.

- If solvent-only calibration is used, a **measurement of matrix effects** must be made to demonstrate equivalence of results by comparing responses of matrix-matched with solvent-only standards.

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Trueness and Recovery

- Trueness is the closeness of **agreement between a test result** and the **accepted reference value**.

  - Trueness is stated quantitatively in terms of "**bias**," with smaller bias indicating greater trueness. Bias is determined by comparing the response of the method to a certified reference material.

- Recovery refers to the proportion of **analyte determined** in the final result compared with the **amount added**, generally expressed as a percentage.

  - Routine recovery refers to the determination(s) performed in quality control spikes in the analysis.

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Precision

- Precision is the closeness of agreement between **independent replicates** obtained under stipulated conditions. It is usually specified in terms of **standard deviation** (SD) or **coefficient of variation** (CV).

Two types of precision sets of conditions are relevant:

- **Repeatability**, the variability of measurements within the same analytical sequence
- **Reproducibility**, the variability of results among multiple sets of the same sample

- In single-laboratory validations, precision often varies with analyte concentration (when analyte level approaches LOQ).

- There is no change in precision with analyte level
- The standard deviation is proportional to, or linearly dependent on, analyte level.

Limit of Quantification (LOQ)

- The LOQ is the concentration at which the average **signal/noise ratio (S/N) equals 10** in the analysis.

- The LOQ in practice can **only be estimated** because can change day-to-day due to the performance state of the instrument, among many other factors.

- The LOQ should be **verified via spiking experiments** at the Lowest Validated Level (LVL). Quantification of analytes should not be made below LVL in the same analytical sequence.

- Detection capability (ccβ)

Detection Capability ccβ

- ccβ is the **smallest content of the analyte** that may be detected, identified and/or quantified in a sample with an error probability of β. The β error is the probability that the tested sample is **truly noncompliant** even though a compliant measurement has been obtained.
- ccβ is the concentration at which only ≤ 5 % false compliant results remain
- In the case of analytes for which no Regulatory Limit has been established, ccβ is the lowest concentration at which a method is able to detect truly contaminated samples
- In the case of analytes with an established Regulatory Limit, ccβ is the concentration at which the **method is able to detect** permitted limit concentrations.
  - CCα = MRL+1.64*SD of 20 Fortified blanks at MRL
  - CCβ = CCα+1.64*SD of 20 Fortified blanks at CCα

Analytical Range

- The validated range is the **interval of analyte concentration** within which the method can be regarded as validated.
- Most methods will be validated for at least **two levels of concentration**, but many laboratories choose to validate at a third level to demonstrate linearity.
- For monitoring residue concentrations, the Lowest Validated Level (LVL) for each analyte is at or below the **Maximum Residue Levels (MRLs)**.
  - For **authorised analytes**, the Screening Target Concentration is at or below the MRL.
  - For **prohibited & unauthorised analytes**, the Screening Target Concentration must be at or less than the Minimum Required Performance Limits (MRPL).
- For analytes for which Maximum Residue Levels (MRLs) have not been established according to Council Regulation (EC) No 470/2009, the lower the probability of obtaining false-negative results (**ccβ**).

Ruggedness

- The ruggedness (often synonymous with robustness) of an analytical method is **the resistance to change** in the results produced by the analytical method when deviations are made from the experimental conditions described in the procedure.

- Such permissible deviations should produce no **meaningful change** in the results produced.

  - Small changes in the instrument.
  - Brand/lot of reagent or changes in operator.
  - Concentration of a reagent.
  - pH of a solution.
  - Temperature of a reaction.
  - Time allowed for completion of a process.

Method Documentation

- After validation, the **method documentation** should provide, in addition to performance criteria (data quality objectives), the following information:

  - Identity of the analytes included.
  - Concentration range covered by the validation.
  - Matrices used in the validation.
  - Protocol describing the equipment, reagents, detailed step-by-step procedure including permissible variations (e.g. “heat at 100 ± 5 °C for 30 ± 5 min”), calibration and quality procedures, special safety precautions required, and intended application and critical uncertainty requirements.
  - Quantitative result of the measurement uncertainty (MU) for the method should be calculated in the validation procedure and reported.

Performance criteria of screening methods

- Screening methods are usually either **qualitative or semi-quantitative** in nature, with the objective being to discriminate samples which contain no residues above a threshold value ("negatives") from those which may contain residues above that value ("indicated positives").

- Screening methods should also be checked in terms of **selectivity and sensitivity**.

  - The **selectivity of screening methods** must be able to distinguish the presence of the target compound, or group of compounds, from other substances that may be present in the sample material.

  - The validation of a screening method based on a screening detection limit (SDL) can be focused on **detectability**. a minimal validation should involve analysis of at least 5 samples spiked at the estimated SDL.

Performance criteria of quantitative methods

- Selectivity. The method needs to provide a signal response that is free from interferences.

  - Range of different pesticide residues in one extraction increases the potential for compromised selectivity compared to single residue methods.

- Validation. Recommended that a minimum of 5 replicates be analysed (to check the recovery and precision) at LOQ

  - When the residue definition includes two or more analytes, the method should be validated for all analytes.

- Trueness. Analysis of a certified reference material.

- Recoveries. it is necessary to recognize that analyte spiked into a test sample may not behave in the same manner as the biological.

  - The amount of an extracted residue is less than the total

Definitions

- **Applicability**: The analytes, matrixes, and concentrations for which an analytical method can be used satisfactorily.
- **Coefficient of Variation (CV)**: A measure of precision in quantitative studies comparing the variability of sets with different means.
- **Confirmation**: The combination of two or more analyses that are in agreement with each other, at least one of which meets identification criteria.
- **Confirmatory method**: A method that is capable of providing complementary information in agreement with a previous result.
- **False positive:** A result wrongly indicating that the analyte is present or exceeds a specified concentration.
- **False negative:** A result wrongly indicating that the analyte is not present or does not exceed a specified concentration.

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Definitions

- **Fortification**: Addition of analytes for the purposes of determining the recovery (also known as spiking).
- **Interference**: Intrinsic or extrinsic response unrelated to an analyte (e.g. noise) due to electronic, chemical, or other factors related to the instrumentation, environment, method, or sample.
- **Interferent**: A chemical or other factor causing an interference
- **Internal standard (IS)**: A chemical added at a known amount to samples and/or standards in a chemical analysis, including the blank and calibration standards. This substance can then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the concentrations.
- **Limit of Detection (LOD)**: The lowest concentration or mass of the analyte that can be detected (but not quantified) in a sample. In practice, this is typically the analyte concentration at which the average signal/noise is 10.

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Definitions

- **Limit of quantification (LOQ)**: The smallest concentration of the analyte that can be quantified. It is commonly defined as the minimum concentration of the analyte in the test sample that can be determined with repeatability and accuracy under the stated conditions of the test.
- **Linearity**: The ability of a method of analysis, within a certain range, to provide an instrumental response or results, proportional to the quantity of analyte to be determined
- **Lowest Calibrated Level (LCL):** The lowest concentration which the determination system is successfully calibrated.
- **Lowest Validated Level (LVL):** The lowest validated spiking level meeting the method performance criteria.
- **Matrix effect:** An influence of the one or more undetected components from the sample on the measurement of the analyte concentration or mass.

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Definitions

- **Matrix-matched standards**: Standard solutions prepared in final extracts of matrix blanks similar to that of the sample.
- **Multiresidue method (MRM):** A method which can determine a large number of compounds typically from different chemical classes.
- **Precision:** Degree of variability of a measurement around a mean.
- **Quantitative method**: A method capable of producing analyte concentration (determinative) results with trueness and precision that comply with established criteria.
- **Recovery:** Amount measured as a percentage of the amount of analyte(s) originally added to a sample of the appropriate matrix, which contains either no detectable level of the analyte or a known detectable level. Recovery experiments provide information on both precision and trueness and thereby the accuracy of the method.

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Definitions

- **Relative Standard Deviation (RSD)**: The standard deviation, divided by the absolute value of the arithmetic mean, expressed in percentage. It refers to the precision of the method (also known as coefficient of variation-CV).
- **Repeatability**: Precision usually expressed as RSD, obtained from the same measurement procedure or test procedure; the same operator; the same measuring or test equipment used under the same conditions; the same location and repetition over a short period of time.
- **Reproducibility:** Precision (typically expressed as RSD) where independent test/measurements results are obtained with the same method on identical test/measurement items in different test or measurement facilities with different operators using different equipment.
- **Ruggedness:** A measure of the capacity of an analytical procedure to remain unaffected in method parameters and provides an indication of its reliability during normal usage.

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Definitions

- **Screening Detection Limit (SDL):** Lowest level of fortification that has been shown to have certainty at a 95% confidence level.
- **Selectivity:** The extent to which a method can determine particular analyte(s) in a mixture(s) or matrices(s) without interferences from other components of similar behaviour.
- **Sensitivity**: Quotient of the change in the indication of a measuring system and the corresponding change in the value of the quantity being measured.
- **Trueness:** The closeness of agreement between the average of an infinite number of replicate measured quantity value and a reference quantity value.
- **Uncertainty:** A parameter associated with the result of a measurement that characterizes the dispersion of values that could reasonably be attributed to the measurement “the true value can be expected to lie”

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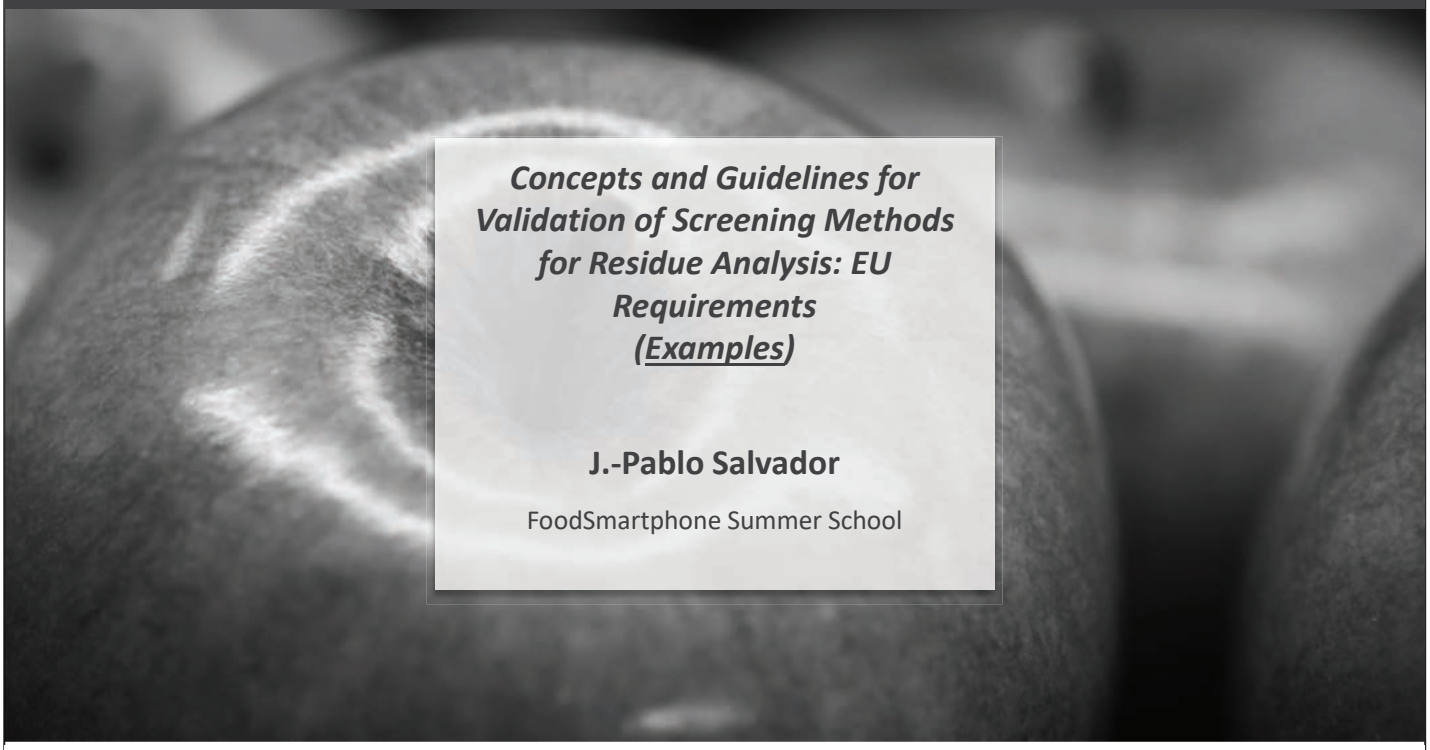

Concepts and Guidelines for Validation of Screening Methods for Residue Analysis: EU Requirements (Examples)

J.-Pablo Salvador

FoodSmartphone Summer School

OUTLINE

- **KEY CONCEPTS**
- **MICROBIOLOGICAL METHODS**
- **ELISA**
- **LFIA**
- **BIOSENSORS**

According to 2002/657/EC

Classification by detection principle

- Biological methods (Cellular response)
- Biochemical methods (molecular interactions)
- Physicochemical methods (separation techniques)

Classification by degree of quantification

- Qualitative
- Semiquantitative
- Quantitative

As a general principle, there has to be a sufficient margin of difference between the Screening Target Concentration and the Regulatory Limit. Therefore CCβ (detection capability) must be less than or equal to the Regulatory Limit.

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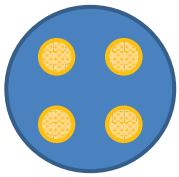
Screening Test for Antibiotic Residues (STAR)

Five plate test

Ten different groups of antibiotics were studied: macrolides, aminoglycosides, cephalosporins, penicillins, quinolones, tetracyclines, sulphonamides, lincosamides, phenicolated and miscellaneous drugs

Bacillus subtilis at pH 7.2 (**Bs7.2**).
Kocuria varians at pH 8 (**Kv8**).
Bacillus cereus at pH 6 (**Bc6**).
Escherichia coli at pH 8 (**Ec8**).
Bacillus stearothermophilus at pH 7.4—DST (**Bst**).



Samples Ø 9mm
Bs7.2 and Bc6 at 30°C (18 h)
Kv8 and Ec8 at 37°C (at 24 and 18 h, respectively).
Bst at 55°C (12–15 h)

Measurement: anular zone

Gaudin et al. Food Additives and Contaminants, Vol. 21, No. 5 (2004), pp. 422–433

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VALIDATION OF MICROCROBIOLOGICAL METHODS

STAR protocol

Validation in milk
Three interlaboratory profiency test

All antibiotics tested were spiked at 0.5x, 1x, 2x MRL

Table 2. Mean inhibition zones (mm), standard deviation and coefficient of variation (%) for each control disc. Data are shown for control discs compiled during the 7 months of the study: antibiotic disc concentration ($\mu g\,l^{-1}$), plates, mean inhibition zones (mm), standard deviations (mm), coefficients of variation (CV%) and the number of replicates (n).

	Chlortetracycline 200 $\mu g\,l^{-1}$	Streptomycin 2000 $\mu g\,l^{-1}$	Tylosin 1000 $\mu g\,l^{-1}$	Ciprofloxacin 100 $\mu g\,l^{-1}$	Sulphamethazine 1000 $\mu g\,l^{-1}$
Plate	Bc6	Bs7.2	Kv8	Ec8	Bst
Mean (mm)	5.47	4.67	5.13	4.83	5.33
SD (mm)	1.07	1.20	0.85	1.36	1.68
CV (%)	19.7	25.8	16.6	28.3	31.5
n	30	30	30	30	30

21 antibiotics ≤ MRL
27 antibiotics ≤ 4*MRL

Gaudin et al. Food Additives and Contaminants, Vol. 21, No. 5 (2004), pp. 422–433

STAR protocol

Sensitivity was defined as the lowest concentration for which positive results (inhibition zones more than 2 mm) were obtained for the four discs.

Bs7.2, Ec8, Bc6 and Bst plates = 0 mm
Kv8 = 0 83%
0<Ø<1 mm 7%
1< Ø<2 mm 10%
=2 mm 0%
N=30

Antibiotic	⩽ MRL	MRL ⩽ 4*MRL	>4*MRL
Macrolides	Erythromycin Neospiramycin Tilmicosin	Spiramycin Tylosin	
Penicillins	Penethamate Oxacillin Nafcillin	Ampicillin Amoxycillin Penicillin G Cloxacillin Dicloxacillin	
Aminoglycosides	Neomycin Framycetin	Gentamicin	Spectinomycin Streptomycin Dihydrostreptomycin Kanamycin
Cephalosporins	Ceftiofur Cephalexine	Cephapirine Cephalonium Cefquinome Cefoperazone	Cephazoline Cephacetril
Tetracyclines	Chlortetracycline	Tetracycline Oxytetracycline	
Quinolones	Danofloxacin Enrofloxacin Ciprofloxacin Marbofloxacin		Flumequine
Sulphonamides	Smethoxazole Sdiazine Schloropyridazine	Squinoxaline Sdimethoxine Smonomethoxine Snilamide Spyridine Sphenazole Smethizole Sdoxine Smerazine Sguanidine Scetamide	Smethazine Sthiazole Smethoxypyridazine
Lincosamides	Pirlimycin	Lincomycin	
Phenicolated			Thiamphenicol
Miscellaneous	Trimethoprim Baquiloprim	Rifaximin	Colistin Bacitracin Novobiocin

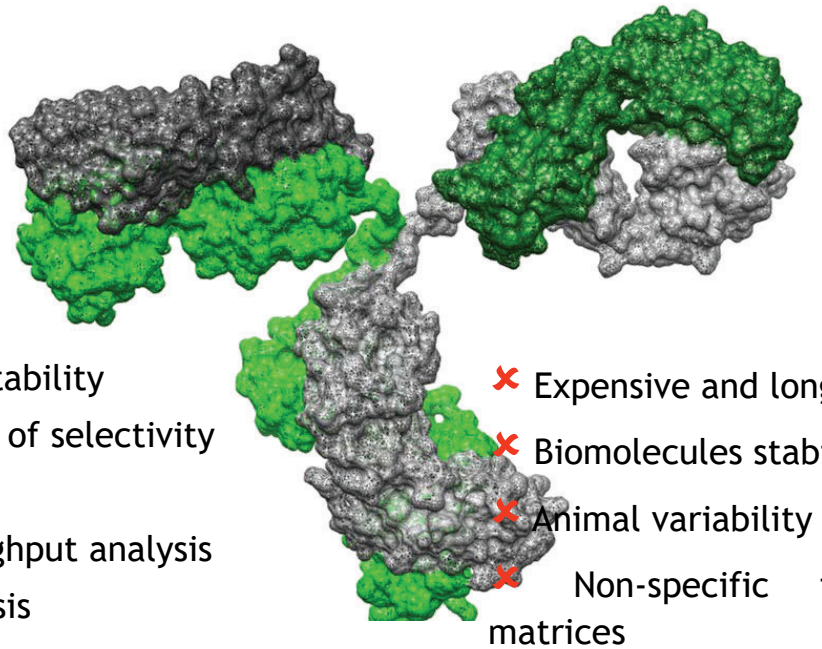
Gaudin et al. Food Additives and Contaminants, Vol. 21, No. 5 (2004), pp. 422–433

- KEY CONCEPTS
- MICROBIOLOGICAL METHODS
- **ELISA**
- LFIA
- BIOSENSORS

Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

IMMUNOCHEMICAL TECHNIQUES

THE ANTIBODY



- ✓ High detectability
- ✓ Wide range of selectivity
- ✓ Simple
- ✓ High-throughput analysis
- ✓ Field analysis

- ✗ Expensive and long development
- ✗ Biomolecules stability
- ✗ Animal variability in PAbs
- ✗ Non-specific interaction in matrices

HOW TO RAISE ANTIBODIES FOR SMALL MOLECULES????

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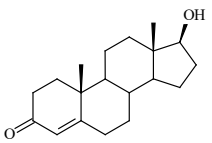
Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

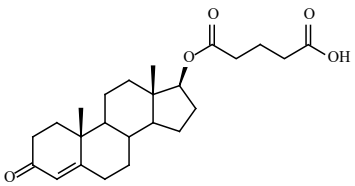
IAs FOR SMALL MOLECULES DETECTION

Small Molecules are not immunogenic by itself. Therefore, to produce antibodies against **SM** a derivative has to be synthesized to be able coupling to a carrier protein to inoculate into host animal.

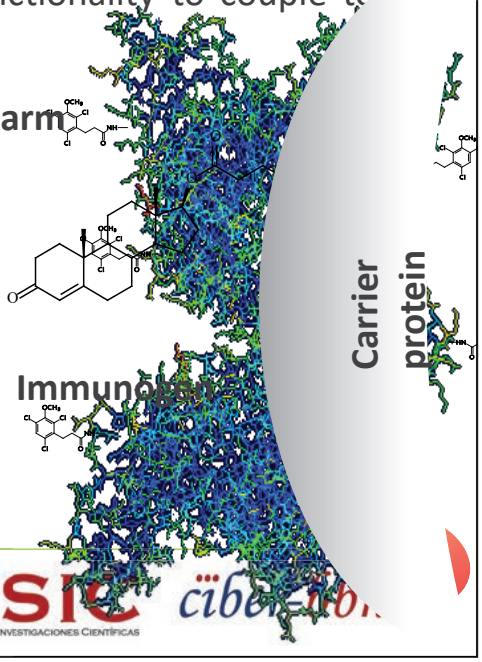
An **immunizing hapten** is a mimic of target analyte that has a similar physico-chemical properties with a additional functionality to couple to carrier protein.



Analyte



Hapten



Immun

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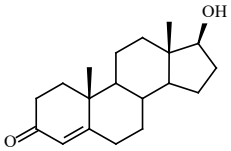
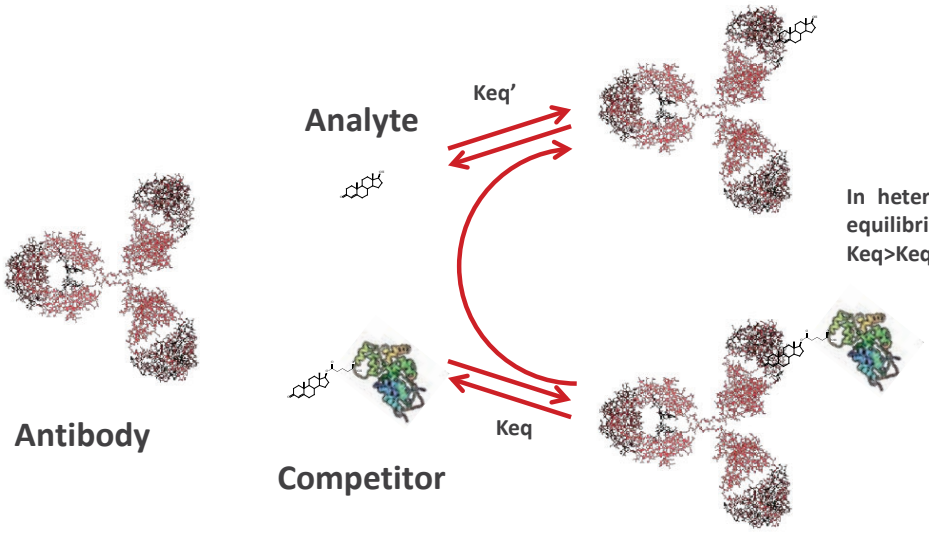
ELISA: HETEROLOGY



The detection usually takes place under **competitive** configurations.



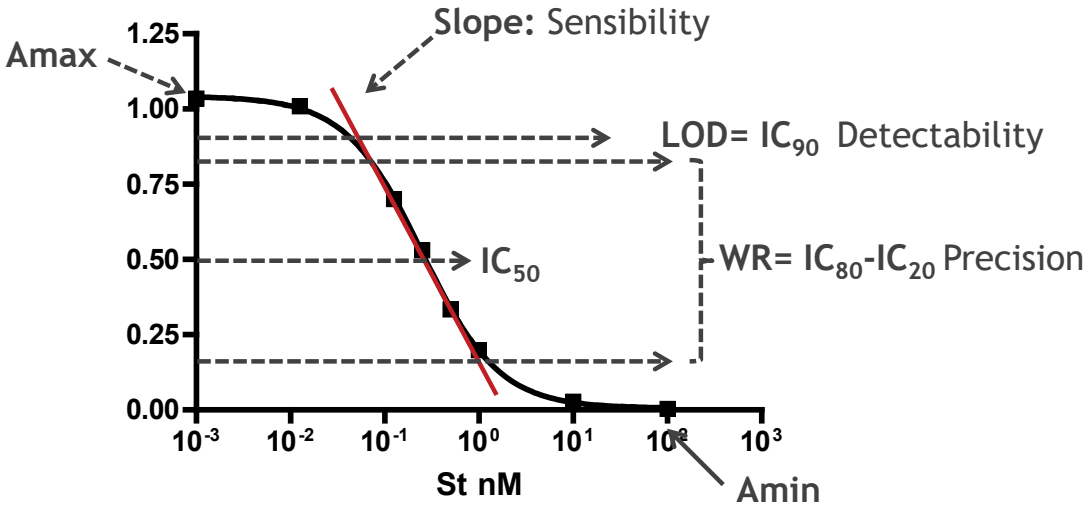
In heterologous conditions, the equilibrium is displaced due to Keq>Keq'

Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

ELISA: ANALYTICAL PARAMENTERS

Adjustment: Four logistic parameter equation

$$Y=(Amax - Amin)/[1 - (X/IC_{50})^{Slope} + Amin$$



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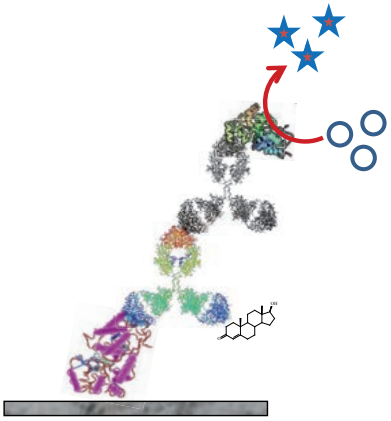
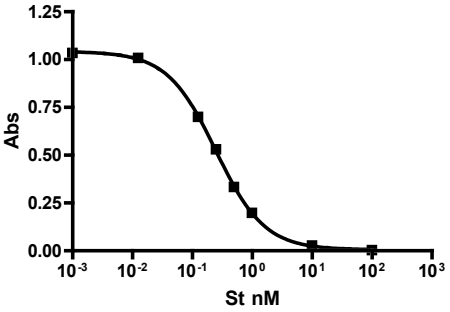
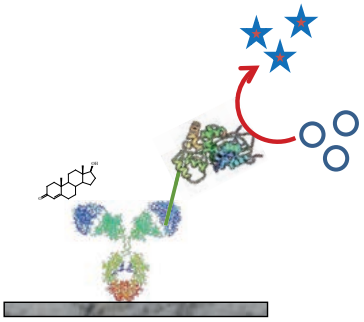
ELISA: COMPETITIVE FORMAT

Indirect Competitive ELISA



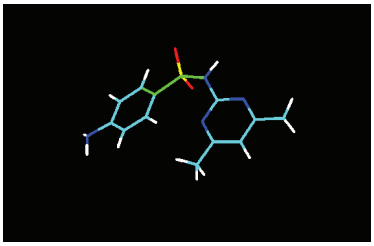


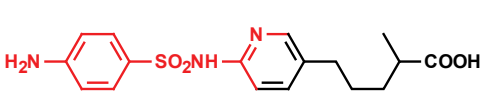
Direct Competitive ELISA

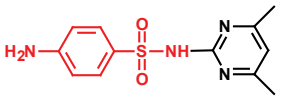


HAPTEN DESIGN: RAISE ANTIBODIES FOR FAMILY SAs DETECTION

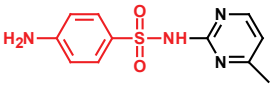
Example 2: SULFONAMIDE ANTIBIOTICS



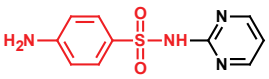




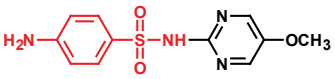
Sulfamethazine / Sulfadimidine



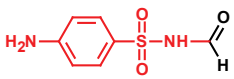
Sulfamerazine



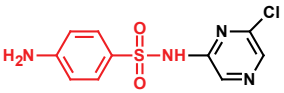
Sulfadiazine



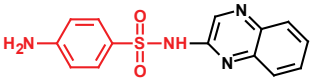
Sulfamethoxydiazine



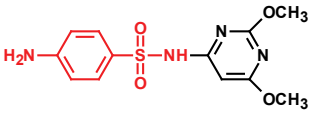
Sulfacetamida



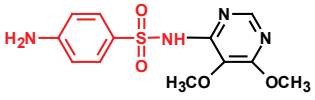
Sulfachloropirazine



Sulfaquinoxaline



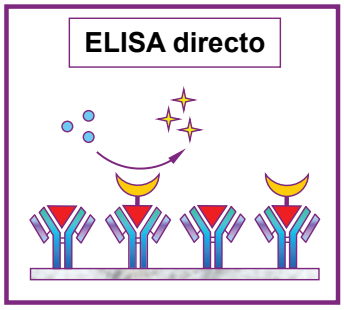
Sulfadimethoxine

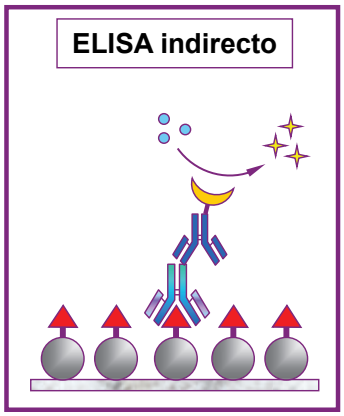


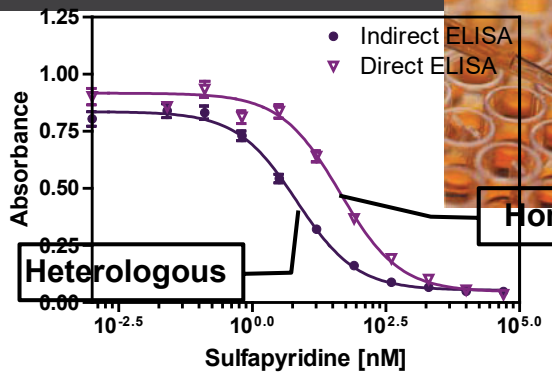
Sulfadoxine

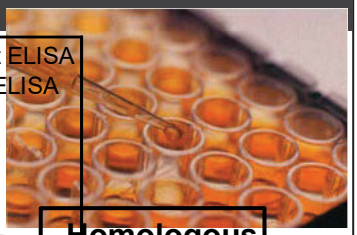
Adrian et al. J. Agric. Food Chem., 2009, 57 (2), pp 385–394

DEVELOPMENT OF AN ELISA





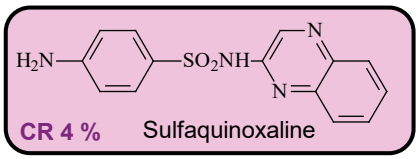


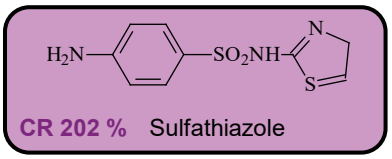


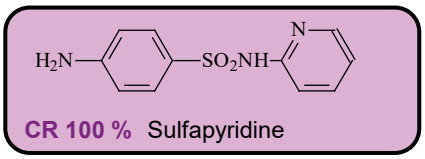
	HETEROLOGOUS	HOMOLOGOUS
	Direct ELISA As155/SA1	Indirect ELISA As155/SA2
A*max*	0.047	0.836
Am*in*	0.917	0.054
Slope	0.78	0.77
IC50, µg L^{-1}	**11.1 ± 1.3**	**1.69 ± 0.23**
LOD, µg L^{-1}	**0.49 ± 0.15**	**0.10 ± 0.06**
N	*11*	*11*

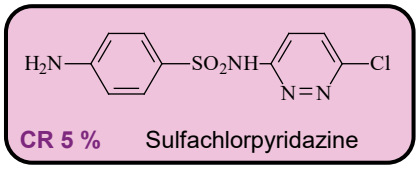
Adrian et al. J. Agric. Food Chem., 2009, 57 (2), pp 385–394

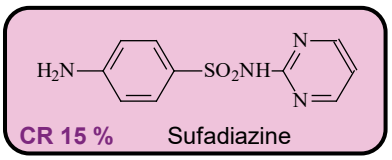
HAPTEN DESIGN: SELECTIVITY PROFILE

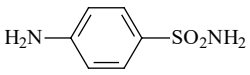

CR 4 % Sulfaquinoxaline

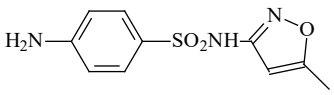

CR 202 % Sulfathiazole

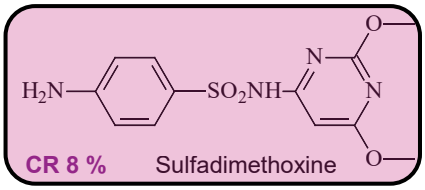

CR 100 % Sulfapyridine

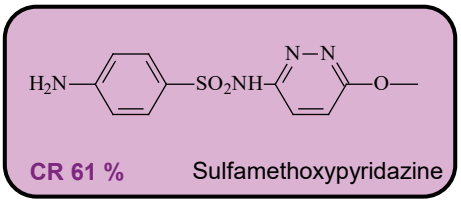

CR 5 % Sulfachlorpyridazine

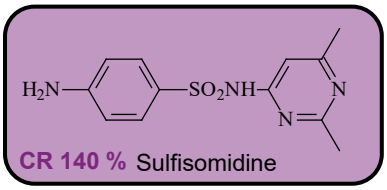

CR 15 % Sufadiazine

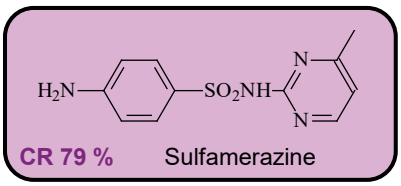

Sulfanilamide

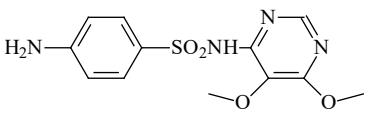

Sulfamethoxazole

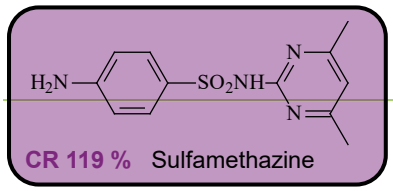

CR 8 % Sulfadimethoxine

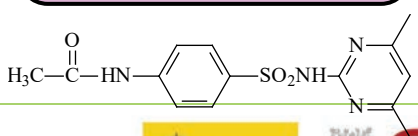

CR 61 % Sulfamethoxypyridazine

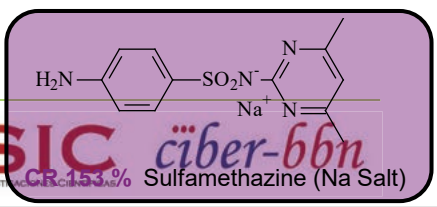

CR 140 % Sulfisomidine


CR 79 % Sulfamerazine


Sulfadoxine


CR 119 % Sulfamethazine


N4-Acetyl Sulfamethazine


CR 153 % Sulfamethazine (Na Salt)

HAPTEN DESIGN: DETECTABILITY PROFILE

Detectability achieved

Heterologous Competitor

Regulación 2377/90
MRL, maximum residue levels
Sulfonamides: **100 µg L^{-1}**

Immunochemical Analysis of Milk

	Indirect ELISA	
Compound	IC50(µg/L)	LOD(µg/L)
Sulfapyridine	2.25	0.15
Sulfaquinoxaline	79.15	1.17
Sulfachloropyridazine	61.81	1.34
Sulfamethoxazole	>>> MRL	>>> MRL
Sulfisomidine	2.10	0.23
Sulfathiazole	1.30	0.13
Sulfadiazin	14.05	0.38
Sulfadimethoxine	41.22	0.99
Sulfamerazine	3.73	0.43
Sulfadoxin	>>> MRL	>>> MRL
Sulfamethoxypyridazine	4.95	0.33
Sulfamethazine	1.78	0.15
N4-Acetyl-Sulfamethazine	>>> MRL	>>> MRL
Sulfamethazine (Sodium Salt)	1.49	0.09
Sulfanilamida	>>> MRL	>>> MRL

Adrian et al. J. Agric. Food Chem., 2009, 57 (2), pp 385–394

VALIDATION OF SULFONAMIDES ELISA

Validation must be in concordance **according** to the provisions of Council Decision **2002/657/EC**
Performance characteristics [specificity, accuracy, robustness, and **detection capability (CCß)**] were determined

Protocol of the assay

1g feed ---→ MRLs SAs in tissues 100 µg/g
Feeds no limits, but unauthorized
So, should be 0

+ 10 mL 95:5 ($ACN:H_2O$)

Centrifugation

Extract

ELISA **HPLC**

Jiménez, V. *et al*. *JAFC* **2010**, *58*, 7526-7531

VALIDATION OF SULFONAMIDES ELISA

Method of validation (CCß determination)

Threshold (T) = A – 2.33*SD

→ Average 20 blank samples

CCβ= T – 1.64*SD

→ Detection capability

Each day (N=4):

- 20 blank feed extracts
- 6 extracts spiked at T value for SDZ and SMZ
- 20 blank feed spiked at CCβ

Analytes

Single analyte with lowest CR*
Sulfapyridine (SPY) =100%
Sulfamethazine (SMZ)= 119%
Sulfadiazine (SDZ)= 15%

* Guidelines for the validation of screening methods for residues in veterinary medicines (2002/657/EC)

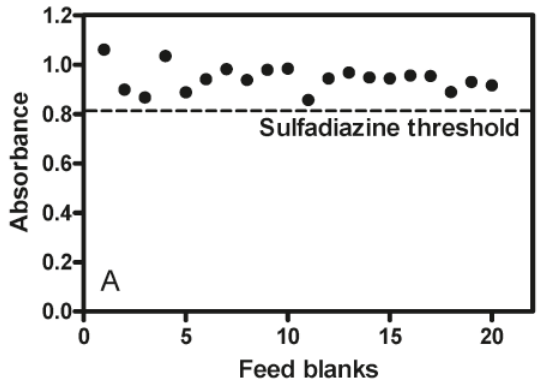
Jiménez, V. *et al*. *JAFC* **2010**, *58*, 7526-7531

Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

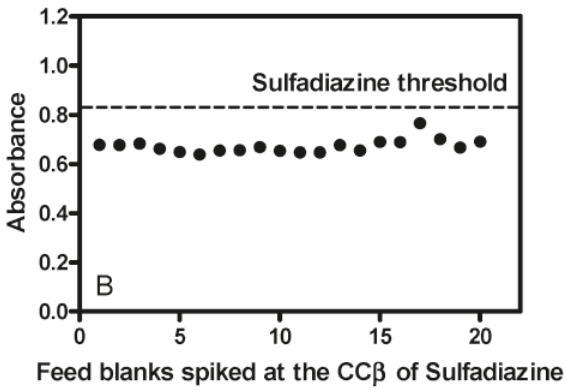
LOD, Threshold and CCβ

µg/g	LOD	T	CCβ
Sulfamethazine	0.03	0.06	0.1
Sulfadiazine	0.12	0.4	0.8

No false non-compliant verification for sulfadiazine



No false compliant verification for sulfadiazine



Jiménez, V. *et al*. *JAFC* **2010**, *58*, 7526-7531

VALIDATION LC vs ELISA

Table 2. Results Obtained by LC and ELISA for Feed Samples

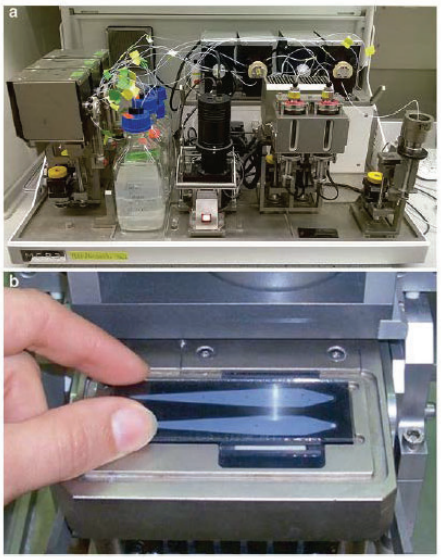
	LC		ELISA	
sample	μg of sulfadiazine/g of feed	% RSD	μg of sulfadiazine equiv/g of feed	% RSD
S1	3.3	4	4.2	25
S2	0.82	8	0.84	22
S3	8.7	5	7.7	12
S4	0.85	8	0.77	14
S5	53	9	45	7
S6	415	5	498	19
S7	5559	5	5177	24

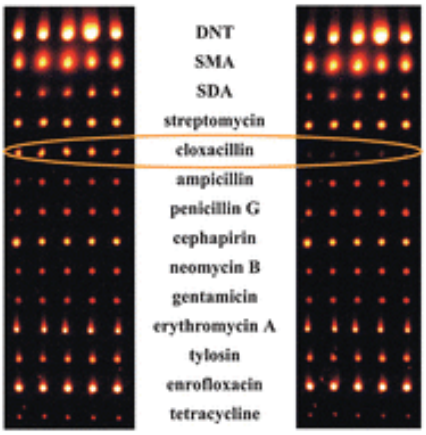
Jiménez, V. *et al*. *JAFC* **2010**, *58*, 7526-7531

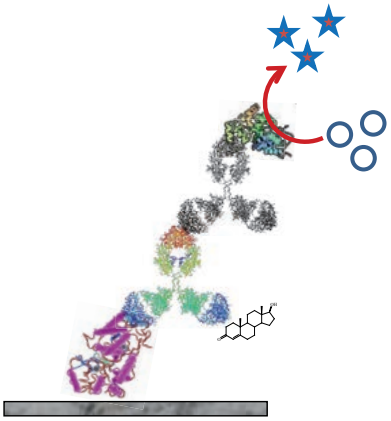
Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

MULTIPLEXED ANTIBIOTIC IMMUNOASSAY

MCR3 setup and chip







Meyer, V. K.; et al. In Small Molecule Microarrays: Methods and Protocols, 2017, pp 195-212

Validation according to **2002/657/EC**
Considering precision, specificity, accuracy, ruggedness, repeatability, recovery, decision limit (CCα) and detection capability (CCβ)

Protocol of the assay

Raw milk---→

↓

dilution

CLIA

Antibiotic	MRL [ng/mL]
Ampicillin	4
Penicillin G	4
Cloxacillin	30
Nafcillin	30
Ceftiofur	100
Enrofloxacin	100
Sulfamethazine	100
Sulfadiazine	100
Streptomycin	200
Neomycin	1500
Gentamicin	100
Tylosin	50
Cephapirin	60

Kloth, K. et al. Analyst **2009**, 134, 1433-1439.

Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

VALIDATION OF MULTIPLEXED CLIA

Road map for validation:

1. Repeatability: Determine calibration curves at several days and on different chips. CV< 10%
2. Determination of LoD as: 20 blanks and calculate $\bar{x} \pm 3*SD$. **LOD < MRL**
3. Specificity of signal: Comparison cocktail vs individual antibodies
4. Stability of milk: 4ºC, -20ºC (1d, 3d, 1w and 4w)
5. Determination of CCα, 20 blanks spiked at MRL value and calculate as $\bar{x} \pm 1.64*SD$
6. Determination of CCβ, 20 blanks spiked at MRL value and calculate as $\bar{x} \pm 1.64*SD$
7. Confirm deternination of CCβ by measuring 20 blanks samples spiked at CCβ. Should be < 5%
8. Recovery at 0.5*MRL, 1*MRL and 1.5*MRL
9. Ruggedness, measuring MRL at different times, T, sources of milk and N=2

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Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

OUTLINE

- KEY CONCEPTS
- MICROBIOLOGICAL METHODS
- ELISA
- **LFIA**
- BIOSENSORS

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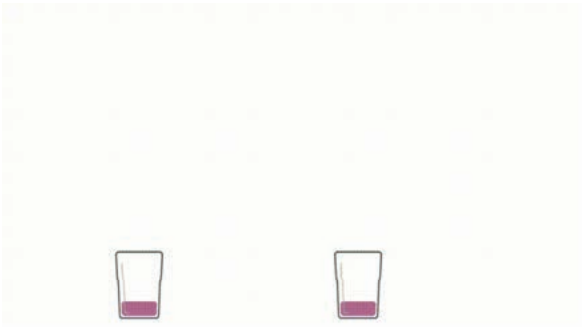
Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

VALIDATION OF MULTIPLEXED LFIA

Lateral flow immunoassay (AFLA-V AQUA™)



Unisensor

Aflatoxin B1 (AFB1)

Protocol of the assay

5g maize ---→ **LC-MS/MS protocol**

AOAC official method 991.31

+25 mL AQUA premix solution)

↓

Filtration

Extract

Add 100 uL LFIA

Lattanzio et al. Anal. Methods, 2018, 10, 123–130

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Validation design

Considering

EU 2002/657/EC
EU 519/2014/EC

Test samples

a) **Blank samples**
b) **Spiked AFB1 at 2 ppb**
c) **Spiked AFB1 at 4 ppb**

DEFINE
compliant vs suspect non-compliant

5 days
three maize batches
Two of them per duplicate

} 25 measurements/day/concentration

Screening target concentration (STC)

Cut-off value = $R_{STC} - t_{value(0.05)} * SD_{STC}$

Reybroeck et al. Food Additives & Contaminants: Part A, 2014, Vol. 31, No. 12, 2080–2089

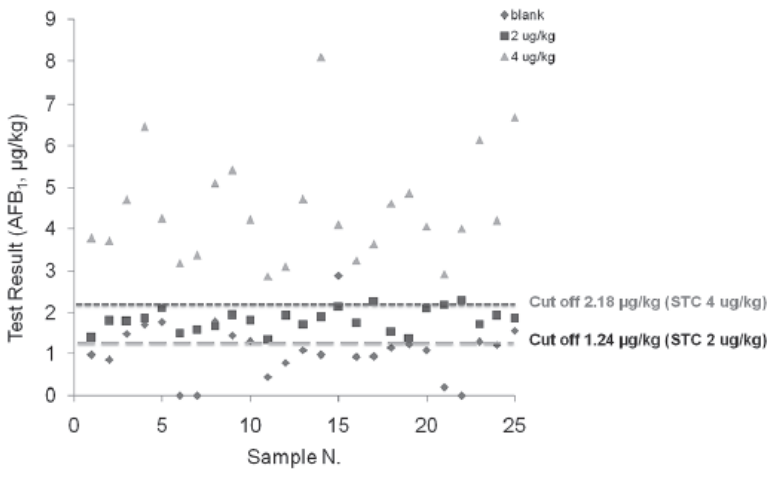
Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

VALIDATION AFB1 LFIA

Validation design

Considering

EU 2002/657/EC
EU 519/2014/EC



Reference value (µg kg^{-1})			STC 2 µg kg^{-1}	STC 4 µg kg^{-1}
5.8 ± 0.4	Mean response (µg kg^{-1})[a]	7.9		
	RSD_{ip} (%)[b]	14.0		
	Rate of false negative%		0.01	0.03
7.7 ± 1.3	Mean response (µg kg^{-1})[a]	8.9		
	RSD_{ip} (%)[b]	21.3		
	Rate of false negative%		0.14	0.06

	Reference value (µg kg^{-1})	Test result[a] (µg kg^{-1})
Trilogy A-C-285	5.9 ± 1.2	5.8 ± 0.2
Trilogy A-C-274	7.3 ± 0.9	9.3 ± 0.4
Trilogy A-C-276	1.7 ± 0.3	2.3 ± 0.4
Biopure BRM003027	4.52 ± 1.20	4.9 ± 0.2

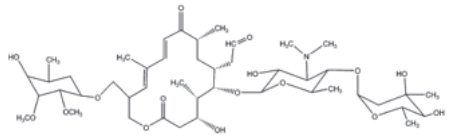
Reybroeck et al. Food Additives & Contaminants: Part A, 2014, Vol. 31, No. 12, 2080–2089

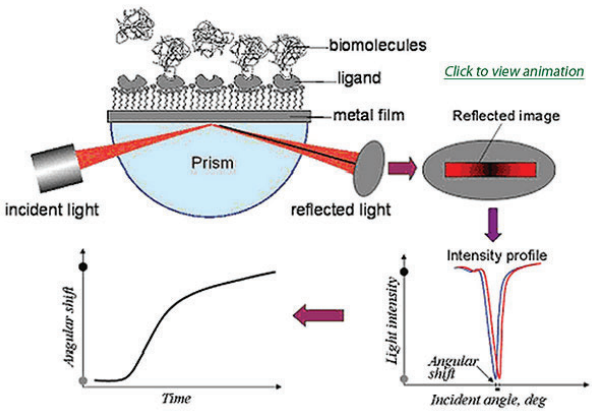
OUTLINE

- KEY CONCEPTS
- MICROBIOLOGICAL METHODS
- ELISA
- LFIA
- **BIOSENSORS**

VALIDATION SRP BIOSENSORS

SPR FOR TYLOSINE A DETECTION





MRLs in tissues
Honey no limits, but unauthorized
So, should be 0

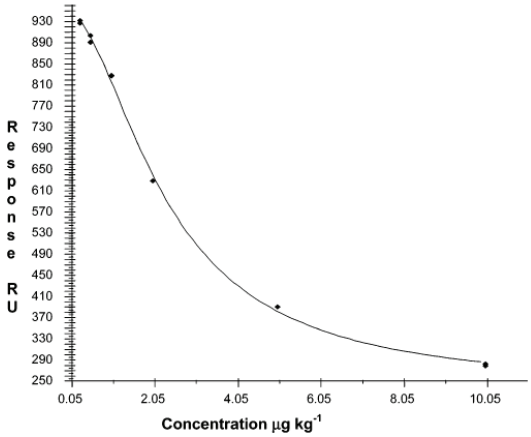
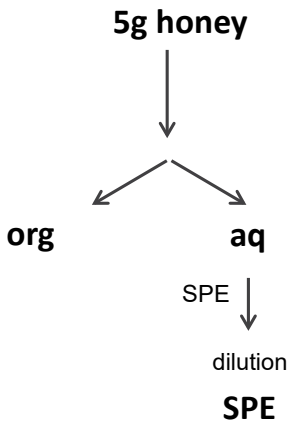


Figure 2. SPR biosensor calibration curve using matrix-matched standards in the range 0.05–10 μg kg^{-1}.

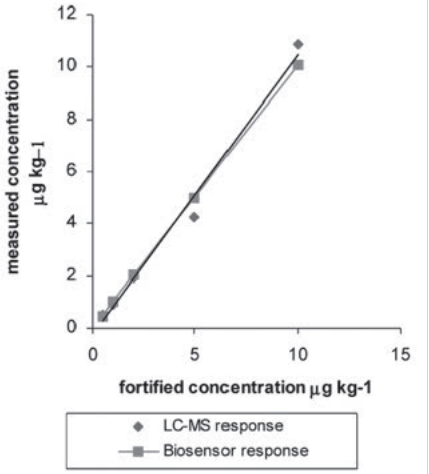
Protocol of the assay



- **Determination of CCß**

LOD: 0.5 µg/Kg
CCβ: 20 samples of known honey blank were spiked with tylosin at 2.5 µg kg-1.

Recovery 60.3% (CV 9.3%)
Repeatability 61.5% (CV 7.5%)
Robustness
Specificity
Accuracy



Jiménez, V. *et al*. *JAFC* 53 (19) 2005, 7367

Take into consideration current directives on the Validation of

Screening meethods: Directive 2002/657/EC

Be updated about MRL and MRPL

Calculate CCβ

Establish your STC and QC

QUEEN'S UNIVERSITY BELFAST | THE INSTITUTE FOR GLOBAL FOOD SECURITY | FoodSmartphone.eu

Workshop: Development of Fit-for-Purpose Validation Protocols for Smartphone-based Assays

Dr. Cuong Cao

Lecturer in Advanced Micro- and Nanodiagnostics

Institute for Global Food Security

School of Biological Sciences, Queen's University Belfast

E-mail: c.cao@qub.ac.uk

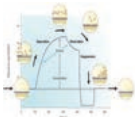
Advanced Micro- and Nano-Diagnostics Laboratory

PI: Cuong

PDRA: Seong Ying/ Casy

PhD student: Natasha, Javier, Michaela, Brendan

MSc: Alex



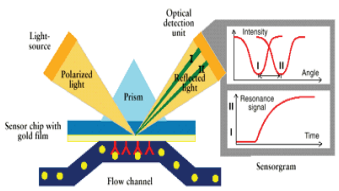
"Designing and realizing novel biosensing platforms exploiting micro- and nanotechnologies for diagnosis"

T1. Multifunctional Micro- and Nanostructures
1. Synthesis
2. Mechanism
3. Functionalization

T2. Advanced Plasmonic Biosensing Platforms
1. SPR
2. LSPR and SERS
3. Metamaterials

T3. Integrated Point-of-Care Analysis
1. Microfluidics
2. Paper formats
3. Slide formats

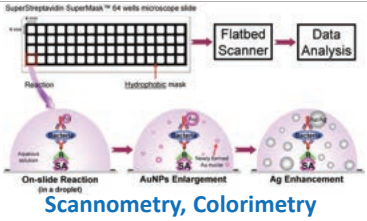
Detection and identification of drugs, toxins, pathogens, biomarkers



SPR Biosensor

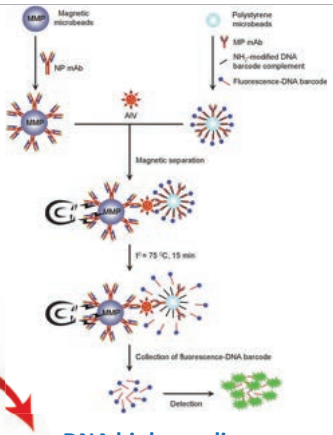
PSA: *Biosens. Bioelectron. 21 (2006) 2106*

Anti-GAD Ab: *Biosens. Bioelectron. 22 (2007) 1874*



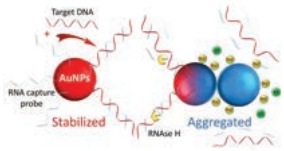
Scannometry, Colorimetry

Whole cell: *Small 2011, 7, 1701*



DNA biobarcoding

Virus: *Analyst, 2010, 135, 337*

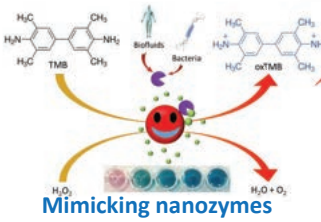


Plasmonic Gold Nanoparticles-LSPR

DNA: *Biosens. Bioelectron. 92 (2017) 502*

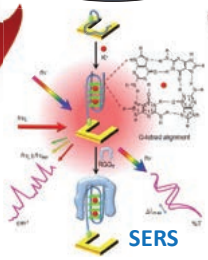
K-Ras gene: *Biosens. Bioelectron. 63(2015) 472*

Technological Platforms for Diagnostics



Mimicking nanozymes

Enzyme biomarkers (protease, lipase (*submitted*))

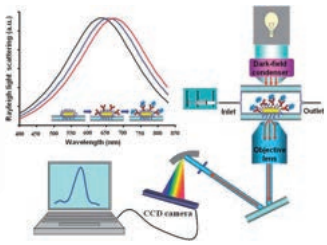


SERS

DNA, nucleolin: *ACS Nano, 2013, 7, 7583*

Small molecules: *Small 2014, 10, 3252; Adv. Mater. 2014, 26, 2431*

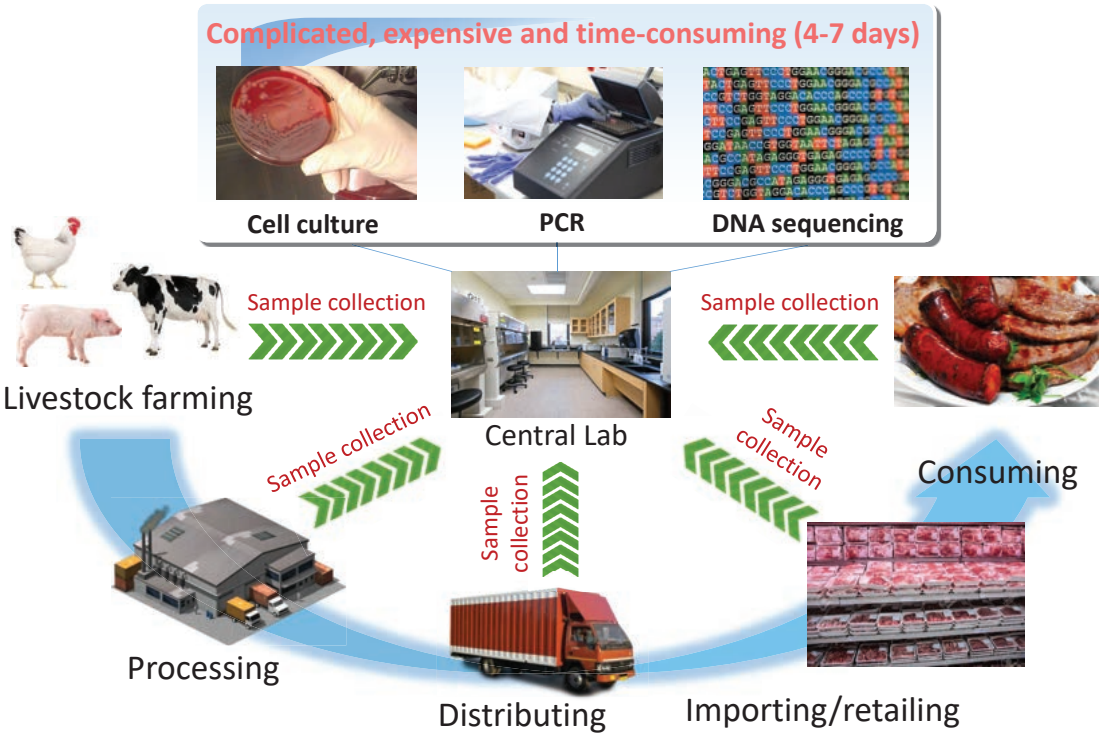
ACS Nano 2014; 8: 3796-3806



Single Particle Spectroscopy

Protein: *Lab Chip, 2009, 9, 1836; Lab Chip, 2011, 11, 2591*

… Moving Toward Mobile Health Technology



… Moving Toward Mobile Health Technology

Detection of food-borne pathogens and AMR

Funded by

Invest Northern Ireland

Collaborating with

Short analysis time: less than 90 min

Detection of food spoilage micro-organisms

Funded by

MARIE CURIE ACTIONS

European Commission | Horizon 2020 European Union funding for Research & Innovation

Collaborating with:

FoodSmartPhone Colleagues

Smartphone-based Assays: State-of-the-Art

(Photo adapted from http://www.emfnews.org)

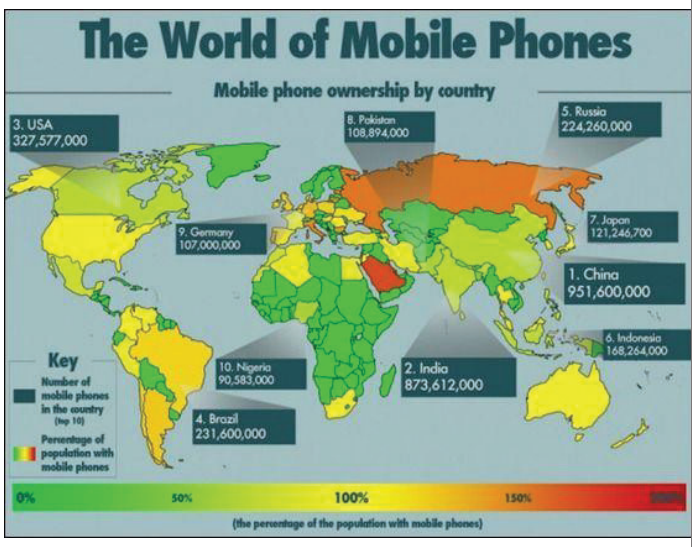
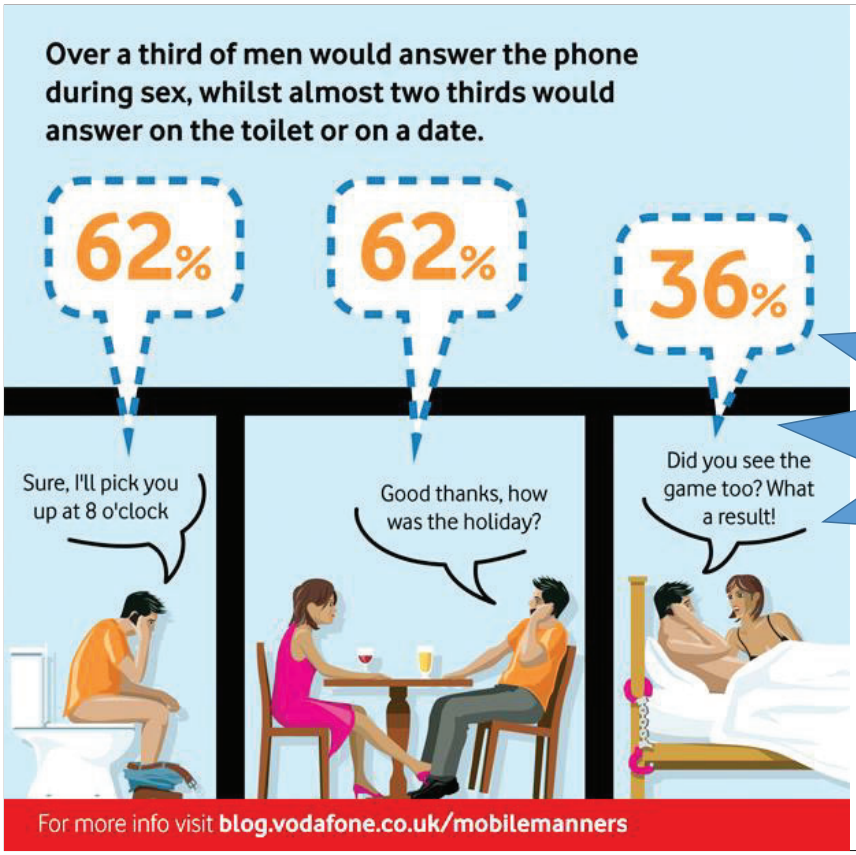


Image adapted from https://www.trendhunter.com)

The world is home to 7.2 billion gadgets, and they're multiplying five times faster than we are!



We are infinitely connected anywhere, at any time!!!

Mobile Phone: Not only for Making Phone Call

Improvements in Smartphone Allowing It to Play as On-Site and On-Line Diagnostic Devices

Powerful hardware

- On-board processing
- Memory capacity
- Dual camera
- GPS
- Wifi, wireless connectivities

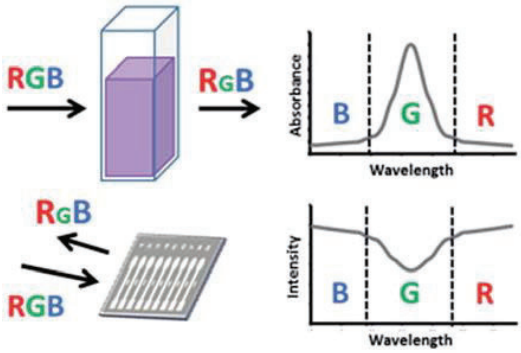
Ultraportable optical sensing platform

- Fully integrated sensor
- Detector
- Data processor
- Instrument interface
- Data storage and communication

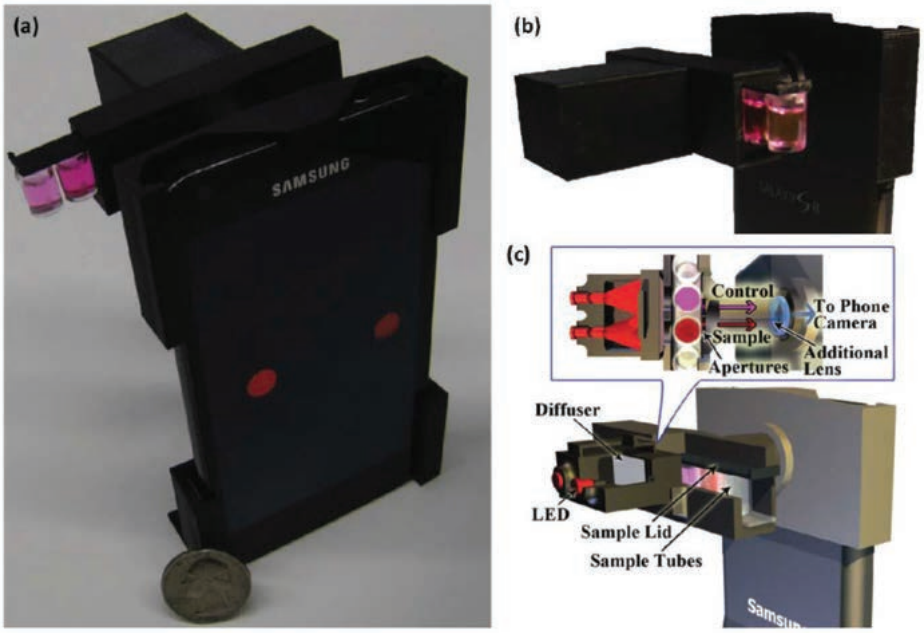
Onsite and online diagnostics

- Low cost
- Responsive
- Suitable for resource-limited settings

Colorimetric Smartphone Based Platform

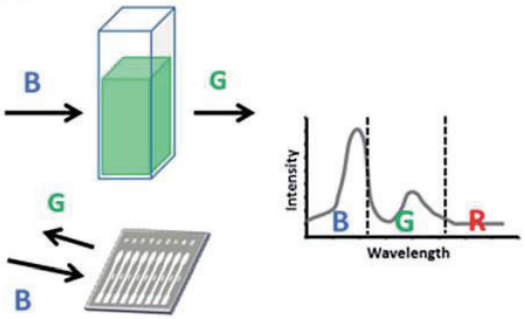


Typical optical detection for both light transmitted through liquid sample platforms (i.e. cuvette, well plate) and light reflected from solid sample platforms (i.e. test strip, cassette) using colorimetric assays
Anal. Methods, 2016, 8, 6591–6601

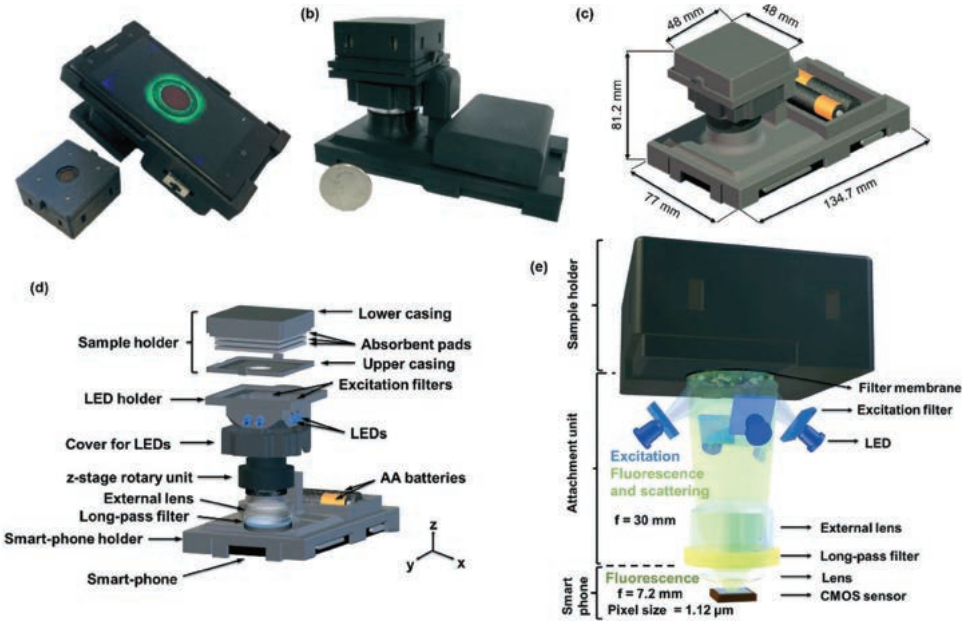


Colorimetric smartphone based platform for allergens
Lab Chip, 2013, 13, 636–640

Fluorescence Platform

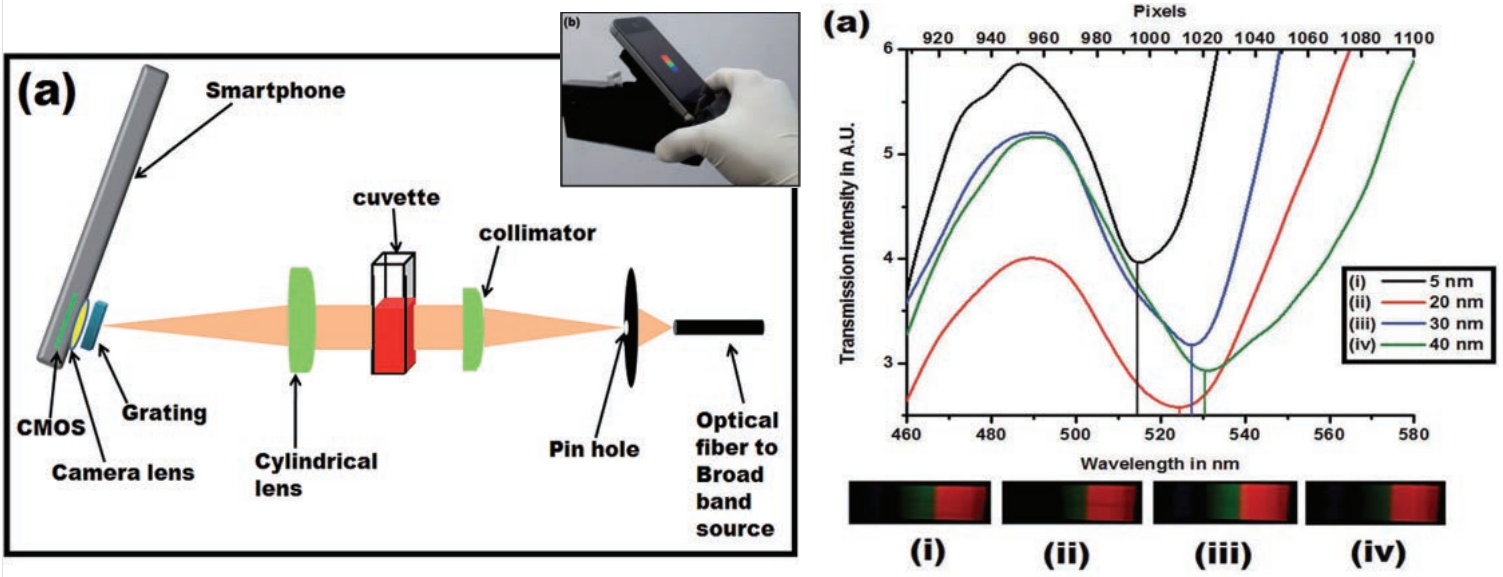


Typical configuration for fluorescence assays
Anal. Methods, 2016, 8, 6591–6601



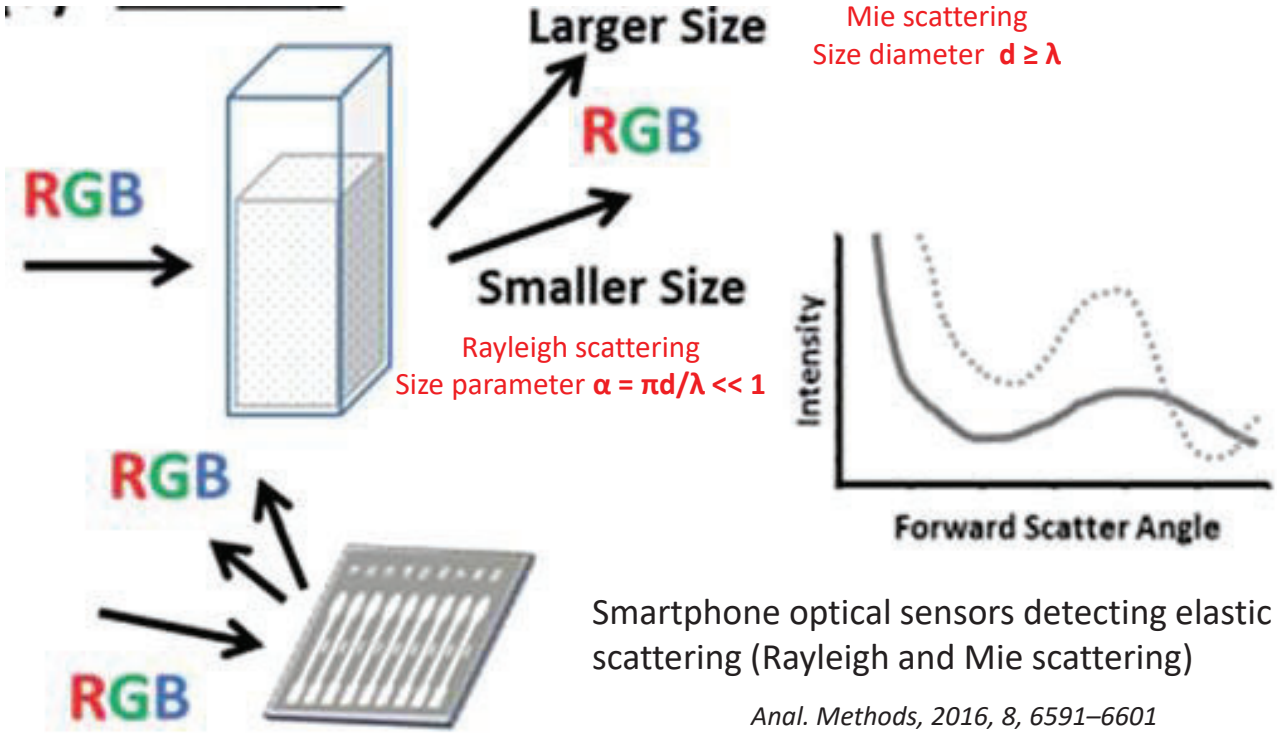
Fluorescence combined with microscopy for the detection of *Giardia lamblia* (a–b) Digital photographs of smart-phone based fluorescent microscope, including a disposable sample cassette. (c) Schematic illustration demonstrating the dimensions of the detection platform. (d) Expanded view of the design. (e) Schematic illustration of the illumination/ excitation path (*Lab Chip, 2015, 15, 1284–1293*)

LSPR Spectroscopy-based Smartphone Platform



LSPR spectroscopy-based smartphone platform using gold nanoparticles
RSC Adv., 2016, 6, 21871–21880

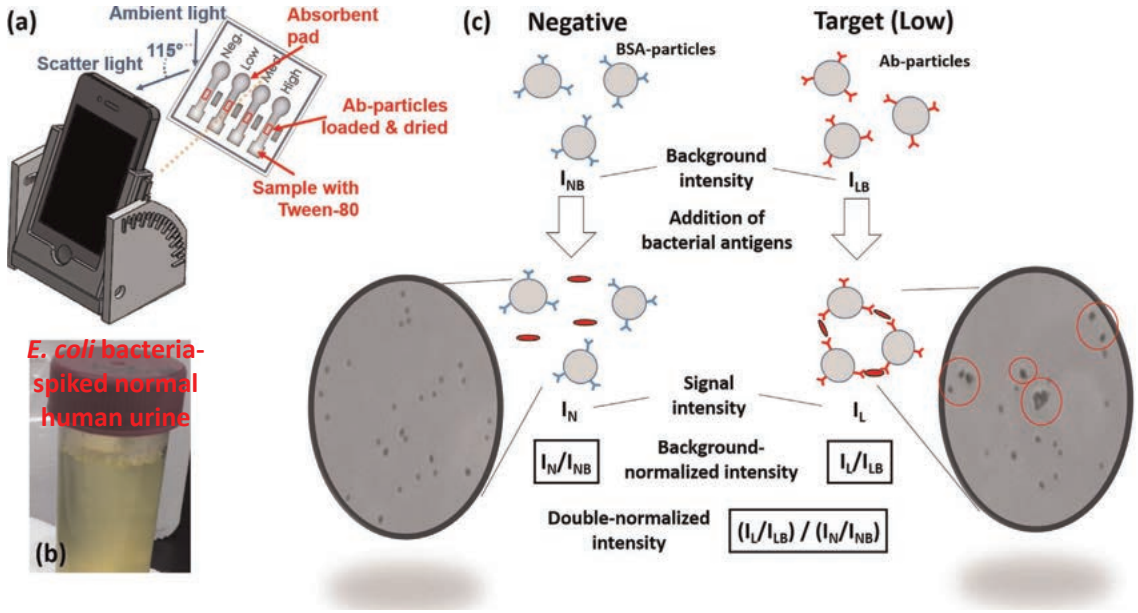
Scattering-based Smartphone Platform



Smartphone optical sensors detecting elastic light scattering (Rayleigh and Mie scattering)

Anal. Methods, 2016, 8, 6591–6601

Scattering-based Smartphone Platform



Antibody-conjugated particles (Ab-particles) are loaded on the mPAD (a) in the loading site next to horizontal marker, located at the center of each channel. Sample with Tween80 is loaded onto the inlet that flows through each channel towards the loading site. Target antigens in the sample induce immunoagglutination of Ab-particles. This causes an increase in light scattering, which is captured by a smartphone (*Biosensors and Bioelectronics 74(2015), 601–611*)

Possible Innovation Aspects and Benefits

Resource-poor settings
- Electricity
- Controlled lighting
- Sanitation
- Accredited precision equipment
- Trained personnel

ASSURED Diagnostics

- **A** Affordable
- **S** Sensitive
- **S** Specific
- **U** User-friendly
- **R** Rapid and Robust
- **E** Equipment-free
- **D** Delivered to those who need it

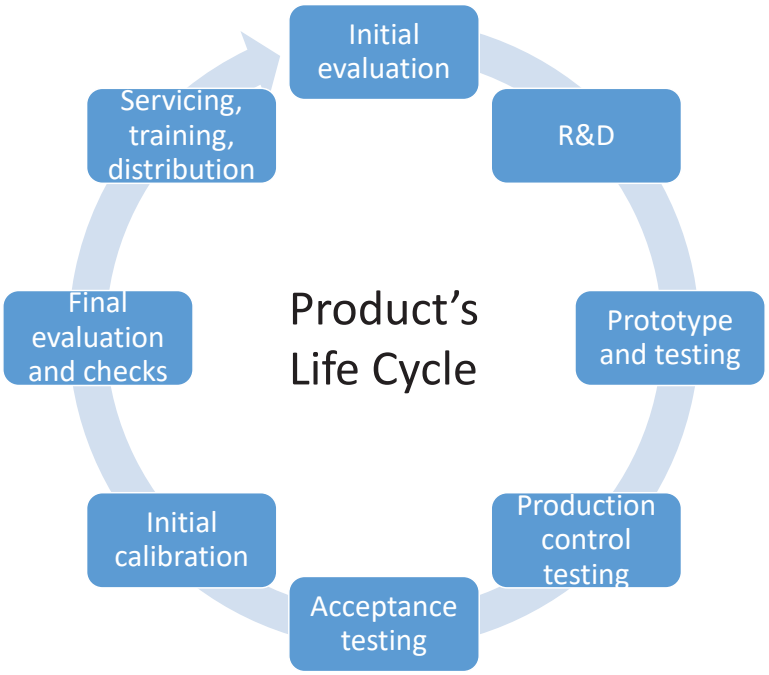
But… there're inherent drawbacks and challenges …

Interference factors

- **Environment**
  - Lighting
  - Temperature
  - Humidity
- **Complexity of sample matrix**
  - Dust
  - Algae, untargeted cells
  - DNA, protein
- **Adaptability/ Versatility**
  - Proof-of-concept, R&D stage
  - Not yet clinically validated
- **Sensitivity**
  - Lack full sensitivity of designated sensor instrumentation

Smartphone-based bioassays need to be validated so that they can be fit-for-purpose

Life Cycle of Medical Device Development and Production



- ✓Smartphones used for medical purposes should be considered as medical devices
- ✓Therefore, development of smartphone-based biosensors/bioassays must adhere to quality control requirements of regulatory authorities (i.e. EN ISO 13485)

What is Validation?

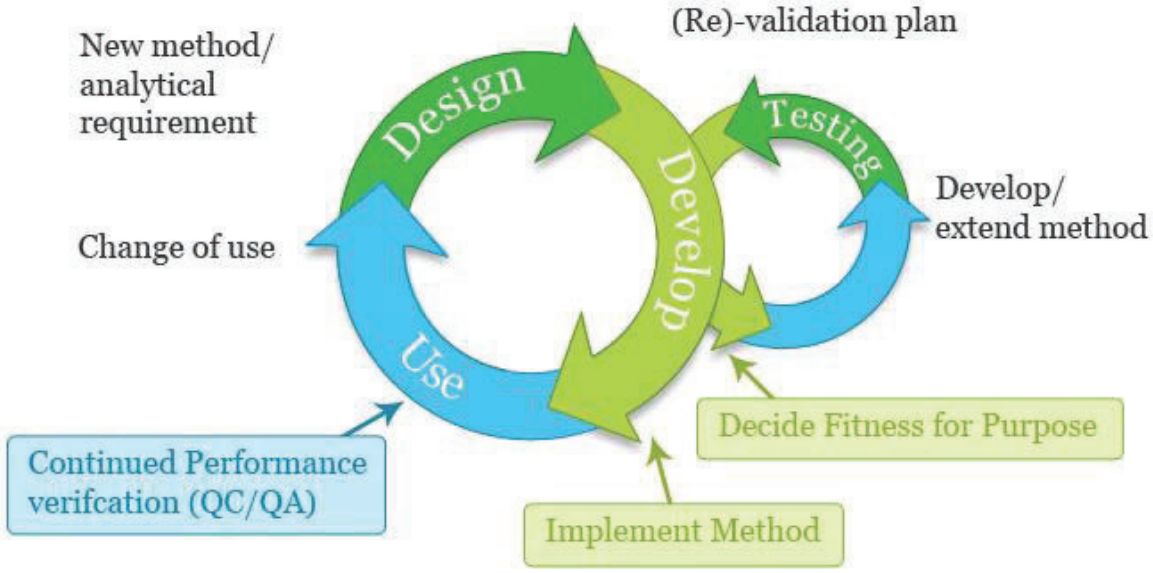
"Validation is the confirmation by examination and the provision of **objective evidence** that the particular requirements for **a specific intended use** are fulfilled" (***ISO/IEC 17025:2005 cl. 5.4.5.1***)

Analytical System = Analytical procedure/ protocol + target analyte + sample matrix

- **Specific intended use:** The performance characteristics
- **Objective evidence:** experimental data that help judge the system suitability
- Closely tied to development stage

Validation Vs Verification

ISO 9000: "Verification is the confirmation, through provision of objective evidence, that specified requirements have been fulfilled"



Validation or Verification: When?

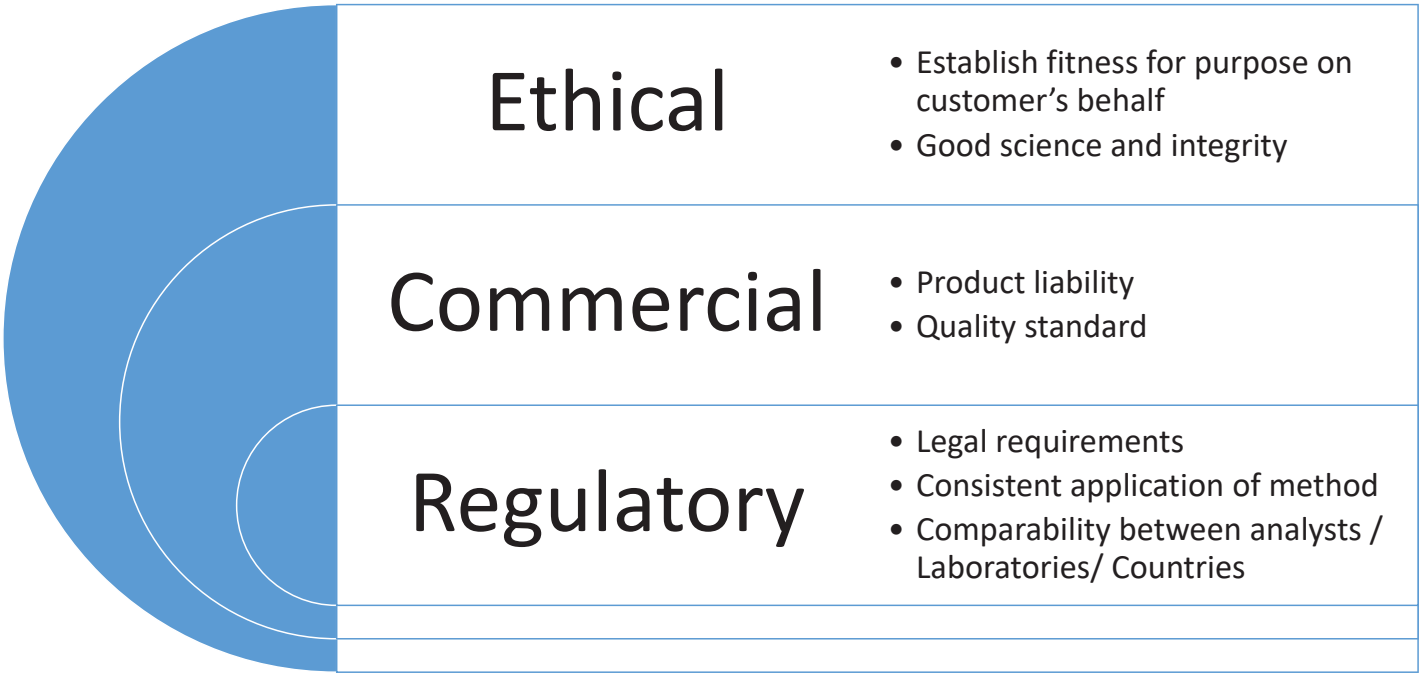
Validation

- Non-standard methods: new methods, laboratory designed/developed methods
- Standard methods used outside their intended scope (e.g. new targets, new problems)
- Amplifications and modifications of standard methods
- Demonstration of the equivalence between two methods, e.g. a new method vs. a standard method

Verification

- Standard(ised) methods (i.e. published by Iso, AOAC, etc.)
- There is an important change such as a new but similar instrument, an instrument is updated with new software, relocation of equipment etc.
- Quality control indicates that the performance of an established method is changing with time

Importance of Validation



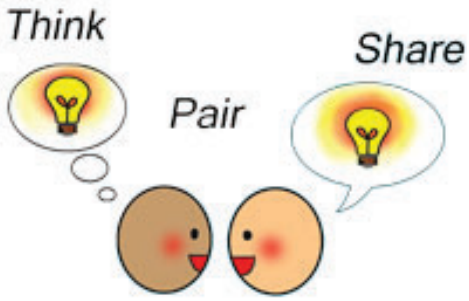
A new person is employed in your lab and she will replace a colleague in doing certain analyses. Does this require any changes concerning validation?

A. Yes, new data for important validation parameters should be collected and compared/pooled with the existing data.

B. Yes, the methods should be validated from scratch.

C. No, method parameters do not need to be determined again after the validation has been completed once by the previous employee.

Exercise 1: 10 min



Work in pair. Tell your friend about:

1. What are the analytical system (i.e. analytical procedure/ protocol + target analyte + sample matrix) you working on in relation to validation?

2. What is to be accomplished by the study ?

3. Outline the purpose, e.g. full validation of a new method/device, verification of performance of a standardised method/device, extension to method scope, etc.?. The **ANTICIPATED** extent of the validation work, i.e. the performance characteristics which will be investigated and any associated requirements?

Vice versa, your friend will need to share with you the same

Performance Characteristics to Be Validated?

Selectivity	Accuracy, Precision, and Recovery	The calibration curve
Sensitivity: Limit of detection and limit of quantification	Range and linearity	Robustness-Ruggedness

Selectivity

Selectivity: "*the extent to which the method can be used to determine **particular analytes** in **mixtures or matrices** without **interferences from other components of similar behaviour***"
*(Selectivity in analytical chemistry (IUPAC recommendations 2001), Pure Appl. Chem., 2001, **73**(8), 1381)*

An analyte may exist in the sample in more than one form:

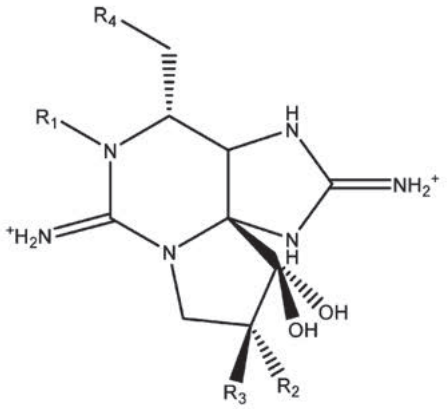
- ✓ Different analogues;
- ✓ Bound or unbound;
- ✓ Inorganic or organometallic;
- ✓ Or different oxidation states

Different matrices have different degrees of interference, and thus ..**X**.. ?

Selectivity

There are greater than 20 main analogues of saxitoxin, each with a different toxicity factor, responsible for Paralytic shellfish poisoning (PSP) toxins in shellfish

(Anal. Chem. **2010,** *82,* 2977–2988)



			Carbamate Toxins	*N*-Sulfocarbamoyl toxins	Decarbamoyl toxins	Deoxydecarbamoyl toxins
R_1	R_2	R_3	R_4- $OCONH_2$	R_4- $OCONHSO_3^-$	R_4- OH	R_4- H
H	H	H	STX	B1 (GTX 5)	dc-STX	do-STX
H	H	OSO_3^-	GTX 2	C1	dc-GTX 2	do-GTX 2
H	OSO_3^-	H	GTX 3	C2	dc-GTX 3	do-GTX 3
OH	H	H	NEO	B2 (GTX 6)	dc-NEO	
OH	H	OSO_3^-	GTX 1	C3	dc-GTX 1	
OH	OSO_3^-	H	GTX 4	C4	dc-GTX 4	

Examples of Food Commodities Containing Allergens

Table 1. Food commodities that should be included in cross-reactivity testing for ELISA methods targeting egg

Adzuki beans	Almond	Barley	Beef	Brazil nut
Buckwheat	Cashew	Chestnut	Chick peas	Chicken
Cocoa	Coconut	Corn	Crustacean/prawn/shrimp	Duck
Fish	Gelatin (bovine)	Hazelnut	Kidney beans	Kiwi
Lecithin	Lentils	Lima beans	Linseed	Macadamia nut
Milk	Oats	Octopus	Peanut	Peas
Pecans	Pine nut	Pistachio	Poppy seeds	Pork
Pumpkin seed	Rice—white and brown	Rye	Sesame	Soybean
Split peas	Sunflower seed	Turkey	Walnut	Wheat

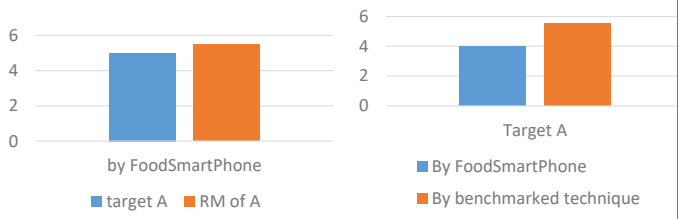
Table 2. Food commodities that should be included in cross-reactivity testing for ELISA methods targeting milk

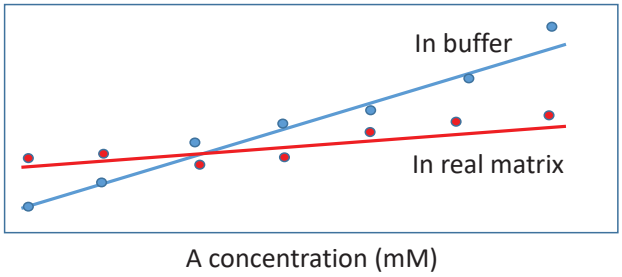
Almond	Barley	Brazil nut	Beef	Buckwheat
Cashew	Chick peas	Cocoa	Corn meal	Crustacean/prawn
Egg	Fish	Hazelnut	Lecithin	Lima bean
Oats	Peas	Peanut	Pecan	Pine nut
Pistachio	Poppy seed	Pumpkin seed	Rice–white and brown	Rye
Sesame seed	Soy bean	Split peas	Sunflower seed	Walnut
Wheat				

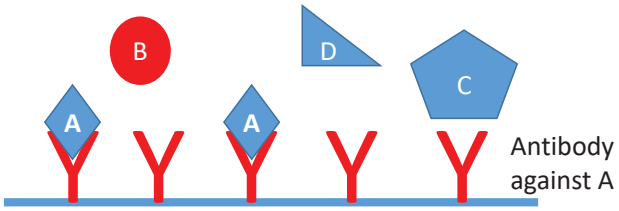
(ABBOTT ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 93, NO. 2, 2010)

Selectivity

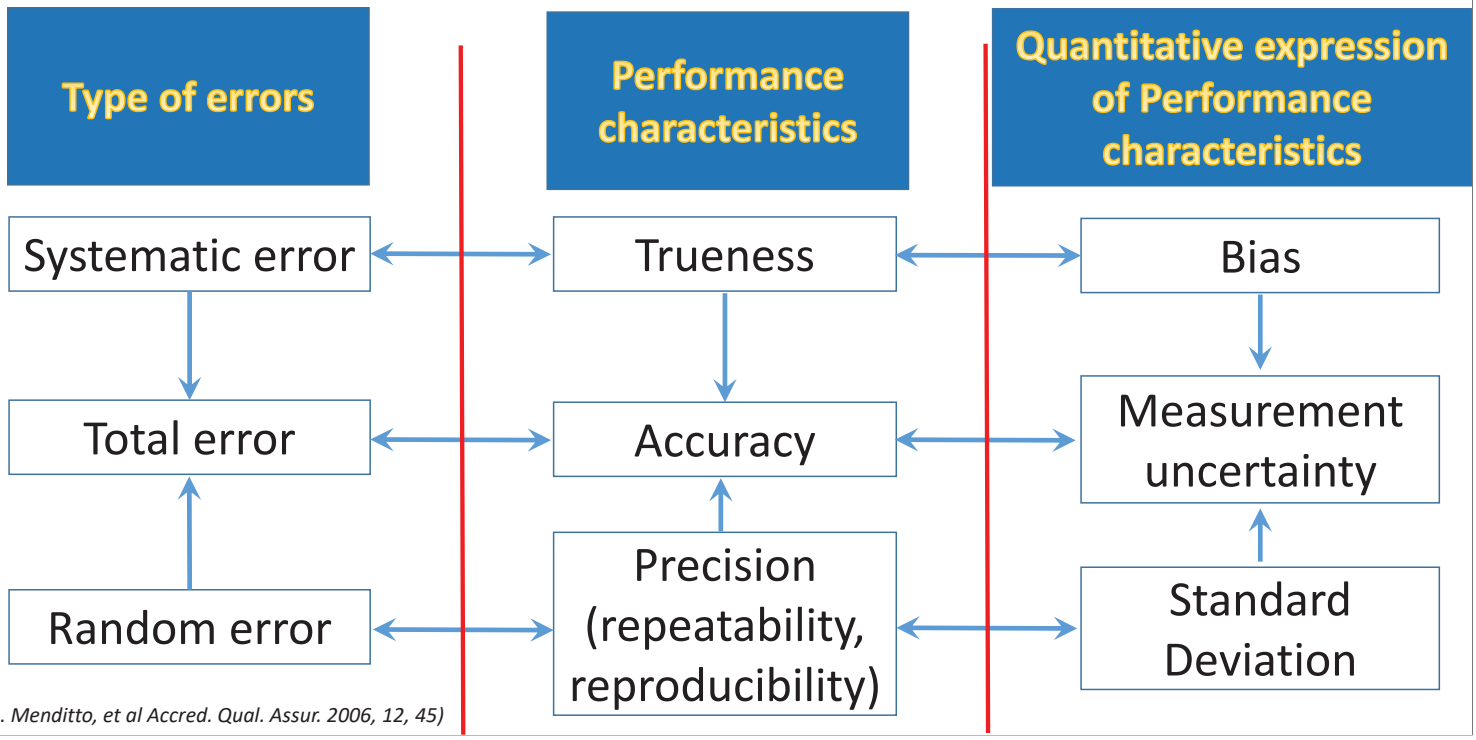
- ✓ Substances physiochemically similar to the analyte (those thought likely to present in samples) should be evaluated individually and in combination with the analyte of interest. This analysis can be performed in parallel with confirmatory techniques to assess the selectivity ability
- ✓ Matrix effects should be evaluated. The calibration curve in different matrices should be compared with calibrators in buffer to detect matrix effects
- ✓ Nonspecific binding should be determined







Accuracy, Precision and Recovery



(A. Menditto, et al Accred. Qual. Assur. 2006, 12, 45)

Accuracy, Precision and Recovery

Accuracy = Trueness = how close are the results to the right answer

"Accuracy is the closeness of agreement between **a test result** and the **accepted reference or true value** of the property being measured"

Can be evaluated as 'Bias' by comparing the mean of the results from the candidate method with a suitable reference value. 3 General approaches:

1. Analysis of reference materials (RM): i.e. measure concentration of RM (10 times) using candidate method. Compare mean measured concentration ($C_{measured}$) with reference concentration of RM (($C_{ref.}$).

Bias b = $C_{measured} - C_{ref}$

Relative bias b (%) = $\frac{C_{measured} - C_{ref}}{C_{ref}} x\ 100$

Relative % recovery = $\frac{C_{measured}}{C_{ref}} x\ 100$

Accuracy, Precision and Recovery

2. Recovery experiments using spiked samples:

1. Measure the mean value of a blank, negative matrix: C_0
2. Spike an analyte over a range of concentrations (C_{known}) into the matrix. Measure mean concentration of the spiked analyte: C_{spiked}
3. The relative spike recovery R(%) at various concentrations:

$$R(\%) = \frac{C_{spiked} - C_0}{C_{known}} x100$$

Example:

Spike 5 mg of analyte into a confirmed negative real matrix. So the final spiked sample should contain 5 mg of the analyte.

If not, then the value obtained from the measurement is not accurate

90-110% recovery generally considered OK

Accuracy, Precision and Recovery

3. Comparison with results obtained with another method: i.e. measure concentration of the target analyte (10 times) using candidate method ($C_{measured}$) and the alternative (standard) method ($C_{benchmarked}$) . Compare mean measured concentration ($C_{measured}$) with the mean $C_{benchmarked}$:

Bias b = $C_{measured} - C_{benchmarked}$

Relative bias b (%) = $\frac{C_{measured} - C_{benchmarked}}{C_{benchmarked}} x\ 100$

Relative % recovery = $\frac{C_{measured}}{C_{benchmarked}} x\ 100$

Accuracy, Precision and Recovery

Precision = Repeatability: The closeness of agreement between independent test results obtained under stipulated conditions

Combination of **Within batch (Internal)** and **Between batches variability**

- ✓ Influenced by changes in analyst, instrument conditions, reagents etc.
- ✓ Can be assessed (in simplest way) by analysis at least 10 times of known material singly or in replicate.
  - The **SD of single measurements** gives an estimate of **precision within batch**
  - The **SD of replicate measurements** gives and indication of **precision between batch**

Limit of detection (LOD) and limit of quantification (LOQ)

The most controversial part of any method validation

Different definitions:

- The lowest concentration that can be measured with reasonable statistical certainty (AOAC)
- The lowest concentration of analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test (NATA Tech, Note #3)
- The smallest concentration that can be determined statistically different from a blank at a specified level of confidence (typically 95%). This corresponds to the critical level. (Currie, 1988 Am. Chem. Soc)
- The output signal or value above which it can be affirmed with a stated level of confidence, for example 95 %, that a sample is different from a blank sample containing no determinant of interest. (ISO 13530 :2009)

Limit of detection (LOD) and limit of quantification (LOQ)

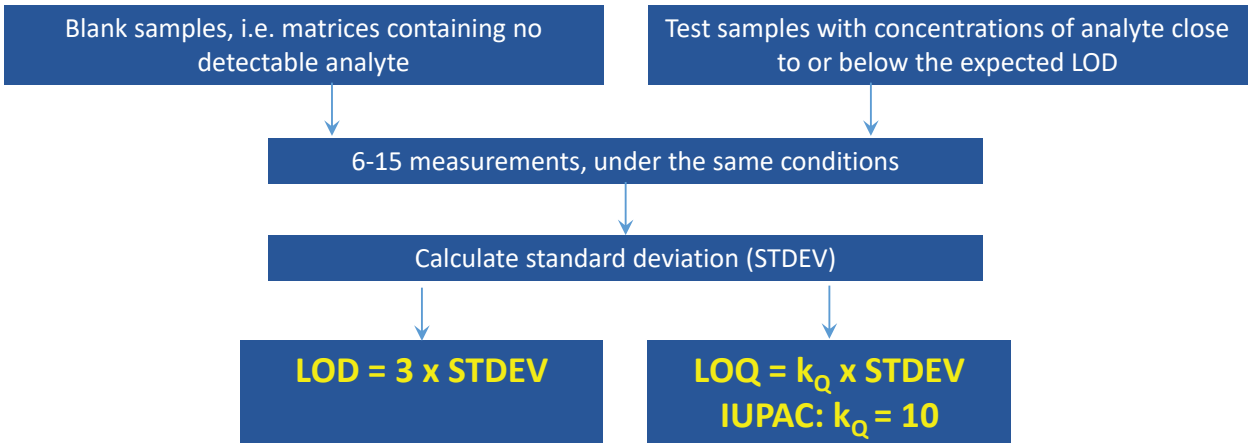
The most controversial part of any method validation

Different definitions
Different approaches for determining LOD/LOQ:

- Based on visual evaluation: The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected (LOD), or the analyte can be quantified with acceptable accuracy and precision (LOQ).
- Based on Signal-to-Noise Approach (generally accepted, next slide)
- Based on the Standard Deviation of the Response and the Slope:
  LOD = 3 x STDEV (zero or lowest conc.) / slope of the calibration line
  LOQ = 10 x STDEV (zero or lowest conc.) / slope of the calibration line

Limit of detection (LOD) and limit of quantification (LOQ)

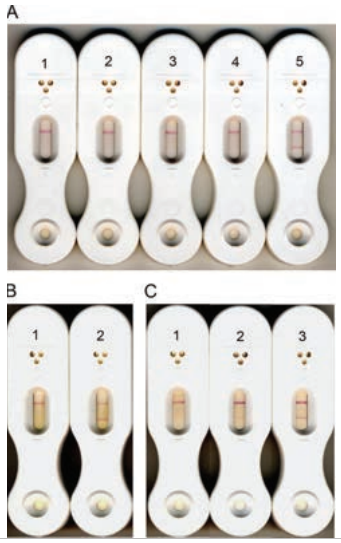
Both LOD and LOQ are normally calculated by multiplying a standard deviation by a suitable factor

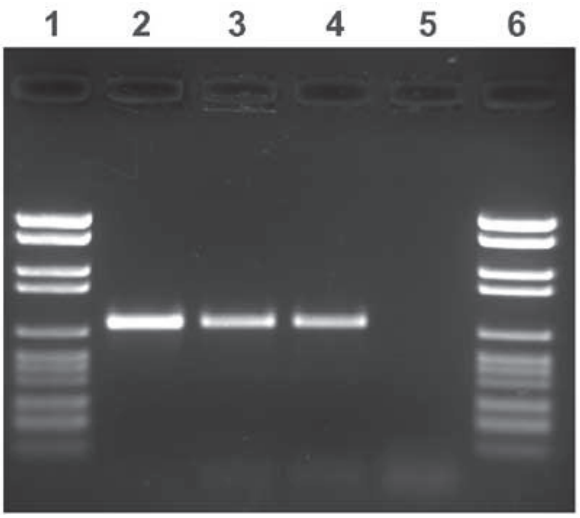


*(Pure Appl. Chem., 1995, **67**, 1699; EURACHEM, "The Fitness for Purpose of Analytical Methods", 2nd edition, 2014)*

Capacity of Detection for Qualitative/ Semi-quantitative Analysis

Qualitative analysis involves identification or classification of substances and is effectively a 'yes'/'no' answer at a given cut-off concentration of an analyte





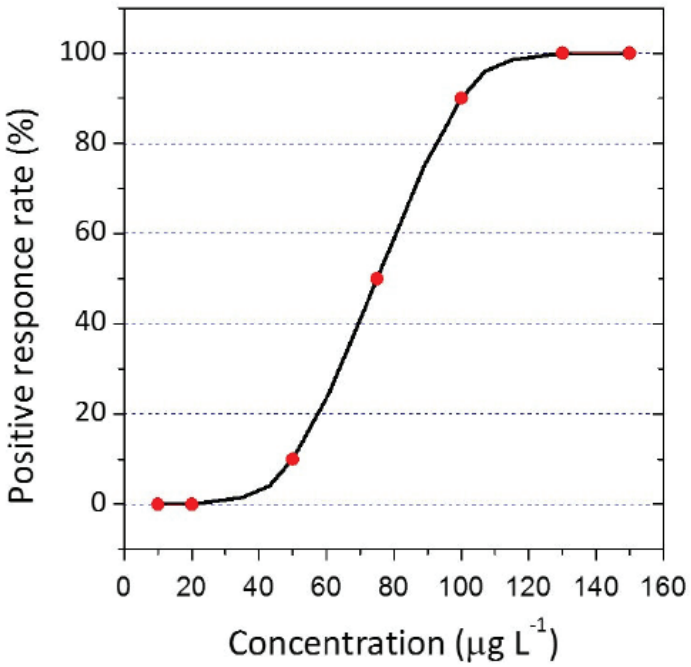
Precision cannot be expressed as a standard deviation, but may be expressed as true and false positive and negative rates

The cut-off limit is where false negative rates for concentrations above the limit are low – with a stated probability, e.g. 5 %.

Capacity of Detection for Qualitative/ Semi-quantitative Analysis

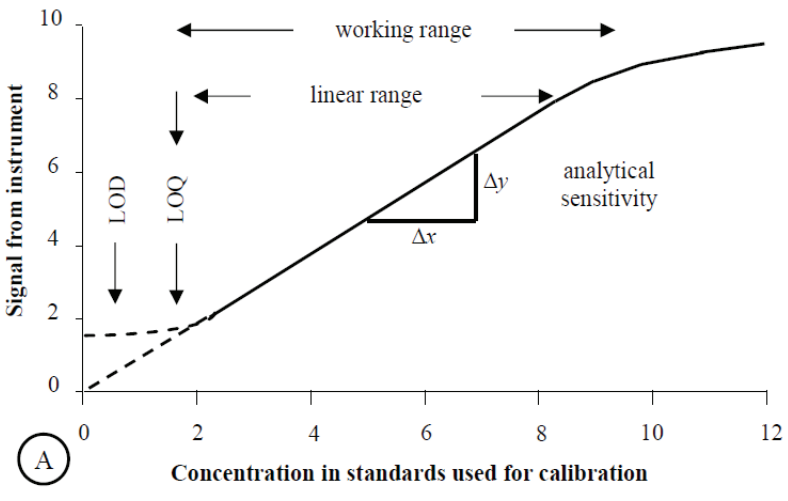
C (µg L^{-1})	No. of positive/negative results
150	10/0
130	10/0
100	9/1
75	5/5
50	1/9
20	0/10
10	0/10

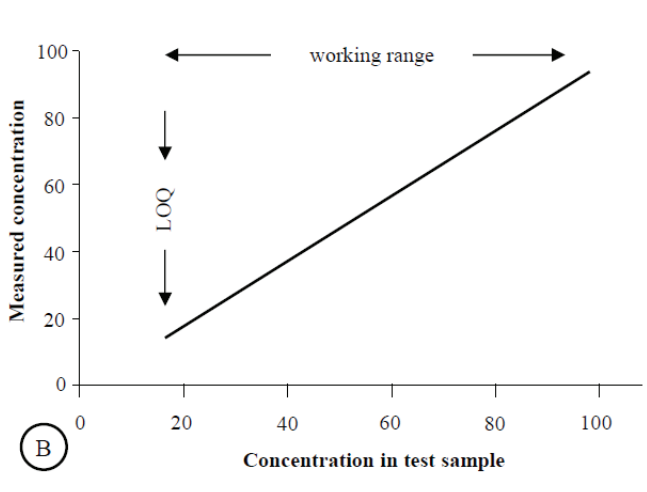
Determination of cut-off concentration for a qualitative method. 10 observations were recorded at each level. A response curve with fraction (in %) of positive results versus concentration was constructed, from which it was possible to determine, by inspection, the threshold concentration at which the test becomes unreliable. With a criterion of < 5 % false negative results, the cut-off concentration is between 100 and 130 µg/L.



Range and Linearity

Working range: the interval over which the method provides results with an acceptable uncertainty. The lower end of the working range is bounded by the limit of quantification LOQ. The upper end of the working range is defined by concentrations at which significant anomalies in the analytical sensitivity are observed. An example of this is the plateauing effect at high absorbance values in UV/VIS spectroscopy.



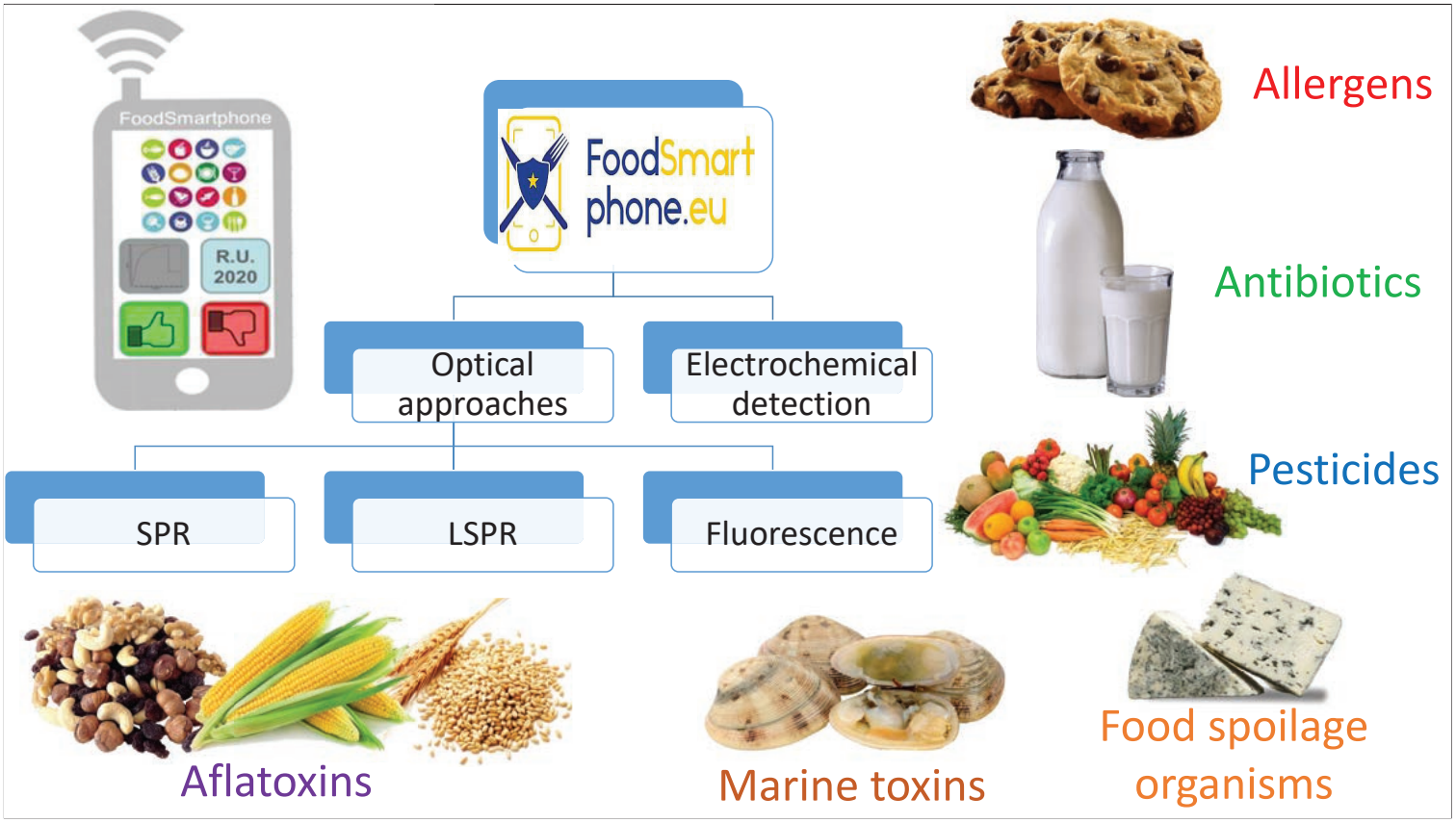


Ruggedness- Robustness

The 'ruggedness' ('robustness'): "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Ruggedness provides an indication of the method's reliability during normal usage"

What to do:

- Identify variables which could have a significant effect on method performance: flowrate (i.e. in SPR), incubation temperature, incubation time, etc.
- Set up experiments (analysing RMs or test samples) to monitor the effect on measurement results of systematically changing the variables.



What is 'Fitness for Purpose'?

"The degree to which data produced by a measurement process enables the user to make **technically and administratively correct decisions** for a **stated purpose**"

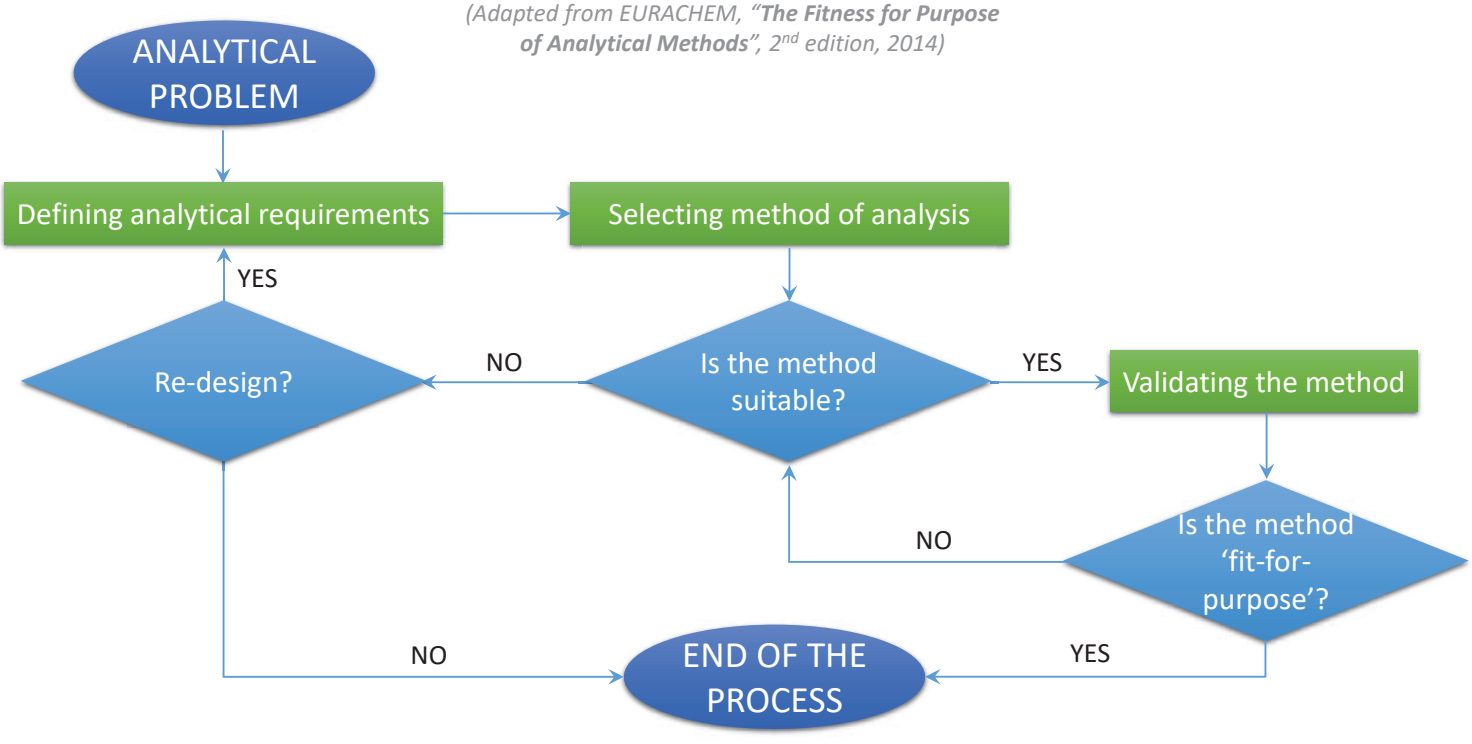
(M. Thompson and M. H. Ramsey, Analyst, 1995, 120, 261–270)

Performance characteristics should be adequate to meet the needs of the user / Regulator as appropriate

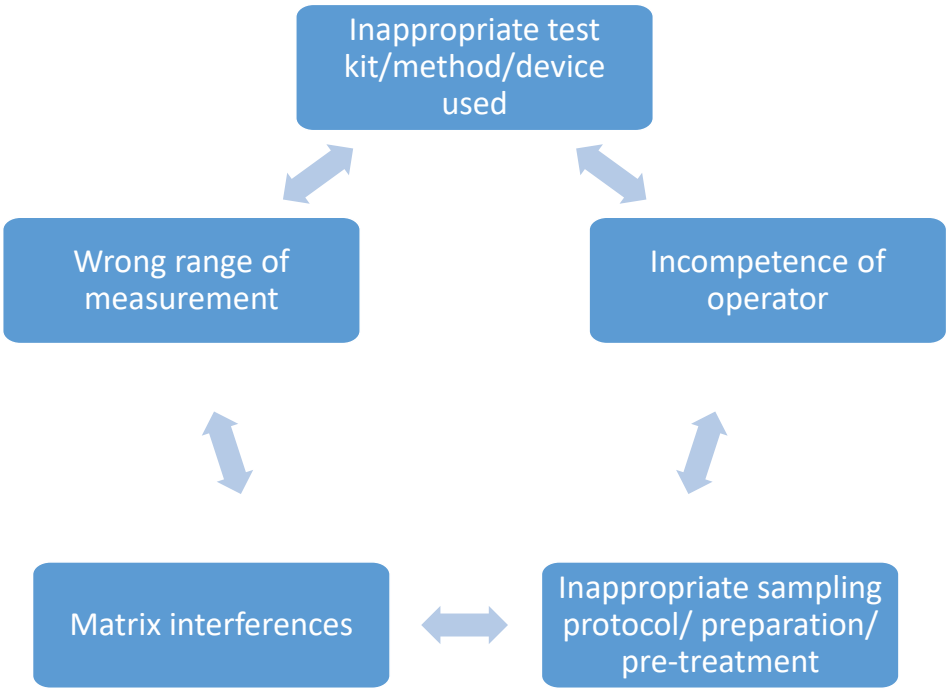
Determined by evaluating actual performance and not just that stated by the supplier / manufacturer

'Fitness for Purpose': Procedure

*(Adapted from EURACHEM, "**The Fitness for Purpose of Analytical Methods**", 2nd edition, 2014)*



What Causes 'Unfit-for-Purpose' Results?



Exercise 2: 15 min

You are grouped into 3 sub-groups. Discuss with your friends to select an example of an analytical system to be validated. Then write an outline for a validation protocol with categories as described in the table below. Then each group will report back to the whole participants for further discussion

Tittle	identify an analytical system for validation; when and who is performing the work?
Purpose	a short description of what is to be accomplished by the study
Overview	This section should outline the purpose, e.g. full validation of a new method/device, verification of performance of a standardised method/device, extension to method scope, etc. The extent of the validation work should be indicated, i.e. the performance characteristics which will be investigated and any associated requirements.
Performance characteristics	give a brief explanation of the performance characteristics (selectivity, linearity, LOD, LOQ, trueness, precision, recovery, robustness, etc.), repeat any specific requirements, outline the experiments which will be done and how the results are to be evaluated.

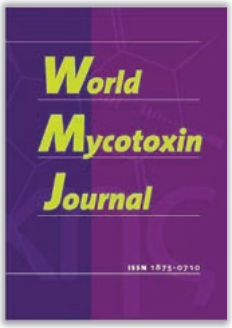
References for Further Reading

1. **Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices**. JOURNAL OF AOAC INTERNATIONAL VOL. 93, NO. 2, 2010
2. **Harmonized guidelines for single-laboratory validation of methods of analysis**. *Pure Appl. Chem.*, Vol. 74, No. 5, pp. 835–855, 2002
3. **Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics**, (2nd ed. 2014). ISBN 978-91-87461-59-0. Available from www.eurachem.org
4. **AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces**. 2002. Available at: http://www.aoac.org/aoac_prod_imis/AOAC_Docs/StandardsDevelopment/AOAC_Validation_Guidelines_for_Food_Microbiology-Prepub_version.pdf
5. **A practical guide to immunoassay method validation**. Available at: https://doi.org/10.3389/fneur.2015.00179

INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

Cross-reactivity in immunochemistry-based methods applied in mycotoxins analysis

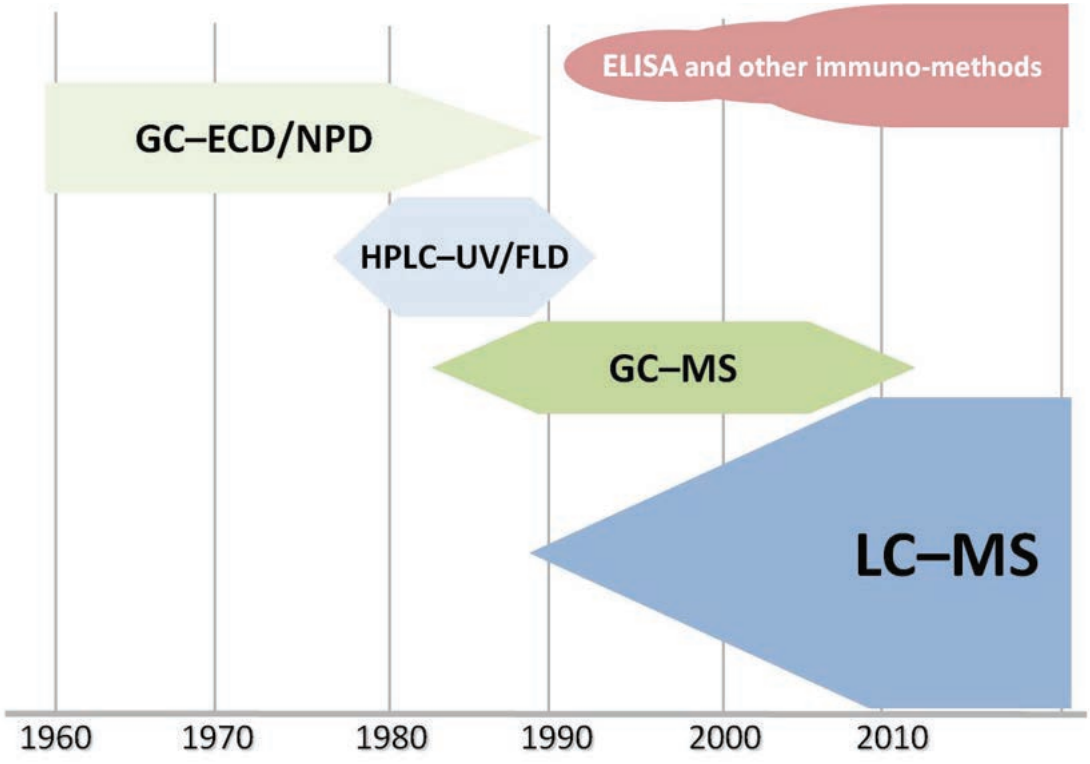
Milena Zachariasova, Jana Hajslova



REVIEW

***Zachariasova et al., 2014**. Cross reactivity of rapid immunochemical methods for mycotoxins detection towards metabolites and masked mycotoxins: the current state of knowledge*. *World Mycotoxin Journal 7: 449-464*

METHODS FOR MYCOTOXINS ANALYIS



Hajslova J., Zachariasova M., Cajka T. **Analysis of multiple mycotoxins in food. In:** ***Mass Spectrometry in Food Safety, Methods and Protocols, Methods in Molecular Biology,*** Springer, 2011, 233–258.

Fast screening immunoassay tools for monitoring of mycotoxins

Quantitative / semiquantitative methods

- ELISA
- Lateral flow devices
- Fluorescence polarization immunoassay

Immunosensors

- X-MAP multi-analyte profiling
- Surface plasmon resonance
- Electrochemical immunosensors

- ***Mostly develpled for mycotoxins with maximum limits*** *(1881/2006 EC ammended by 1126/2007 EC)*
  - *High throughput analysis*
  - *Cheaper instruments (portable devices)*
  - *Highly skilled laboratory staff not needed*

ELISA (Enzyme-linked Immunosorbent Assay)

Rapid screening of mycotoxins in various food / feed commodities

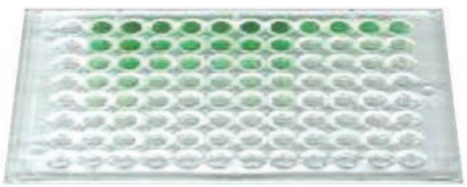
Very easy to handle by following the manufacturers' instructions

(APPARENT) BENEFITS:

- **No clean-up of the sample extract** is required
- For results calculation **pure solvent standards** are always used
- Sample spiking for recoveries determination is usually not recommended

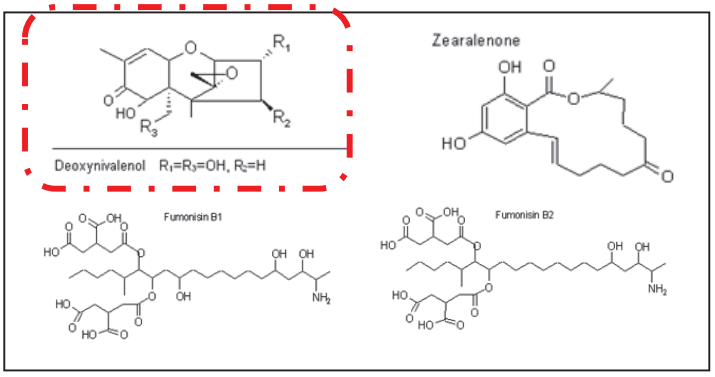
DRAWBACKS:

- **Cross-reactivity** of antibodies
- **Biassed (overestimated) results** can be obtained

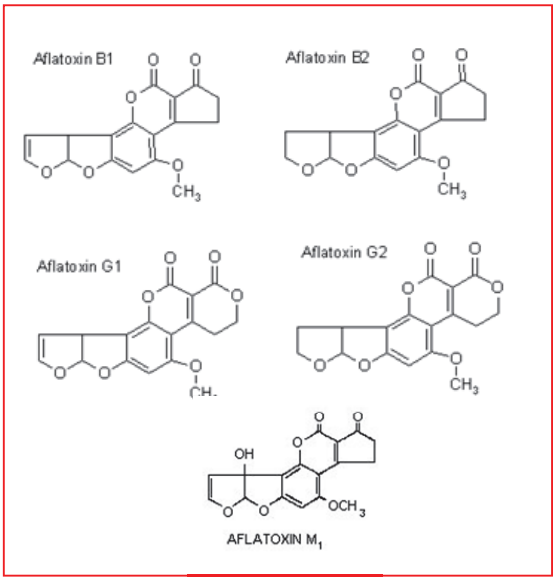


ELISA FOR SCREENING OF REGULATED MYCOTOXINS

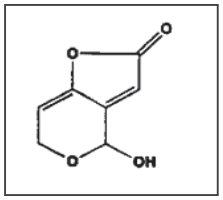
1881/2006 EC ammended by 1126/2007 EC



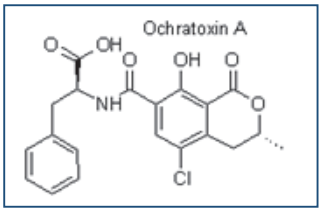
Deoxynivalenol, zearalenone, fumonisins



Aflatoxins

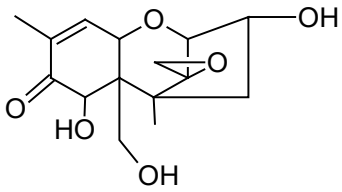


Patulin



Ochratoxin A

STUDIES DISCUSSING THE SUITABILITY OF COMMERCIALLY AVAILABLE DON-DEDICATED ELISA FOR THE ANALYSIS OF REAL-LIFE SAMPLES



Food Additives and Contaminants, Vol. 21, No. 6 (June 2004), pp. 607–617

Screening survey of deoxynivalenol in beer from the European market by an enzyme-linked immunosorbent assay

A. Papadopoulou-Bouraoui†, T. Vr…, S. Valzacchi§, J. Stroka† and E. …

† European Commission, DG Joint Research … Reference Materials and Measurements, B… ‡ National Center of Hygiene, Medical Ec… Sofia 1431, Bulgaria; §European Commission … Centre, Institute for Health and Consumer … Ispra, Italy

(Received 5 September 2003; revised 8 F… accepted 13 February 2004)

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Deoxynivalenol and its conjugates in beer: A critical assessment of data obtained by enzyme-linked immunosorbent assay and liquid chromatography coupled to tandem mass spectrometry

…lanska[a], Jan Poustka[a],

…Republic … 5, Czech Republic … Republic

Anal Bioanal Chem (2014) 406:505–514
DOI 10.1007/s00216-013-7463-3

RESEARCH PAPER

Enzyme-linked immunosorbent assay in analysis of deoxynivalenol: investigation of the impact of sample matrix on results accuracy

Zbynek Dzuman · Marta Vaclavikova · Ivana P…
Zdenka Veprikova · Marie Fenclova · Milena Zachariasova · Jana Hajslova

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

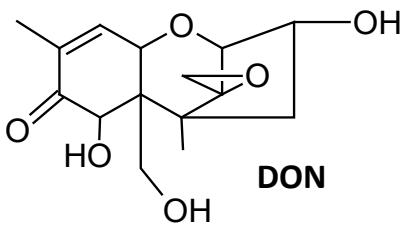
J. Agric. Food Chem. 2010, 58, 12625–12633 12625
DOI:10.1021/jf103025e

Cross-Reactivity of Antibodies in Some Commercial Deoxynivalenol Test Kits against Some Fusariotoxins

Emmanuel K. Tangni,* Jean-Claude Motte, Alfons Callebaut, and Luc Pussemier

Veterinary and Agrochemical Research Centre (CODA-CERVA), Operational Directorate of Chemical Safety of the Food Chain, Unit of Toxins and Natural Components, Leuvensesteenweg 17, 3080 Tervuren, Belgium

OVERVIEW OF STUDIES PROVIDING THE DETAILED COMPARISON OF COMMERCIALLY AVAILABLE DON-ELISAS WITH THE REFERENCE METHODS

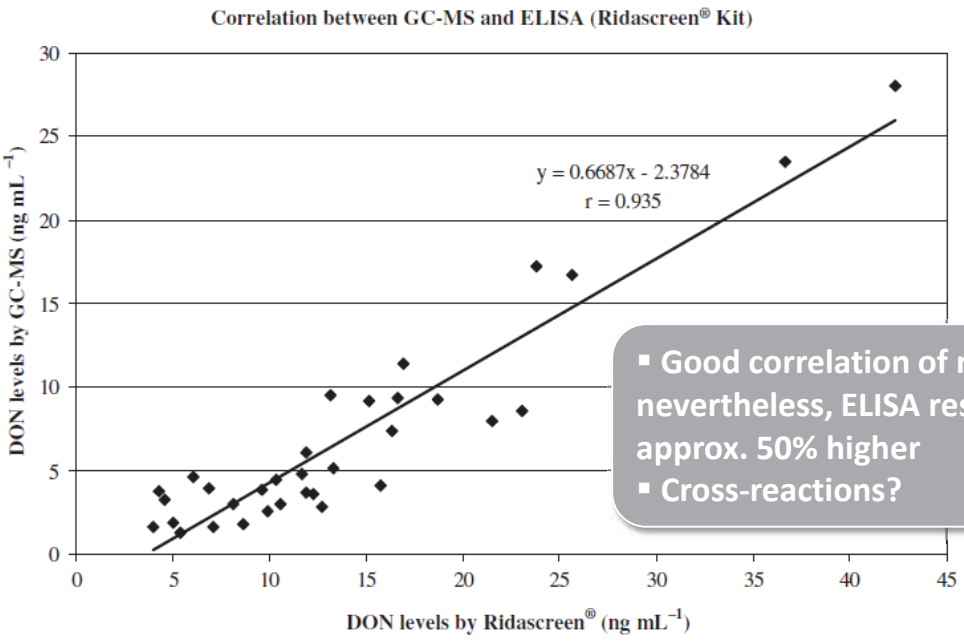


DON

Mycotoxin (reference)	*Matrix measured*	*Reference method*	*Name of the ELISA kit*	*Over-estimation of results*
DON *(Papadopoulou-Bouraoui et al., 2004)*	Beer (real samples)	GC–MS	**Ridascreen DON (R-Biopharm)**	**Yes**
			EZ-Quant HS DON (Diagnostix)	
DON *(Zachariasova et al., 2008)*	Beer (real samples, spikes), wheat (certified reference material)	LC–MS/MS	**Ridascreen DON (R-Biopharm)**	**Yes**
			DON EIA (Euro-Diagnostica)	**Yes**
			Veratox DON 5/5 (Neogen Corporation)	**Yes**
			AgraQuant DON 0.25/5.0 (Romer Labs)	**Yes**
DON *(Kostelanska et al., 2009)*	Barley, malt (real samples, spikes, matrix matched standards)	LC–MS/MS	**Ridascreen DON (R-Biopharm)**	**Yes**
			DON EIA (Euro-Diagnostica)	**Yes**
			Veratox DON 5/5 (Neogen Corporation)	**Yes**
			AgraQuant DON 0.25/5.0 (Romer Labs)	**Yes**
DON *(Dzuman et al., 2013)*	Wheat, barley, malt (real samples, spikes, matrix matched standards)	LC–MS/MS	**Ridascreen DON (R-Biopharm)**	**Yes**
			Ridascreen FAST DON (R-Biopharm)	**Yes**
			DON EIA (Euro-Diagnostica)	**Yes**
			Veratox DON 5/5 (Neogen Corporation)	**Yes**
			Veratox DON HS (Neogen Corporation)	**Yes**
			AgraQuant DON 0.25/5.0 (Romer Labs)	**Yes**
DON *(Aamot et al., 2012)*	Wheat, oat (real samples, certified reference material)	LC–MS/MS	**Ridascreen DON (R-Biopharm)**	**Yes**
			Ridascreen FAST DON (R-Biopharm)	**Yes**

OVERESTIMATION OF ELISA WHEN COMPARED TO REFERENCE GC-MS

BEER

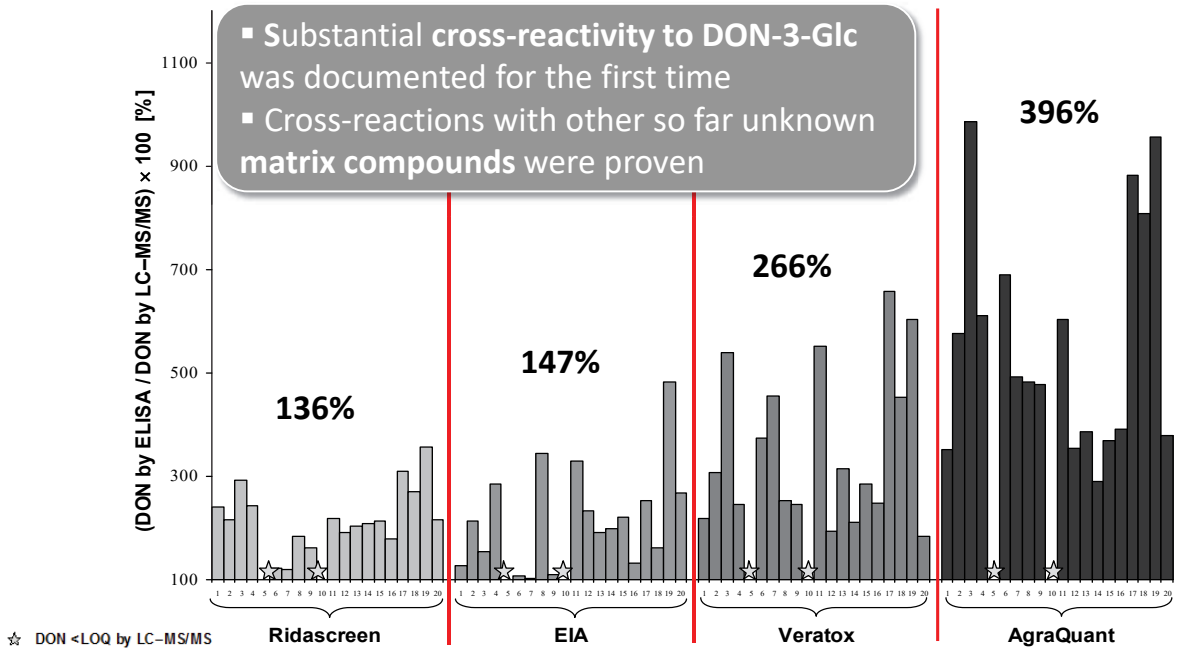


- Good correlation of results, nevertheless, ELISA result were approx. 50% higher
- Cross-reactions?

Papadopoulou-Bouraoui, et al., 2004. *Screening surfy of deoxynivalenol in beer from the European market by an enzyme-linked immunosorbent assay. Food Additives and Contaminants 21: 607-617.*

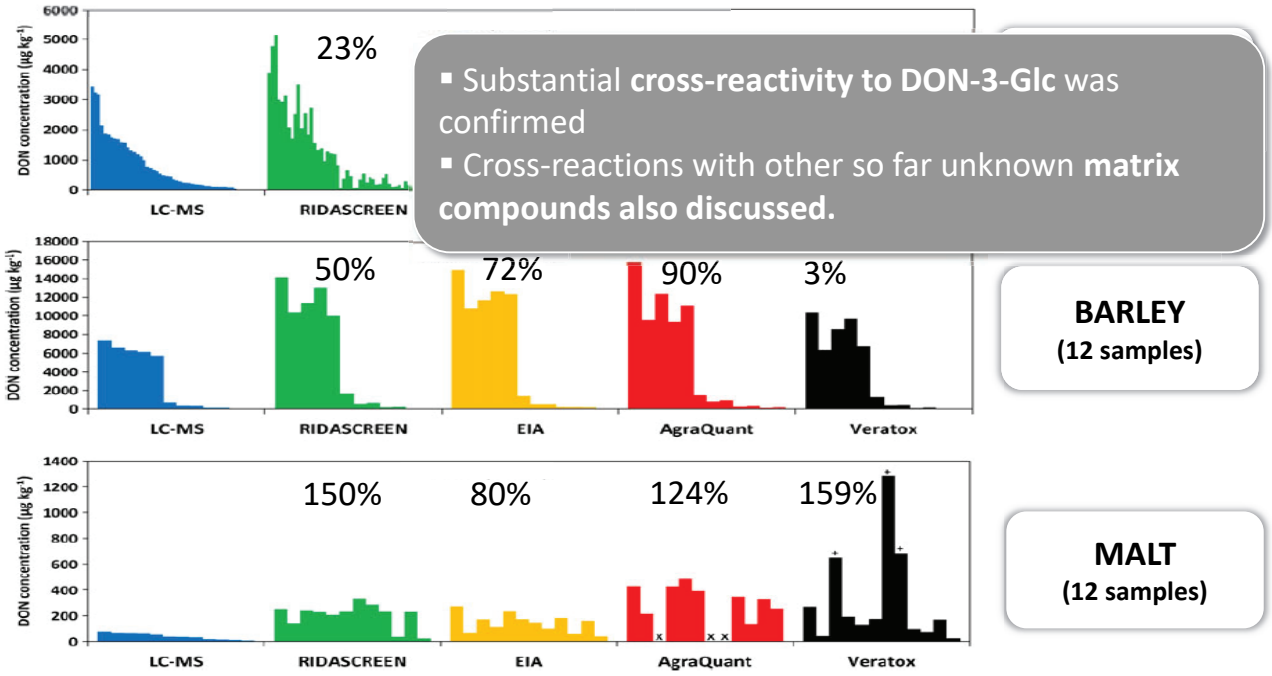
OVERESTIMATION OF ELISA WHEN COMPARED TO REFERENCE LC-MS

BEER



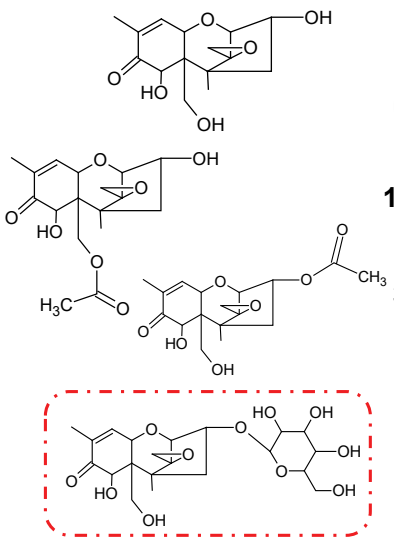
Zachariasova, M. et al., 2008. *Deoxynivalenol and its conjugates in beer: A critical assessment of data obtained by enzyme-linked immunosorbent assay and liquid chromatography coupled to tandem mass spectrometry. Analytica Chimica Acta 625: 77-86.*

OVERESTIMATION OF ELISA WHEN COMPARED TO REFERENCE LC-MS



Dzuman, Z., et al., 2014. *Enzyme-linked immunosorbent assay in analysis of deoxynivalenol: investigation of impact of sample matrix on results accuracy. Analytical Bioanalytical Chemistry 406: 505-514.*

CROSS-REACTIVITY TO DON METABOLITES DOCUMENTED IN THE ELISA KITS *...standards in neat solvent*



	Cross-reactivity declared	Cross-reactivity measured
DON	100%	100%
15-ADON	<0.1 – 19%	0 – 10%
3-ADON	>100%	94 - 498%
DON-3-Glc	Not declared	32-157%

Differences in measured cross-reactivities in time

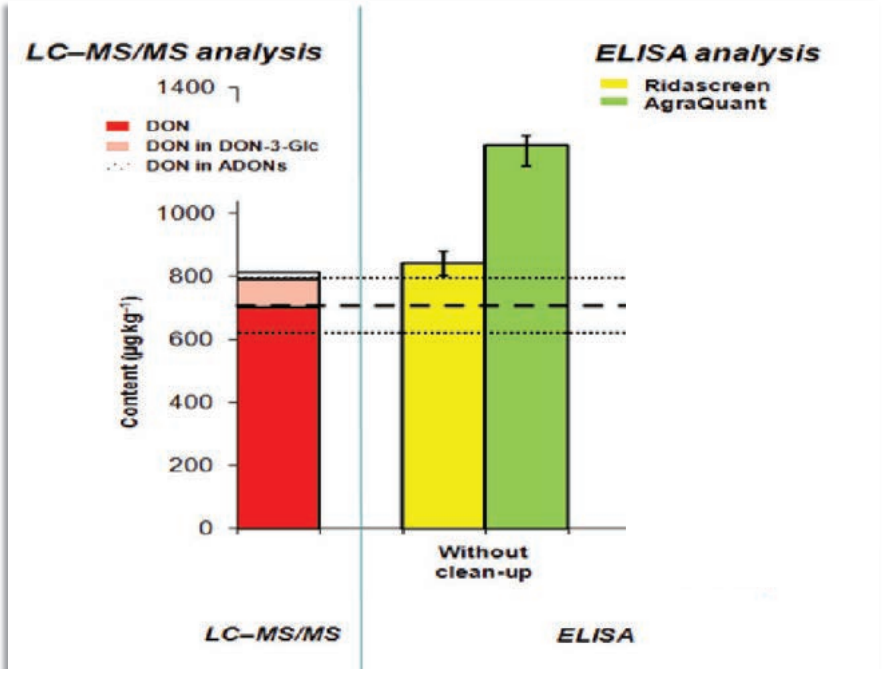
Dzuman, Z., et al., 2014. *Enzyme-linked immunosorbent assay in analysis of deoxynivalenol: investigation of impact of sample matrix on results accuracy. Analytical Bioanalytical Chemistry 406: 505-514.*

Tangni, E.K., et al., 2010. Cross-Reactivity of Antibodies in Some Commercial Deoxynivalenol Test Kits against Some Fusariotoxins. Journal of Agricultural and Food Chemistry 58: 12625-12633.

Zachariasova, M., et al., 2008. *Deoxynivalenol and its conjugates in beer: A critical assessment of data obtained by enzyme-linked immunosorbent assay and liquid chromatography coupled to tandem mass spectrometry. Analytica Chimica Acta 625: 77-86.*

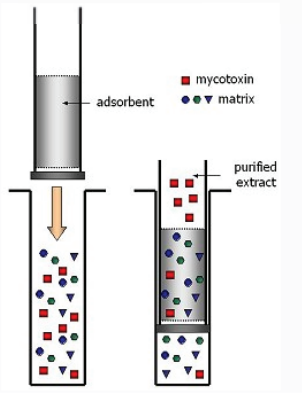
CROSS-REACTIVITY TO MATRIX COMPONENTS

DON in certified reference material of wheat



Certified concentration:
700 ± 100 µg/kg

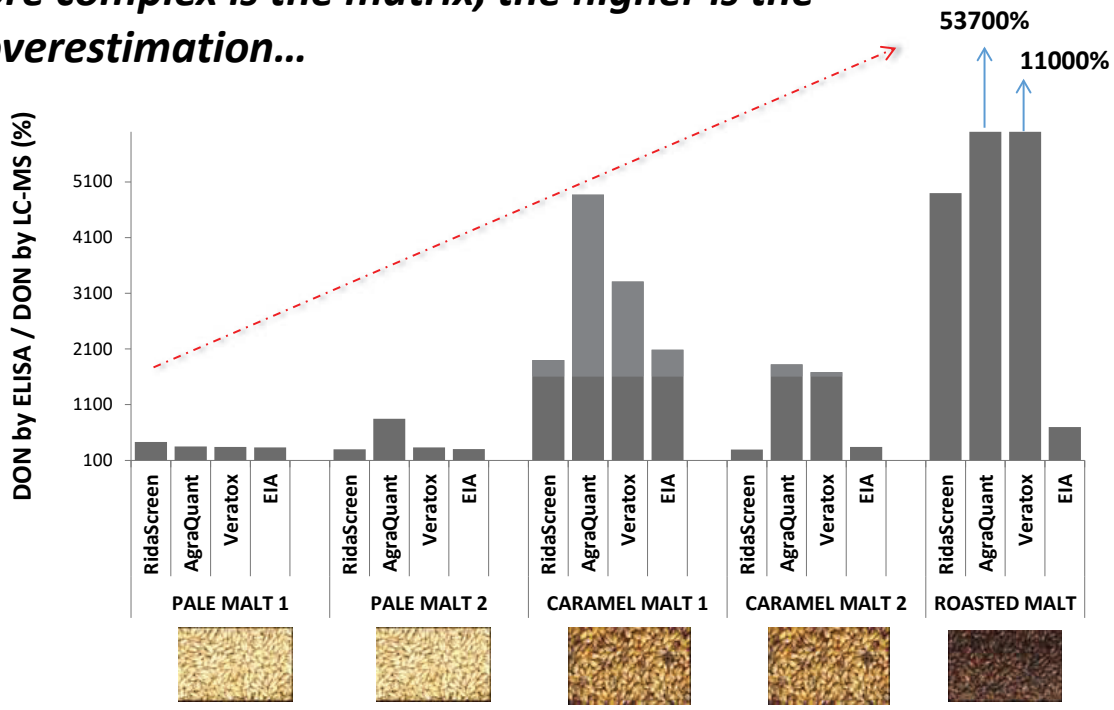
MycoSep clean-up



Zachariasova, M. et al., 2008. *Deoxynivalenol and its conjugates in beer: A critical assessment of data obtained by enzyme-linked immunosorbent assay and liquid chromatography coupled to tandem mass spectrometry. Analytica Chimica Acta 625: 77-86.*

CROSS-REACTIVITY OF MATRIX COMPONENTS

The more complex is the matrix, the higher is the ELISA overestimation…



Kostelanska, M. et al., 2009. Occurrence of Deoxynivalenol and Its Major Conjugate, Deoxynivalenol-3-Glucoside, in Beer and Some Brewing Intermediates. Journal of Agricultural and Food Chemistry 57: 3187-3194

OVERESTIMATION OF RESULTS OF OTHER MYCOTOXINS CAUSED BY THE NON-SPECIFIC CROSS-REACTIVITY

… In several studies, the ELISA overestimation effects were also described for OTA (OTA) (***Koller et al., 2006, Afshar et al., 2013***), aflatoxins (***Rodríguez-Cervantes et al., 2013***), and fumonisins (***Rodríguez-Cervantes et al., 2013, Dall'Asta et al., 2008***)….

Zachariasova et al., 2014. *Cross reactivity of rapid immunochemical methods for mycotoxins detection towards metabolites and masked mycotoxins: the current state of knowledge. World Mycotoxin Journal 7: 449-464*

OVERVIEW OF STUDIES PROVIDING THE DETAILED COMPARISON OF COMMERCIALLY AVAILABLE ELISAs WITH THE REFERENCE METHODS

Aflatoxins
Ochratoxin A
HT-2 / T-2 toxins

Mycotoxin	*Matrix measured*	*Reference method*	*Name of the ELISA kit*	*Over-estimation of results*	*Reference*
Aflatoxins	Tilapia feed	HPLC–FLD (IAC clean-up)	Ridascreen Aflatoxin total (R-Biopharm)	Yes	**Rodríguez-Cervantes et al., 2013**
Aflatoxins	Grains and grain products	HPLC	AgraQuant Total Aflatoxin (4–40 ppb) ELISA (Romer Labs)	No	**Zheng et al., 2005a**
OTA, AFM1	Human breast milk	HPLC–FLD	Veratox for Ochratoxin #8610 (Neogen) Aflatoxin M1 #5121 ELISA for AFM1 (EuroProxima)	Yes No	**Afshar et al., 2013**
HT2, T2	Oat (real samples, certified reference material)	LC–MS/MS	Ridascreen FAST T-2 Toxin (R-Biopharm)	No	**Aamot et al., 2013**

OVERVIEW OF STUDIES PROVIDING THE DETAILED COMPARISON OF COMMERCIALLY AVAILABLE ELISAs WITH THE REFERENCE METHODS

Ochratoxin A
Zearalenone
Fumonisins

Mycotoxin	*Matrix measured*	*Reference method*	*Name of the ELISA kit*	*Over-estimation of results*	*Reference*
OTA	Human blood serum	HPLC–FLD (IAC clean-up)	Ridascreen OTA 30/15 (R-Biopharm)	No	**Dohnal et al., 2013**
OTA	Human blood serum	Capillary electrophoresis with laser-induced FLD	Veratox® for ochratoxin (Neogen)	Yes	**Koller et al., 2006**
OTA	Corn, milo, barley, wheat, soybeans and green coffee	HPLC	AgraQuant ELISA OTA (Romer Labs)	No	**Zheng et al., 2005b**
ZEA	Maize-based food and feed	HPLC–FLD (IAC clean-up)	Ridascreen Zearalenone (R-Biopharm)	No	**Nuryono et al., 2005**
Fumonisins	Tilapia feed	HPLC–FLD (IAC clean-up)	Ridascreen Fumonisin (R-Biopharm)	No	**Rodríguez-Cervantes et al, 2013**
Fumonisins	Thermally treated and untreated maize-based foods	LC–MS/MS	Ridascreen Fummonisin (R-Biopharm)	Yes	**Dall'Asta et al., 2008**

INFLUENCE OF ANTIBODY PRODUCTION ON ITS SPECIFICITY / CROSS-REACTIVITY

….. **Cross-reactivities** of antibodies used in immunoassays may **largely differ…..** depending on the overall **immunisation strategy** which actually opens up the perspectives for assay specificity….

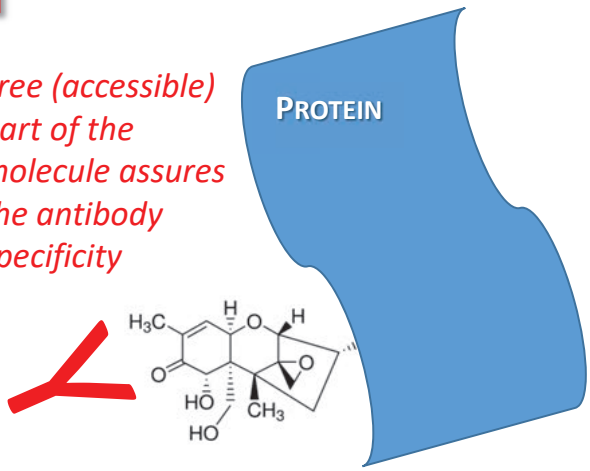
Zachariasova et al., 2014. *Cross reactivity of rapid immunochemical methods for mycotoxins detection towards metabolites and masked mycotoxins: the current state of knowledge. World Mycotoxin Journal 7: 449-464*

IMPORTANT FACTOR(S):

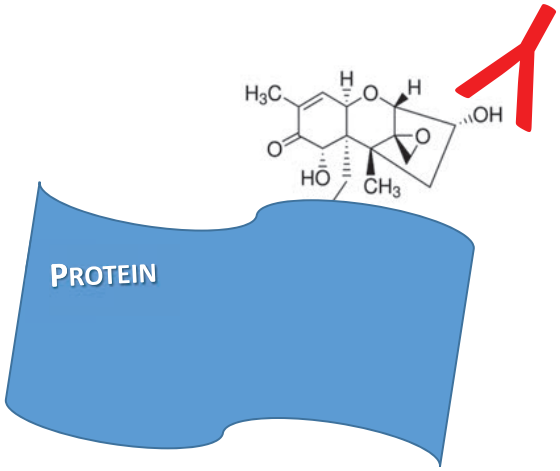
- **Binding location** of mycotoxin within the mycotoxin-carrier protein complex
  - Type of **carrier protein**
  - Type of **cross-linking activator**

STRATEGIES FOR PRODUCTION OF SPECIFIC ANTIBODIES FOR (MASKED) MYCOTOXINS DETECTION

Free (accessible) part of the molecule assures the antibody specificity



Proposed cross-reactivity to DON-3-Glc and to DON-3-derivates

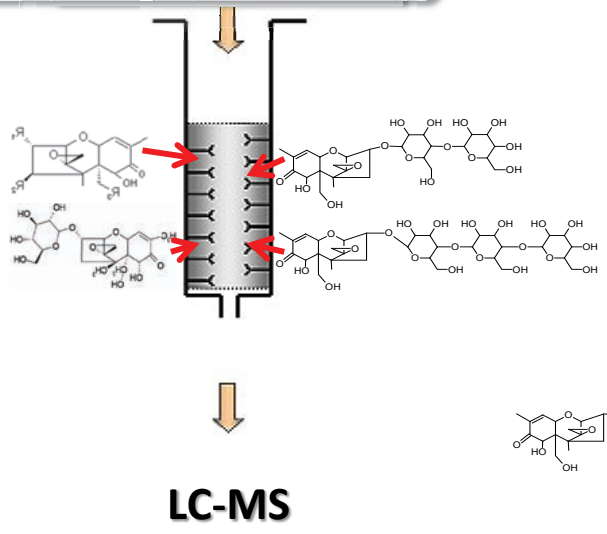


No cross-reactivity to DON-3-Glc and to DON-3-derivates

If mycotoxin is conjugated to a protein through the same functional group as is conjugated the mycotoxin derivate, high cross-reactivity to this mycotoxin derivate could be expected.

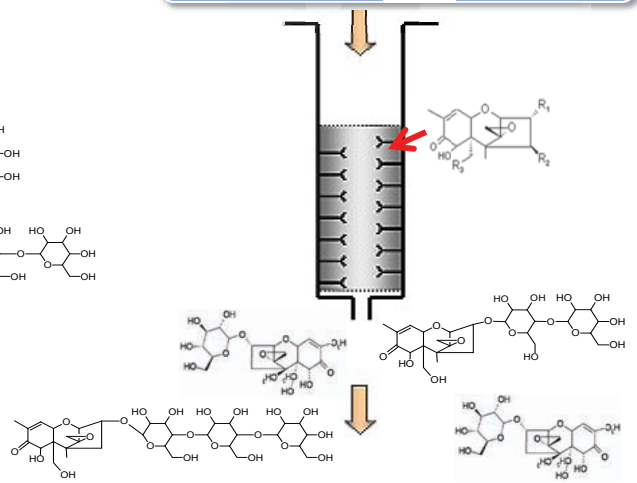
EXPLOITING OF ANTIBODIES FOR PRE-CONCENTRATION OF GLYCOSILATED MYCOTOXINS

CROSS-REACTIVE



LC-MS

NON CROSS-REACTIVE



LC-MS

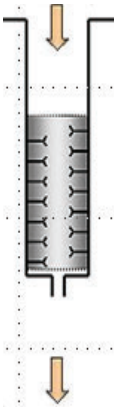
➧ ***Not for the purpose of utilizing in rapid immunochemical based test, but for the more sophisticated instrumental LC-MS analysis***

EXPLOITING OF ANTIBODIES FOR PRE-CONCENTRATION OF GLYCOSILATED MYCOTOXINS

CROSS-REACTIVE

r-biopharm

DON-Prep
HT2/T2 Easy Extract



NON CROSS-REACTIVE

DON-Test
DON-NIV WB
ZearalaTest

VICAM

DON-Star

ROMER
Romer Labs®

HT2/T2 Easy Extract

r-biopharm

EXPLOITING THE IMMUNOAFFINITY COLUMNS FOR PRE-CONCENTRATION OF GLYCOSILATED DON

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Deoxynivalenol Oligoglycosides: New "Masked" *Fusarium* Toxins Occurring in Malt, Beer, and Breadstuff

Milena Zachariasova, Marta Vaclavikova, Ondrej Lacina, Lukas Vaclavik, and Jana Hajslova*

Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Technicka 3, 166 28 Prague 6, Czech Republic

Supporting Information

ABSTRACT: The co-occurrence of deoxynivalenol-3-glucoside with its parent toxin, deoxynivalenol, has been recently documented in many cereal-b
masked mycotoxin in the hu
selective immunoaffinity-bas
with high-resolution orbitrap
oligoglycosylated deoxynival
origination, and fate of thes
investigated. Special attentio
enzymatic preparations. To
deoxynivalenol issue.

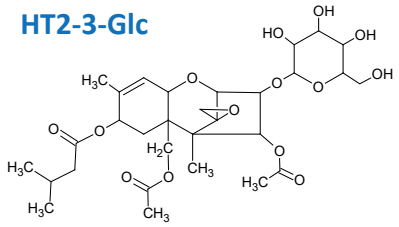
- For **structure characterization of DON-oligoglycosides**, their **selective pre-concentration** before LC-MS was needed
- **Both cross-reactive, and non-cross-reactive** IACs are combined

IMMUNOAFFINITY COLUMNS FOR PRE-CONCENTRATION OF GLYCOSILATED HT2/T2

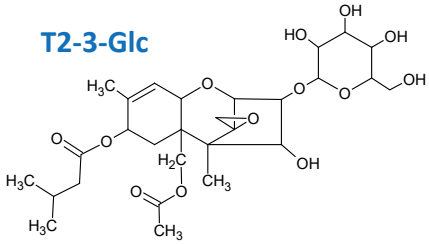
r-biopharm

HT2, T2 – EasyExtract

HT2-3-Glc



T2-3-Glc



World Mycotoxin Journal, August 2012, 5 (3): 231-240

Wageningen Academic Publishers

Occurrence of mono- and di-glycosylated conjugates of T-2 and HT-2 toxins in naturally contaminated cereals

Z. Veprikova, M. Vaclavikova, O. Lacina, Z. Dzuman, M. Zachariasova and J. Hajslova

Institute of Chemical Technology, Department of Food Analysis and Nutrition, Technicka 3, 166 28, Prague 6 – Dejvice, Czech Republic; jana.hajslova@vscht.cz

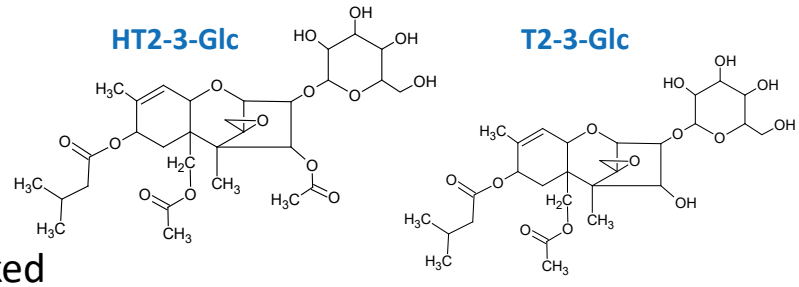
Received: 13 May 2012 / Accepted: 16 July 2012

Abstract

An increasing incidence of T-2 and HT-2 toxins (T-2 and HT-2) representing group A trichothecenes has been observed in *Fusarium* infected small grain cereals in the last decade. Quite recently, the existence of glucosides of T-2 and HT-2 has also been proved next to the common parent forms. There is a strong desire to deepen the knowledge of the occurrence of these conjugates and the possibilities of their analytical determination. A new analytical procedure has been developed for monitoring T-2/HT-2 conjugates in cereal samples. Four different sample preparation methods based on crude acetonitrile:water extraction, QuEChERS, solid phase extraction and immunoaffinity clean-up, were tested. The latter approach employing dedicated immunoaffinity cross-reactive cartridges was shown to be the best option for selective isolation and pre-concentration of the target conjugated analytes. The samples obtained in this way were examined by ultra-high performance liquid chromatography hyphenated to high resolution tandem mass spectrometry that enabled the confirmation of the presence of conjugated T-2 and HT-2. In addition to mono-glycosylated forms of T-2 and HT-2 detected in naturally contaminated barley, wheat and oats, we have also documented for the first time the existence of diglucosides of HT-2 in barley.

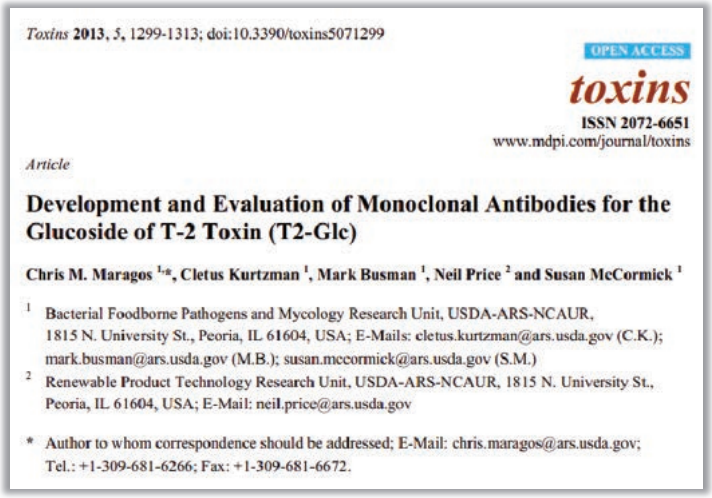
Keywords: masked mycotoxins, HT-2 toxin, T-2 toxin, high resolution mass spectrometry, UPLC-QqTOF MS

RAPID IMMUNOCHEMICAL DETECTION OF MASKED MYCOTOXINS



Number of known masked mycotoxins continues to expand

Necessity to screen the overall mycotoxins content, including their masked forms as potential toxicity reservoirs, have started to be reflected



Toxins **2013**, *5*, 1299-1313; doi:10.3390/toxins5071299

OPEN ACCESS

toxins

ISSN 2072-6651

www.mdpi.com/journal/toxins

Article

Development and Evaluation of Monoclonal Antibodies for the Glucoside of T-2 Toxin (T2-Glc)

Chris M. Maragos [1,*], Cletus Kurtzman [1], Mark Busman [1], Neil Price [2] and Susan McCormick [1]

1 Bacterial Foodborne Pathogens and Mycology Research Unit, USDA-ARS-NCAUR, 1815 N. University St., Peoria, IL 61604, USA; E-Mails: cletus.kurtzman@ars.usda.gov (C.K.); mark.busman@ars.usda.gov (M.B.); susan.mccormick@ars.usda.gov (S.M.)

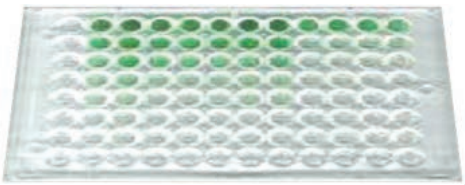
2 Renewable Product Technology Research Unit, USDA-ARS-NCAUR, 1815 N. University St., Peoria, IL 61604, USA; E-Mail: neil.price@ars.usda.gov

* Author to whom correspondence should be addressed; E-Mail: chris.maragos@ars.usda.gov; Tel.: +1-309-681-6266; Fax: +1-309-681-6672.

PROFICIENCY TESTING

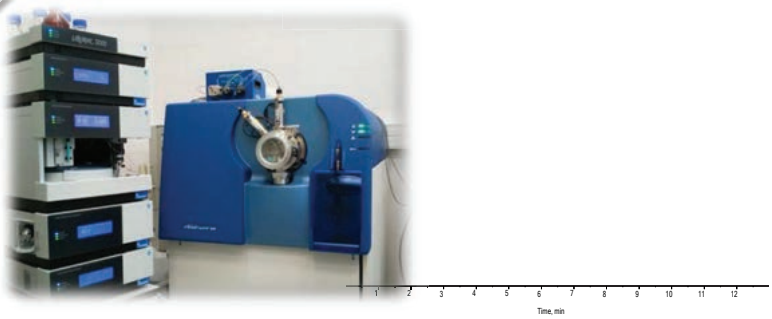
ELISA

(Enzyme Linked Immunosorbent Assay)



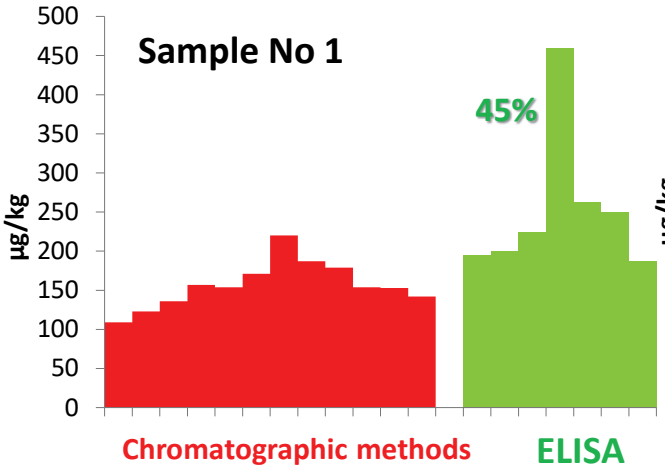
LC-MS

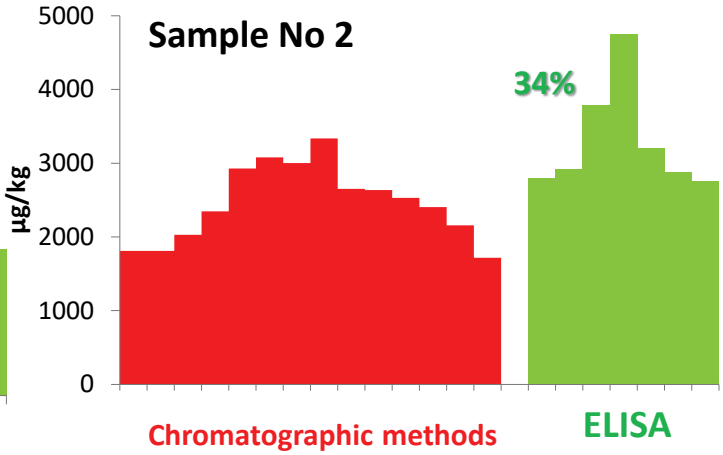
(Liquid Chromatography – mass spectrometry)



Proficiency testing results influence…

DON

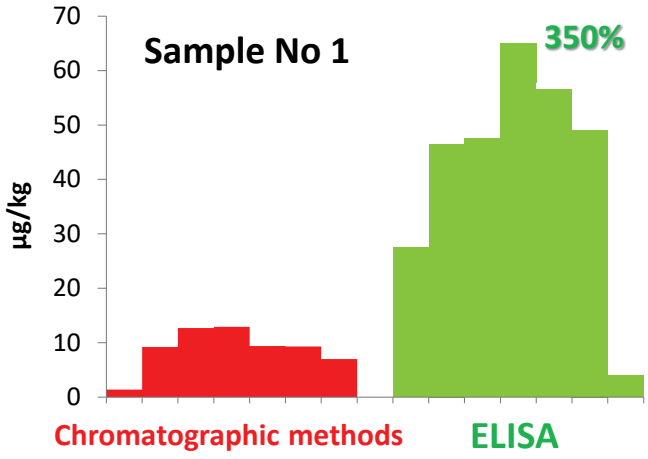




- **Overestimation of ELISA** when compared to chromatographic methods
- **Bimodal distrubution of results… How to calculate the Z-score?**

Proficiency testing results influence…

ZEA

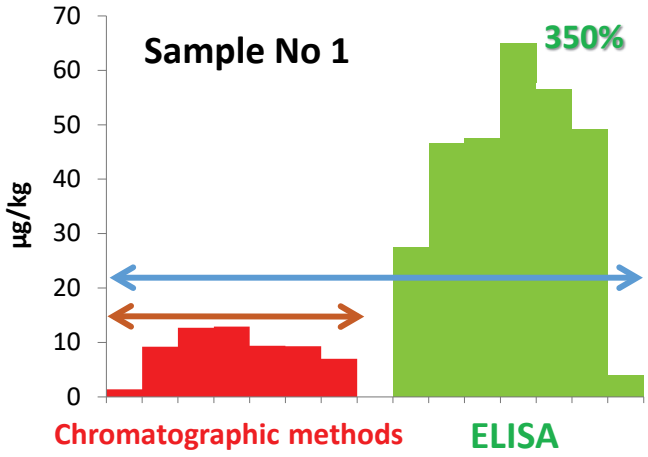


$$Z\ score = (x - X) / sd$$

x – measured value
X – assigned value (mean)
sd – standard deviation

Proficiency testing results influence…

ZEA



- Assigned value based on **all** the reported results: **20.5 µg/kg**
- Assigned value based on the **chromatographic** data: **9.9 µg/kg**

Z score = (x – X) / sd

x – measured value
X – assigned value (mean)
sd – standard deviation

Z-SCORE CALCULATION:

Laboratory No 12 submitting **9.3 µg/kg**

- z-score: **–0.8**
- z-score: 0.02

Conclusions and remarks

Drawbacks and benefits related to the cross-reactivity issue…

- **Cross-reactivities of antibodies may pose a risk of biased results** – one should be aware when official control / proficiency testing are performed

- **More realistic toxicological profile** of the investigated food/feed samples can be obtained

the systems approach

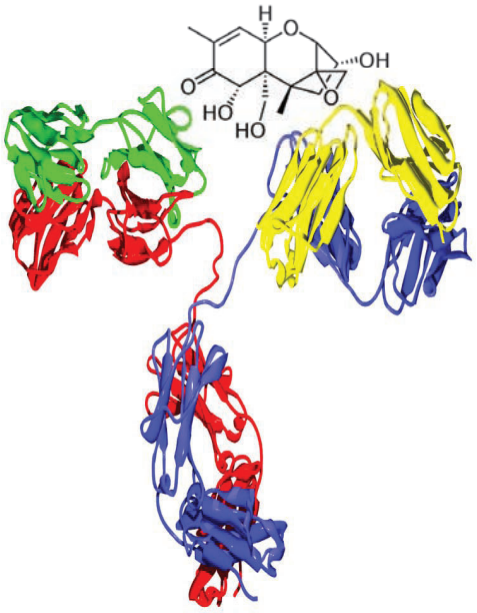
- As far as the **laboratory operators** keep in mind the **purpose of analysis,** and are familiar with the **risk of potentially biased results**, as well as with **ways of their compensation** when needed, then **immunochemical methods pose an highly effective tool** in food safety compliance control.

- **Open communication between users and producers** on the troublesome issues and their solutions is an **important assumption for growing use of these techniques**.

THE World Mycotoxin Forum

Thank you for your attention…

milena.zachariasova@vscht.cz
jana.hajslova@vscht.cz



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Collaborative Validation Studies

Katerina Mastovska, Ph.D.
Covance Laboratories, Madison, WI, USA

katerina.mastovska@covance.com

Collaborative (Interlaboratory) Study

- **Coordinated evaluation of an analytical method by multiple laboratories**
- Test of the method – not test of the labs!
- Collaborators should be representative end-users of the method and the method should be applied under conditions of the intended use (*e.g.* field vs. laboratory)

Purpose of Collaborative Studies

- Provide **confidence** in the method performance (especially important for official/standard methods)
- Demonstrate **reproducibility**
- Opportunity for method **improvements** (clarity, performance-based parameters, elimination of external factors etc.)

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Standard Setting Organizations - Examples

- ISO (International Organization for Standardization)
- CEN (Comité Européen de Normalisation; European Committee for Standardization)
- AOAC International

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Type of Methods and Validation Requirements

Method Classification	Quantitative	Qualitative	Identification
Single-Laboratory Validation	Applicable range Limit of quantitation (LOQ) Bias Recovery Precision (RSD_r)	Probability of detection (POD) Inclusivity/selectivity Exclusivity/cross-reactivity Environmental interference Laboratory variance	Probability of identification (POI) Inclusivity/selectivity Exclusivity/cross-reactivity Environmental interference
Collaborative Study	Reproducibility (RSD_R)	POD (0) POD (c) Laboratory POD	POI (c) Laboratory POI

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Collaborative Study Design

Minimum requirements:

- **Quantitative methods:**
  - Recruit 10–12 collaborators
  - 8 valid data sets
  - 2 blind duplicate replicates
  - 5 materials = 5 analyte (test components)/matrix/concentration combinations

- **Quantitative methods:**
  - 10 testing sites, each reporting at least 6 valid replicate analyses per concentration (at least 3 concentrations)

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Collaborative Study Start – Must Have:

- **Well-optimized method**
- **Clearly written method SOP**
- **Single-laboratory validation (ideally multiple analysts/multiple days)**
- **Collaborative study protocol (based on fit-for-purpose study design)**
- **Suitable study materials (homogenous and stable)**
- **Qualified (and committed) collaborators**
- **Dedicated (and committed) study director (study direction team)**

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Collaborative Study Start – Good to Have:

- **Limited interlaboratory study (3-4 labs)**
  - To work out potential issues and make improvements before the actual collaborative study
- **Kick-off meeting with collaborators**
  - To answer any potential questions, explain any critical steps in the method or protocol etc.
- **Laboratory qualification test**
  - Analysis of qualification samples to make sure that the lab is able to follow the method

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Method SOP

- Specification of reagents, chromatographic materials, enzymes, antibodies and other performance-critical materials
- Description and explanation of every step so as to discourage deviations (*use imperative directions and avoid subjective and conditional expressions as options*)
- Safety precautions
- System suitability
- Performance-based parameters for instruments, columns etc.
- Critical steps
- Convenient stopping points

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Collaborative Study Protocol

- Based on fit-for-purpose study design
- Clear instructions to collaborators
- Description of study phases and expectations
- Material receipt and storage conditions
- Documentation of potential deviations
- Timelines/deadlines/milestones
- Reporting format (report templates)
- Statistical data analysis – description, treatment of outliers etc.

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Collaborative Study Protocol

Recommended statement:

"THIS IS A STUDY OF THE METHOD, NOT OF THE LABORATORY. THE METHOD MUST BE FOL LOWED AS CLOSELY AS PRACTICABLE, AND ANY DEVIATIONS FROM THE METHOD AS DE SCRIBED, NO MATTER HOW TRIVIAL THEY MAY SEEM, MUST BE NOTED ON THE REPORT FORM."

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Collaborative Study Test Samples

- Must be homogeneous
- Must be stable (freezing, dehydrating, antioxidants, preservatives etc.)
- Inert containers
- Randomly coded
- Appropriate analyte concentrations
- Representative matrices
- Sample size only needed for the study
- Free of contamination
- Typically blind duplicates

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Suitable Test Samples

- Single batch, homogeneous, stable material
- Reference materials – expensive but sometimes available through a collaboration with the certification organization (JRC, NIST etc.)
- Synthetic materials/special formulations
- Spiked materials (for residues: include at least some incurred samples)
- Unknown test solutions - for direct addition by the collaborators to blank matrix samples (last resort - for unstable analytes)
- Blanks

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Examples of Other Materials Provided to Collaborators

Practice samples	Qualification samples	Quality control samples
Reference standards	Test kits	Special reagents

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Sending Collaborative Study Materials

- Notify collaborators of shipping arrangements (tracking numbers, expected delivery time etc.)
- Label samples legibly
- Pack samples well and label properly to avoid damage and transportation delays
- Include all necessary certificates
- If necessary, use sufficient amount of dry ice (to last several days longer than anticipated)
- Provide clear storage instructions

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Obligations of Collaborators

- Analyze samples according to the protocol
- Follow method exactly!!!
- Conduct exactly the number for required tests
- Report individual values (use reporting templates)
- Carefully review reported results and calculations (transcription errors, use of wrong units etc.)
- Supply raw data, graphs and other documentation

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Statistical Analysis – Initial Review (Data Audit)

- Major discrepancies (decimal points, wrong units etc.) and trends (consistently low or high lab results – based on ranking)
- Only valid results included in statistical analysis
- Invalid results:
  - Method not followed
  - Unexpected calibration function (non-linear when linear expected)
  - System suitability specifications not met
  - Resolution or other performance characteristics not adequate
  - Unexpected reactions occur
  - Other atypical phenomena

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Statistical Analysis – Outliers

- Some outliers expected in collaborative studies
- Rejection of more than 2/9 of the data for a given material is considered excessive
- Required number of valid data points has to be maintained (8 for quantitation methods)
- Determine the probability that the apparent aberrant value(s) is part of the main group of values considered as normal population:
  - Cochran test
  - Single Grubbs test
  - Double Grubbs test

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Statistical Analysis – Outlier Tests

- **Cochran Test**
  - For removal of laboratories (or extreme individual values from a set of laboratory values) showing significantly greater variability among replicate (within-laboratory) analyses than other laboratories for a given material
  - Calculates distribution of all the differences between the duplicates and then tests each individual difference to see if it fits in that distribution (homogeneity of variance)

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Statistical Analysis – Outlier Tests

- **Grubbs Tests**
  - For removal of laboratories with extreme averages
  - First apply single-value test, then if no outlier found, apply pair value test (2 values at the highest end, 2 values at the lowest end, and 2 values – one at each end)

Use statistical tools for outlier tests and other calculations, such as AOAC Int. statistical worksheet:

https://www.aoac.org/aoac_prod_imis/AOAC_Docs/NEWS/08trad03_AOAC_BlindDup_v2-1.xls

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Statistical Analysis – Precision

- **Repeatability**
  - Within laboratory
  - RSD_r
- **Reproducibility**
  - Among laboratories
  - RSD_R

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Statistical Analysis – HorRat

$$HorRat = \frac{RSD_R}{PRSD_R}$$

$PRSD_R$ – predicted reproducibility relative standard deviation

$$PRSD_R = 2C^{-0.1505}$$

C – estimated mean concentration expressed as a decimal fraction – e.g. 100% = 1; 1% = 0.01; 1 ppm = 0.000001

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Statistical Analysis – HorRat

Guidelines for evaluation reproducibility results based on HorRat:

- **HorRat ≤ 0.5**: may be in question (lack of study independence, unreported averaging, or consultations)
- **0.5 < HorRat ≤ 1.5**: as normally expected
- **HorRat > 1.5**: higher than expected – study director needs to critically evaluate reasons (homogeneity or stability of samples etc.)
- **HorRat > 2**: problematic, may result in method rejection (some organization only accept methods with HorRat ≤ 2)

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Collaborative Study Example - Quant

RESIDUES AND TRACE ELEMENTS

Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Seafood Using Gas Chromatography-Mass Spectrometry: Collaborative Study

KATERINA MASTOVSKA and WENDY R. SORENSON
Covance Laboratories Inc., Nutritional Chemistry and Food Safety, 3301 Kinsman Blvd, Madison, WI 53704
JANA HAJSLOVA
Institute of Chemical Technology, Faculty of Food and Biochemical Technology, Department of Food Chemistry and Analysis, Technická 3, 166 28 Prague 6, Czech Republic

Collaborators: J. Betzand; J. Binkley; K. Bousova; J.M. Cook; L. Drabova; W. Hammack; J. Jabusch; K. Keide; R. Lizak; P. Lopez-Sanchez; M. Misunis; K. Mittendorf; R. Perez; S. Perez; S. Pugh; J. Pulkrabova; J. Rosmus; J. Schmitz; D. Staples; J. Stepp; B. Taffe; J. Wang; T, Wenzl

AOAC Int. Official Final Action Method 2014.08

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Validation of Qualitative Methods

- **Probability of Detection (POD)** as a statistical model for validation of qualitative methods
  - Proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration
  - Concentration dependent
  - Combines sensitivity, specificity, false-positive and false-negative parameters in one single parameter (POD)
  - AOAC Int. OMA, Appendix H (http://www.eoma.aoac.org/app_h.pdf)
  - AOAC Int. OMA, Appendix N (http://www.eoma.aoac.org/app_n.pdf)
  - AOAC Int. OMA, Appendix F (http://www.eoma.aoac.org/app_f.pdf)

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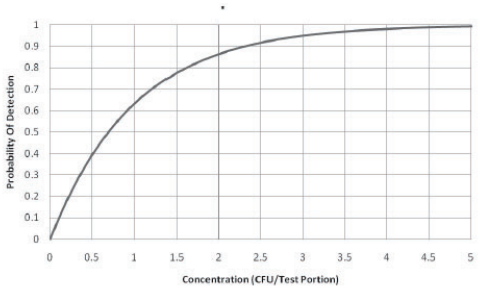
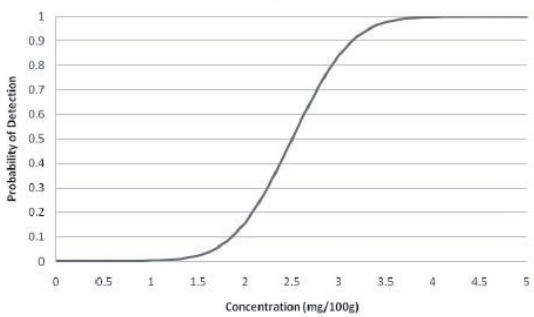
POD Curves - 2 Types of Qual Methods



POD response curve of a microbiological method where Poisson sampling variation dominates.



Theoretical POD response curve for a method that uses a threshold value

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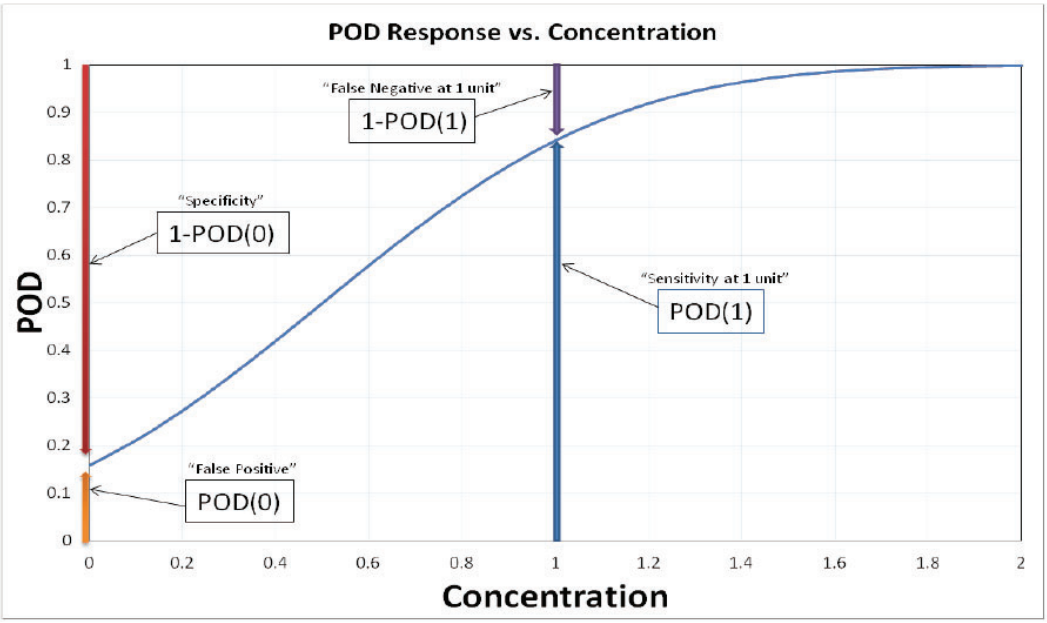
POD Model vs. Other Terminology



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POD Model vs. Other Terminology

Traditional terminology	Concept	POD equivalent	Comment
False positive	Probability of the method giving a (+) response when the sample is truly without analyte	POD(0) POD at conc = 0	POD curve value at conc = 0; "Y-intercept" of the POD curve
Specificity	Probability of the method giving a (-) response when the sample is truly without analyte	1-POD(0)	Distance along the POD axis from POD = 1 to the POD curve value
False negative (at a given concentration)	Probability of a (–) response at a given concentration	1-POD(c)	Distance from the POD curve to the POD = 1 "top axis" in the vertical direction
Sensitivity (at a given concentration)	Probability of a (+) response at a given concentration	POD(c)	Value of the POD curve at any given concentration
True negative	A sample that contains no analyte	C = 0	Point on concentration axis where c = 0
True positive	A sample that contains analyte at some positive concentration	C > 0	Range of concentration where c > 0

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Collaborative Study Example - Qual

Table 3. Example of collaborative data for detection of *Salmonella* in ground beef

			Candidate method			Reference method				
Concn, MPN/25 g[a]	Laboratory	*n*	*x*[b]	PODc or LPODc	95% CI[c]	*x*	PODr or LPODr	95% CI	dPODc or dLPODc	95% CI
0	1	6	0	0		0	0		0.00	
0	2	6	0	0		0	0		0.00	
0	3	6	0	0		0	0		0.00	
0	4	6	0	0		0	0		0.00	
0	5	6	0	0		0	0		0.00	
0	6	6	0	0		0	0		0.00	
0	7	6	0	0		0	0		0.00	
0	8	6	0	0		0	0		0.00	
0	9	6	0	0		0	0		0.00	
0	10	6	0	0		0	0		0.00	
0	11	6	0	0		0	0		0.00	
0	All	60	0	0	(0.0, 0.060)	0	0	(0.0, 0.060)	0.00	(–0.060, 0.060)
0.75	1	6	1	0.17		2	0.33		–0.17	
0.75	2	6	1	0.17		1	0.17		0.00	
0.75	3	6	0	0.00		3	0.50		–0.50	
0.75	4	6	1	0.17		3	0.50		–0.33	
0.75	5	6	3	0.50		5	0.83		–0.33	
0.75	6	6	0	0.00		1	0.17		–0.17	
0.75	7	6	1	0.17		2	0.33		–0.17	
0.75	8	6	5	0.83		4	0.67		0.17	
0.75	9	6	0	0.00		4	0.67		–0.67	
0.75	10	6	2	0.33		2	0.33		0.00	
0.75	11	6	0	0.00		2	0.33		–0.33	
0.75	All	60	14	0.23	(0.06, 0.41)	28	0.47	(0.33, –0.60)	–0.233	(–0.45, –0.014)

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Collaborative Study Example - Qual

0.75	1	6	1	0.17		2	0.33		–0.17	
0.75	2	6	1	0.17		1	0.17		0.00	
0.75	3	6	0	0.00		3	0.50		–0.50	
0.75	4	6	1	0.17		3	0.50		–0.33	
0.75	5	6	3	0.50		5	0.83		–0.33	
0.75	6	6	0	0.00		1	0.17		–0.17	
0.75	7	6	1	0.17		2	0.33		–0.17	
0.75	8	6	5	0.83		4	0.67		0.17	
0.75	9	6	0	0.00		4	0.67		–0.67	
0.75	10	6	2	0.33		2	0.33		0.00	
0.75	11	6	0	0.00		2	0.33		–0.33	
0.75	All	60	14	0.23	(0.06, 0.41)	28	0.47	(0.33, –0.60)	–0.233	(–0.45, –0.014)
10.75	1	6	4	0.67		6	1.00		–0.33	
10.75	2	6	5	0.83		4	0.67		0.17	
10.75	3	6	5	0.83		5	0.83		0.00	
10.75	4	6	5	0.83		6	1.00		–0.17	
10.75	5	6	6	1.00		6	1.00		0.00	
10.75	6	6	0	0.00		2	0.33		–0.33	
10.75	7	6	6	1.00		6	1.00		0.00	
10.75	8	6	6	1.00		6	1.00		0.00	
10.75	9	6	6	1.00		5	0.83		0.17	
10.75	10	6	4	0.67		6	1.00		–0.33	
10.75	11	6	4	0.67		6	1.00		–0.33	
10.75	All	60	51	0.85	(0.76, 0.94)	56	0.93	(0.84, 0.97)	–0.083	(–0.18, 0.048)

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Collaborative Study Example - Qual

Table 4. Statistical summary for collaborative study for *Salmonella* in ground beef

	Candidate method		
	Low level	Mid level	High level
Concentration	0.00 MPN/25 g	0.75 MPN/25 g	10.75 MPN/25 g
No. laboratories (reported/used)	(11/10)	(11/10)	(11/10)
N total replicates	60	60	60
LPODc	0.00	0.233	0.850
LPODc 95% CI	(0.00, 0.060)	(0.040, 0.384)	(0.757, 0.943)
S_r^a	0.00	0.3568	0.3606
S_L^b	0.00	0.2144	0.000
S_R^c	0.00	0.4162	0.3606

AOAC Int. Statistical Worksheet for Binary (Qualitative) Methods:

https://www.aoac.org/aoac_prod_imis/AOAC_Docs/NEWS/09trad04_AOAC_binary-v2-3.xls

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AOAC Int. Collaborative Study on PAHs in Seafood

Katerina Mastovska[1], Wendy Sorenson[1], and Jana Hajslova[2]

[1]Covance Laboratories, NCFS, Madison, WI, USA
[2]Institute of Chemical Technology, Prague, Czech Republic

INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

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AOAC Int. Response to the Oil Spill

- Stakeholders Panel on Seafood Contaminants
- Fitness-for-purpose statement
- Call for methods for PAHs in seafood
- Selection of a candidate method
- Collaborative study

AOAC INTERNATIONAL
The Scientific Association Dedicated to Analytical Excellence®

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Call for Methods

NEWS FLASH

Methods for Measurement of Polycyclic Aromatic Hydrocarbon (PAH) Compounds in Gulf of Mexico Seafood

AOAC INTERNATIONAL is inviting method developers to submit methods for consideration and possible evaluation through the AOAC *Official Methods*SM program. Prospective methods must be able to quantify polycyclic aromatic hydrocarbons (PAHs) in seafood.

Acceptable methods must be able to demonstrate a **Limit of Quantification of 1 ppb** (ng/g) **for benzo(a)pyrene** in seafood. Currently accepted analytical methods require 96 to 120 hours to complete. Evaluation of analytical methods that **significantly reduce the time-to-signal** (including sample preparation and extraction**) is a primary goal of this call for methods**.

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Call for Methods: PAHs of Concern

PAH	FDA/NOAA	US EPA	EFSA
Anthracene	x	x	
Benz[*a*]anthracene	x	x	**x**
Benzo[*a*]pyrene	x	x	**x**
Benzo[*b*]fluoranthene		x	**x**
Benzo[*k*]fluoranthene		x	x
Benzo[*g,h,i*]perylene		x	x
Chrysene	x	x	**x**
Dibenz[*a,h*]anthracene		x	x
Fluoranthene	x	x	
Fluorene	x	x	
Indeno[1,2,3-*cd*]pyrene		x	x
Naphthalene	x	x	
Phenanthrene	x	x	
Pyrene	x	x	

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Method Selection Process

PAH Working Group on Quantitative Methods (chaired by Gina Ylitalo, NOAA)

- reviewed about 30 methods submitted as a response to the call for methods or found in literature
- selection criteria:
  - Fitness-for-purpose requirements (LOQ, speed, scope)
  - Identification and quantification (compatibility with MS)
  - Quality of data to meet the AOAC Int. Single Laboratory Validation (SLV) requirements (e.g. accuracy, precision, analysis of reference materials)
  - Practical considerations, e.g. availability of used equipment

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Selected Method

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L. Drabova, K. Kalachova, J. Pulkrabova, T. Cajka, V. Kocourek and J. Hajslova: Rapid Method for Simultaneous Determination of Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated Biphenyls (PCBs) and Polybrominated Diphenyl Ethers (PBDEs) in Fish and Seafood Using GC-TOF MS, ICT document, Prague, Czech Republic, 2010.

- developed within a European integrated project **CONffIDENCE**

Contaminants in food and feed:
Inexpensive detection for control of exposure

www.conffidence.eu

SEVENTH FRAMEWORK PROGRAMME

To develop a simplified sample preparation strategy for simultaneous determination of a wide range of contaminants in food and feed focused on fish and cereal based food.

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Selected Method

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10 g of homogenized sample
- Add ISTDs (^{13}C-PAHs), vortex, equilibrate (15 min)

↓

Extraction:
- Add 5 mL water and 10 mL ethyl acetate (EtOAc), shake (1 min)
- Add 4 g MgSO4 and 2 g NaCl, shake (1 min), centrifuge
- Evaporate 5 mL aliquot of extract, reconstitute in 1 mL hexane

↓

Silica-SPE clean-up:
- Condition 1g silica with 6 mL hexane:DCM (3:1, *v/v*) and 4 mL hexane
- Apply sample
- Elute with 10 mL of hexane:DCM (3:1, *v/v*)
- Gently evaporate and reconstitute in 0.5 mL isooctane

↓

GC-MS analysis

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Selected Method: Validation Results

Analytes:
- 32 polycyclic aromatic hydrocarbons (PAHs)
  - including 16 US EPA and several of their methylated homologues
- 18 polychlorinated biphenyls (PCBs)
- 7 polybrominated diphenyl ethers (PBDEs)

Spiking levels:
- 1 and 5 µg/kg for PAHs, PCBs and PBDEs
- 5 and 25 µg/kg for major PCB 138, 153, 180 and for PBDE 47

PERFORMANCE CHARACTERISTICS - TROUT			
Analytes	Recovery [%]	RSD [%]	LOQ [µg/kg]
PAHs	73-97	2-13	0.05-0.25
PCBs	74-113	4-18	0.1-0.5
PBDEs	82-107	5-9	0.5

PERFORMANCE CHARACTERISTICS - SHRIMP			
Analytes	Recovery [%]	RSD [%]	LOQ [µg/kg]
PAHs	73-109	2-15	0.05-0.25
PCBs	93-124	5-21	0.1-0.5
PBDEs	79-122	4-11	0.5

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Selected Method: NIST SRM Analysis

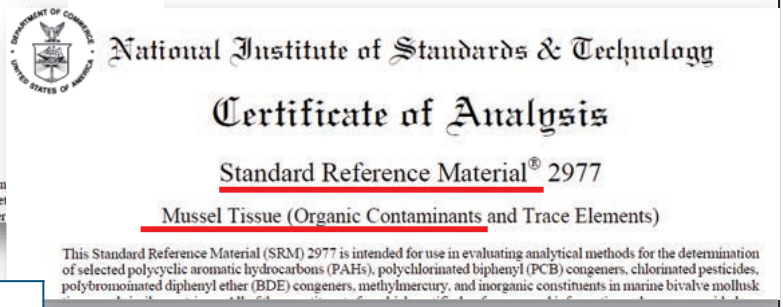
National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1947

Lake Michigan Fish Tissue

Standard Reference Material (SRM) 1947 is a frozen fish tissue homogenate, which was prepared from from Lake Michigan, and is intended primarily for use in evaluating analytical methods for the det selected trace elements, methylmercury, total mercury, polychlorinated biphenyl (PCB) congener



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2977

Mussel Tissue (Organic Contaminants and Trace Elements)

This Standard Reference Material (SRM) 2977 is intended for use in evaluating analytical methods for the determination of selected polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, polybromoinated diphenyl ether (BDE) congeners, methylmercury, and inorganic constituents in marine bivalve mollusk

SRM 1947 – Lake Michigan Fish Tissue			
Analytes		Determined value [µg/kg]	Certified value [µg/kg]
Mono-ortho PCBs	PCB 105	53.2 ± 4.8	50.3 ± 3.7
	PCB 118	115.1 ± 10.4	112 ± 6
	PCB 156	15.0 ± 0.5	13.3 ± 0.9
	PCB 157	3.8 ± 0.3	4.08 ± 0.77
Major PCBs	PCB 138	167.0 ± 13.4	162.0 ± 6.9
	PCB 153	204.6 ± 10.2	201 ± 3
	PCB 180	83.3 ± 9.2	80.8 ± 5.0
PBDEs	PBDE 47	70.7 ± 6.4	73.3 ± 2.9
	PBDE 99	18.4 ± 1.3	19.2 ± 0.8
	PBDE 100	17.7 ± 1.4	17.1 ± 0.6
	PBDE 153	3.8 ± 0.3	3.83 ± 0.04
	PBDE 154	6.3 ± 0.5	6.88 ± 0.52

SRM 2977 – Mussel Tissue		
Analytes	Determined value [µg/kg]	Certified value [µg/kg]
B[*a*]A	20.42	20.34 ± 0.78
B[*b*]Fln	10.86	11.01 ± 0.28
B[*a*]P	8.19	8.35 ± 0.72
B[*ghi*]P	9.22	9.53 ± 0.43
I[*1,2,3cd*]P	4.19	4.84 ± 0.81
DB[*ah*]A	1.29	1.41 ± 0.19
Chr	50.57	49 ± 2
B[*j*]Fln	4.48	4.6 ± 0.2
B[*f*]Fln	3.46	4 ± 1

COVANCE

AOAC Int. Collaborative Study

Study participants that completed the study (10 out of 16 labs):

- Adpen Laboratories (FL, USA)
- Canadian Food Safety Inspection Agency (AB, Canada)
- Covance Laboratories (WI, USA)
- EU PAH Reference Laboratory (Belgium)
- FL Dept. of Agriculture and Consumer Services (FL, USA)
- Institute of Chemical Technology (Czech Republic)
- LECO Corporation (MI, USA)
- MI Dept. of Community Health (MI, USA)
- State Veterinary Institute (Czech Republic)
- Thermo Fisher Scientific FSRC (Germany)

Study direction team:

- Co-study directors: K. Mastovska, W. Sorenson, and J. Hajslova
- Technical advisors: J. Schmitz (Covance), J. Pulkrabova (ICT)

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AOAC Int. Collaborative Study

- **19 analytes included in the study:**

Name	Abbreviation
Anthracene	Ant
Benz[*a*]anthracene	BaA
Benzo[*a*] pyrene	BaP
Benzo[*b*]fluoranthene	BbF
Benzo[*k*]fluoranthene	BkF
Benzo[*g,h,i*]perylene	BghiP
Chrysene	Chr
Dibenz[*a,h*]anthracene	DBahA
Fluoranthene	Flt
Fluorene	Fln
Indeno[1,2,3-*cd*]pyrene	IcdP
Naphthalene	Naph
Phenanthrene	Phe
Pyrene	Pyr
3-Methylchrysene	3-MC
1-Methylnaphthalene	1-MN
1-Methylphenanthrene	1-MP
2,6-Dimethylnaphthalene	2,6-DMN
1,7-Dimethylphenanthrene	1,7-DMP

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AOAC Int. Collaborative Study

Study design:

- 3 matrices: mussel, oyster, shrimp
- total of 5 different levels of BaP (2 - 50 µg/kg)
- other studied PAHs at varying levels from 2 to 250 µg/kg that mimic typical PAH patterns
- each matrix fortified at 3 different concentration levels in duplicate + one blank for each matrix
- total of 7 x 3 = 21 study samples

Study phases:

(1) Laboratory qualification
(2) Test sample analysis

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Laboratory Qualification

Why?

- Performance-based criteria (GC-MS instrument, GC column and conditions, silica-SPE, evaporation technique and conditions)
- Optimization of GC-MS, silica-SPE clean-up and solvent evaporation conditions
- Check of potential reagent blank contamination
- Familiarization with the method

Qualification steps:

(1) GC separation test
(2) Calibration range test
(3) Solvent evaporation test
(4) PAH and fat elution profiles
(5) Procedure blank test
(6) Low-level spike test
(7) Practice sample analysis

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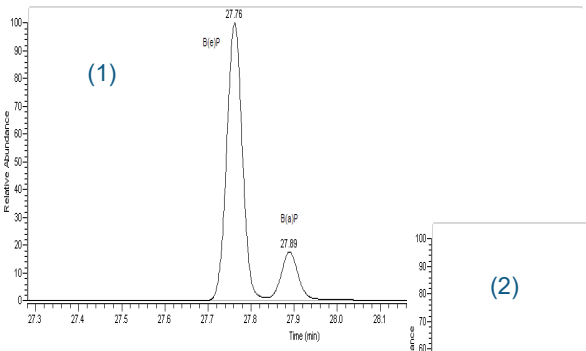
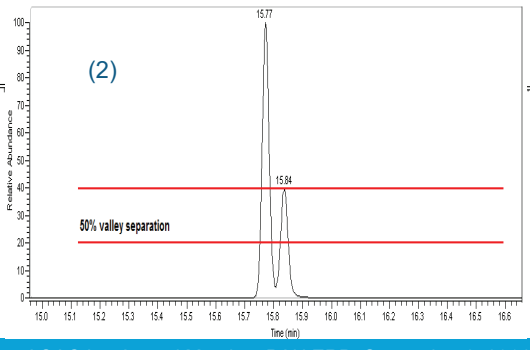
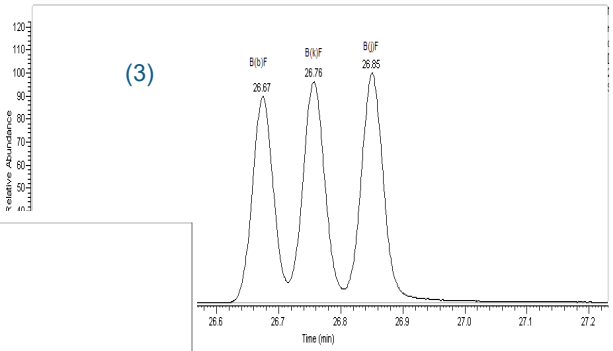
Step 1: GC Separation Test

Criteria:

(1) baseline separation of benzo[*a*]pyrene and benzo[*e*]pyrene (concentration ratio of 1:5)
(2) at least 50% valley separation of anthracene and phenanthrene (concentration ratio 1:2.5; % valley evaluated for the anthracene peak)
(3) at least 50% valley separation for benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene (concentration ratio of 1:1:1)







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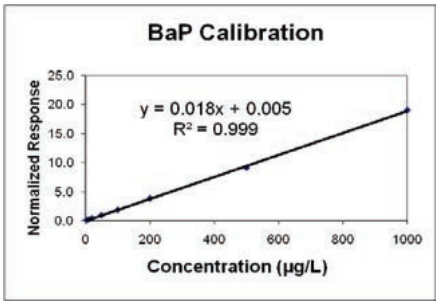
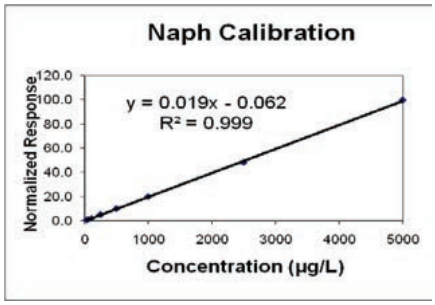
Step 2: Calibration Range Test

- Determine linear range for analyte responses normalized to respective ^{13}C-PAHs
- Optimize injection conditions
- Test carry-over (response in the solvent blank < 0.5% of the highest standard)

Calibration Level	Concentration in µg/L				Equivalent concentration in µg/kg			
	BaP and others[1]	Chr and others[2]	Naph[3]	^{13}C-PAHs	BaP and others[1]	Chr and others[2]	Naph[3]	^{13}C-PAHs
1	5	12.5	25	50	0.5	1.25	2.5	5
2	10	25	50	50	1	2.5	5	5
3	20	50	100	50	2	5	10	5
4	50	125	250	50	5	12.5	25	5
5	100	250	500	50	10	25	50	5
6	200	500	1000	50	20	50	100	5
7	500	1250	2500	50	50	125	250	5
8	1000	2500	5000	50	100	250	500	5

Analytes at (1) 10 µg/mL, (2) 25 µg/mL and (3) 50 µg/mL in the Mixed Stock Standard Solution.





r^2 > 0.990

Max residuals ± 20%

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Step 3: Solvent Evaporation Test

Determine absolute recoveries of PAHs and ^{13}C-PAHs during two evaporation experiments simulating the two evaporation steps in the method:

(a) evaporation of 5 mL of an PAH/^{13}C-PAH solution in EtOAc and reconstitution in isooctane

(b) evaporation of 10 mL of an PAH/^{13}C-PAH solution in hexane:DCM (3:1, *v/v*) and reconstitution in isooctane

Criteria:

Recovery of all PAHs and ^{13}C-PAHs > 70%

Recommendations:

- use isooctane as a keeper in both evaporation steps
- add 1-2 mL of EtOAc prior to the second evaporation step to improve recoveries of volatile PAHs

Evaporation techniques employed in the study:

- nitrogen blown-down
- rotary vacuum evaporation

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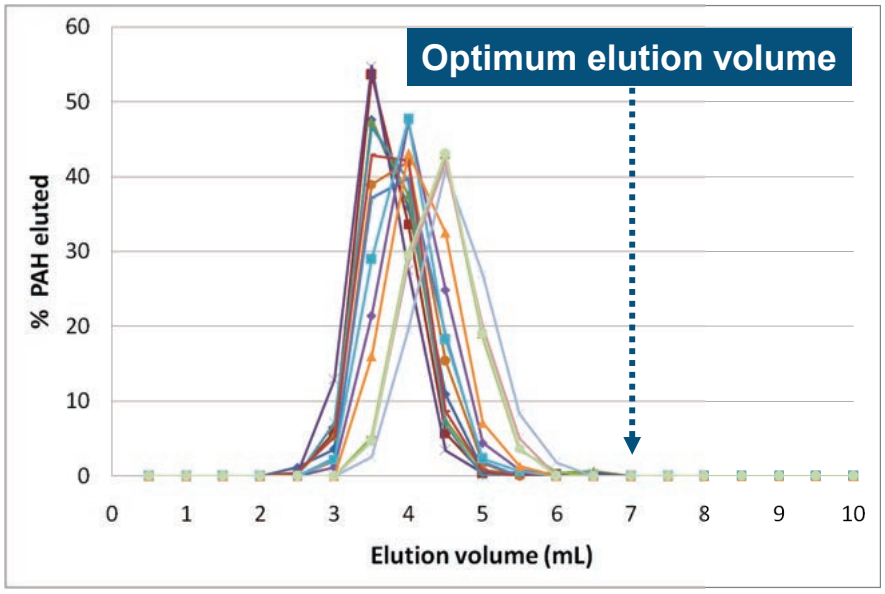
Step 4: PAH and Fat Elution Profiles

- The amount of water in silica gel (silica deactivation) can affect PAH retention
- Determine elution profile of target PAHs and optimum elution volume
- Check gravimetrically that fat (fish oil) is not eluting in the PAH fraction



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Step 5: Procedure Blank Test

- Check concentration of target PAHs in procedure (reagent) blank
- Eliminate source of potential contamination

Criteria:

Concentration of PAHs in blank < lowest calibration level

Concentration of Naph < 50 µg/mL (equivalent to 5 µg/g sample)

Potential contamination sources:

- Laboratory air
- Solvents
- Salts (have to be muffled)
- Glassware
- Extraction tubes (certain PAHs released from contaminated tubes when heated by the exothermic reaction caused by addition of anhydrous $MgSO_4$ to the aqueous extract)

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Practice Sample Analysis

- 2 shrimp samples fortified at different concentrations with PAHs
- NIST SRM 1974b: mussel tissue – recoveries vs. mean certified values:

		NIST SRM 1974b - Recoveries (%)									
PAH	**µg/kg**	**Lab 1**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5**	**Lab 6**	**Lab 7**	**Lab 8**	**Mean**	**RSD (%)**
BaA	**4.74**	90	83	80	86	73	93	85	89	**85**	**7.4**
BaP	**2.80**	99	80	79	76	72	79	76	89	**81**	**11**
BbF	**6.46**	93	81	78	81	69	82	84	88	**82**	**8.6**
BghiP	**3.12**	103	99	93	96	84	100	101	109	**98**	**7.7**
BkF	**3.16**	97	71	78	74	63	74	86	88	**79**	**13**
Flt	**17.1**	104	102	93	102	85	100	103	103	**99**	**6.5**
IcdP	**2.14**	98	66	66	78	65	59	104	104	**80**	**24**
Phe	**2.58**	95	87	101	119	89	85	104	92	**97**	**12**
Pyr	**18.04**	100	99	90	96	85	103	93	92	**94**	**6.2**

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Collaborative Study Results

- 10 laboratories completed the collaborative study
- 8-10 valid results for the majority of determinations

Results:

- Mean recoveries for all analytes (5 levels) – in the 70-120% range:
  - **Shrimp**: 83.8 – 115%
  - **Mussel**: 77.3 – 107%
  - **Oyster**: 71.7 – 94.6%, except:
    - BaA: 68.6% recovery at 25 µg/kg in oyster (RSD_r: 5.84%, RSD_R: 21.1%)
    - **Ant**: 50.3-56.5% recovery in oyster (RSDr: 8.78-9.96%; RSD_R: 44.5-64.7%; HORRAT: 1.56-1.94)
    - **BaP**: 48.2-49.7% recovery in oyster (RSD_r: 6.43-11.9%; RSD_R: 40.6-43.5%; HORRAT: 1.10-1.45)

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Collaborative Study Results

- Repeatability, reproducibility and HORRAT value:
  - Shrimp:
    - RSD(r): 1.40 – 26.9%;
    - RSD (R): 5.41 – 29.4%;
    - HORRAT: 0.22 – 1.34
  - Mussel:
    - RSD(r): 2.52 – 17.1%;
    - RSD (R): 4.19 – 32.5%;
    - HORRAT: 0.17 – 1.13
  - Oyster (except Ant and BaP):
    - RSD(r): 3.12 – 22.7%;
    - RSD (R): 8.41 – 31.8%;
    - HORRAT: 0.34 – 1.39

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Results: Oysters

- Lab A: samples stored at -70°C
- Recoveries (%) in oysters study samples SFC O1-O7:

PAH	SFC O1	SFC O2	SFC O3	SFC O4	SFC O5	SFC O6	SFC O7	Mean	RSD (%)
1,7-DMP	94	99	108	95	98	106	96	**100**	**5.4**
1-MN	107	109	-	109	110	101	101	**106**	**3.8**
1-MP	94	100	-	100	92	102	88	**96**	**5.5**
2,6-DMN	85	90	-	89	80	77	78	**83**	**6.8**
3-MC	99	101	-	102	96	100	93	**99**	**3.5**
Ant	92	91	-	89	87	91	84	**89**	**3.3**
BaA	88	90	-	90	87	91	81	**88**	**4.0**
BaP	90	87	-	86	85	88	82	**86**	**3.0**
BbF	90	93	-	93	87	92	83	**90**	**4.2**
BghiP	94	95	-	94	89	94	90	**93**	**2.7**
BkF	91	92	-	91	90	94	85	**90**	**3.1**
Chr	90	92	-	92	88	93	84	**90**	**3.8**
DBahA	95	96	-	95	92	96	89	**94**	**3.1**
Fln	93	94	-	93	88	92	87	**91**	**3.2**
Flt	95	92	-	94	88	92	86	**91**	**4.1**
IcdP	94	93	-	93	87	92	87	**91**	**3.5**
Naph	94	99	-	96	93	96	88	**94**	**3.8**
Phe	90	92	-	91	89	93	84	**90**	**3.6**
Pyr	92	94	-	93	90	94	86	**91**	**3.3**

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Results: Oysters

- Lab B: samples stored at -20°C
- Recoveries (%) in oysters study samples SFC O1-O7:

PAH	SFC O1	SFC O2	SFC O3	SFC O4	SFC O5	SFC O6	SFC O7	Mean	RSD (%)
1,7-DMP	93	95	93	102	90	107	92	**96**	**6.3**
1-MN	102	102	-	98	124	98	111	**106**	**9.5**
1-MP	97	99	-	100	93	109	94	**99**	**5.8**
2,6-DMN	85	77	-	91	65	71	73	**77**	**12.6**
3-MC	92	94	-	98	96	97	96	**95**	**2.1**
Ant	**41**	**47**	**-**	**51**	**50**	**53**	**44**	**48**	**9.6**
BaA	74	75	-	80	78	81	76	**77**	**3.5**
BaP	**47**	**51**	**-**	**56**	**52**	**54**	**50**	**52**	**6.3**
BbF	82	88	-	92	87	89	86	**87**	**3.7**
BghiP	87	83	-	87	82	84	90	**86**	**3.4**
BkF	86	85	-	92	89	89	88	**88**	**2.9**
Chr	86	85	-	91	87	88	87	**87**	**2.5**
DBahA	87	84	-	89	82	84	86	**85**	**2.7**
Fln	86	86	-	93	83	77	86	**85**	**6.3**
Flt	85	84	-	90	87	88	91	**87**	**3.2**
IcdP	88	85	-	88	86	86	89	**87**	**1.6**
Naph	93	93	-	101	95	92	94	**95**	**3.4**
Phe	89	87	-	93	90	89	88	**89**	**2.2**
Pyr	86	85	-	89	88	88	85	**87**	**2.2**

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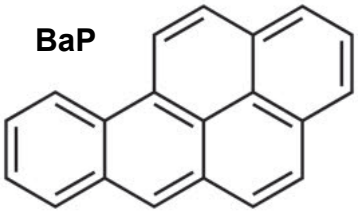
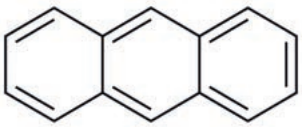
Results: Oysters

- Lower recoveries of BaP and Ant (and BaA) in oyster samples stored at -20°C

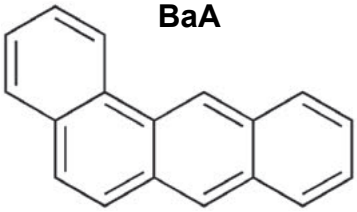
BaP



Ant



BaA



- Confirmed by analysis of a second set of oyster test samples in Lab B and by results from a third lab.
- Extracts from the first set of oysters analyzed by Lab B were all **dark green** but the second set of extracts (prepared 1.5 months later) produced a dark green extract only for the blank sample and all the extracts of fortified samples were **yellow-brown** in color.

Long-term storage of oyster samples at -20°C:

➧ **Potential matrix changes caused by the presence of PAHs and accompanied by selective losses of BaP and Ant**

COVANCE

Acknowledgments

- Study participants
- Method working group and method committee
  - chairs: Gina Ylitalo, Tom Phillips
  and special thanks to Jack Cochran, Restek
- Stakeholders and sponsors
  (Shrimp Alliance, Cambridge Isotope Laboratories)
- Scott Coates and other AOAC Int. staff
- Study team
  ICT: Jana Pulkrabova, Lucie Drabova
  Covance: John Schmitz, Jack Jabusch and numerous other people that helped preparing and shipping the samples
- Covance Nutritional Chemistry & Food Safety management

COVANCE

3rd Summer School on

Smartphone Analyzers for on-site testing of food quality and safety

10-14 June 2019

Queen's University, Belfast, Northern Ireland

Course: Smartphone analyzers for on-site testing of food quality and safety

10-14 June 2019
H2020 Marie-Curie project FoodSmartphone in co-operation with Queen's University, Belfast
Course Director: Professor Chris Elliott
Course Organiser: Ms Ciara Sarsfield / Professor Karen Rafferty
Co-Organizer: Ms Ciara Sarsfield / Mrs Joanna Scott
Course Venue: Training Room 6, Graduate School, Queen's University, Belfast

Monday 10 June 2019	**Topics: (1) market needs and technology drivers, (2) user-interfaces, multimedia and the concept of Citizen Science**	QUB Chair: Chris Elliott/Karen Rafferty
09:00	Registration with Tea/Coffee	
09:00 – 09:30	Welcome to Queen's	Professor Chris Elliot (IGFS, QUB)
	Introduction to Concepts of Citizen Science	Professor Chris Elliot (IGFS, QUB)
09:30 – 10:30	Entrepreneurship – You need it all to win	Professor Alistair Fee (Management, QUB)
10:30 – 11:00	**Break**	
11:00 – 12:00	Mental Maze: - In and Out of the Box	Professor Alistair Fee (Management, QUB)
12:00 – 13:00	**Lunch**	
13:00 – 15:00	Innovation	Professor Alistair Fee (Management, QUB)
15:30 – 16:00	**Break**	
16:00 – 18:00	Workshop: Individual Solution sketching and Idea Generation	Ms Helen Keys (Entrepreneur)
18:00 – 18:30	Reflection	QUB

Tuesday 11 June 2019	**Topics: (3) introduction to software engineering, (4) integration of data from different sources, (5) secure web interfaces**	**QUB Chair: Cuong Cao**
09:00 – 10:00	Masterclass - Introduction to Software Engineering	Dr John Bustard (EEECS, QUB)
10:00 – 10:30	Masterclass - Integration of data and Analytics	Dr John Bustard (EEECS, QUB)
10:30 – 11:00	Break	
11:00 – 12:00	Masterclass - Web and cloud security	Prof Sakir Sezer (ECIT, QUB)
12:00 – 13:00	Lunch	
13:00 – 14.00	Bringing it all Together TEST2ALL	Evelyn Fussel (Zeulab)
14:00 – 14:30	Demo	Evelyn Fussel (Zeulab)
14:30 – 15:30	Business Planning	Prof Roger Woods (QUB)
15:30 – 16:00	Break	
16:00 – 18:30	Group forming	

Thursday 13 June 2019	**Topics: (7) workshop on App design, (8) introduction to- and exploitation of IPR, (9) entrepreneurship in an innovation or software SME**	**QUB Chair: Karen Rafferty**
09:00 – 10:00	Masterclass: An overview of App design	Dr John Busch (QUB)
10.00-- 10.30	Designing your App	Dr John Busch (QUB)
10:30 – 11:00	Break	
11:00 – 12:00	Workshop: Developing your App	Dr John Busch (QUB)
12:00 – 13:00	Lunch	
13:00 – 14:00	IPR – Protecting your idea	Dr Jacob Baggerman (Aquamarijn)
14:00 – 15:00	Innovation in a Microsieve SME	Dr Jacob Baggerman (Aquamarijn)
15:00 – 15.30	Break	
15.00 -18.00	Individual research & pitch development	QUB Facilitation (Karen Rafferty)

Friday 14 June 2019	Final FSP Pitches	QUB Chair: All- panel
09:00 – 10:30	Group Work: Putting it all together	QUB
10:30 – 11:00	**Break**	
11:00 – 12:00	Final Pitches	Students
12:00 – 13:00	**Lunch**	
13:00 – 15:00	Final Pitches	Students
15:00 – 16:30	**Break**	
16:00 – 16:30	Future Outlook and Closing	QUB

FoodSmartphone.eu

QUEEN'S UNIVERSITY BELFAST

FoodSmartphone Summer School 2019

Queen's University Belfast - Graduate School
Week commencing 10th June 2019

AIM:

This advanced course aims to provide knowledge regarding *software design* and *smartphone exploitation* and their applicability to **complex food systems**.

PROGRAM:

The programme of the summer school is based on software design and smartphone exploitation for food analysis. There will be a focus on end-user acceptance and exploitation potential of smartphone-based pre-screening tools as well as user-friendly data handling and App software solutions and secure web-interfaces. Vehicles for commercial exploitation will be explored through new start-ups and existing innovation small and medium-sized enterprises (SMEs).

TOPICS:

- Market needs and technology drivers,
- Introduction to user-interfaces, multimedia and the concept of Citizen Science,
- Introduction to chemometric data handling,
- Introduction to software engineering,
- Integration of data from different sources,
- Secure web interfaces,
- Workshop on App design,
- Introduction to- and exploitation of IPR,
- Entrepreneurship in an innovation or software SME,
- Workshop: designing a fit-for-purpose business model for smartphone-based pre-screening solutions.

SPEAKERS:

Prof Christopher Elliott, IGFS, QUB
Dr John Busch, EEECS, QUB
Dr John Bustard, EEECS, QUB
Prof Alister Fee, School of Management, QUB
Ms Helen Keys, Entrepreneur in Residence, QUB
Dr Karen Rafferty, EEECS, QUB
Dr Huiyu Zhou, University of Leicester
Mr Luis Mata, Zeulab, Spain
Prof Maire O'Neill, ECIT, QUB
Prof Roger Woods, Analytics Engines & QUB
Dr Terry McGrath, IGFS, QUB
Dr Cuong Cao, IGFS, QUB
Dr Jacob Baggerman, Aquamarijn, The Netherlands

IGFS: Institute for Global Food Security
QUB: Queen's University Belfast
EEECS: School of Electronics, Electrical Engineering and Computer Science
ECIT: Institute of Electronics, Communications and Information Technology

ORGANISERS:

This course will be organised by QUB in collaboration with partner organisation CSEM and successful entrepreneurs from Aquamarijn and ZEU.

QUEEN'S UNIVERSITY BELFAST IGFS
AQUAMARIJN Micro Filtration B.V.
csem
ZEU

FoodSmartphone is funded by the European Community's Horizon 2020 Framework Programme under Grant Agreement - 720325

Participants' Evaluation of FoodSmartphone Summer School

Term of FoodSmartphone Summer School: 10-14 June 2019

Name:

_____

1. The Summer School was organized in accordance with my expectations.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

2. All topics covered were of interest and relevant to me.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

3. The content / programme was well organized and easy to follow.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

4. This training experience will be useful in my work.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

5. The documents distributed were helpful.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

6. The trainers were knowledgeable about the training topics.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

7. The time allocated for the training was sufficient.

1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Comment:

8. Can you suggest any changes / improvements / other topics for future FoodSmartphone Summer School?

_____

_____

_____

9. Was there any content or topics missed in the programme?

_____

_____

_____

Thank You for Your Feedback!

ESR N3 Summer School Feedback

Summary of Feedback

Overall the FSP summer school was deemed a successful and interactive week for the ESRs.

100% of the ESRs agreed that the content of the summer school was well organised and easy to follow

82% agreed that the summer school was organised in line with their expectations

All of the ESRs agreed that the trainers were knowledgeable on their training topic, with 55% strongly agreeing on this.

Improvements

More hands on experience, demos' workshops for topics such as app development, software development where ESR knowledge may not be as strong

More time for pitch idea development

Longer time for ESR Meeting, excursions

More information on Intellectual Property concept

Learning

36% were neutral about the relevancy of the topics – this relates to the cloud security, which they found intense, not relevant and hard to put into practice with little knowledge/experience.

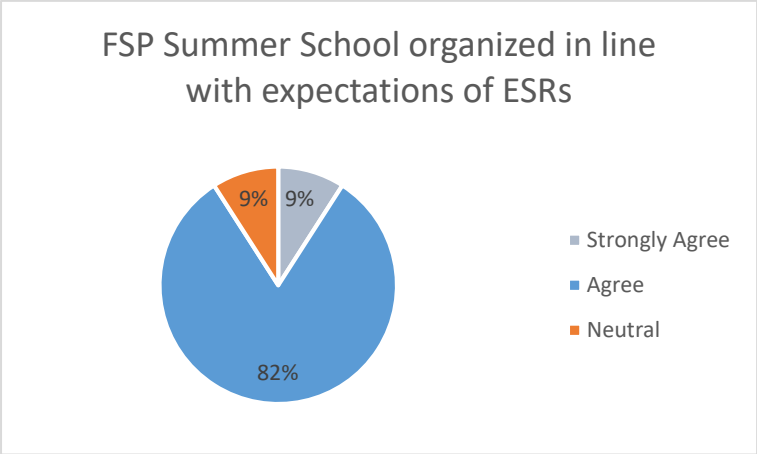
Number of participants

12 Participants (Jordi feedback not included)

1. The Summer school was organized in accordance with my expectations

82% of participants felt their expectations had been met

1 strongly agreed, 9 participants agreed and 1 was neutral



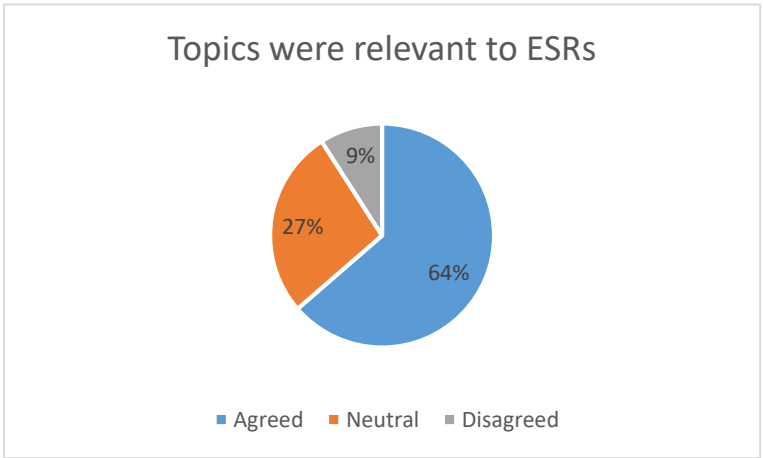
2. All topics were of interest and relevant to me

64% agreed, however 36% were neutral or disagreed

7 Agreed, 3 were neutral and 1 disagreed

Feedback comments were as follows:

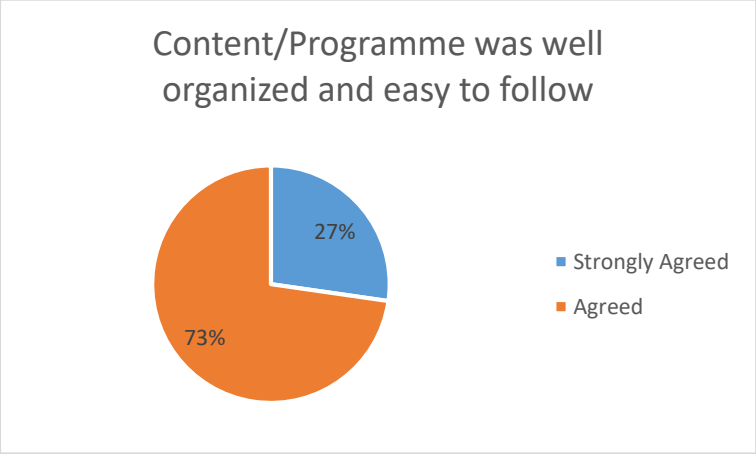
- Cloud security was a bit intense
- Software development wasn't relevant to me. A lot of information but no applicable examples
- Some topics were technical to understand



3. The content of the programme was well organized and easy to follow

All participants agreed that the content of the FSP programme was well organised and easy to follow, with 73% agreeing

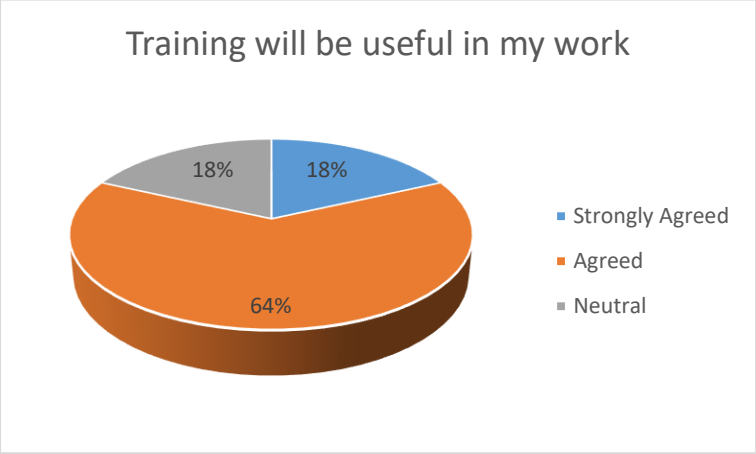
3 Strongly Agreed, 8 Agreed



4. This training experience will be useful in my work

64% agreed this training will be useful in future work

2 Strongly agreed, 7 Agreed and 2 were neutral



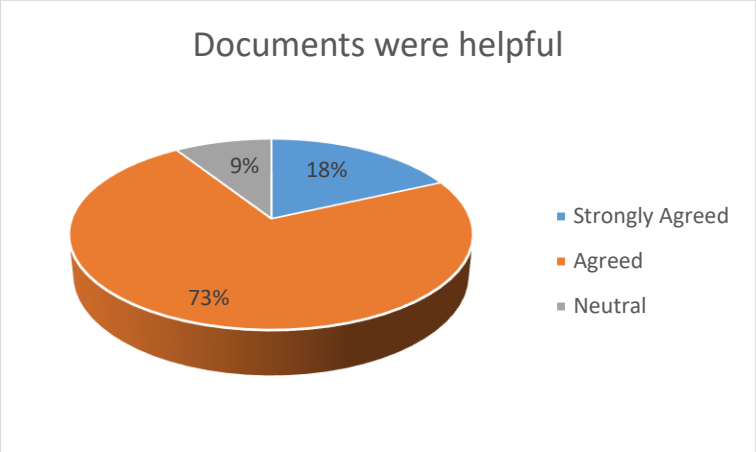
5. The documents distributed were helpful

73% agreed that the documents were helpful

2 Strongly Agreed, 8 Agreed and 1 was neutral

Feedback comment:

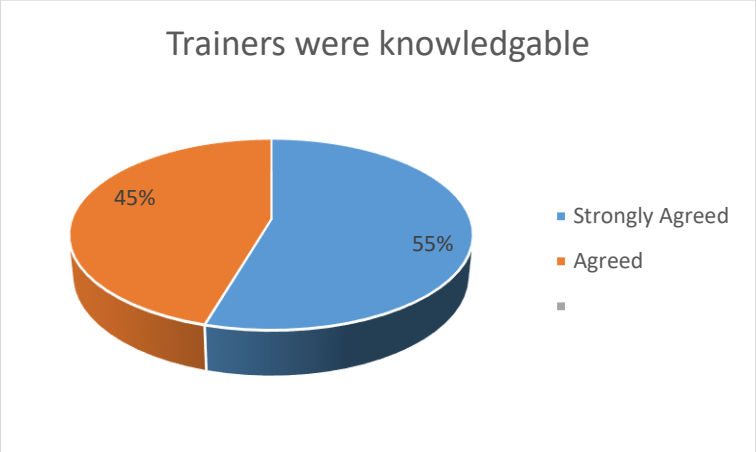
- It would be helpful to have the presentations prior to each session



6. The trainers were knowledgeable about the training topics

55% strongly agreed that the trainers were knowledgeable on their training topics

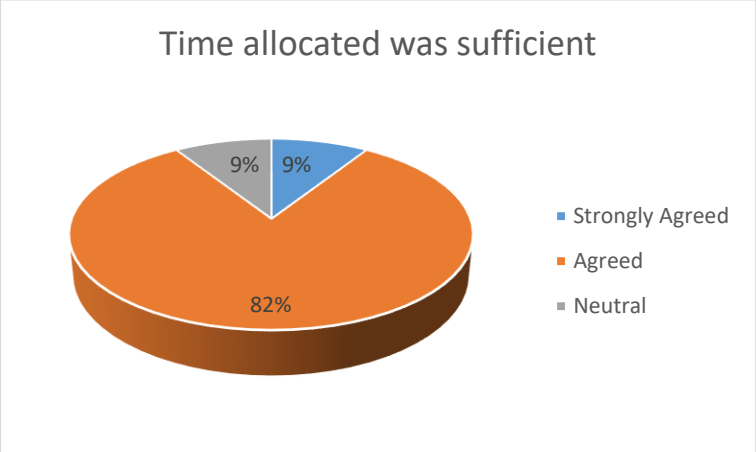
6 Strongly agreed, 5 Agreed



7. The time allocated for the training was sufficient

82% agreed that the time for the training was sufficient

1 Strongly agreed, 9 Agreed and 1 was neutral



8. Can you suggest any changes/improvements /other topics for future FSP summer school?

- Local tours are great
- Longer ESR Meeting times – excursions
- More sessions on App development and design
- More hands on experience – demos, workshops
- It was interactive which is important – keep this up
- More time to work on developing an idea
- More time for app development
- Longer ESR meeting time

9. Was there any content or topics missed in the programme?

- More information on Intellectual property concept

Pitch Competition Scoring Sheet

Judge: _____ Team: _____

The team's goal is to get you excited about the value of their business idea within 10 minutes using various content and how they deliver the content (e.g. clear, enthusiastic)

Pitch Content

If the information was provided or not, please enter a checkmark in the Yes/No as applicable. If yes, please also score using a checkmark using a scoring between 1 and 5.

	Criteria	Yes	No	Score 1 (bad)	2	3	4	5 (good)
1.	Was there a 'hook' – compelling message at the start							
2.	Need - information about the problem/opportunity							
3.	Approach - product/service info, how it will solve the problem or take advantage of the opportunity							
4.	Benefit – what are the benefits to the customer, investor and partner? What does it cost?							
5.	Competition and competitive advantage							
6.	Had a closing/ask for the audience							
	Total: (out of 30)							

Pitch Delivery

If the criteria for delivery was provided or not, please enter a checkmark in the Yes/No as applicable. If yes, please also score using a checkmark using a scoring between 1 and 5.

	Criteria	Yes	No	Score 1 (bad)	2	3	4	5 (good)
1.	Spoke clearly							
2.	Told a story *(not a list)*							
3.	Provided examples							
4.	Used easily understood language							
5.	Related to the audience							
6.	Enthusiastic, passionate and full of energy							
	Total: (out of 30)							

Please turn over

Positive Feedback

Please provide at least 1 **positive** piece of feedback.

- ☐ Solid business idea
- ☐ Addressing a real, sizeable and/or important problem/opportunity
- ☐ Creative solution to the problem/opportunity
- ☐ Knowledgeable about the problem/opportunity
- ☐ Strong value proposition for the customer
- ☐ Knowledgeable about their business idea
- ☐ Understood the market
- ☐ Understood the competition
- ☐ Understood their competitive advantage
- ☐ Solid team to take advantage of the business idea
- ☐ Understood the financial aspects of the business idea (e.g. price, costs, margins, funding needs)
- ☐ Talked very clearly about the business idea
- ☐ Told a good story
- ☐ Used examples
- ☐ Talked in language that everyone could understand
- ☐ Showed a lot of excitement and passion for idea
- ☐ Had a closing that could be remembered
- ☐ Other: _____

_____

Negative Feedback

Please provide at least 1 **negative** piece of feedback, i.e. things the team need to work on.

- ☐ Business idea not well thought out
- ☐ Addressing a small, non-existent, and/or unimportant problem/opportunity
- ☐ Didn't have a creative solution to the problem
- ☐ Weak value proposition to the customer
- ☐ Wasn't knowledgeable about the problem
- ☐ Wasn't knowledgeable about business idea
- ☐ Didn't understand the market
- ☐ Didn't understand the competition
- ☐ Didn't understand their competitive advantage
- ☐ Need to improve founding team member skills
- ☐ Didn't understand the financial aspects of the business idea (e.g. price, costs, margin, funding)
- ☐ Didn't talk very clearly about the business idea
- ☐ Didn't tell a good story
- ☐ Could have used examples to explain idea better
- ☐ Used language that is difficult to understand
- ☐ Didn't show excitement and passion for idea
- ☐ Didn't have a closing/ask the audience
- ☐ Other: _____

_____

Overall Score

In the following boxes, please enter the overall score for this team (**add content and delivery from the previous page)** and enter a rank based on the total number of teams.

	Score		**Rank**
Overall Score for the Pitch (out of 60)		**Rank Order** (1 is the highest)	

Notes: _____

_____

_____

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INVENT 2017

FINAL PITCH TEMPLATE

Thursday 11th June 2019

Catalyst Inc

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The Goal is…

- To communicate the company's story as clearly as possible
- To create excitement for the opportunity to attract further resources

"Every presentation will be unique and have its own flow, but a template is a really good start."

INVENT 2017

Catalyst Inc

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Presentation Musts…

- Less Is More
  - 13-15 Minutes only
  - Graphics; Keep It Simple
- 12-15 slides with key sound-bites (3-4 per slide...the rest is your story!)
- Utilize back-up slides to pull out during the Q&A session (anticipate questions and prepare)

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Slide 1 – Introduction

- Presenter and company introduction
  - Who you are
  - What market you address
  - What does your business do?
  - Significant milestones to date

  } 30s Elevator Pitch
- Anchor Points (2-3 questions to focus the panel)
  - Business model?
  - Go-to-Market strategy?
  - Funding?

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Slide 2 – Opportunity

- Define the opportunity *(The Pain)*
  - Who is the customer?
  - What is the big problem?
  - How important is a resolution?
  - How do/will you turn "need" to "want"?

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Slide 3 – Your Solution

- Compelling description of your solution
  - Graphics, illustrations pictures, video
  - What is it, what does it do?
  - KISS (Keep It Simple Stupid)
  - Key Benefits v Features (don't go into technical detail)
  - USPs

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Slide 4 – Market Frame

- Frame your market
  - Type (who are the customers)
  - Size (Annual Available Market)
  - Growth (AGR)
  - Maturity (Buying Cycle)
- How will your solution be positioned?
- Demonstrate market fit
- Market penetration
  - What is your unfair advantage?

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Slide 5 – Technology (only if appropriate)

- In layman's terms
- Graphs and pictures work
- Assume that the audience does not know the technological field you are in
- Give a compelling description without using abbreviations, acronyms or techy terms

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Slide 6 – Competitive Market

- What does the competition landscape look like?
- Are there alternative solutions?
- Barriers to Entry
- Who are your target customers?
  - How do they buy?
- What's holding you back?

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Slide 7 – Competitive Advantage

- What is your "unfair advantage"?
- What are your differentiators that "hook" the decision makers?
- What is your solutions life cycle?
- Why should the customer pay for it
- What is your value proposition?
  - Quantify the solution
  - Why will the customer pay?

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Slide 8 – Go to Market Strategy

- Price (value)
  - What is your Pricing model?
- Place (where and how will you make sales)
  - Channel to market
- Promotion (branding, lead generation)
  - PR
  - SEO
  - Advertising
  - Media

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Slide 9 – Traction

- Your Team
  - Corporate Governance
  - Financial
  - Marketing and sales
  - Technical
- Demonstrate the team's ability to deliver
  - Build confidence in team
  - Track record
  - Traction to date
- Milestones achieved against objectives set

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Slide 10 – Financials

- Financial Plan (overview)
  - P&L (in appendix)
  - Cash Flow (in appendix)
  - B/S (in appendix)
- 5 Year forecast
- Highlight critical assumptions/milestones
  - Highlight risk mitigation
- Don't provide details
  - But be prepared to discuss

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Slide 11 – Funding requirement

- How much money do you need?
- Where will the money be put to use?
  - R&D
  - Marketing
  - Sales
  - Production
  - Administration
- How long will the money last?
- What is your exit plan and valuation?

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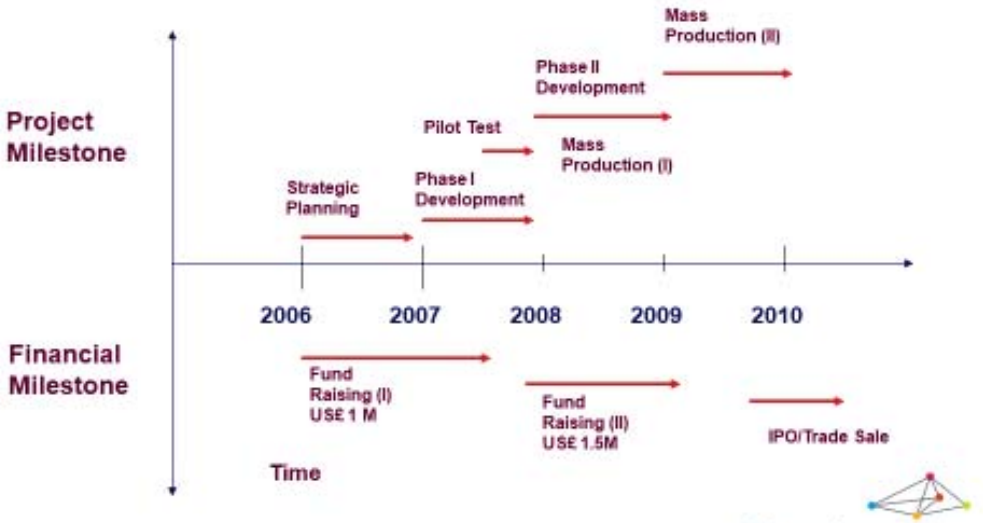
Slide 12 – Chronology



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Slide 13 – Assumptions & Risks

List of Critical Assumptions	What is the negative impact?	How you have mitigated?
In order, risk size / priority	…	…
2		
3		
4		

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Slide 14 – Summary

- The Opportunity
- Your Solution
- Your uniqueness
- The Prognosis

} The Wow Factor

"List your USPs, leave the audience with a few "deep" lingering thoughts and repeat the 3 questions you want the panel to focus on…"

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Agenda

- Introduction
- The Shared Responsibility Model of Cloud Security
  - Security of the cloud
  - Security in the cloud
    - AWS Security Services
    - APN Security Services
    - Next Generation Cloud Security Architecture (NexGenCSA)
  - Security from the cloud…
- Cloud-assisted Security

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Agenda

- Introduction
- The Shared Responsibility Model of Cloud Security
  - Security of the cloud
  - Security in the cloud
    - AWS Security Services
    - APN Security Services
      - Virtual Infrastructure Security Solutions
      - Visibility Solutions: Logging & Monitoring
      - Configuration & Vulnerability Analysis Solutions
      - Data Protection Solutions
      - Access & Control Solutions
      - Security Consultation & Penetration Testing Service
    - Private Cloud Datacenters Security Solutions

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Agenda (Cont.)

- Next Generation Cloud Security Architecture (NexGenCSA)

- Security from the cloud
  - APN Security Services
    - Cloud Access Security Broker (CASB)

- Cloud-assisted Security

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Introduction

- Cloud computing has experienced such an extraordinary growth over the last years. Its global industry market is projected to increase from $209.2B in 2016 to $383.3B in 2020.
- Service cloudification (moving a service to the cloud) is a major rising IT trend. Cloudifiying FoodSmartphone can be one of the too many use cases.

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Introduction (Cont.)

- Despite this great success, the potentials of cloud computing have not yet been fully unleashed mainly due to security concerns.
- According to the 2016 Cloud Security Spotlight Report, cloud security concerns not only top the list of perceived barriers to cloud adoption, they are further increasing with 53% up from 45% in previous year's survey.
- Many businesses are still reluctant to shift their valuable assets to the public clouds. This is one of the main reasons why, according to the RightScale 2017 State of the Cloud Report, only 22% of cloud adoption scenarios are on the public clouds while the hybrid adoption model covers 67%.

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Introduction (Cont.)

The special security concerns of cloud systems are mainly due to the following three points:

- Cloud has a larger attack surface. It inherits the same set of vulnerabilities exist in conventional systems, further, its dynamicity, heterogeneity, elasticity, and multi-tenancy properties pose additional security threats. This makes business assets on the cloud more exposed to risk.
- Many critical businesses do not like to delegate the security of their assets to a cloud service provider who may not precisely fulfil all of the security needs.
- Protecting assets at the cloud against the curiosity of the cloud service provider itself is another important concern.

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Introduction (Cont.)

Cloud security is enforced based on a shared responsibility model.

- **Security of the cloud** which refers to the safety of the cloud platform itself. It is usually the responsibility of the cloud service providers.
- **Security in the cloud** that deals with securing the business assets hosted on the cloud, e.g., VM instances, and it is usually the customer's responsibility who needs to adopt security solutions offered by 3rd party security providers in order to secure their assets on the cloud.
- **Security from the cloud …**

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Security of the cloud

Attack Type	Target	Countermeasure
Distributed Denial of Service	Network | Application	- Use SYN cookies and emplace IDS/IPS systems to thwart DDoS. - Use the Software Defined Perimeter (SDP) security architecture developed by CSA which ensures that an SDP client is authenticated and authorized before being able to access any resource.
Buffer Overflow Attack	Application	- Use advanced compiler options to emit extra code to check for buffer overflows. - Use Address Space Layout Randomization (ASLR) to randomize where core kernel modules are loaded into memory.
Code Injection Attack	Application	- Web Application Firewall (WAF). - Use CPU NX/XD technology to isolate areas of non-executable data in memory from areas of executable instructions.
BootKit Attacks	Application	- Use a Trusted Platform Module (TPM or vTPM) along with a secure boot protocol, e.g., a Unified Extensible Firmware Interface (UEFI).
VM Migration Security Issues	Network | Virtualization	- Use the Trusted Cloud Computing Platform (TCCP) that enables consumers to attest IaaS providers and ensure service security before they launch/migrate their VMs.
VM Image Sharing Security Issues	Virtualization	- Use of VM Image Management System (IMS) that regulates the publishing and retrieval of VM images with a properly enforced access control.
VM Rollback Security Issue	Virtualization	- Secure logging of VM operations, e.g., disabling credentials, is needed in order to be applied after every rollback process to bring the VM back to a secure state as one of the functionalities of an IMS.

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Security of the cloud (Cont.)

Attack Type	Target	Countermeasure
VM Sprawl	Virtualization	- Monitored and enforced policies that strongly govern who can create a VM.
VM Escape	Virtualization	- Applying software patches on regular basis. - Use Para Virtualization (PV) and Hardware Assisted Virtualization (HAV) technologies which are less-vulnerable to this exploit than Full Virtualization (FV) as they are virtualization-aware. - Enforce strong VM isolation policies by network-based solutions, e.g., Virtual Extensible LAN (VXLAN), software-based solutions, e.g., HyperSafe, or hardware-based solutions, e.g., HyperCheck, HyperGuard, HyperSentry, and Copilot.
In-Cloud Data Breaches	Hardware | Network | Virtualization | Application	- Use encrypted TLS channels to protect in-transit data - Use a full disk encryption service, e.g., BitLocker, with the key sealed by an SE solution and a hardware implementation of the AES algorithm, e.g., Intel Advanced Encryption Standard New Instructions (AES-NI), for better performance. - Regular patching to protect against flows such as Spectre and Meltdown chip flows.
DMA Attack	Hardware | Application	- Use of signed device drivers only with restricted access. - Use IOMMU technology, e.g., Intel VT-d & AMD-Vi, to block a device from accessing I\O and memory regions that is not allowed to access acting as a hardware firewall. - Store crypto keys in special devices, e.g., CloudHSM.

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Security of the cloud (Cont.)

Attack Type	Target	Countermeasure
Cross-VM Side-Channel Attacks	Hardware | Virtualization	- Software tools based on *Access Control* (VM Secure Runtime Environment), *Nested Virtualization* (CloudVisor), or *Secure Processor* (HyperCoffer). - Memory encryption techniques, e.g., AMD SEV, to provide page-granular memory encryption. - Partitioned Cache where the cache is split into protected regions allocated exclusively to each VM. - Use Intel SGX, a set of CPU instructions that allows a user-level code to allocate private regions of memory (enclaves) that is protected from processes running at a higher privilege level.
Post-VM Memory Scan	Virtualization	- Keep data encrypted in memory - Keep sensitive data always out of memory, e.g., use a CloudHSM solution. - Emplace a mechanism by which a VM can register the memory locations holding confidential data so it can wiped out before releasing the VM memory.
Virtual Machine Introspection Attacks	Virtualization	- A VMI attack trying to alter the VM kernel code can be detected by a vTPM-based solution - Placing kernel structures in a read-only segment whenever possible. - Kernel memory access monitoring.
AV Storm Security Issue	Virtualization	- Use an agentless antivirus by grouping VMs behind a single antivirus gateway. - Use the agentless VM Introspection model. - Use lightweight AV agents installed on every VM and orchestrated by a master agent as proposed in NexGenCSA.

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AWS Security Services

Authentication & Authorization:
- Identity & Access Management (IAM), AWS Organizations, & AWS Multi-Factor Authentication (MFA).
- AWS Directory Service, Amazon Cloud Directory, & Amazon Cognito

Auditing:
- AWS CloudTrail, CloudWatch, and AWS Config
- AWS Artifact

Certificate & Key Management:
- AWS Certificate Manager (ACM), AWS CloudHSM and Key Management Service (KMS)

Security Assessment Services:
- Amazon Inspector
- Amazon Macie

Limited Proactive Protection Services:
- Amazon EC2 Security Groups, AWS Web Application Firewall (WAF), and AWS Shield
- Amazo GuardDuty

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AWS Security Services (Cont.)

- AWS provides customers with a set of security services that is limited to **logging**, **monitoring**, and **assessment** sort of services beside protection against special kind of attacks, such as **DDoS**.
- Real-time protection against **viruses**, **Trojans**, **worms**, etc. requires a global and up-to-date **threat intelligence network** that is out of the cloud service provider business scope.
- For that, many cloud customers on AWS deploy solutions offered by **AWS Security Partners**.
- As of today, there are more than 45 security partners offering SECurity as a Service (**SECaaS**) on AWS, e.g., TrendMicro, Symantec, Sophos, Alert Logic, Armor, etc..

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TrendMicro DSaaS

- TrendMicro offers Deep Security as a Service (DSaaS) on AWS following an **agent-based** model.

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TrendMicro DSaaS (Cont.)

DSaaS absolutely represents an interesting case study of shifting a security service onto the cloud (**cloud-migrated**), however, the proposed model suffers from the following limitations:

- It is a heavy-agent based model that may cause an AV storm (Table 1). Trend Micro has worked with VMware to prevent AV storms in private datacenters.
- It looks like a security service elegantly moved onto the cloud rather than being specifically designed for the cloud.

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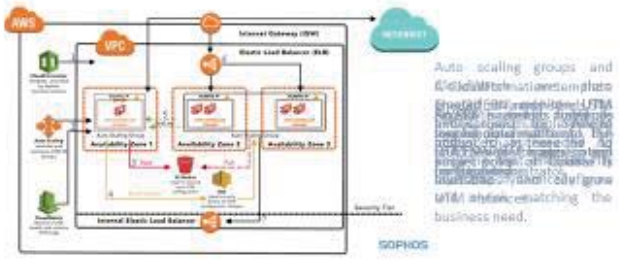
Sophos UTM

- Sophos offers its Unified Threat Management (UTM 9) on AWS. It encompasses multiple security tools, i.e., NGFW, WAF, IPS, and Advanced Threat Protection (ATP).
- It is a **cloud-aware** security service designed with AWS Best Practices in mind.



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Sophos UTM (Cont.)

- Sophos has effectively migrated its security services to AWS leveraging its ready-made cloud services, i.e., CloudFormation, Auto Scaling, CloudWatch, S3, SNS, and ELB.

- Despite its efficient utilization of various cloud services, being a cloud-aware is different from being originally designed for the cloud. Though this model can scale efficiently based on business need, the core instance, the UTM node, is not designed for the cloud rather it is being wrapped within a cloud deployment model.

- It requires two more extra switches between the guest OS level and the virtualization platform level, vSwitch → UTM node and UTM node → vSwitch, before the packet can reach its target VM.

- Moreover, Sophos UTM 9 uses an agentless gateway model of security enforcement. Thus, there is no fear of an AV Storm, however, it lacks to a clear visibility on an end-to-end SSL traffic. Hence, it can be suitable to protect servers, e.g., web servers, while it can be inadequate to protect end-users' VM instances.

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Alert Logic Cloud Defender

Alert Logic offers its Cloud Defender as a **cloud-native** suite of security solutions that provides active monitoring with threat detection, log management, and vulnerability assessment to cloud datacentres leveraging its **Active Threat Analytics Platform** where threat data is collected, aggregated and analysed.

- The **Threat Manager (TM)** is a virtual appliance working as a network IDS leveraging an agent methodology to get a replica of the protected resources' network traffic. TM inspects the replicated traffic, in case of any malicious payload is found, it is sent to the active analytics platform for a deeper and correlated review.

- The **Log Manager (LM)** is responsible for collecting system and applications log data, signing, compressing, encrypting and sending it to the active analytics platform to get investigated.

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Alert Logic Cloud Defender (Cont.)

Despite its deep environmental visibility, and well-established active analytics platform, Cloud Defender has the following architectural issues:

- It is an overhead on the virtual network to copy network traffic to TM and log files to LM to be inspected. This also encompasses lots of switching between the guest OS level of context and the virtualization layer.
- TM represents a single point of failure and a proper fail-over policy has to be defined to ensure the availability.
- It is also an overhead on the active analytics platform to analyze all of the malicious traffic captured by every registered TM. Even if it is supported by an excellent resource capacity, it is still an expensive task when compared to a local decision making model.

From the mentioned limitations, it can be concluded that **Cloud Defender is designed to provide security from the cloud (the active analytics platform) rather than being designed for the cloud.**

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Next Generation Cloud Security Architecture (NexGenCSA)

Currently offered security services on AWS can be classified fundamentally into three categories:

- **Cloud-migrated** security services, e.g., TrendMicro DSaaS, which adopt a deployment model on the public clouds similar to that followed on their traditional system deployment.
- **Cloud-aware** security services, e.g., Sophos UTM, which know that they are running on the cloud and leverage various cloud services, i.e., ELB, auto scaling, etc., in their deployment model to provide elastic security based on business demand.
- **Cloud-native** security services, e.g., Alert Logic Cloud Defender, which are security services serving from the cloud to secure traditional or cloud systems.

Nevertheless, public cloud environments are in need for security services that are originally designed for the cloud rather than being migrated to it or even aware of it. This requires a new security architecture that pervades various layers down from the hardware level up to the guest software layer.

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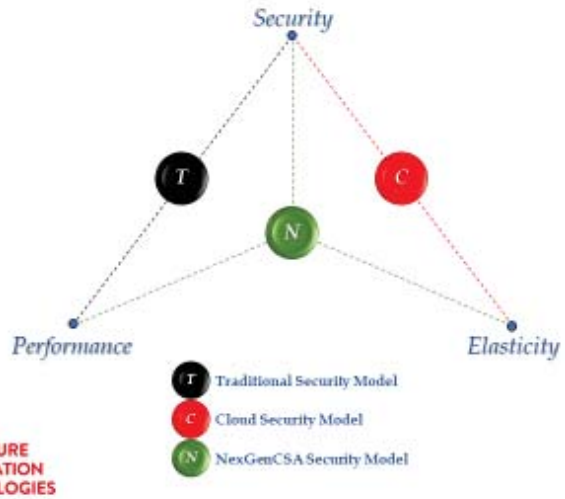
Next Generation Cloud Security Architecture (NexGenCSA) (Cont.)



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Next Generation Cloud Security Architecture (NexGenCSA) (Cont.)

- On traditional systems, we used to design a specific hardware to run a specific service with a better performance, e.g., F5 ADC is used to run on purpose-built hardware.
- This is no longer valid for a cloud environment which virtualizes every purpose-built hardware turning it into a virtual software appliance to provide elasticity and business agility, however, it also scarifies the performance/cost.
- We believe that a proper security architecture should hold the stick from the middle. In that, instead of having a special hardware for every security service, the cloud has to be supported by a suite of hardware and software security services specifically designed for its scalable computing model.
- There are a few examples of a hardware level optimization that have been done so far to enhance cloud security. However, they have a limited application area, i.e., CloudHSM, which is a hardware module specifically designed for secure key management on the cloud, and Intel SGX, which is a new ISA designed to solve some cloud security issues.

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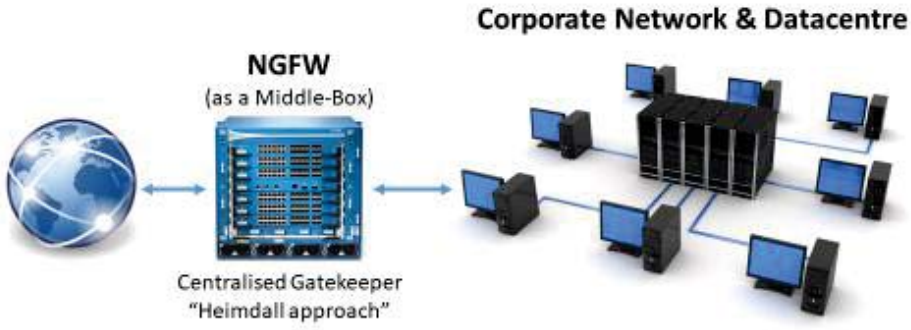
Next Generation Cloud Security Architecture (NexGenCSA) (Cont.)



- Centralised, difficult to scale
- Locked to one specific vendor
- Vulnerable to vendor specific DDoS attacks
- Cannot be easily extended into the cloud
- Single point of failure

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Next Generation Cloud Security Architecture (NexGenCSA) (Cont.)

Virtual Security Appliance
AWS Instance + AWS Marketplace App

Virtual Security Appliance Vendors on AWS

TREND MICRO, Check Point, intel Security, Barracuda, SOPHOS, splunk>enterprise, HYTRUST, citrix, Symantec, FORTINET, IBM Radar

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Security becomes a heavyweight inefficient software-based virtual appliance

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Next Generation Cloud Security Architecture (NexGenCSA) (Cont.)

Taking into consideration the analysis and concerns discussed, hereby, the major characteristics of an efficient security solution for public clouds is identified to be:

- It should not require a deep support from the virtualization layer. Service providers are usually not willing to integrate their virtualization platform with any 3rd party tool to reduce their attack surface and ultimately reducing the risk.
- It should implicates minimal number of context switchings between the guest and the virtualization layers to avoid unnecessary latency.
- It should provide a deep visibility into the workload. Ultimately, this requires an endpoint security agent.
- Endpoint security agents have to be well orchestrated in order to avoid AV Storms.
- It should be accelerated with proper hardware support.

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Next Generation Cloud Security Architecture (NexGenCSA) (Cont.)

NexGenCSA is offered in two deployment models, the agent-based and the gateway-based models. It satisfies the characteristics of an efficient security solution for public clouds through the followings:

- It encourages the adoption of a SmartNIC technology, which allows a virtual security gateway to offload a part of the security control overhead onto the NIC level. Simple security controls, e.g., blocking access to certain ports, can be enforced down from the SmartNIC level instead of pushing it up to the destined VM.

- A security gateway is used, in case of the gateway deployment, and a suit of VM-level orchestrated agents are used, in case of the agent-based deployment, to enforce the security controls which cannot be offloaded onto the SmartNIC, e.g., inspecting an end-to-end encrypted traffic. The orchestration is essential to avoid AV storms.

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Next Generation Cloud Security Architecture (NexGenCSA) (Cont.)

- To reduces the number of context-switching, it uses the IVCom techniques instead of the TCP/IP protocol for the communication between the security gateways and the monitored VMs, in case of the gateway deployment, or between the master and the orchestrated agents, in case of the agent-based deployment.

  However, this requires the service providers to enable IVCom while they may not be willing to do so as it increases the attack surface. For that, NexGenCSA defines a new two-levels enclaving policy in order not to expose more attack surface by enabling IVCom than what is already exposed by TCP/IP.

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Next Generation Cloud Security Architecture (NexGenCSA) (Cont.)

NexGenCSA software suit is a cloud-agnostic, distributed, lightweight agent-based system that is composed of the following components:

- A set of Elementary Agents (EAs) installed on the monitored VMs.
- A set of vSwitch Master Agents (MAs) installed on an elected VM per every virtual switch broadcast domain.
- The Master Service (MS) that manages and controls all of the MAs across different vSwitches.

- A set of VMM Agents (VAs) installed on every hypervisor. They are controlled only by the service provider and can be monitored by the customers' MS services to provide transparency into the overall security posture.
- The VAs Master Service (VMS) to control all of the VAs across the datacenter. It is managed by the service provider.

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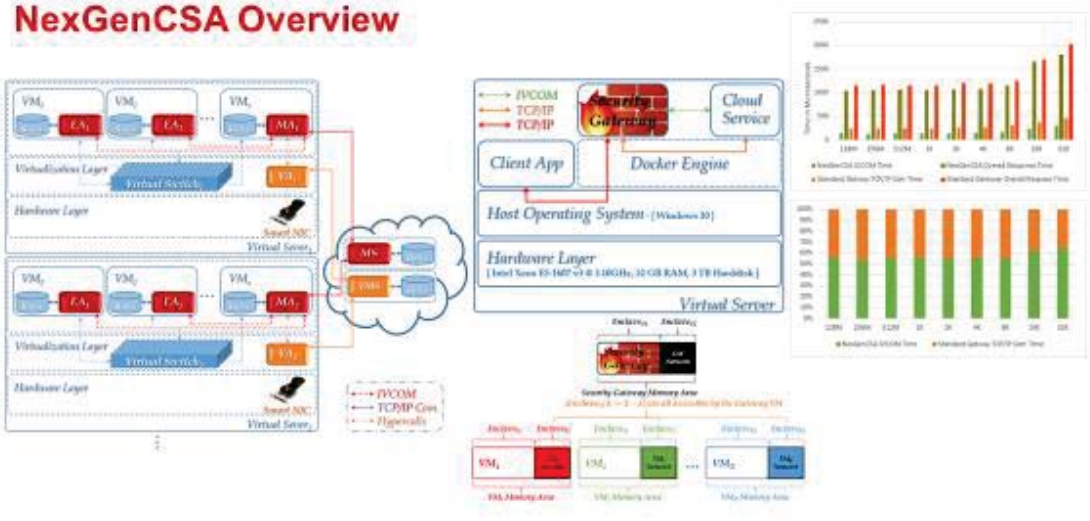
NexGenCSA Overview



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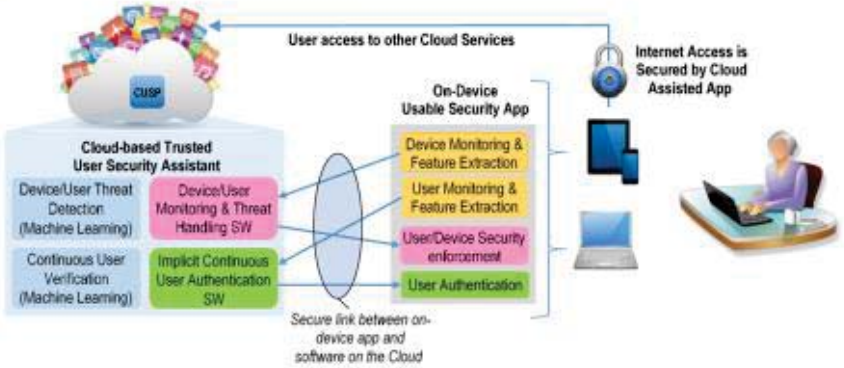
Cloud-assisted Security

This research direction tries to define a new model of security assisted by a set of cloud services to make security more resistance and usable at the same time especially for the non-techie users.



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Cloud-assisted Security (Cont.)

- **SSIDaaS: Self-Sovereign Identity as a Service**

  It is a promising architecture that is going to revolutionize the way in which we present & prove our identities while maintaining usability and security at a higher level.

  It offers a scalable, hands-free, and smart platform to implement self-sovereign identity. It further takes it to the next step by offering expert advices that we believe to be a crucial feature for a world-scale identity management system where most users are non-techies.

	Centralized	Federated	User-Centric	Self-Sovereign (Yoti)	Self-Sovereign (Blockchain)	SSIDaaS
Usability	✗	?	✓	✓	✓	✓+
Updatability (SPU)	✗	?	?	✗	?	✓
Portability	✗	?	?	?	✓	✓+
User-Controllability	✗	✗	?	✓	✓	✓+
Smooth R2F	✗	✗	?	✗	?	✓
Scalability	✓	✓	✓	?	?	✓
No SPD	✗	✗	✗	?	?	✓
User-Advisability	✗	✗	✗	✗	✗	✓
Trustworthiness	?	?	?	?	?	✓
Collusion-Proofness	✗	✗	✗	?	?	✓

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Cloud-assisted Security (Cont.)

- **C-Pay: A Cloud-assisted Payment System (CPS)**

  C-Pay is a new payment system, where C stands for being: Cloud-assisted, Contactless, Customizable, Controllable, Central Point for Update, and Cost effective.

  C-Pay cloudifies the payment process through a novel architecture offering several advantages over existing systems, i.e., EMV Chip Cards, Contactless Cards, Secure Element & Host-based Card Emulation, and QR code payments, which have suffered from lots of frauds. These advantages can be summarized in offering Enhanced Security (MFIA), Improved Usability (Hands-free, Central Point for Update, & Virtual Loyalty Cards), Stronger Privacy (Isolation), Market-based profile (Customizable), and User-based profile (Controllable).

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Thank you
Q & A

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Intellectual Property Rights

Dr Rosi Armstrong

Armstrong IPR Ltd

Innovation Centre, Catalyst Inc, Queen's Road, Belfast BT3 9DT

E: ra@armstrongipr.com T: 028 9078 5870 M: 07548 978888

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Purpose of IP Rights

IP Rights are legal rights which give you ownership of your ideas.

Your IP Rights can:

- protect your work and reputation
- create barriers for your competitors
- generate income streams as your IP rights can be sold or licensed
- help raise finance by attracting investment / buy-out

Examples:
https://www.youtube.com/channel/UCb_52rKv06VJ8Ehyhvygdpw/featured

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Copyright

Protects	literary works, drawings, video, music and software
Provides	protection against copying of the work BUT independent creation of the same or a similar work will not infringe copyright
Criteria	work must be original i.e. not copied
Duration	approximately life of the creator of the work + 70 years
To get	copyright is granted automatically as soon as an original work is created, there is no registration scheme to protect your copyright works – mark with © and year and keep good records of the works and their dates of creation

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Trade Marks

Protects	signs which designate the origin of the goods/services for which they are used sign can be a word, logo, shape, colour, sound, smell
Provides	protection for brand and reputation
Criteria	sign should be distinctive - when choosing a trade mark, avoid descriptive words, common surnames, place names...
Duration	initially 10 years, renewable indefinitely
To get	a trade mark can be used without registration registration ® provides better protection your chosen trade mark needs to be free for use

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Trade Mark Databases

European Union Trade Mark Database:

https://euipo.europa.eu/eSearch/

UK Trade Mark Database:

www.gov.uk/search-for-trademark

US Trade Mark Database (TESS):

www.uspto/trademark

Trade Mark goods and services classification system:

www.wipo.int/classifications/nice/en/

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Design Rights

Protects	shape and/or surface decoration of aesthetic & industrial designs computer screenshots
Provides	Unregistered Design Right - protection against copying only Registered Design Right - protection against copying of the design AND independent creation of the same or similar design
Criteria	shape/decoration of the design must not be commonplace
Duration	Unregistered Design Right - approximately 5-10 years Registered Design Right - up to 25 years
To get	Unregistered Design Right - granted automatically on creation of a design Registered Design Right - must be applied for, gives extra protection, a design can be disclosed before registration

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Patents

Protects	inventions of a technical nature products such as hardware and software processes such as services BUT products and processes for business are difficult to patent
Provides	the right to prevent others from using the invention (e.g. making or selling the invention)
Criteria	invention must be new and not obvious
Duration	20 years from the date of patent application
To get	patent protection must be applied for in each country there must be no non-confidential disclosure of the invention before an application has been filed

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Software

- Copyright - protects only the code of the software (narrow protection)
- Patent - protects the method of the software (broader protection)

- Open Source Software - check the terms and conditions to see how this can be used; cannot be the subject of a patent application

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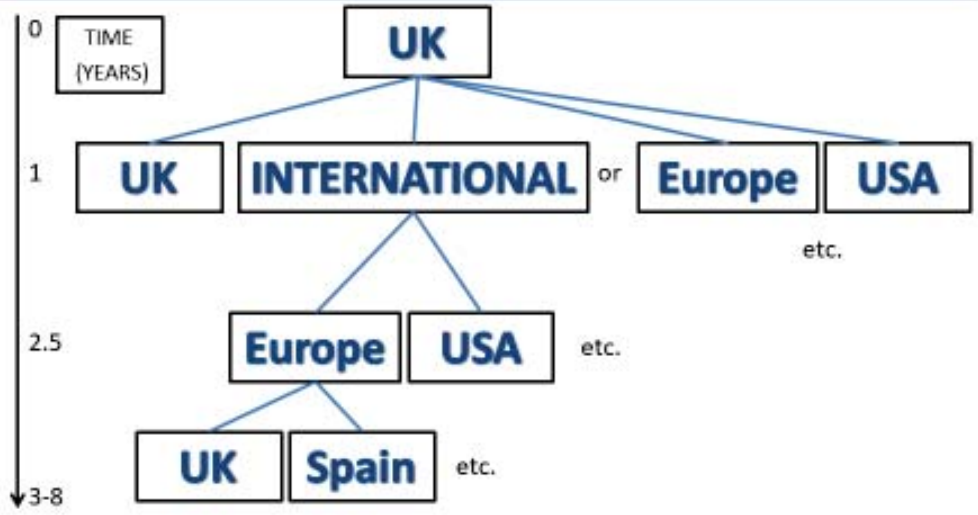
Typical Patent Process



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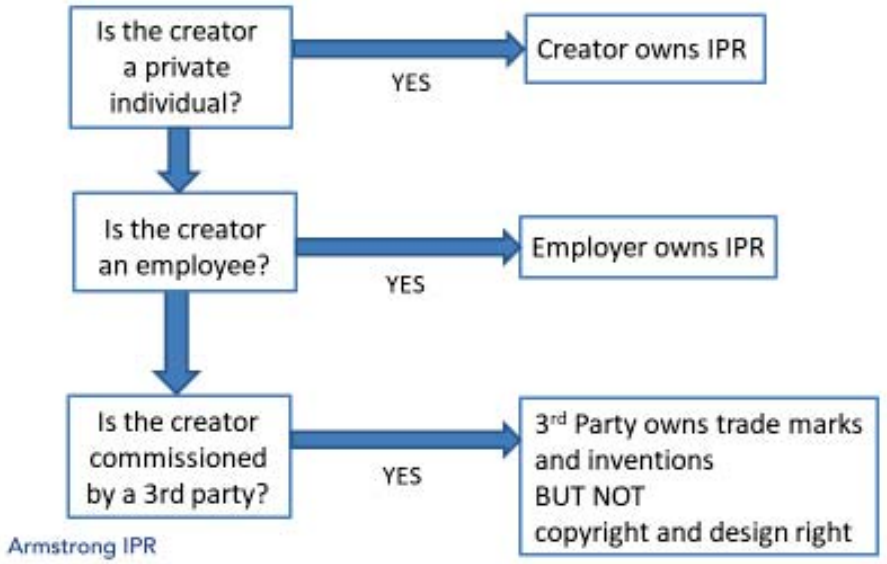
IP Rights Ownership



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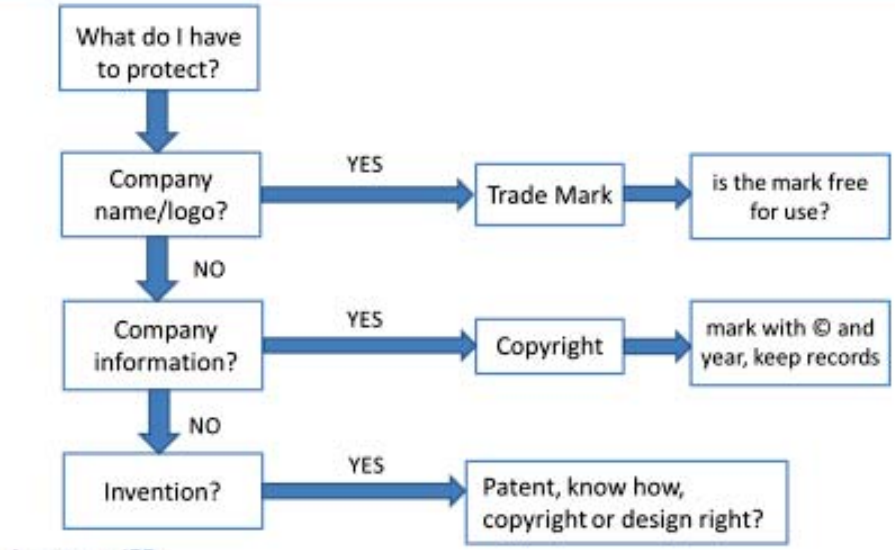
Protecting your IP Rights



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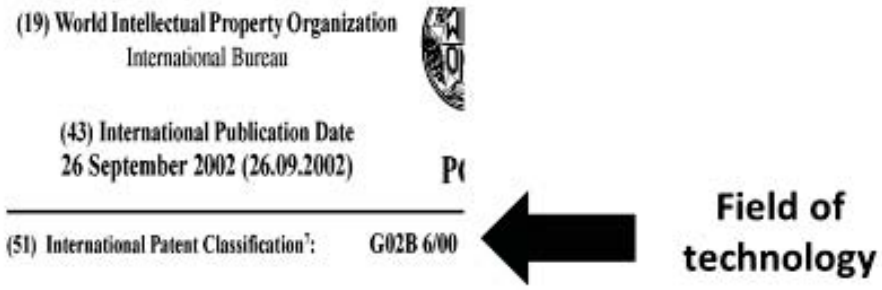
Information from Patents


(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 September 2002 (26.09.2002)

(51) International Patent Classification⁷: G02B 6/00

Field of technology

www.wipo.int/classifications/ipc/en

Patent Databases

www.espacenet.com

- In advanced search option, use 'Keywords in title and abstract' field, and/or company names in 'Applicant' field

www.google.com/patents

www.ipo.gov.uk

- UK Intellectual Property Office

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Technical Invention Protection

- Which types of IP Rights are relevant?

  product – patent, design right, (trade secret)

  process – patent, (trade secret)

  software – copyright, patent, (trade secret)

  service – patent, (trade secret)
- Is the invention easy to copy?

  YES – patent, design right, NO – trade secret
- Is the invention life cycle short e.g. <3 years?

  NO – patent, design right
- Is the invention new? YES – patent etc.
- Is the invention obvious? NO – patent etc.

} use searches to decide

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Introduction to Software Engineering and Data Analysis

Dr John Bustard

j.bustard@qub.ac.uk

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Management

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Project Management

How to make something awesome:

Knowing what to make (Focus)

Knowing how to make the product (Technical Skills)

Knowing what is needed to create a professional product (Standards)

How to ensure it actually gets made (Productivity)

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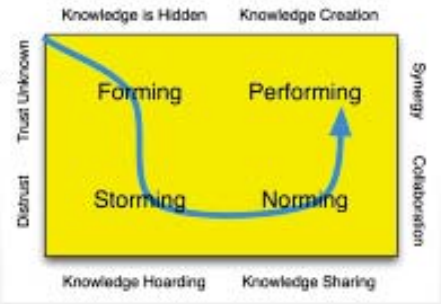
Forming, Storming, Norming, Performing

Forming - Largely behaving independently

Storming - Start to judge one another and disagree

Norming - Common goal is the priority, tolerate each other

Performing - Team members support and understand one another, have got used to working together and so react quickly



You might not get past Storming
The more you work together in the same room the faster you will progress through the stages.

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Being supportive

By far the **most important factor** in a well functioning team is that people **don't feel embarrassed or humiliated** by showing their work.

It must be safe to:

Take risks

Make mistakes

Ask for help

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Google's Empirical Factors for Top Teams

Psychological safety: Can we take risks on this team without feeling insecure or embarrassed?

Dependability: Can we count on each other to do high quality work on time?

Structure & clarity: Are goals, roles, and execution plans on our team clear?

Meaning of work: Are we working on something that is personally important for each of us?

Impact of work: Do we fundamentally believe that the work we're doing matters?

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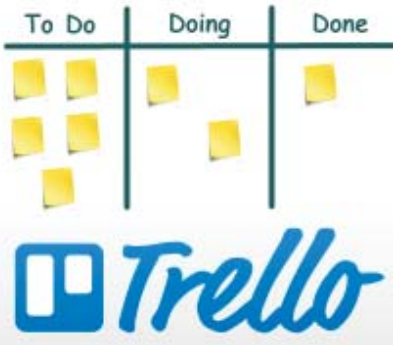
Kanban (Trello)

To keep track of what you have to do and to manage allocating things to different people on the team a Kanban board is useful.

If you are aiming high enough you will have to drop some of your planned features, Kanban can help with this.

It's also a great format for dealing with bugs at the end of a project.

An example of a Kanban board you can use is trello.com



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Source Control

Use Github for your project (check ip issues)

It has free private repositories where projects can be added

It can be integrated into all major tools and will ensure that your work is not lost and that no-one in the team can accidentally break the project in an unrecoverable way

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Requirements

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Focus

Prioritising your work to ensure it is valuable and to minimise how much has to be changed later:

Knowing your **end user** and **customer**: ensure there is someone who will want it

Learning from **competitors**

Clear selling points (Features)

Starting with a rough version of everything and progressing all of it together (Tracer bullet)

Learning from Users

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Development Methodologies

Agile Method

Waterfall Method

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Agile

The approach used by most software companies is called "Agile" it prioritises:

Individuals and interactions over *processes and tools*

Working software over *comprehensive documentation*

Customer collaboration over *contract negotiation*

Responding to change over *following a plan*

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Iteration based on customer feedback!

The more iteration **based on feedback from a real customer** you put into a product the better it will be

The better you will be

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Don't just do what your customers ask for

Professional product design requires working with customers who don't really know what they want until they are using it.

If your product has a detailed specification from a user, the product is either:

Unoriginal - they want a copy of something else, your success from this is very limited

Very simple - unlikely to change an organisation and so not create much value

Based on imagination - unlikely to fit well with a customer in reality

Iterating on an original design based on user feedback and with real market research is important to doing well on a commercial project.

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Use Cases

Use Cases are complete stories about users desires and their complete interaction with a product to fulfil them.

They aren't single features

They typically contain many steps to complete

Good use cases demonstrate the key features of a product

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Tracer Bullet

A tracer bullet is about breadth over depth

The absolute minimum of every important part of the project

It is to ensure that everything you do is in context of the whole project. To help prioritise your work.

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Iterate fast with rough versions

Software quality is all about iteration. You can get faster iteration by making each iteration smaller and rougher.

For example, most professional firms iterate user interface design using paper mockups.

The more roughly you make something the more willing you are to change it.

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Artists borrow, Geniuses steal

Don't reinvent the wheel. Unless you are trying to make something better (i.e. it is one of your 1/2 key features).

Your user testing will tell you if it is better.

Always replicate the best practice of the best products. Use their iteration, build on what is familiar.

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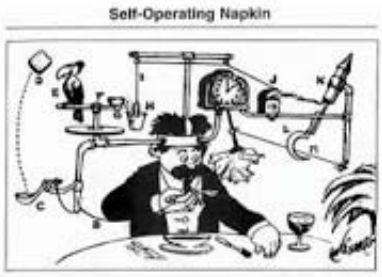
User Wants and Needs vs Difficult Features

Creating a successful product is not about creating something that was technically difficult to make

It is about creating something that the user wants

Don't add features just to show how technically skilled you are



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Development

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Technical skills

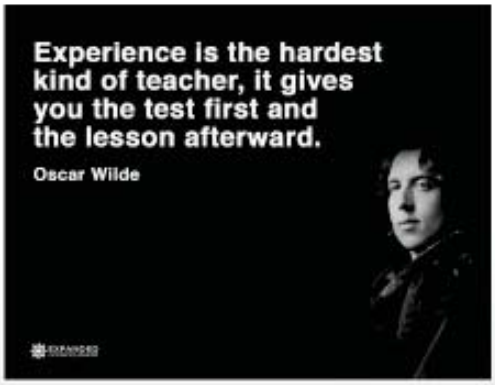
Picking it up as you go along:

Not learning everything in advance

Using online courses, Google and Stack Overflow to help **solve your problems** as you face them

Not avoiding features because you don't know how to implement them (yet)

Asking for help and supporting one another



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Pair Programming

This is a very useful technique for learning new software technology

One person types the code, the other watches and discusses the code, then after a period of time they swap over.

I've spent a lot of time pair programming and I have always been glad I did:

- With peers - Built a real time physics engine (Mythos Games)
- With mentors - Learning to use complex build tools (Sony)
- With students - Teaching students how to wrestle C++ libraries (Computer vision research with PostDoc)

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Code and Design Review

Many companies use internal reviews for the work that they do.

This typically means a more experienced member of a team or possibly the whole team go through an individuals work on a product and discuss suggestions on how to improve it.

Some companies will not allow code to be added to the project until the review is complete.

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Usability

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Simplicity

Good usability takes lots of time and effort

It can be difficult because you are often trying to communicate something complex in a simple way. This is why products with less features are often more popular than products with loads of features.

The less features you have, the more you can polish them.

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Simplicity for the User not the Programmer

The user and their common use cases are what the program should reflect. Not your abstraction.

Simple code does not necessarily mean a simple user experience

Minimise the number of steps users need to take to complete common actions

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This also applies to software design

Software can be thought of as a reflection of the real world.

Classes represent real objects (or at least how people think about real objects)

Great software is about elegantly capturing the real world in simple abstractions

The best programmers will tend to create relatively small and simple programs.

They learnt how to do this by examining lots of different ways of doing something (reading source code) and trying lots of different approaches to learn what is the cleanest, simplest representation

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Quality

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Treat the product as if it is real

User testing should **judge** products against other existing **commercial products**

Ultimately that is the real **standard** that your work will be judged against

An important part of becoming a **professional** is switching from the "I could do this" to the "**The user wants/expects this**" mentality

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The final bits of polish are the hardest

You will often avoid creating a feature because it seems too technically difficult

Small improvements in user experience can require very technically challenging features

These features are the ones that make a product beat their competitors

This is where your expertise makes a difference

SMALL CHANGES EVENTUALLY ADD UP TO HUGE RESULTS.

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Quality is about consistency

The more detailed and controlled your style, the more work is needed to add each new element (the same goes for code)

Things look professional if all elements look like they have been deliberately chosen to go together:

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Quality is about consistency

This applies to the look of something. It also applies to the functionality of something:

People will tend to judge the quality of a product based on the it's worst part

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Polish: 80/20 rule

Your product will look 80% complete after you have put in 20% of the effort needed to finish it

Or in other words the last 20% of a product will take 80% of the total time to complete

This happens because you develop the easier bigger wins first

Professional quality products are about a large number of small changes driven by User feedback

These changes typically take 80% of the time needed to complete the product

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80/20 Case Study: World of Goo

It is very rare to get a detailed honest case study of how long something took to make and what it was like at the beginning

This is partly because showing something after a long period of work without the bits in-between creates a more dramatic impression on people. When they see how it was created it often seems less impressive

Indie game development is a rare situation where some people have shared their real experiences

From 31% to 63% was mostly making changes based on user feedback not adding content

63% to 100% was the difference between polished product and actually for sale

https://2dboy.com/2009/03/06/the-world-of-goo-wasnt-built-in-a-day-part-1-of-7/

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Testing

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User Testing

1. Validating that the design is something the user wants
2. Testers talk about their expectations and impressions as they use the site in a normal way
3. Testers are given high level objectives and asked their impressions of a product

Testing

1. Validating that the program is working without error
2. Testers try to break the website by using it in unexpected way
3. Testers are asked to examine detailed parts of a product and to try to find problems with them. When problems are found testers provide detailed set of steps to replicate the problem they find

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Debugging is a core skill

Key to reading and understanding code

Key to testing

When programming gets really hard, you're debugging more than you're creating

At the end of the project, you'll be debugging all the time

If you get really good at it, your team mates will love you (you solve their biggest problem)

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From "See spot run" to "Sherlock Holmes"

You will feel stupid, everyone does:

When you are first learning to debug

When you are learning some new technology

You're looking up every word

It's often confusing because every part is new and its hard to keep it all in your head

It's very tiring, pace yourself

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Write comments as you go

Treat it like a foreign language. Write your "translation" as comments, explain what is happening in a form that you can understand

It will help you feel like the code is "yours" - this is very important, you need to feel in control and to have a sense of pride and ownership of the work you are involved with

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Logging

Printing out text almost always works

If you don't have a debugger (or the bug relies on timing) then this is mostly what you will do

Useful for debugging problems due to parallel programs (things running at the same time that might create nasty bugs that are hard to replicate while in the debugger)

```
System.out.println("KEEP");
System.out.println("CALM");

System.out.println("AND");

System.out.println("CODE");
System.out.println("ON");
```

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Step through code

Stepping through code with the debugger is the best possible way to learn about your code or someone else's.

Along with trying to write your own programs this is the fastest way to learn about programming.

This is particularly true of advanced programming skills where there are no courses/textbooks to teach you.

GitHub

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Validation checks

Narrow down where a bug could be by creating small checks in the code that ensure that certain things definitely aren't the cause of the problem.

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Magical Thinking

You know when you have lost control of your code base when you start trying to fix/change your program by randomly trying things to see if they work. (GUIs and big libraries/frameworks like Apache are a big problem for this)

This is a sign that your program is too complicated for the task you are trying to perform and that you need to understand it in detail and (usually) rewrite it.

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Resist the urge to rewrite everything

However, avoid the temptation to rewrite something rather than understanding it. When rewriting code you want to very carefully check that the new version produces the same correct results as the old version.

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Unit Tests

- Used for testing components of a system
- Can turn each class into an application to test them (or place them in another class)
- Useful for dealing with complex isolated problems like evaluating a data structure like a list or tree
- Also useful for complex algorithms such as mathematical simulations
- Most bugs aren't detected by this process, they are due to parts of the program being used in unfamiliar ways

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Record and Replay

- Being able to reproduce a bug is the first step in solving it
- Can artificially create a set of inputs as part of testing
- Use the log to record the inputs of a real user interaction
- You could automate this process by reading the log from a file and automatically constructing inputs to simulate the user actions

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Refactoring

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Refactoring: Split

As functions get bigger cut code into smaller parts

The main way to do this is to take chunks of code that do single meaningful steps and put them in functions

Then your program looks like a summary

```
String toBrowser = "";
toBrowser += printHeader();
toBrowser += printJavascript();
toBrowser += startBody();
toBrowser += printNavBar();
toBrowser += printMainContent();
toBrowser += printFooter();
toBrowser += stopBody();
```

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Merge and generalise

Further simplify the codebase by finding functions that are repeated (or close enough that they can be merged with parameters)

```
String toBrowser = "";
toBrowser += Index.printHeader();
toBrowser += printJavascript();
toBrowser += Index.startBody();
toBrowser += Index.printNavBar("This  page
title");
toBrowser += printMainContent();
toBrowser += Index.printFooter();
toBrowser += Index.stopBody();
```

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Extract

As you complete more projects you will encounter certain problems/functions that you will **face over and over again** in different projects

Extracting this code into **reusable functions** that you can add to projects will help you increase the **speed** with which you can develop new projects

You will also be able to develop **larger projects** as these components will be so **familiar** to you

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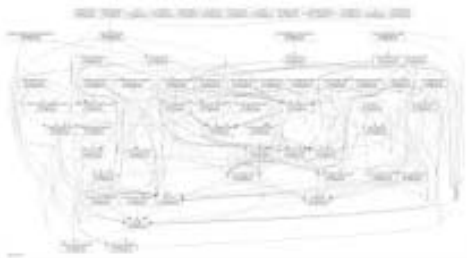
Dependencies

The ability to extract code and reuse it is limited by how linked together it is

Dependencies between pieces of code limit your ability to extract it

Functions that take **basic types as input** are often preferable to functions that take objects (as they are much easier to extract)



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Complexity

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Overgeneralisation

It is very easy to make code more complicated without increasing its **used functionality**

Overgeneralisation (A type of Software Bloat) will also make code that is very heavily linked together with a lot of extra classes

To avoid this only generalise to **simplify code** you are using

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More programs

The ultimate form of extraction is to create **multiple separate programs** that you link together to create your project

Powerful **small programs that do one thing well** are a great way to increase the speed and complexity of the projects you can make

Linux is built this way and it is a very robust and powerful way to develop software which can be **maintained for decades**

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Keep things as simple and open as possible

Use simple file formats that will continue to be supported. **Avoid custom file formats** (after a couple of years you will find it very hard to read them again)

Keep the **structure of your data** as **simple** as possible. Don't store code in databases.

Minimise the number of languages your program uses.

Use **simple language features** to ensure code can be ported to different languages e.g. from Java to Javascript

Avoid using any library you don't have the source code for. Use '**minimal**' libraries when possible

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Further Reading

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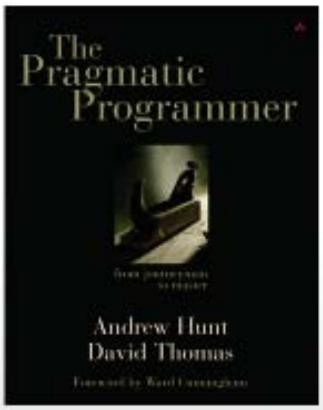
Further Reading/Watching

The Pragmatic Programmer has lots of good material on how to develop and refine software.

Search for "minimal X library Y" where X is whatever you want to do and Y is the language you are using. See the difference between how it is programmed and how "heavy weight" code is written e.g. Apache or standard Java libraries.



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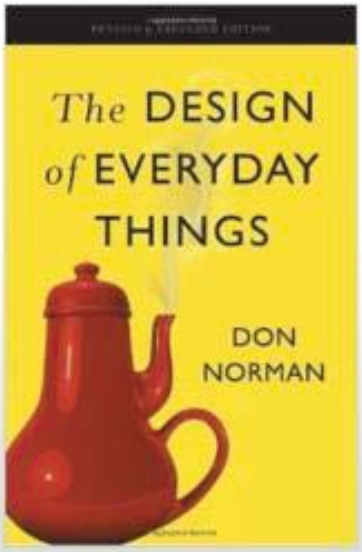
Further Reading/Watching



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Further Reading/Watching

https://en.wikipedia.org/wiki/Tuckman%27s_stages_of_group_development

http://agilemanifesto.org/

https://rework.withgoogle.com/blog/five-keys-to-a-successful-google-team

www.trello.com

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Data Analysis

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Software tools

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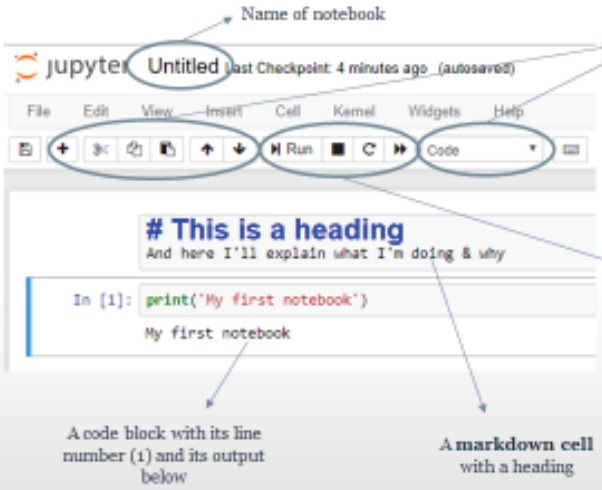
Jupyter Notebook: A quick summary



Cell Controls

Cells are blocks of code or text (markdown) in your notebook. This means you can divide your program into stages a label these sections.

As well as ordering cells appropriately you should also change the cell type (code/markdown) with the dropdown

To run the notebook you can use **run** or **restart the kernel** (the fast forward button) which will re-run the entire notebook and reset variables.
This is useful if you go back and make changes to earlier stages in code

Jupyter notebook will be explained in greater detail later. For now you can use this or an IDE of your choice for running python.

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Loads of great online teaching materials

Your next lab is continuing the learning python course at codecademy. This is a great introduction to python and is accessible to those of you who are uncomfortable with programming.

Data analytics is more about **manipulating** and **understanding data** than creating large software systems. However, it is also a relatively new and changing field that you will need to keep studying to stay on top of. Thankfully there are lots of great online materials.

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Why Python?

3 main tools used by the majority of professional data analysts:

- Excel
- Python
- R

Excel is used in many organisations but usually in a relatively simple way to sort and display data and graphs. It can be useful to have some experience with it but you will learn a lot of what you need by watching this:
https://www.youtube.com/watch?v=0nbkaYsR94c&ab_channel=JoelSpolsky

R is mostly used by people with a mathematical background. It has powerful visualization tools but is a little hard to connect with or deploy as an application.

After Java and JavaScript, **Python** is the most popular language in the world. It has very powerful libraries that make it the tool of choice for software developers working in data analytics
Python is the primary language for **deep learning**

For very large datasets and applications like processing images/video there are additional tools and technologies but these are beyond the scope of this class.

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Gather Data

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Data - 80%-90% of the job

- Finding data
- Cleaning data
- Structuring data

Is generally considered 80-90% of the time that professional data analysts spend their time on. Producing graphs, applying advanced statistics and machine learning are only a small fraction of the more core data acquisition and structuring work.

The number one factor that changes what analysts can do and what academics can produce in machine learning is access to high quality comprehensive data on something.

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Systematic Taxonomy of Variation

Don't start by thinking about what you can **easily gather** as a dataset

First think about **all the factors** that could cause variation in your system's accuracy

Assume you **don't know** which of these factors are most important

In real deployments commercial organisations often use physical constraints, best practices, additional sensors etc. to **make variations controllable**. You don't need to make a perfect 'human-level' AI system

The key thing is to properly understand **how variations affect your problem**.Ensure you are **deliberately choosing** which variations you are addressing and the ones you aren't and that these reflect the priorities of your application. If possible include a systematic small scale evaluation of these factors.

All commercial deployments are immediately **tested** to see where they fail.

You may want to consider how you can **automatically detect** if the system is being used 'properly' so that accurate results are obtained.

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Statistics

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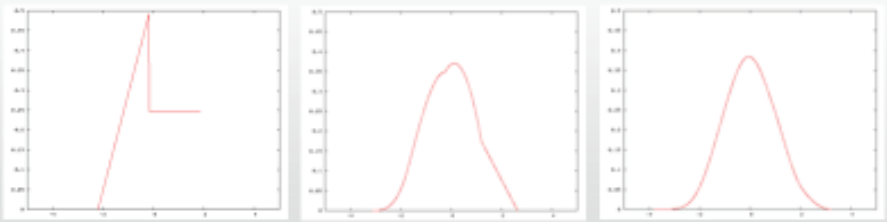
Statistical outliers - Central Limit Theorem

- Most numerical measurements of properties will form a "normal distribution" shape. This is because most measurements will be the result of a number of factors being added together.

For example a person's measured height might be the result of: the length of their feet, legs, torso, neck and head as well as any shoes they might be wearing and possibly their hair.

- If each of these factors vary amongst the population in a complex way their sum will tend to form a normal distribution.



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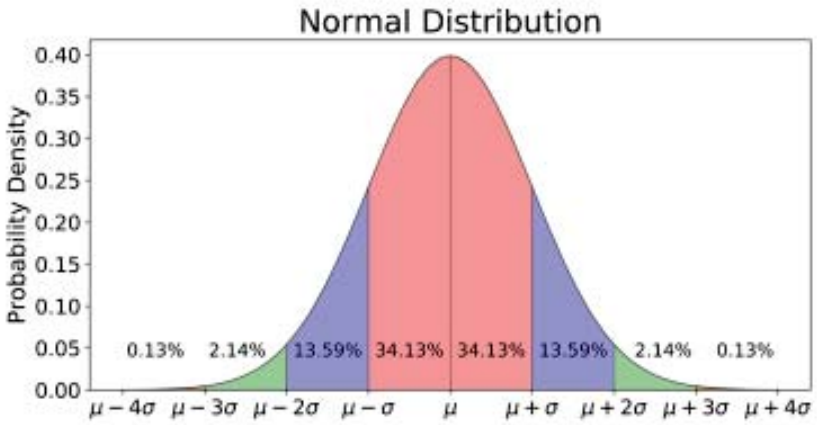
Statistical outliers - Normal distribution

- If the data comes from a normal distribution you can estimate the probability that a certain value will be obtained



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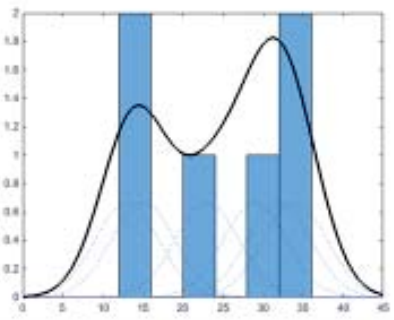
Probability Distribution

- A histogram is an approximation of a probability distribution
- If your samples are not in clear discrete bins then you need a way of creating a continuous probability distribution
- It doesn't take many dimensions before the number of samples needed to create a probability estimate is impossible - less than 100 binary choices
- Can't escape unjustified assumptions



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How does one property change relative to another?

- Covariance
- A measure of how common it is for values to be above average or below average at the same time and to the same amount.
- E.g. Violent Crimes in a month, Number of Ice Cream Sales

$$\operatorname{cov}(X,Y) = \mathbf{E}\left[(X - \mathbf{E}[X])(Y - \mathbf{E}[Y])\right]$$

- Positive covariance means that the expectation is that when one goes up so does the other
- Negative covariance means that the expectation is that when one goes up the other goes down (and vice versa)

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Pearson Correlation Coefficient

- This takes into account the range of values the two properties can have. It produces a number that indicates how strongly two values change together.

$$\rho_{X,Y} = \operatorname{corr}(X,Y) = \frac{\operatorname{cov}(X,Y)}{\sigma_X \sigma_Y} = \frac{E[(X - \mu_X)(Y - \mu_Y)]}{\sigma_X \sigma_Y},$$

- This way you can have a number that says whether two different pairs are more linked than another e.g.
- Ice Creams & Violent Crime
- Poverty & Violent Crime

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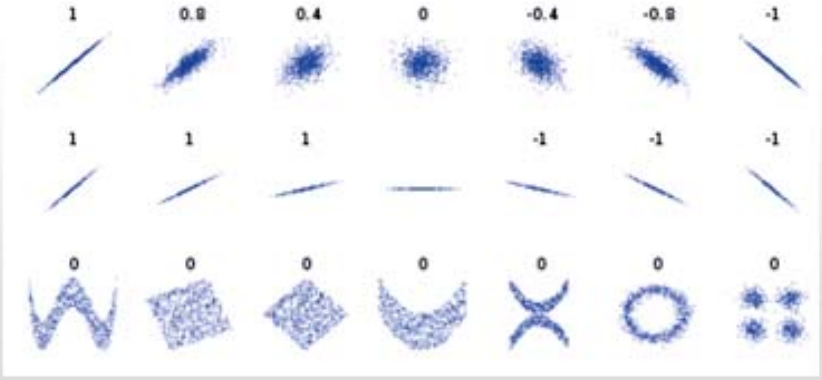
Correlation only works for simple relationships

- Measurements like correlation are a hint that a factor may be linked (or even cause) another one but it will miss any more complex relationship. That is why visualisation is so important.



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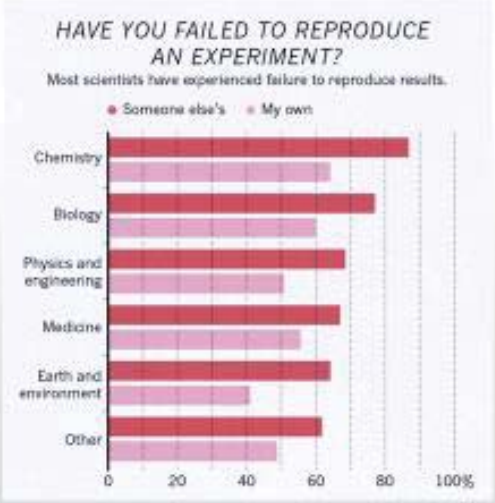
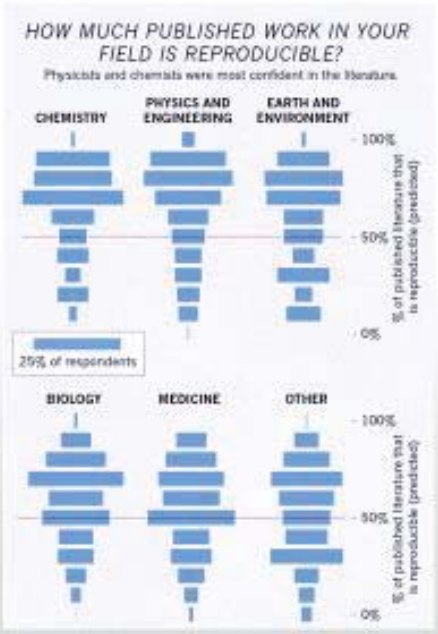
Uses and abuses of statistics

- Unreproducible results in science are very common





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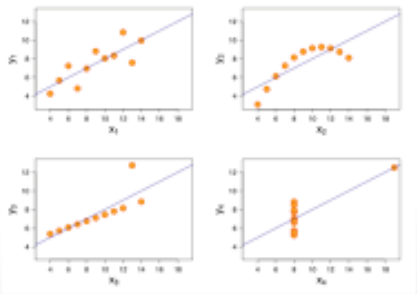
Misleading statistics (Anscombe's quartet)

All example datasets have same simple statistics
Highlights the need to analyse data visually



Property	Value	Accuracy
Mean of *x*	9	exac
Sample variance of *x*	11	exac
Mean of *y*	7.50	to 2 decimal place
Sample variance of *y*	4.125	plus/minus 0.00
Correlation between *x* and *y*	0.816	to 3 decimal place
Linear regression line	*y* = 3.00 + 0.500*x*	to 2 and 3 decimal places, respectivel
Coefficient of determination of the linear		

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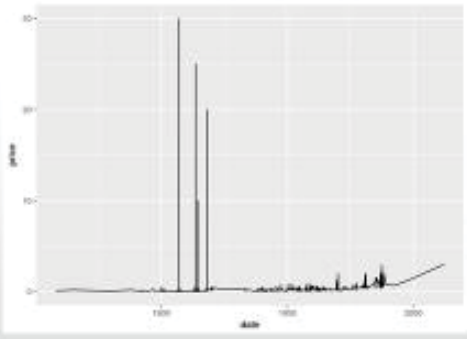
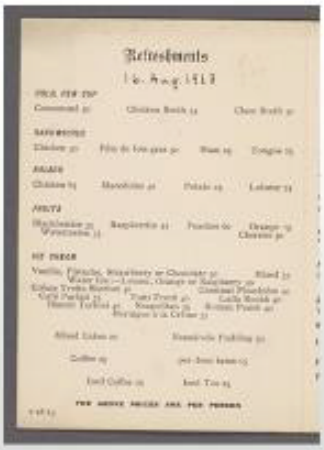
Numerical outliers

- For example the New York Public Library recently digitised and collated a collection of menus from the 1840's to the present day. An analysis of this data revealed price outliers.
- To validate the prices, the original digitised data was examined. This revealed that transcribers had been recording prices as dollars when they were in cents.



Values over time will reveal spikes



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Machine Learning

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Simplify the world to analyse it

- Statistics and probability are focused on provably correct inferences about data given simplifying assumptions.
- In other words, **pretend** that the situation is much **simpler** than it is and then use mathematics to **prove** properties about it.
- This is different from Machine Learning. Machine Learning is focused on developing computer algorithms that are shown to be **effective** through **experiments** that assess how well the algorithms predict results.
- Machine Learning techniques are used because they have been shown to be **practically useful**, they do not always have a mathematical justification for why they are effective.
- Statistics are typically mathematically justified but not necessarily effective. Because of the theoretical justification it can be easier to **understand** what an effective statistic means about the causes of the data you are analysing.

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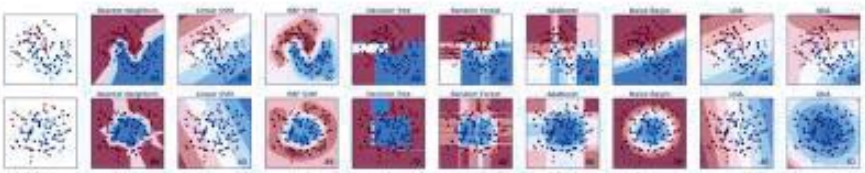
Machine Learning is like smoothing

- All machine learning is basically the same problem
- How to take a relatively small number of samples and blend between them to guess the values at points between the samples



- Different machine learning techniques use different assumptions about a 'good' way of blending the values. In practice many algorithms produce similar results.
- However, for some applications deep learning methods appear to be a lot more capable. Unfortunately they can require a lot of training data and so are more useful for problems like computer vision than general data analysis. Modern transfer learning techniques are starting to change this however.

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A good baseline: AutoML and/or Fast.ai

- Regardless of your ML problem the first thing you want to do is create a baseline performance. The AutoML or Fast.ai techniques will tend to give you a reasonably good result with minimal effort.
- AutoML techniques automate the selection of a machine learning technique
- There are a number of systems, I recommend starting with something like AutoSKLearn for tabular data
- I would also highly recommend Fast.ai's deep learning course and to use their approach to tabular learning
- For image processing/classification I would also recommend using Fast.ai's systems their defaults are as good as if not better than any other's
- Both systems will do a great job of combining data from very different sources and weighting their influence appropriately

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Analyse and iterate

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Manually analyse your errors

- There are many ways to analyse your results, the most important is to manually examine your worst performing examples
- It is also very useful to experimentally examine how sensitive your predictions are to changes in certain values. Both for your samples that work well and the ones that are in error. This will highlight what your system is prioritising and what kinds of error are likely to cause problems
- This will also tend to reveal if you have any errors in your ground truth labelling, which is very common.
- Its then good practice to try to obtain more data in any error conditions and iterate until you aren't getting any improvements

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Augment, Synthesise, and Constrain

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Augment data

- Add synthetic alterations to real training samples to significantly increase your samples
- Try to use real measured variations to identify plausible augmentations. These can vary from simple reflections, rotations, to more complex image/signal processing and perspective distortions.

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Synthetic data

- Typically you are limited by how much data you have.
- One approach which works well is to gradually create more and more realistic synthetic datasets
- You can often solve an ML problem by learning to reverse a synthesising algorithm.
- For example, the parameters used to synthesise an image of a face can be used to identify a person, by separating out the parameters that are due to environment (lighting, pose, expression etc.) with those of identity (shape etc.).
- By learning from synthetic data and then refining your model with the relatively smaller amount of real data you can often get significant improvements in accuracy

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Constrain data

- Recently we have had success in linking very different sensor sources by treating them as constraints on a generative solution.
- Start by estimating how a generative solution would produce different sensory data
- You can then randomly initialise the generative solution and optimise it to minimise the difference between the synthesised sensory output and the measured result
- Repeat this process for different random initialisations to get a distribution of estimates of what the true sensed data represents.

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End

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So you don't think it could be you…

Roger Woods
Professor, Systems and Sensors Cluster, Queen's University Belfast
Chief Scientist, Analytics Engines Ltd.

Analytics Engines

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Brief bio

- Born in New York, USA
- Moved to North Belfast, aged 3
- Grammar school educated: St Malachy's College
- BSc in Electrical and Electronic Engineering, Queen's University Belfast

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Before the Internet when people read 'paper' papers

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Sunday Times Innovation Page

THE SUNDAY TIMES 25 JUNE 1989

Cellmark

COMPUTERS

Speedy chips skip steps

● Streamlining of computer chips allows more sophisticated processing

TELEVISION pictures com-

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NEWS LETTER

Annual Review of COMMERCE & INDUSTRY

Ulster pushing back frontiers in technology

By COLUM DENNY

Roger Woods, 24, as a toddler learned to walk on the pavements of New York.

Twenty-four-old Ming Yan studied in the bustling city of Nanjing in eastern China, population over two million, and Hussein Kaouri grew up in strife-torn Lebanon. Rajinder Jit Singh spent his early life in Malaysia and Stephen Smyth lived by the sea in Bangor, Co Down.

Yet their different paths led them to join a Ballymoney physicist in Belfast.

They came to form part of Dr John McCanny's team of computer research scientists at Queen's University's department of electrical and electronic engineering.

Thirty-five year old Dr McCanny has been always interested in physics, the application of Mathematics and in computers and has been engaged in research work for the past 14 years.

Five years were spent at the Ulster University in Coleraine where he gained his PhD. Then followed a

One result of the work Dr McCanny and his team has been the development of a new family of advanced digital signal processing chips at Marconi Electronic Devices, Ltd., in Lincoln.

Here, chips designed at Queen's are produced for the commercial market. Indeed Queen's-designed chips are already on the market.

Dr McCanny said: "Marconi Electronic Devices use our design ideas to produce commercial chips for use in satellite communications, digital radio transmissions and

Chicago, Detroit, Washington, Dallas, San Francisco, Philadelphia, Tampa and Orlando — Dr McCanny has visited all these cities in the course of his research. He visits America at least once a year.

He has been to many parts of Europe, including Belgium, France and Portugal and has attended meetings in Japan.

In May this year he is off to San Diego and June will see him in Helsinki, Finland.

At Queen's the research team conducts research on novel circuit design techniques to exploit advances in silicon chip technology. Their designs involve chips containing 100,000 transis-

can be applied to the design of very high performance integrated circuits.

The Queen's team collaborates with many other bodies including the University of Southern California, Princeton University in New Jersey, the universities of Oxford, Warwick, Strathclyde and Glasgow.

"We also work with the Alvey information technology programme set up about five years ago by the Government to encourage collaboration in research between universities, industrial companies and Government research laboratories," said Dr McCanny.

The Queen's men also

INTERNATIONAL DIMENSION: Dr McCanny of the QUB research team at the Department of Electrical and Electronic Engineering with students Ring Yan (left) of China, Stephen Smyth, Bangor, Roger Woods, Belfast, Hussein Kaouri, Lebanon, and Rajinoer Jit Singh, Malaysia.

are from Armagh, but he was born in New York. They returned to Northern Ireland in 1966 when he

and patented their original ideas. They still collaborate closely in their work.

represented one of the major efforts in the world and had placed the United Kingdom in the forefront

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Influences

- Prof. Sir John McCanny, BSc, PhD, DSc, Kt, CBE, FRS, FREng, IEEE Fellow, FIAE, MRIA. FIET, FInstP, FIEI

- Simon Knowles

- Prof. John G McWhirter, FRS FREng FIMA FInstP FIEE FLSW

- Dr Stephen Smyth

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Simon's journey

inmos

Broadcom to acquire Element-14 in $600M deal

Broadcom to acquire Element-14 in $600M deal

4 October 2000

DESIGNLINES | AUTOMOTIVE DESIGNLINE

Nvidia agrees to pay $367 million for Icera

9 May 2011

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Simon's journey

Emergent Tech ▸ Artificial Intelligence

Brit startup Graphcore tossed a £200m early Christmas pressie for machine learning CPU

18 December 2018

Graphcore, a British chipmaker which this month achieved coveted 'unicorn' status of being valued in excess of $1bn (£780m), is being circled by suitors looking to acquire the business, which claims it can make machine learning "faster, easier and more intelligent".

30 December 2018

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Reflection

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Stephen's journey

- Innovative music compression algorithm based on Adaptive Differential Pulse Coded Modulation
- Sunday Times Innovation Page

Solid State Logic
OXFORD · ENGLAND

Qualcomm aptX

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Stephen's journey

dts Digital Entertainment

now Xperi

APT IP used in Bluetooth mobile phones

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Analytics Engines | Nov-18

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My own journey

ESTABLISHED
CapnaDSP spun out of QUBIS with investment capital

New CEO

SAP

2007 ANDOR™ TECHNOLOGY

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My own journey

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2007 ANDOR™ TECHNOLOGY

NEW PHASE
Company renamed as Analytics Engines; focusing on data processing for business

Analytics Engines

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Analytics Engines

INVESTMENT
Venture round secures £1.2 million private funding

New CTO

2014

CLEAR
Solving customers' Big Data problems

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Analytics Engines

INVESTMENT
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2014

CLEAR
Solving customers' Big Data problems

POISED FOR GROWTH
Increasing headcount; targeting growth in international markets

2018

MILESTONE WINS
Further investment secured; National Gallery and RTE among new clients onboarded

2019

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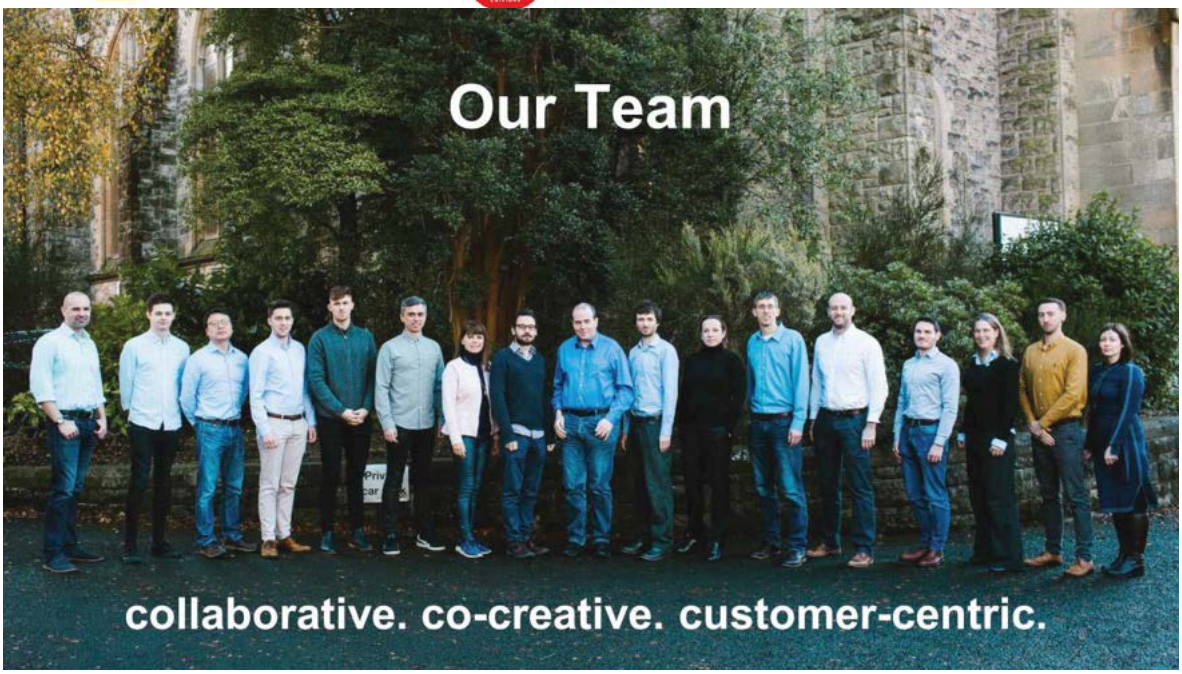

4th Summer School on

Smartphone Analysers for on-site testing of food quality and safety

23-27 November 2020

Consejo Superior de Investigaciones Científicas (CSIC)

The Spanish National Research Council

		CET	DAY 1	
TECHNOLOGICAL APPROACHES, FOOD SURVEILLANCE AND REGULATORY ISSUES	**WELCOME AND PROJECT PRESENTATION**	9:00-9:15	Opening session	Michel Nielen - *Summary of the FoodSmartphone Project, objectives and achievements*
	PLENARY	9:15-10:00	**Plenary session**	**Christopher T. Elliott - Uncovering the cause of a major food safety incident by the application of analytical chemistry**
	Optical sensors *Chairman: Jens Eriksson*	10:00-10:30	Speaker 1	Laura Lechuga - *Photonic nanobiosensors portable platforms for ultrasensitive and fast analysis at the Point-of-Need*
		10:30-10:45	Speaker 2 (ESR)	Chi Xiao - *Lab-on-chip devices for smartphone imaging Surface Plasmon Resonance (iSPR) detection*
		10:45-11:00	Speaker 3 (ESR)	Yunfeng Zhao - *Smartphone analysers for food safety and quality analysis from software perspective*
	Round table	11:00-11:15	**Round table: speakers + chairman**	
	COFFEE BREAK	11:15-11:30	**COFFEE BREAK**	
	Electrochemical Sensors *Chairman: Roger Galve*	11:30-12:00	Speaker 1	César Fernández - *Analytical microsystems for monitoring food production processes and quality*
		12:00-12:15	Speaker 2 (ESR)	Klaudia Kopper - *Electrochemical immunosensors for the detection of pesticides in different food matrices*
		12:15-12:30	Speaker 3 (ESR)	Safiye Jafari - *Test your food for aflatoxin in your smartphone*
	Round table	12:30-12:45	**Round table: speakers + chairman**	
	LUNCH BREAK	12:45-14:00	**LUNCH BREAK**	
	Spectrometric Analysis *Chairman: J.-Pablo Salvador*	14:00-14:30	Speaker 1	Amadeo Rodríguez - *Presence of Pesticide Residues in Fruits and Vegetables in EU. An Analytical Perspective*
		14:30-15:00	Speaker 2	Damià Barceló - *Pharmaceuticals and other emerging contaminants in European seafood samples*
		15:00-15:15	Speaker 3 (ESR)	Ariadni Geballa - *Analysis through Foodsmartphone - mass spectrometry detection*
	Round table	15:15-15:30	**Round table: speakers + chairman**	
	COFFEE BREAK	15:30-15:45	**COFFEE BREAK**	
	Food surveillance & Regulatory issues *Chairman: J.-Pablo Salvador*	15:45-16:15	Speaker 1	Antoni Rubies - *Laboratory of Health Public Agency of Barcelona*
		16:15-16:45	Speaker 2	Frans Verstraete - *Official control of contaminants in the food chain: challenges for innovative analytical techniques*
		16:45-17:15	Speaker 3	Wim Reybroeck - *European food safety legislation with respect to veterinary drugs and the role of rapid tests in its implementation*
		17:15-17:45	Speaker 4	Miquel Paraira - *Experiences and benefits of the implementation of the ISO22000 standard at a large Water Supply System*
	Round table	17:45-18:00	**Round table: speakers + chairman**	
	SB MEETING	18:00-19:00	**SCIENTIFIC BOARD MEETING**	

		CET	DAY 2	
APPLICATIONS	OPEN SESSION	9:00-10:30	VIRTUAL OPEN SESSION (ESR VIDEOS)	
	PLENARY	10:30-11:15	**Plenary session**	**Iñaki Eguileor - Contribution of science and technology to efficient food risk assessment in the EU**
	COFFEE BREAK	11:15-11:30	COFFEE BREAK	
	Natural toxins *Chairman: Cuong Cao*	11:30-12:00	Speaker 1	Anna Jofré - *Predictive microbiology for the in silico simulation of the microbial behaviour. Applications in Food Quality and Safety*
		12:00-12:30	Speaker 2	Michele Suman - *Industrial Food Safety Management of Mycotoxin Issues: the example of Deoxynivalenol*
		12:30-12:45	Speaker 3 (ESR)	Katrina Campbell (on behalf of Jordi Nelis) - *Development of smartphone hyphenated colorimetric, plasmonic and electrochemical biosensors for food contaminant detection*
	Round table	12:45-13:00	Round table: speakers + chairman	
	LUNCH BREAK	13:00-14:00	LUNCH BREAK	
	Food allergens *Chairman: Michel Nielen*	14:00-14:30	Speaker 1	Bert Pöpping - *Portable food safety testing device. The future of food safety testing*
		14:30-15:00	Speaker 2	Patricia Galán - *Multiplex analysis of food allergens: the challenge of developing a test*
		15:00-15:30	Speaker 3	Ronald Niemeijer - *Smart Allergen & Mycotoxin management – the use of smartphone technology in food contaminant management*
		15:30-15:45	Speaker 4 (ESR)	Georgina Ross - *From sample to smartphone: Consumer-Operable Multiplex Allergen Immunodetection*
	Round table	15:45-16:00	Round table: speakers + chairman	
	COFFEE BREAK	16:00-16:15	COFFEE BREAK	
	Antibiotics & Bacteria *Chairman: Luis Mata*	16:15-16:45	Speaker 1	Olga Matveeva - *EXTENSO: a new multiplex and connected platform for antibiotics detection in food*
		16:45-17:15	Speaker 2	Maria Carmen Blanco - *Break Biofilms*
		17:15-17:30	Speaker 3 (ESR)	Javier Lou - *Rapid plasmonic detection of food spoilage organisms in finished dairy products*
		17:30-17:45	Speaker 4 (ESR)	Julián Guercetti - *Multiplex optical biosensor for antibiotic detection in milk*
	Round table	17:45-18:00	Round table: speakers + chairman	
	Pesticides *Chairman: Michele Suman*	18:00-18:30	Speaker 1	Rudolf Schneider - *Immunoanalytical platforms for on-site environmental health and food safety testing*
		18:30-19:00	Speaker 2	Esmeralda Payán - *Quality System and Food Safety*
		19:00-19:15	Speaker 3 (ESR)	Aristeidis S. Tsagkaris - *Smartphone-based enzyme assays for cholinesterase inhibitors screening*
	Round table	19:15-19:30	Round table: speakers + chairman	
		19:30-19:45	Project presentation	Achim Kohler - *PhotonFood Project*
	Closing Session	19:45-20:00	Closing Session	Maria Pilar Marco

SMART TECH for FOOD

The control of your food needs technology that you can trust

The consortium of the "FoodSmartphone" project of the Marie Skłodowska-Curie Innovative Training Networks (H2020-MSCA-ITN-2016) and the Nanobiotechnology for Diagnostics group (Nb4D) of the Institute for Advanced Chemistry of Catalonia (IQAC-CSIC) are glad to announce the

Workshop SMART TECH for FOOD (ST4F)

ON-LINE EVENT from 25th to 26th November 2020

- **Scientific stakeholders (Research Institutes, Diagnostic & Food Companies)**
- **Quality and Safety testing stakeholders. EU regulations**
- **Analytical Device Demonstrations. Open Day**

On-line registration in: http://smarttech4food.activacongresos.com/registration/

ORGANIZERS

This workshop is the 2nd European Workshop on Portable Food Analysis and Citizen Science and, as such, an official RAFA associated event.

FoodSmartphone.eu

Open Day

Smartphone analyzers for on-site testing of food quality and safety

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325.

Meet FoodSmartphone in the

Virtual Open Day

Thursday, November 26, 2020

9:00-10:30 am, online,

part of the Workshop on Smart Tech for Food (ST4F)

- Everything you would like to know about the future of food testing and monitoring practices....
- Watch the videos by the Early Stage Researchers

Register*

***No registration required for the Virtual Open Day: simply follow the live broadcast at https://youtu.be/HsApkr1MQBg**

*Register for the full two-day Workshop on Smart Tech for Food at http://smarttech4food.activacongresos.com/registration/

*Register as a FoodSmartphone stakeholder at www.FoodSmartphone.eu and keep updated on the latest developments!

Contact us

www.FoodSmartphone.eu
www.FoodSmartphone.blog
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WAGENINGEN UNIVERSITY & RESEARCH
UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE
CSIC
QUEEN'S UNIVERSITY BELFAST
AQUAMARIJN Micro Filtration B.V.
csem
LINKÖPING UNIVERSITY
Barilla
ZEU MAKING TESTING EASIER

Summary of the FoodSmartphone project objectives and achievements

Welcome to the ST4F Workshop (N4 final event), November 25-26, 2020

Michel Nielen
WFSR, Wageningen Research, NL

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

1

Welcome!

2

Societal background:

- Information society:
  - health apps, life style apps, food apps, smart packaging, -phone, -watch, ...
- Food related emerging issues:
  - food security (malnutrition), food fraud, food scandals, obesitas, ...

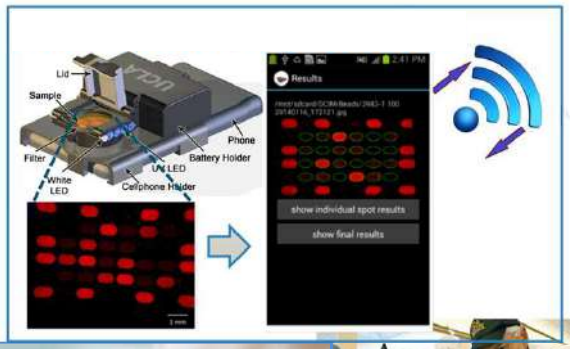
WAGENINGEN
UNIVERSITY & RESEARCH

Increasing societal demand for reliable data and traceability

3

On-site pre-screening of samples: only potentially interesting samples sent to the official lab

- More focus
- Less paper work
- Less transport
- Less storage
- More data
- Involve stakeholders
- **Even involve citizens?**



WAGENINGEN
UNIVERSITY & RESEARCH

4

Personal 'FoodSmartphone' in 2030 to detect 99% of all food safety issues?

Uni- and hyperspectral optical sensors

Biological recognition

Multiple food contaminants

5

5

Imagine.....

- Food Q&S control being more focused and more cost-effective
- Simplified food Q&S screening available on-site
- Everywhere: in the field, at the farm, in food industry, retail, border inspection; ....even at home
- So intuitive, easy and low-cost that everybody can do it, *i.e.*, citizen science ready
- A new generation of PhD students has worked on this!

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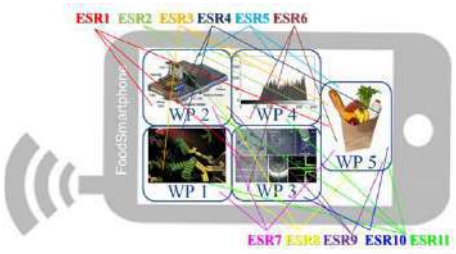
MARIE CURIE ACTIONS

6

The FoodSmartphone objectives

- Smartphone-based (bio)analytical screening tools
- Rapid, user-friendly, fully integrated sample prep
- Image data handling, secure communication, apps
- On-site demonstrators: antibiotics, pesticides, allergens, mycotoxins, food spoilage microorganisms, marine toxins
- Multidisciplinary trained researchers with improved career prospects



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7

The FoodSmartphone achievements

- Can easily fill a two-day workshop program on its own!

DDI microarrays for multiplex detection

Novel antifouling layers for biosensors

Unraveling and early recognition of the Hook effect

Fully tunable LSPR and catalytic nanomaterials

Secure image and video processing plus cloud storage

3D-printed SPR on your smartphone

Concepts for rapid confirmatory analysis

Portable electrochemical detection

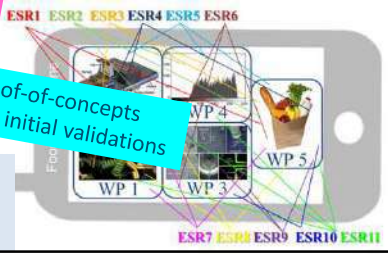
3D-printed paper-hybrid microfluidics

Consumer-operable multi allergen testing

Aptamers or antibodies

Proof-of-concepts and initial validations

Plus all those papers, reviews, lectures, videos, blogs, school visits, etc



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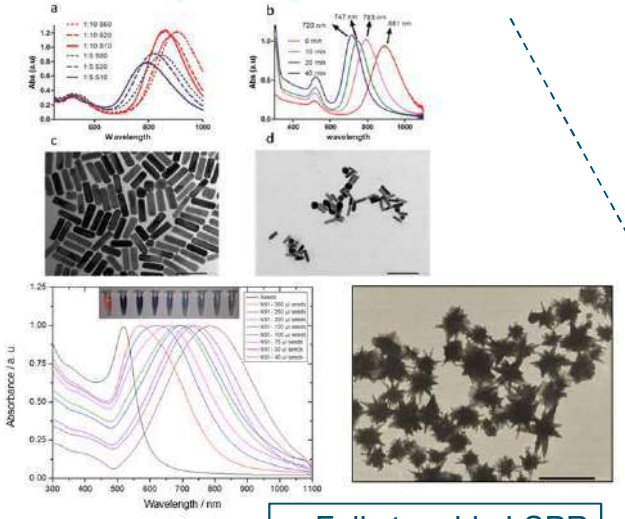
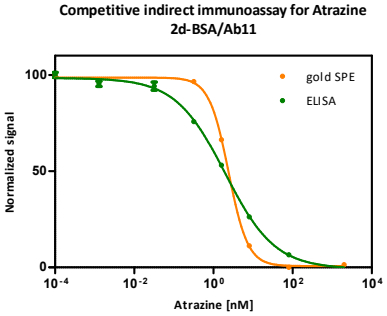
8

Example optical and electrochemical sensing



- Fully tunable LSPR nanomaterials

- Fully characterized SPE surfaces
- Proof-of-feasibility



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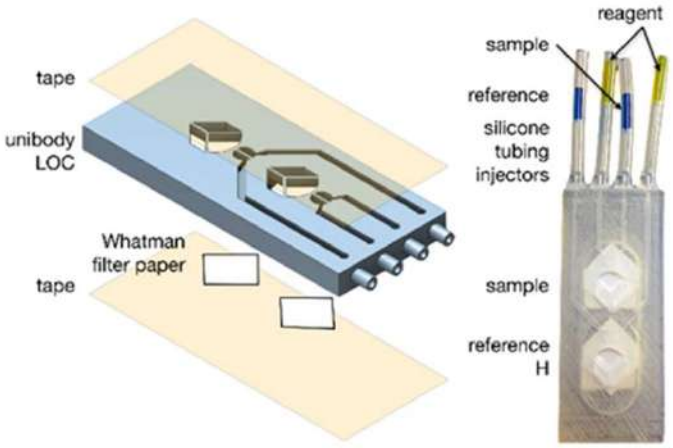
9

Examples in Integrated Sample Preparation



- Unique hybrid paper-ULOC
- Enclosed reagents, sample and reference
- Designed for ACh assay
- Smartphone readout using 3D-printed attachment

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10

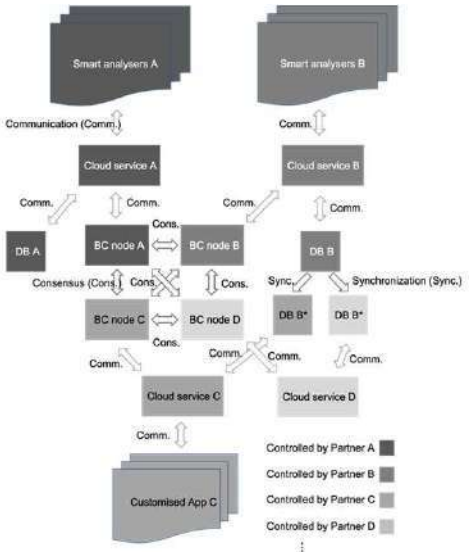
Examples Data Handling and Software

Image and video data software:

- Illumination corrections
- Relative colour constancy achieved
- Machine learning
- Quantitative outcomes
- 'Hook effect' alert for sandwich LFIA

Secure data transmission to stakeholders software:

- Blockchain model for data generation, interpretation, secure cloud storage (FoodTestChain)



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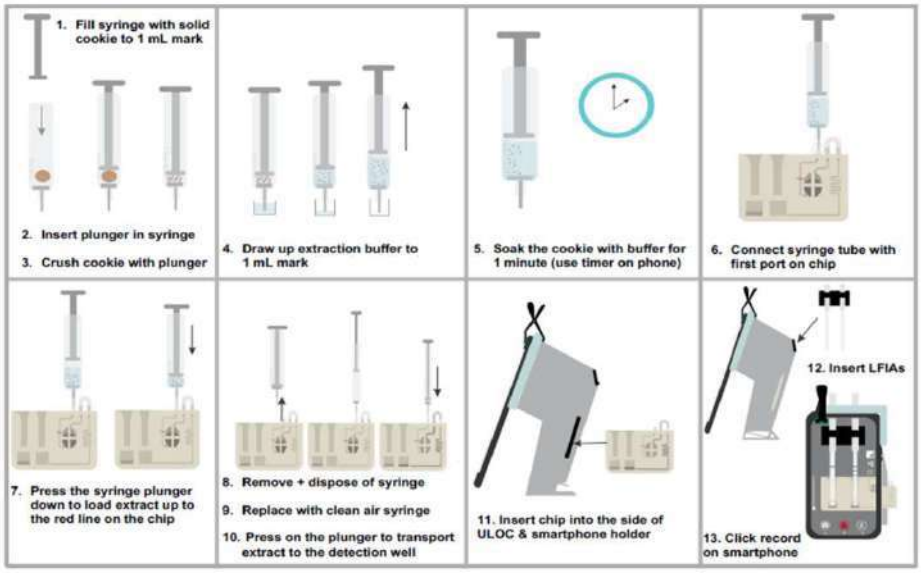
11

Examples in Demonstration of Applicability

Simplified protocol for allergen testing (operated by a teenager)



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12

Smartphone-based diagnostics revisited

FoodSmartphone outcome versus major S&T innovation gaps

- Miniaturization
- Flexibility/adaptability
- Validation, robustness
- Non-expert use
- Low-cost
- Analysis time
- Secure data handling

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13

...to be done and to be considered

- Prototypes developed are at TRL 3-4
- Follow-up needed for full exploitation (kit development, robustness, QA/QC, etc)
- More thorough validation in the field, for use by non-experts
- Stakeholder specific data requierements
- Embedding in regulatory environment
- Communication strategies (fake news)

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14

Acknowledgements

- WFSR, Wageningen, NL
- Queens University Belfast, UK
- Univ. Chem. Tech., Prague, CZ
- CSIC, Barcelona, ES
- Linköping University, SE
- Aquamarijn Microfiltr., Zutphen, NL
- CSEM, Landquart, CH
- *Barilla, Parma, IT*
- *Zeulab, Zaragoza, ES*

REA project officer
Luisa Marconi

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

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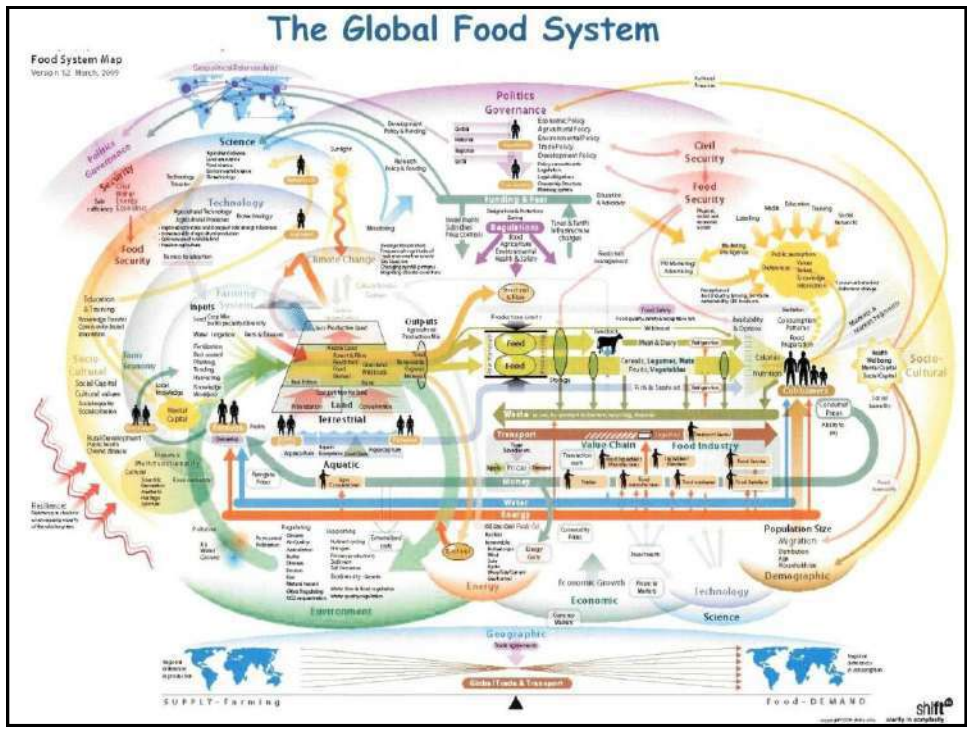
Uncovering the cause of a major food safety incident by the application of analytical chemistry

Chris Elliott (and many colleagues from)
Institute for Global Food Security, Queen's University, Belfast



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Uganda food aid halted over poisoning fears

18 March 2019

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A curious case of poisoning in Uganda's poorest region

SHARE:

The World Food Programme has been accused of negligence after hundreds were poisoned and four people died after eating its food aid.

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The **World Food Programme** (**WFP**) is the food-assistance branch of the United Nations and is the world's largest humanitarian organization addressing hunger and promoting food security.[The WFP provides food assistance to more than 90 million people in 83 countries each year.

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Super Cereal *Plus*

SUPER CEREAL plus
SUPER CEREAL plus
SUPER CEREAL plus

A blend of maize and soy fortified with vitamins and minerals

QUEEN'S UNIVERSITY BELFAST | IGFS THE INSTITUTE FOR GLOBAL FOOD SECURITY

The Ministry of Health in Uganda
The World Health Organization
The Center for Disease Control and Prevention, United States
World Food Programme

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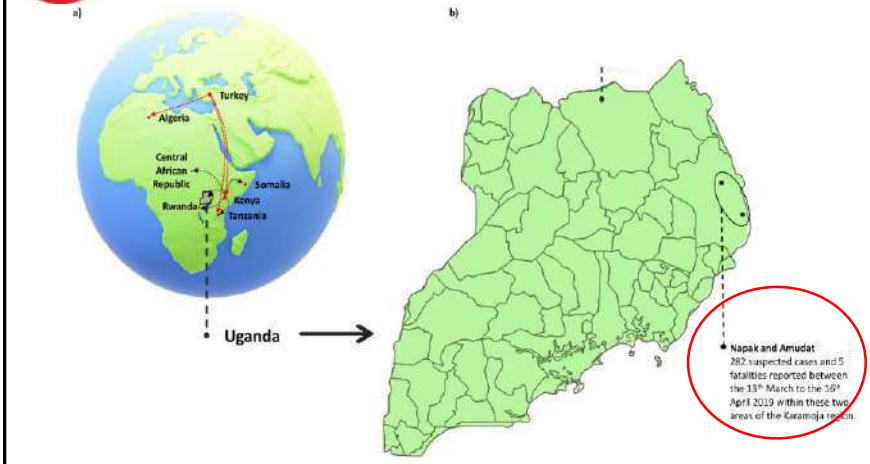

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Symptoms
A rapid onset (one to two hours) after consumption of Super Cereal,

Patients presented with a range of symptoms including – headache, abdominal pain, dry mouths, sticky saliva, body itching, mental confusion dizziness, paleness and dilated pupils.

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The supply chains for SC are complex but this is common with many food products that are processed and transported across national boundaries.

Based on the lot numbers of the contaminated SC the supplier was identified as a Turkish company.

Products with the same batch number were shipped from a port in Turkey to Algeria, Tanzania and Kenya.

A technical inspection of the Turkish company was performed on or around the 17th April. Major systems failures were recorded in quality management procedures.

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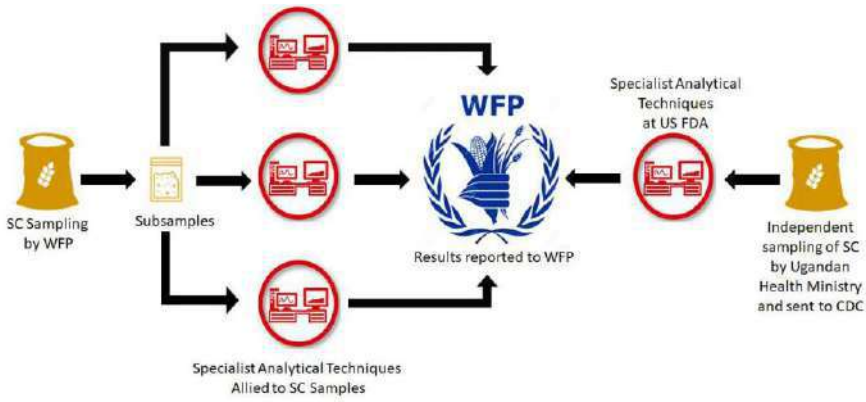

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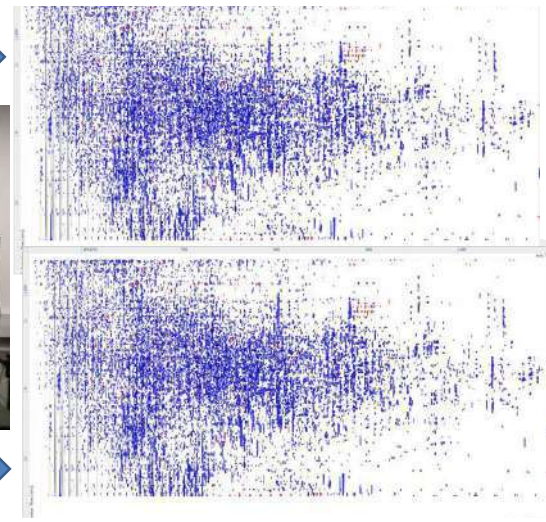
Untargeted Analysis



Untargeted Analysis

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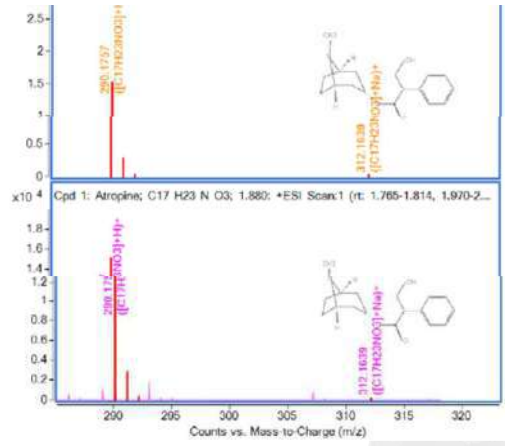
Atropine detected!!

Scopolamine also detected!



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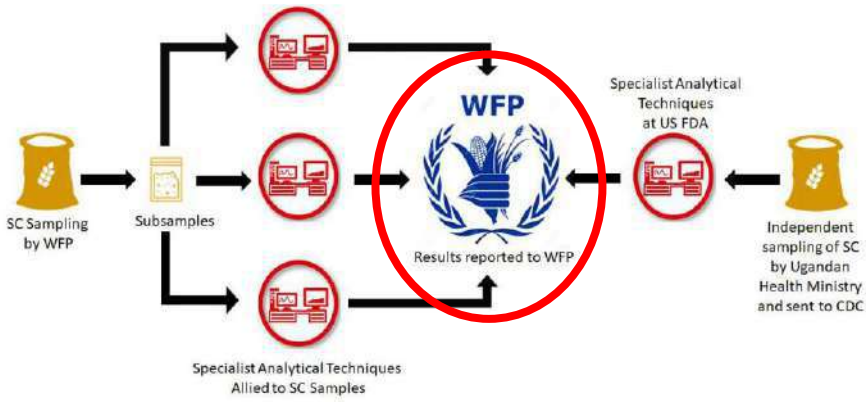

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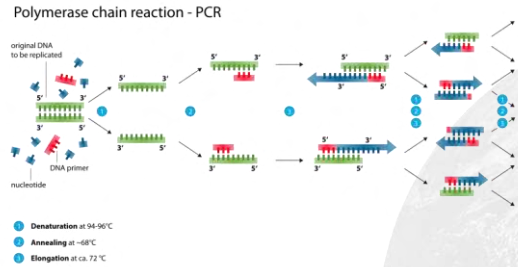
It was confirmed by PCR (by CDC) that Super Cereal samples had been contaminated with the toxic plant species *Datura stramonium* (also commonly referred to as jimson weed or thorn-apple).



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18th July 2019

Joint Statement WHO – WFP Suspected food poisoning outbreak, Karamoja region, Uganda, Executive summary (part 1)

1. The probable source of the outbreak is assessed as WFP fortified maize/soya bean, CSB+

2. A chemical aetiology is considered the most plausible cause of the outbreak.

3. Acute tropane alkaloid poisoning is considered the most plausible chemical agent: concentrations of the alkaloids atropine and scopolamine were identified in samples recovered from suspected cases in the order of parts per million, concentrations at which features of toxicity could be expected.

4. Suspected cases had features of alkaloid poisoning, including delirium; low-grade fevers and signs of central nervous system toxicity. The absence of some classical anticholinergic features, principally dilated pupils and tachycardia, in suspected cases is not apparent.

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18th July 2019

Joint Statement WHO – WFP Suspected food poisoning outbreak, Karamoja region, Uganda, Executive summary (part 2)

5. Reporting bias; the time-course of features and the influence of other alkaloids on the toxidrome are possible explanations. The relative ratios of the alkaloids atropine and scopolamine present in household and CSB+ samples and the identification of Datura stramonium DNA in samples are consistent with botanical contamination.

6. Analytical confirmation of exposure to alkaloids in contemporaneous biological samples from suspected cases could provide further evidence to confirm that alkaloid toxicity was the cause of the Karamoja outbreak.

7. The absence of other chemical agents on non-targeted screening by combined chromatographical and mass spectrometry, including mycotoxins, excludes the presence of a known alternative agent

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Food aid linked to illness again in Uganda after fatal outbreak earlier this year

By Joe Whitworth on September 24, 2019

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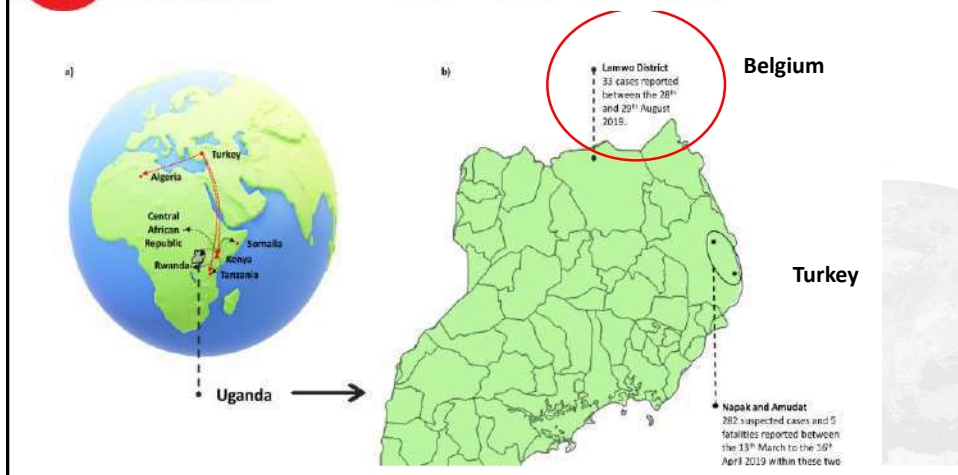

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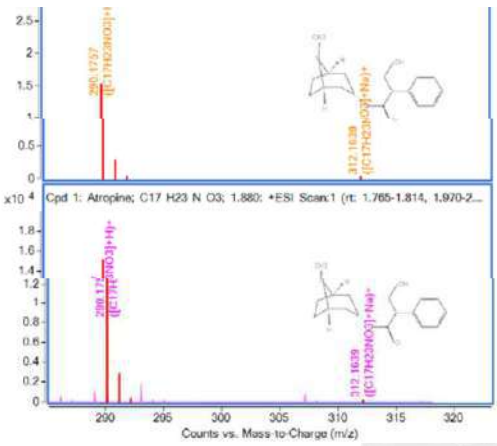
Atropine detected!!

Scopolamine also detected!



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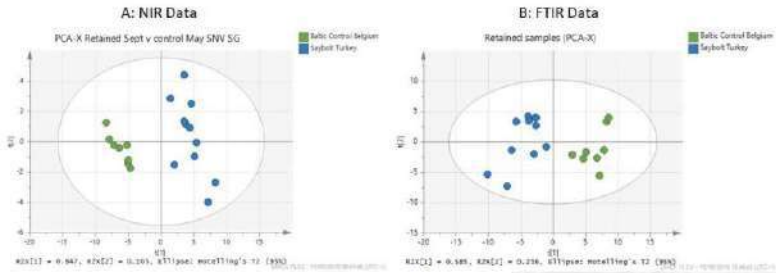
Spectroscopic fingerprinting



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Proximate analysis refers to the quantitative analysis of macromolecules in food.

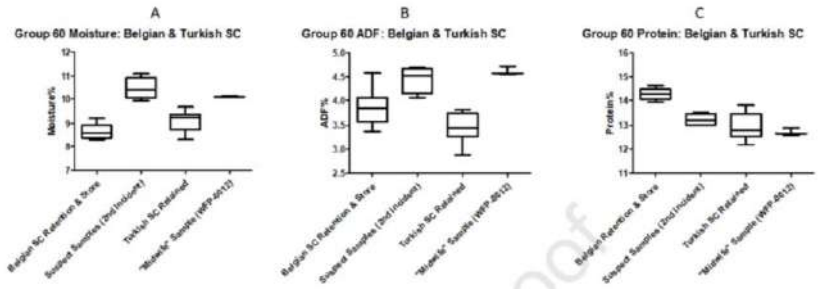
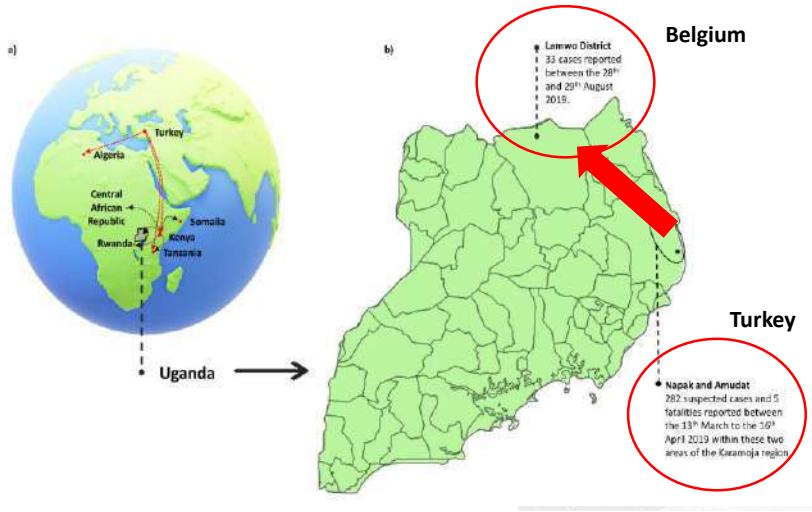


Figure 6 Proximate analysis content (A) Moisture; (B) ADF; (C) Protein



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Suspected food poisoning outbreak, Karamoja region, Uganda, March – April 2019: outbreak investigation report

17 January 2020

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Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Laboratory investigations into the cause of multiple serious and fatal food poisoning incidents in Uganda during 2019

Simon A. Haughey *, Olivier P. Chevallier, Claire McVey, Christopher T. Elliott

ASSET Technology Centre, Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gardens, Belfast, Northern Ireland, BT9 5DL, United Kingdom

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COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Comprehensive REVIEWS WILEY

Tropane alkaloid contamination of agricultural commodities and food products in relation to consumer health: Learnings from the 2019 Uganda food aid outbreak

Wilfred A. Abia[1] | Holly Montgomery[1] | Anne P. Nugent[1,2] | Christopher T. Elliott[1]

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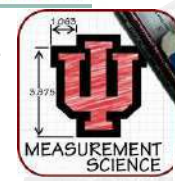
Conclusions

- The incident has shown how weaknesses in a supply chain can have fatal results

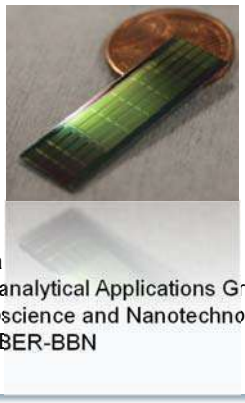
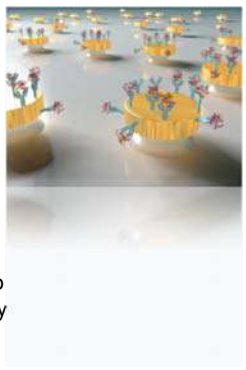
- Good analytical chemistry played a major role in the investigations



- There is a clear need for more exposure data and rapid, low cost, reliable methods to monitor for TA contamination of cereals

Workshop
SMART TECH for FOOD

Photonic Nanobiosensors portable platforms for ultrasensitive and fast analysis at the Point-of-need


Prof. Laura M. Lechuga
Nanobiosensors and Bioanalytical Applications Group
Catalan Institute of Nanoscience and Nanotechnology
(ICN2) CSIC, BIST & CIBER-BBN
Barcelona, Spain

@NanoB2A_group

Nanob2a.icn2.cat

FOOD DIAGNOSTICS: today and tomorrow

Analytical laboratory

- **Limited to centralized labs**
- **Trained personnel, time-consuming**
- **Expensive instrumentation**
- **Not available to everyone** *(resource-constrained settings)*

Bench-top instrument


PCR


Hand-held spectrometers

Point-of-care biosensor (POC)



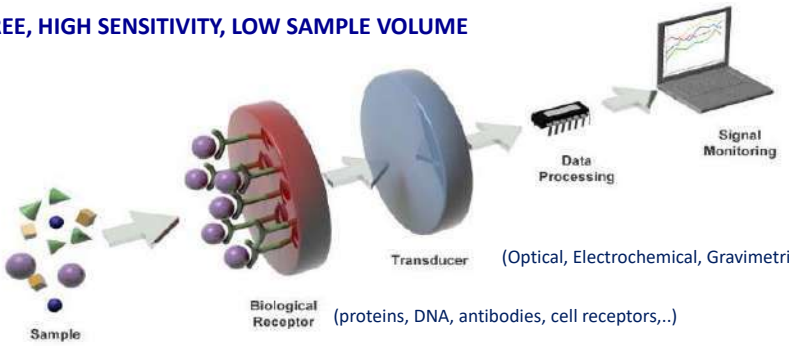

- **Diagnostics in-situ (out of the lab)**
- **High sensitivity, Fast, Label-free**
- **Multiplexing capabilities**
- **User-friendly/minimum operation**
- **Minimum sample**

Future Diagnostics →

BIOSENSORS provide the possibility to create miniaturized **POINT-OF-CARE** devices containing the functionalities of an analytical laboratory

BIOSENSOR DEVICE

FAST, DIRECT, LABEL-FREE, HIGH SENSITIVITY, LOW SAMPLE VOLUME



(Optical, Electrochemical, Gravimetric, mechanical..)

(proteins, DNA, antibodies, cell receptors,..)

Glucose biosensor

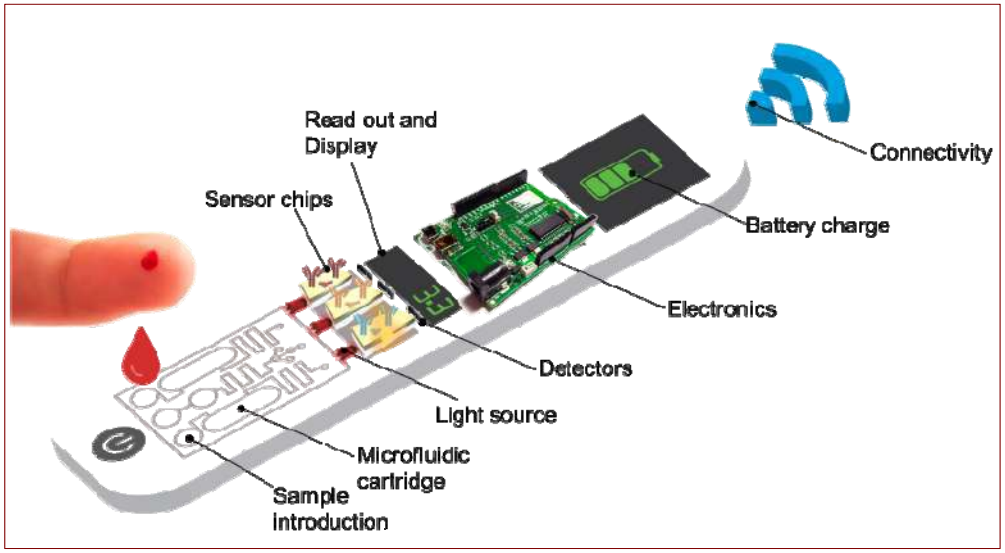
Abbott's FreeStyle Libre

Pregnancy Test

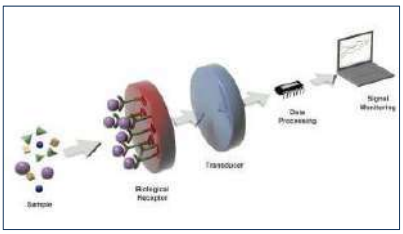
Test COVID-19

Pregnancy Test

Point-of-Care Biosensor



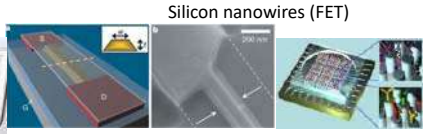
Overview of Biosensor devices for POC diagnostics



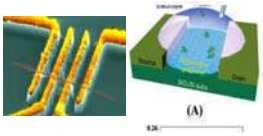
Electrochemical Biosensors

Glucose biosensor

Silicon nanowires (FET)

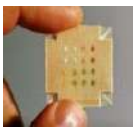


Carbon Nanotubes/Graphene

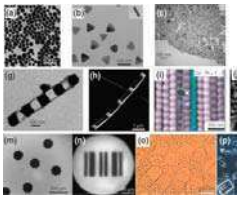


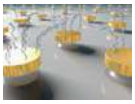
Microfluidic paper-based Biosensors

A 3-Cent HIV Test

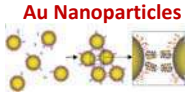


Biosensors based on Nanoparticles

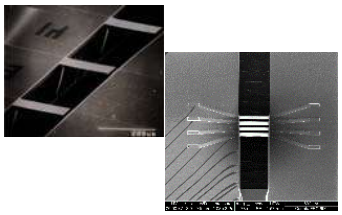




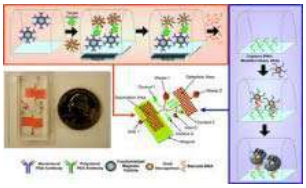
Au Nanoparticles



MEMS-based Biosensors



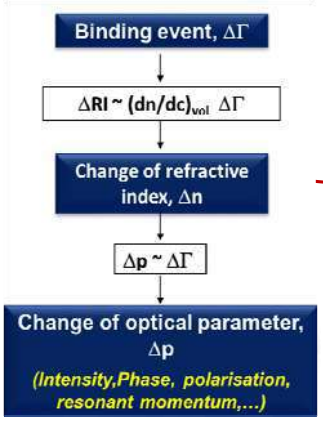
Magnetic Biosensors

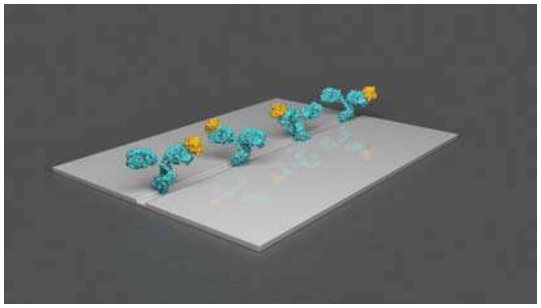


PHOTONIC BIOSENSORS

- Immunity to electromagnetic interferences
- High bandwidth
- Miniaturization
- **HIGH SENSITIVITY**
- Capacity of integration in lab-on-a-chip
- Multiplexing

Optical waveguide biosensors offer an unique opportunity for POC devices





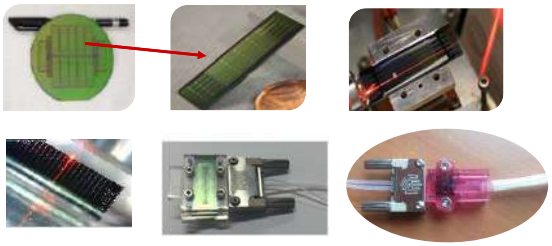
Evanescent wave principle:
refractive index change at the sensor surface

EW Probe: 100-900 nm

- **High sensitivity**
- **Real-time**
- **Label-free**

Biosensores POC Nanofotónicos @ Nanob2a Group

Nanophotonic Waveguide Interferometric Biosensors



Silicon Photonics Technology

LOD: pg-fg/mL

- Complete in-house design, fabrication and assembly
- Miniaturized & compact lab platforms
- User-friendly

NanoPlasmonic Biosensors (SPR & LSPR)

POC- SPR Biosensor

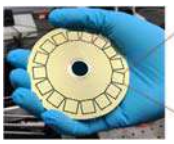
2-channels SPR

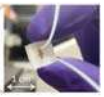
Tablet control

LOD: ng/mL

POC- LSPR Biosensor

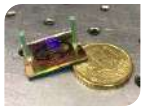
- Nanodiscs
- Nanogratings





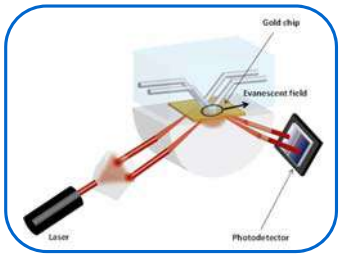
One channel

Multiplex

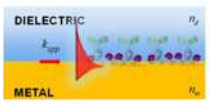


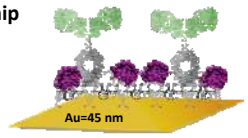
Nanoplasmonics Biosensors (SPR & LSPR)

Surface Plasmon Resonance (SPR)



SPR sensor chip

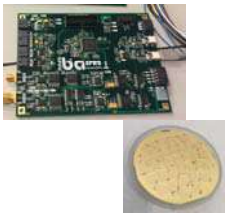




- Versatility: analysis of any type of biomolecular interaction
- Robustness and simplicity
- Sensitivity: LOD pM-nM (~10^{-5}–10^{-7} RIU)
- Affinity and kinetic studies
- Widespread technique and commercially available

POC- SPR Platforms

2-channels SPR

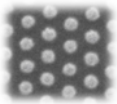


Tablet control

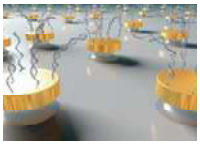
- Complete in-house design and assembly
- Miniaturized & compact platforms
- User-friendly
- Gold Sensor chip production

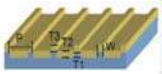
Localised Surface Plasmon Resonance Biosensor (LSPR)

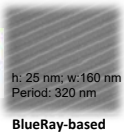
Nanostructures Design and Fabrication



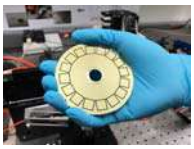
Gold Nanodisks







BlueRay-based Nanogratings

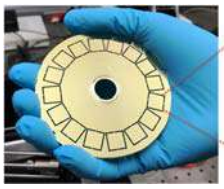


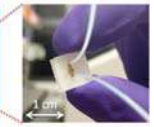
Flexible Blue-Discs

Fabrication techniques: Colloidal Lithography, nanoimprint, ebeam evaporation.

Nanophotonics 6 (1), 123–136 (2017)
Biosens.& Bioelec. 96, 260–267 (2017)
J. Biophotonics 1(8):e201800043 (2018)
Biosens. Bioelect. 119, 149-155 (2018)

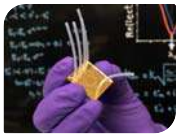
POC Device construction

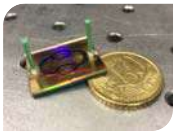


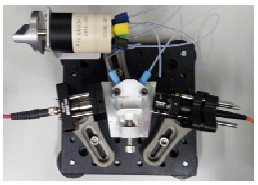


One channel

Multiplex

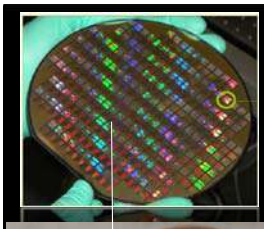


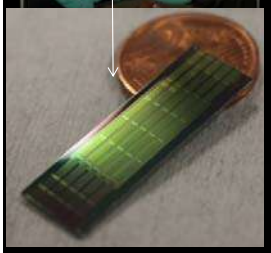




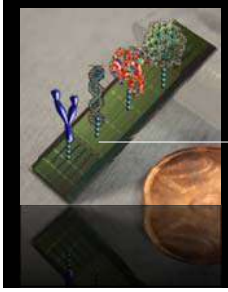
- Complete in-house design and assembly
- Miniaturized & compact platforms

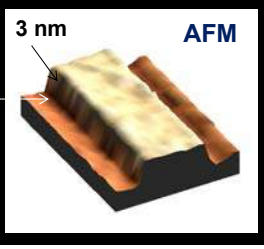
Nanophotonic biosensors





Nanometric waveguides in silicon technology (3 nm)

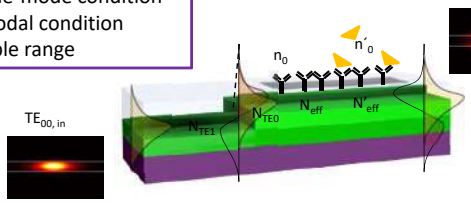




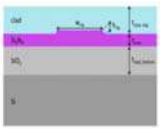
Label-free detection in the picomolar range (pM-fM-aM)

Bimodal waveguide interferometer (BiMW)

- Single-mode condition
- Bimodal condition
- Visible range

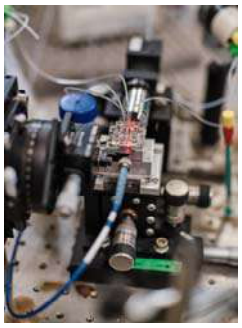


Rib waveguides



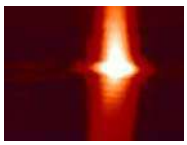
- Si_3N_4 (n_c=2.00) core layer: **150 nm** (single mode)/ **340 nm** (bimodal)
- rib depth: **1- 3 nm**
- Width ≤ 4 μm
- SiO_2 cladding 2 μm thick (n=1.46)/Si

Sensitivity evaluation



$\Delta n_{o,min}$= **2.5 x10^{-7}** $\Delta N_{eff,\ min}$= **2.0x10^{-8}**

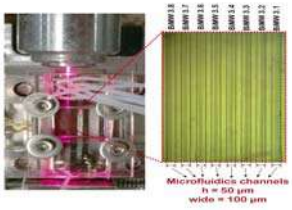
Direct detection of biomolecules at femtomolar-attomolar range possible



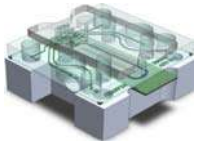
- **One of the most sensitive EW sensors**
- **A simple IO sensor**
- **Visible range**
- **Hundreds of devices per wafer/ low cost**

POINT-OF-CARE: μ-Fluidics integration

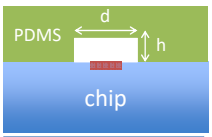
- **Hermetic sealing**
- **No air bubbles**
- **Low cost (disposable)**
- **Affording multiplexing**



integration of pneumatically actuated pumps and valves



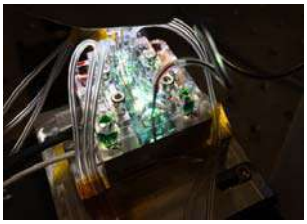
PDMS technology: **Independent flow cells for each sensor within the chip**



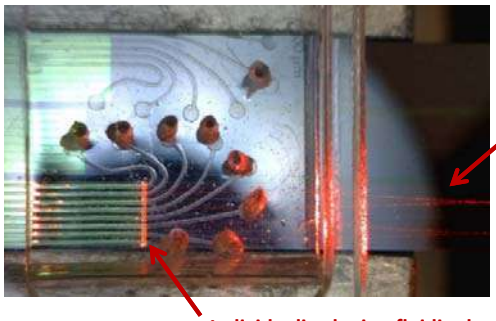
- **Width: 100 μm**
- **Height: 100 μm**
- **Pitch channels: 250 μm**

d → 50 – 150 μm
h → 20 – 100 μm

Automated on-chip fluid handling



Multiplex BiMW configuration



Lab on a Chip 17 (16), 2793-2804 (2017)

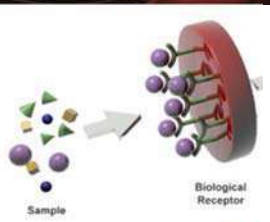
REAL APPLICATIONS

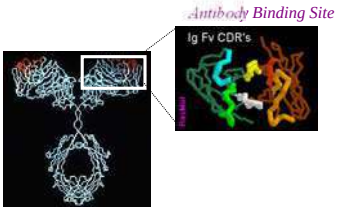
Surface biofunctionalization

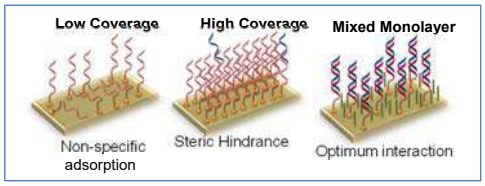
- **Chemical Surface activation (1st step)**
  - Introduction of functional groups to bind to the bioreceptor
- **Surface biofunctionalization (2nd step)**

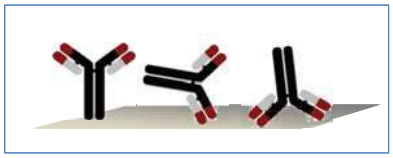
  Maintaining structure and functional properti
  - **Stable** linkage between the biomolecul ace
  - Optimized **density** of functional gr
  - Favorable **orientation**
  - Good **accessibility** to the targ and vertical spacers)
- **Antifouling surfaces (3rd step)**
  - Prevention of non-specific adsorptions from real samples

KEY STEPS







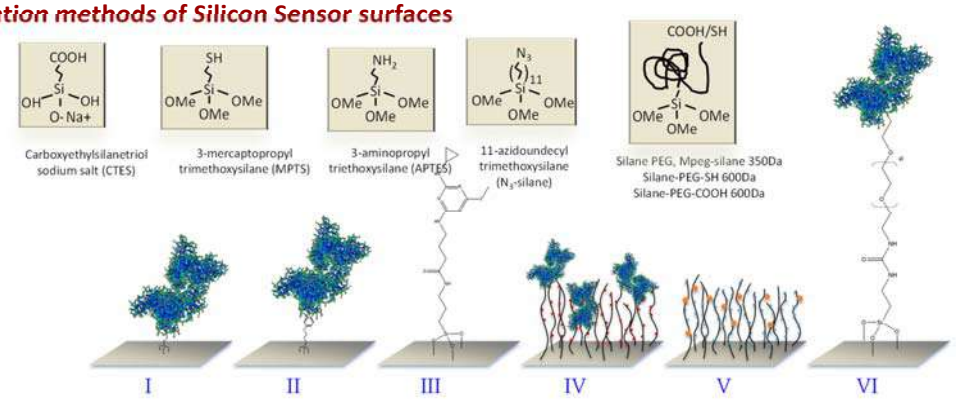


TRAC 79,191-198 (2016)

Surface biofunctionalization

Functionalisation methods of Silicon Sensor surfaces

Silanization protocols



Parameters to be optimized: surface chemistry, pH, ionic strength, receptor and Ab concentration, regeneration solution,…

Antifouling Strategies for real samples evaluation

- Modifying medium composition: surfactants, additives
- Modifying surface behaviour: hydrophilic blocking agents (as PEG)

Real Samples

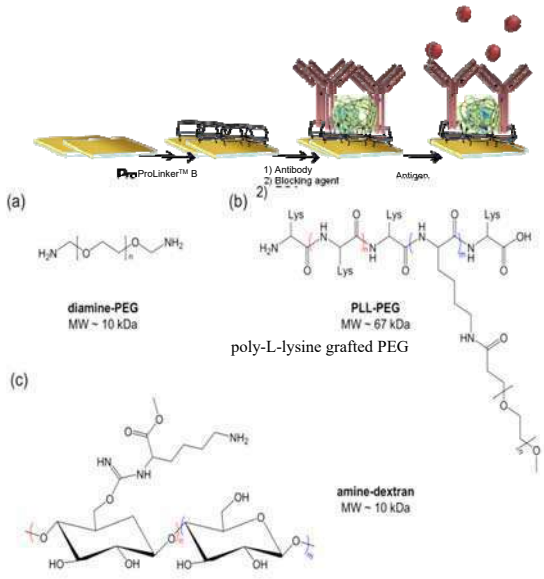
Blood/Serum

Tears

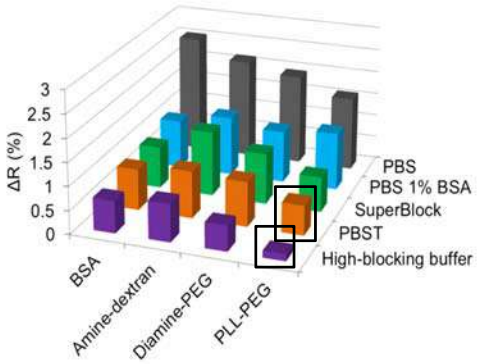
Urine

Analyst , 138 (7) 2023 (2013); *Sensors*, 14(2) 2239 (2014)

Biosensor Analysis in complex matrices



Serum non-specific adsorptions



- PLL-PEG + HBB
- PLL-PEG + PBST 0.5%

Non-specific binding of serum could be reduced up to 94%

Analyst , 138 (7) 2023 (2013)
Sensors, 14(2) 2239 (2014)

POC for Celiac Disease Follow-up

CELIAC DISEASE → **Gluten Intolerance** → Usual ingestion of small quantities of gluten can lead to serious injuries in Celiac Disease patients

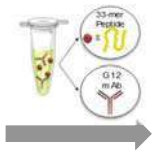
Gliadin
Main component of gluten

Digestion →

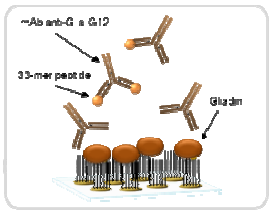
33-mer Gliadin Peptide
- Resistant to digestion process
- Detectable in urine or faeces

Only effective therapy: Gluten-Free Diet (GFD)

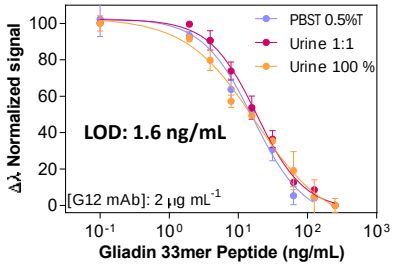
POC Biosensor: Monitoring of gluten immunogenic Peptide in urine (Gliadin 33-mer)



Urine 100 %



Competitive Immunoassay



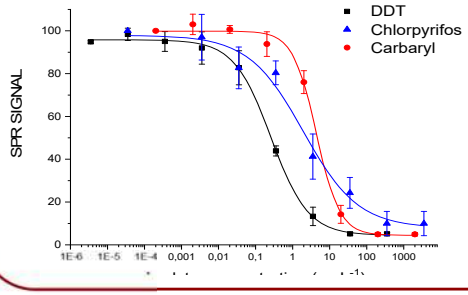
- **High sensitivity and reproducibility**
- **Direct, non-invasive detection in urine**
- **No extraction or purification**
- **Good Correlation with clinical samples**

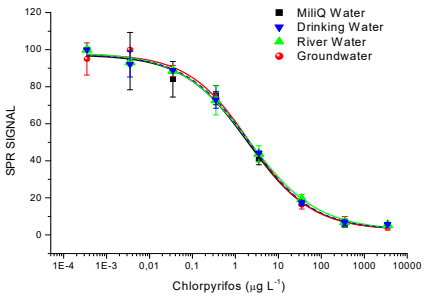
Biosens & Bioelec. 79,158 (2016)

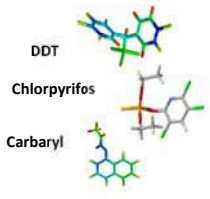
In collaboration with BIOMEDAL, SL (Sevilla)

Biosensors for pesticides detection

Environmental toxic pollutants in real samples (water safety)







Small MW (200Da)

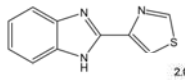
Label-free competitive immunoassay

Detection limit: 0.02-0.05 µg/L
(ppt level, EU legislation: 0.1 µg/L)

- **Direct detection in real water samples**
- **No matrix effects, no pretreatment**
- **Fast analysis**
- **Reusable up to 200 times**
- **Validated results**

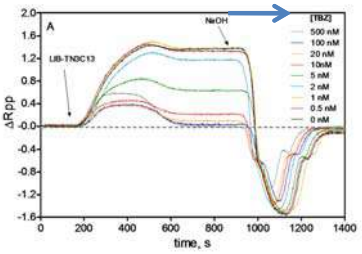
Anal. Bioanal. Chem. 387, 1449 (2007)
Biosens.& Bioelec. 22, 1410 (2007)
Anal. Bioanal. Chem. 388, 207 (2007)
Sens.Actu. B 118, 399 (2006)
Talanta 69 (2), 359 (2006)
Anal. Chim. Acta 561, 40 (2006)
Biosens.& Bioelec.21, 2129 (2006)

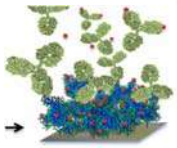
Thiabendazole detection in whole oranges



Thiabendazole (TBZ)

Small MW (200Da)



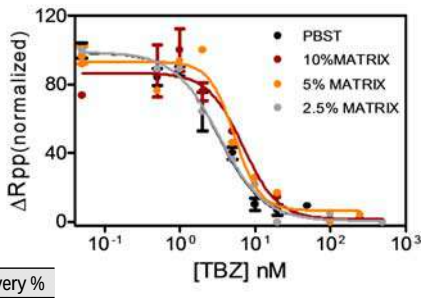


Label-free competitive immunoassay

	LOD (IC_{90})	IC_{50}	Working Range (IC_{80}-IC_{20})
PBST 0.002%	0.61 nM (0.13 µg L^{-1})	3.2 nM (0.64 µg L^{-1})	1.13-9.35 nM (0.23-1.88 µg L^{-1})
PBST 0.05	0.67 nM (0.13 µg L^{-1})	3.2 nM (0.64 µg L^{-1})	1.21-8.5 nM (0.24-1.7 µg L^{-1})
ELISA	0.1 nM (0.02 µg L^{-1})	1 nM (0.2 µg L^{-1})	0.25-4.5 nM (0.05-0.9 µg L^{-1})

• Ground homogenate
• TBZ Extraction (MeOH sonication 1h)

• 10-Fold diluted

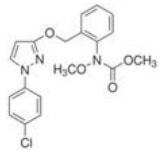


Blank sample

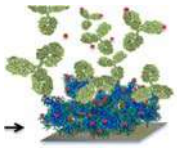
Sample	HPLC, µg L^{-1}	SPR immunosensor, µg L^{-1}	Recovery %
1	371	391.2±52.7	105.2
2	261	288.6±49.1	110.9
3	337	354.1±68.8	105.1

MRL (Maximum Residue Level) permitted= 5mg/Kg

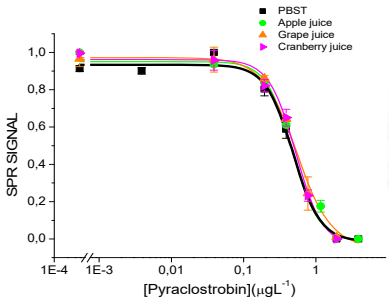
Pyraclostrobin detection in fruit juices



Small MW (388 Da)



Label-free competitive immunoassay



Analytical calibration values

I_{50} ± *SD* (µg L^{-1})	0.44 ± *0.028*
LOD ± *SD* (10% inhibition concentration, µg L^{-1})	0.19 ± *0.068*
Working range ± *SD* (µg L^{-1})	0.26 ±*0.056* - 0,86±*0.16*

Recovery of Pyraclostrobin from spiked apple, grape and cranberry juices

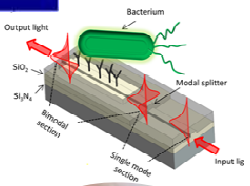
Fruit juice	Pyraclostrobin added, µg L^{-1}	Pyraclostrobin recovered, µg L^{-1}	Recovery (%)	CV (%)
Apple				
	0.0387	0.039	100.6	1
	0.193	0.197	101.6	1
	0.387	0.389	100.3	2
	1.163	1.15	98.8	17
	3.878	3.82	98.5	1
Grape				
	0.0387	0.0384	99	7
	0.193	0.195	100.6	2
	0.387	0.384	99	2
	0.776	0.764	98.5	18
	1.939	1.954	100.8	1
Cranberry				
	0.0387	0.0384	99	5
	0.193	0.197	102.1	4
	0.387	0.388	100	7
	0.776	0.764	98.5	13
	1.939	1.982	102.2	1

BiMW POC Biosensor for direct bacteria detection

Early Identification of infections in cirrhotic patients

- Limited to central laboratories
- Require trained personnel
- Time consuming

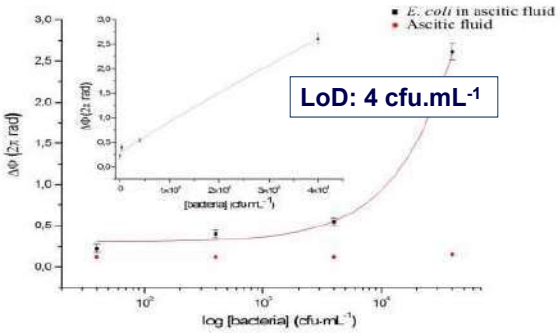
1.- Fast Identification and quantification of bacteria
2.- Detection of the antibiotic resistant profile

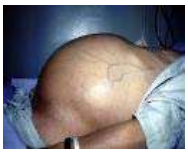


Direct detection of *E. Coli* in patients´ ascitic fluid

LOQ: 40 cfu.mL^{-1}
Analysis cycle: 25 min.
Volume: 250 µL

The POC biosensor detects bacteria at relevant physiological level in ascitic fluid, direct and label-free.



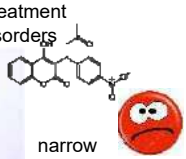


Biosens. Bioelectr., **85**, 310-316 (2016)
Analyst 145 (2), 497-506 (2020)

Vall d'Hebron Hospital

POC for anticoagulant Sintrom® control

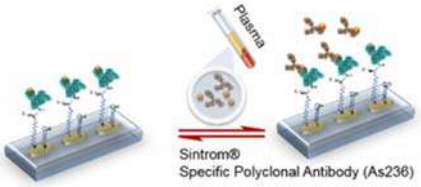
Acenocoumarol (Sintrom®)
anticoagulant for the treatment of thromboembolic disorders

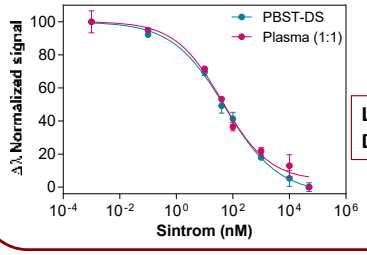


narrow therapeutic range

Difficulty in the dose regulation
Low doses: risk of coagulation
High doses: risk of bleeding

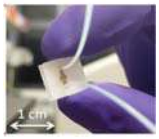
Nanoplasmonics POC Bisensor





POC Biosensor can offer an added value for self-patient monitoring

LOD: 0.77 nM
DR: 3.38 nM - 1154 nM



100 µL of plasma sample

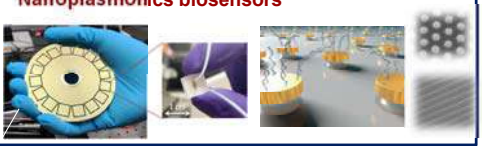
In collaboration with M.P. Marco´s Group

Nanobiosensors and Bioanalytical Applications Group

Technological platforms

Silicon Nanophotonics biosensors

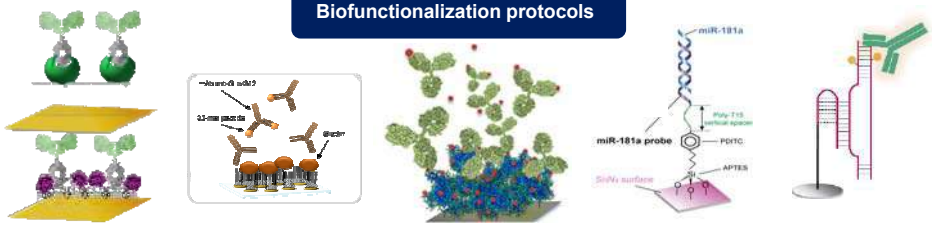
Nanoplasmonics biosensors



Point-of-care devices

- Complete in-house design and assembly
- Miniaturized & compact portable platforms

Biofunctionalization protocols



Summary of Applications @NanoB2A Group

PROTEIN BIOMARKERS

Early detection Colorectal cancer
Gluten consumption
Hormone alteration
Doping control
Tuberculosis
Allergy diagnosis
Growth factors
Sintrom antibiotics

Urine, serum, plasma, tears

NUCLEID ACIDS

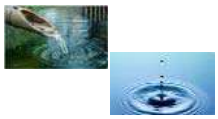
Single DNA cancer mutations
DNA Epigenetics
microRNAs biomarkers

Messenger RNA
Alternative splicing RNA

Urine, serum, plasma, tissue

SMALL POLLUTANTS

Environmental water pollutants
Pesticides, Organo-halogenated compounds, antibiotics, biocides



Food contaminants
Pesticides residues: canned food, oranges

Toxins

Wastewater, tap water, ocean, food

PATHOGENIC BACTERIA

Nosocomial pathogens

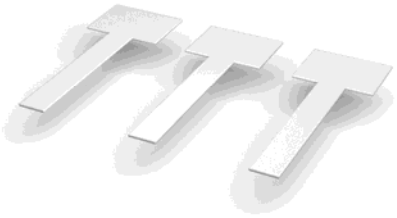
- Chronic liver failure
- Sepsis

Antibiotic susceptibility of bacteria

Water pathogens

Urine, serum, plasma, ascetic fluid

Point-of-care platforms for descentralized analysis



- **Point-of-care biosensors** are required for fast, direct, label-free, high sensitivity, low sample volume and massive diagnostics
- Evanescent field photonic chip sensors are one of the **most competitive technology** (Silicon photonics sensors as MZI, BiMW)
- They leverage on the unique features of photonic chips, such as **stability** and **production scalability**
- Sensors need to be disposable, that means cheap, therefore **mass production** is required
- **Surface chemistry biofunctionalization is the key for sensors specificity**

THANK YOU!!!
GRACIAS!!!

@NanoB2A_group

Nanob2a.icn2.cat

ESR#19: Lab-on-chip devices for smartphone imaging Surface Plasmon Resonance (iSPR) detection

Software design, Citizen Science and FoodSmartphone exploitation

Chi Xiao, ESR#19
Supervisors: Jens Eriksson, Wing Cheung Mak
Linköping University (LIU), Sweden

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

1

Content

2

Objectives of research project

1. To develop low cost advanced reusable accessories to temporarily transform smartphones into iSPR analytical instruments.
2. To adapt innovative fast prototyping 3D printing techniques to fabricate custom lab-on-a-chip devices and disposable coupling optics.
3. To incorporate supporting systems for autonomous sample conditioning and data acquisition.
4. To demonstrate the applicability of the developed iSPR smartphones with the detection of relevant food quality and safety targets.

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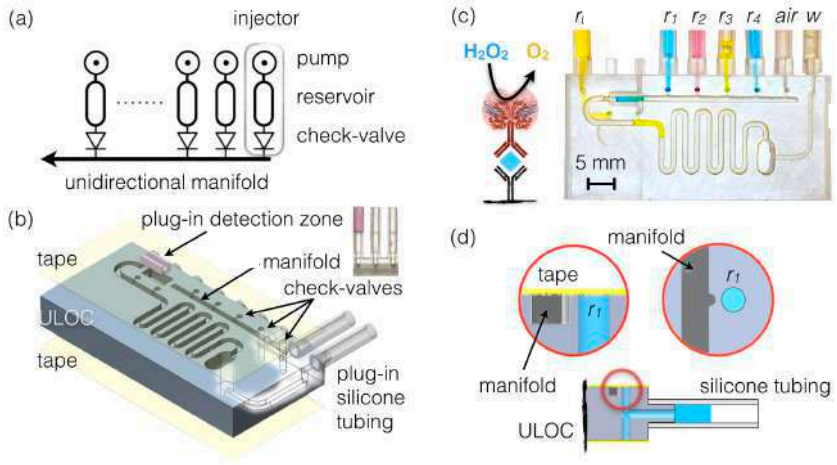
3

design of lab-on-a-chip configuration for imaging SPR assay

Lab-on-a-chip



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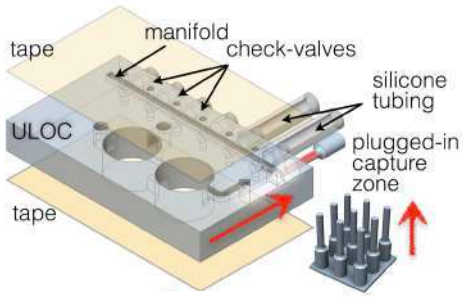
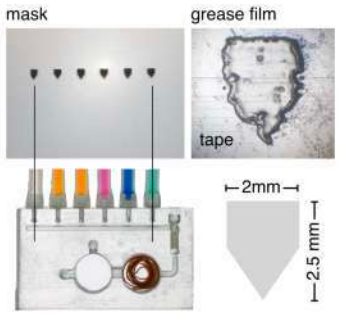
Autonomous lab-on-a-chip generic architecture for disposables with integrated actuation.*Scientific reports* 9.1 (2019): 1-9.

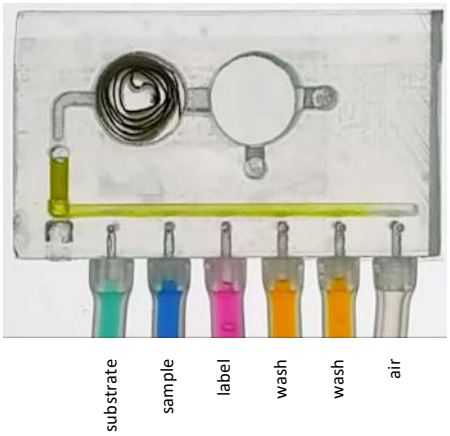
4

design of lab-on-a-chip configuration for imaging SPR assay

Lab-on-a-chip







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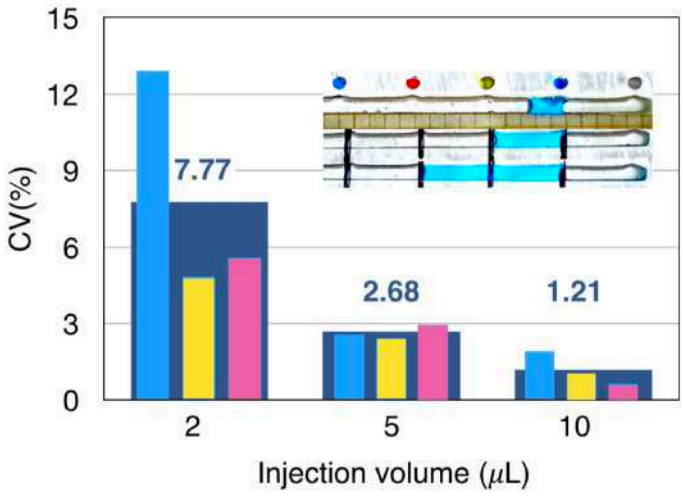
Autonomous lab-on-a-chip generic architecture for disposables with integrated actuation.*Scientific reports* 9.1 (2019): 1-9.

5

design of lab-on-a-chip configuration for imaging SPR assay

Lab-on-a-chip

$$CV(\%) = \frac{std(X)}{mean(X)} * 100$$



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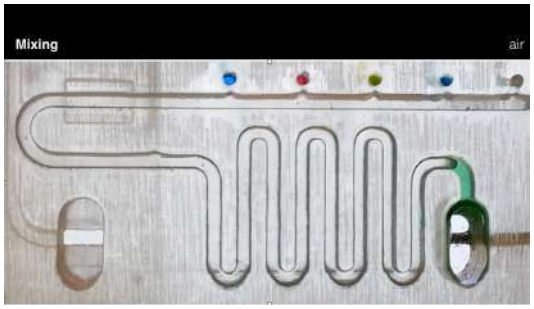
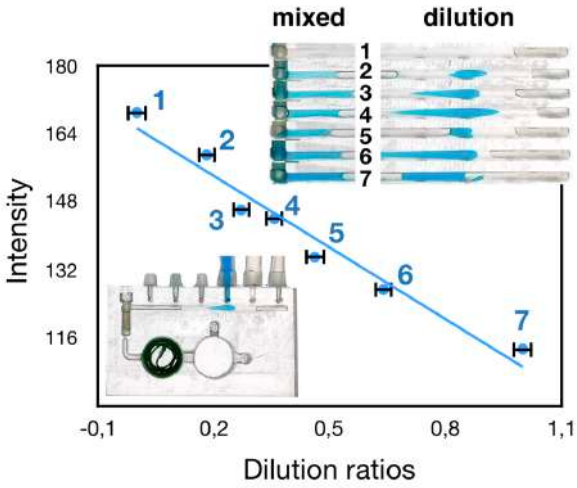
Autonomous lab-on-a-chip generic architecture for disposables with integrated actuation.*Scientific reports* 9.1 (2019): 1-9.

6

design of lab-on-a-chip configuration for imaging SPR assay

Lab-on-a-chip





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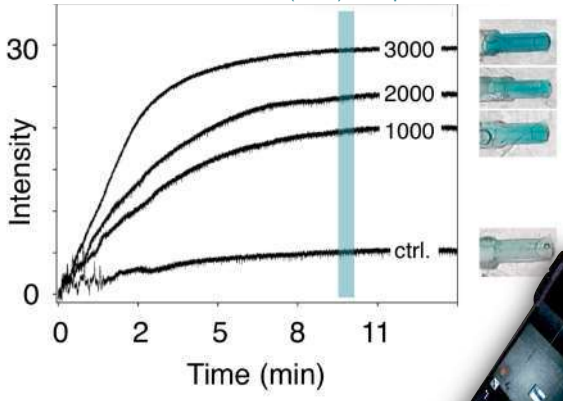
Autonomous lab-on-a-chip generic architecture for disposables with integrated actuation.*Scientific reports* 9.1 (2019): 1-9.

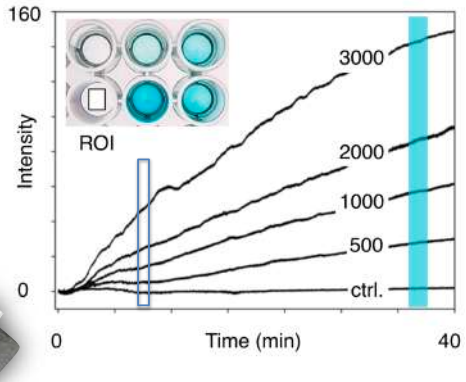
7

design of lab-on-a-chip configuration for imaging SPR assay

Lab-on-a-chip

Tumor Necrosis Factor (TNF) receptor 1





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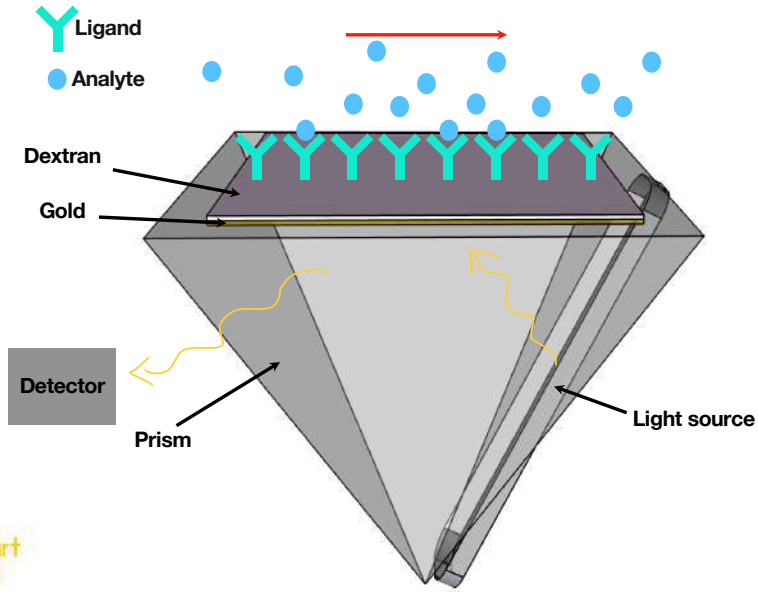
8

Optical design and fabrication of reusable coupling elements for angle-resolved imaging SPR on smartphones.

iSPR optical coupler



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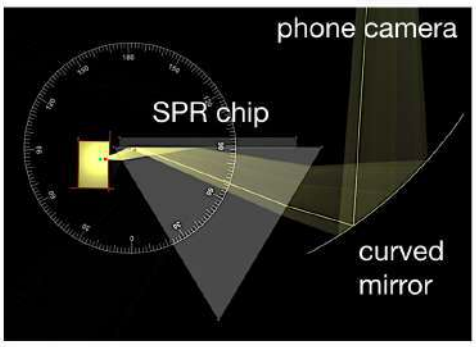
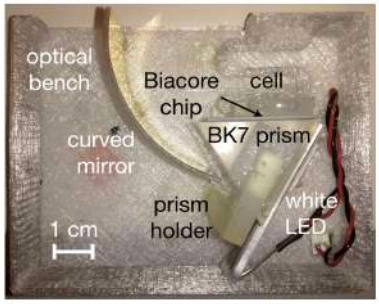
9

Optical design and fabrication of reusable coupling elements for angle-resolved imaging SPR on smartphones.

iSPR optical coupler





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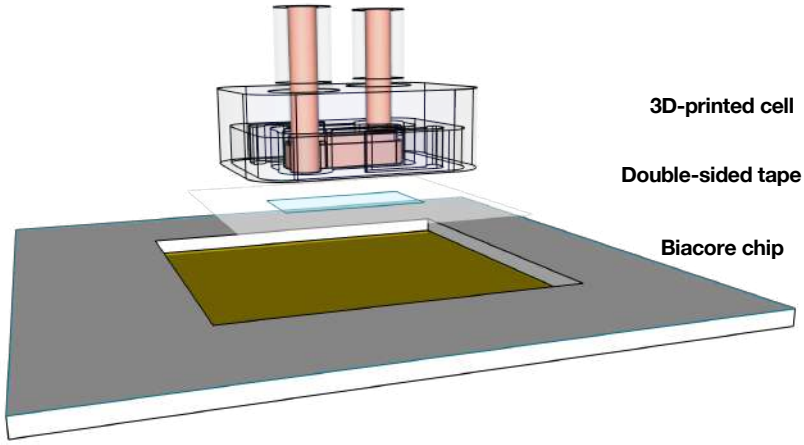
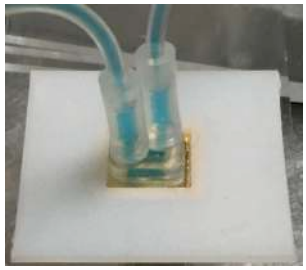
Unpublished results, confidential information

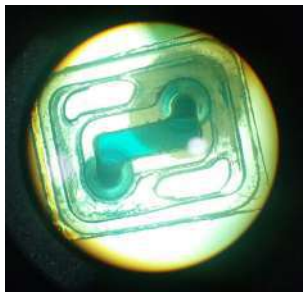
10

Optical design and fabrication of reusable coupling elements for angle-resolved imaging SPR on smartphones.

iSPR optical coupler







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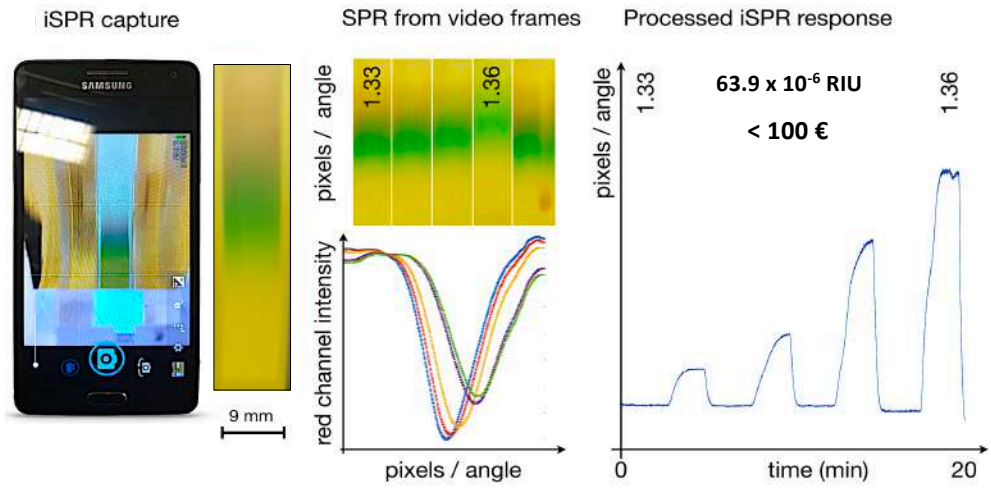
11

Optical design and fabrication of reusable coupling elements for angle-resolved imaging SPR on smartphones.

iSPR optical coupler



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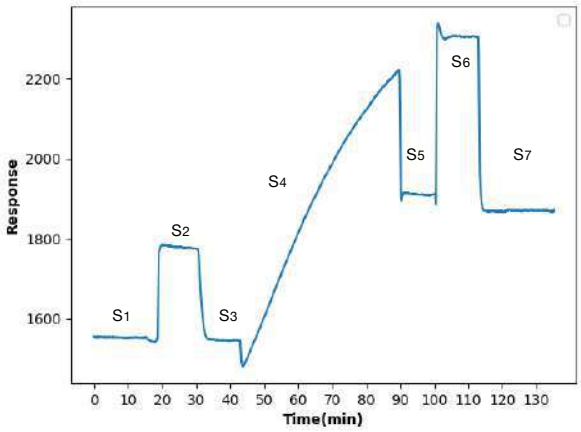
12

Optical design and fabrication of reusable coupling elements for angle-resolved imaging SPR on smartphones.

iSPR optical coupler



S1: Running Buffer

S2: Amine coupling (EDC&NHS)

S3: Running Buffer

S4: Ligand

S5: Running Buffer

S6: Degeneration (Ethanolamine)

S7: Running Buffer

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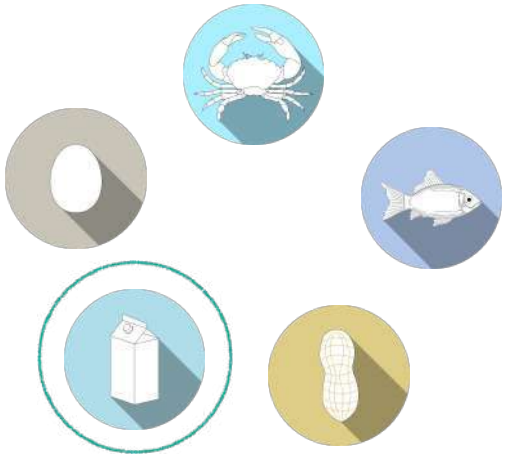
Unpublished results, confidential information

13

The detection of relevant food quality and safety targets based on smartphones SPR platform.

Smartphone SPR for food analysis



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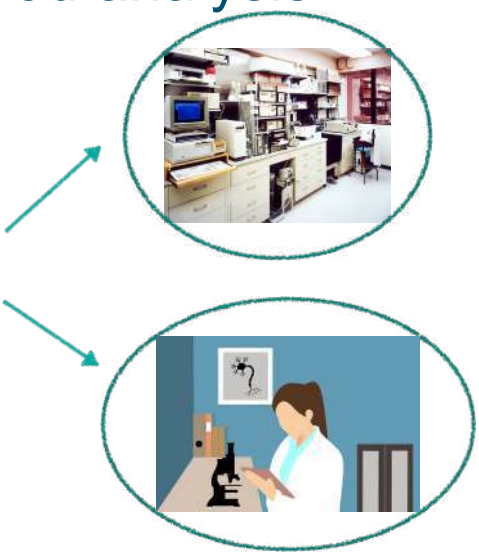
14

The detection of relevant food quality and safety targets based on smartphones SPR platform.

Smartphone SPR for food analysis



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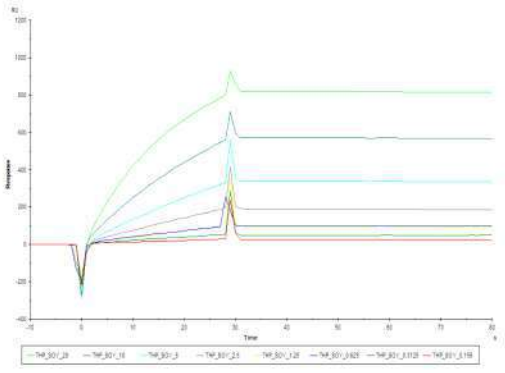
15

The detection of relevant food quality and safety targets based on smartphones SPR platform.

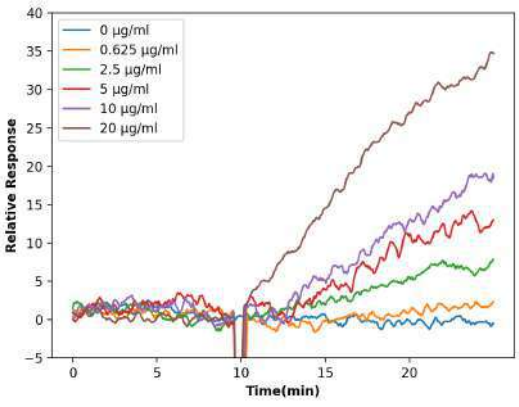
Smartphone SPR for food analysis

Superimposed sensorgrams for total hazelnut protein (THP) in soy milk

Biacore 3000



Smartphone SPR



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Unpublished results, confidential information

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Thank you for your attention!

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

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FoodSmartphone

Smartphone analysers for food safety and quality analysis from software perspective

Yunfeng (Jack) Zhao
Queen's University Belfast

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

- Can smartphone be used for food analysis? ✓
- How can we transfer a smartphone into a food analyser?

Challenge 1:

Various smartphone models, dimensions, and specificities

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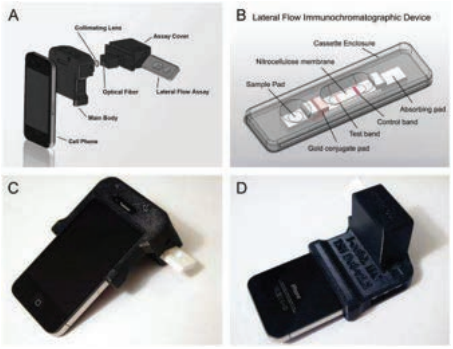
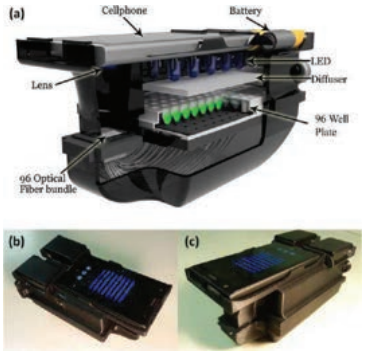
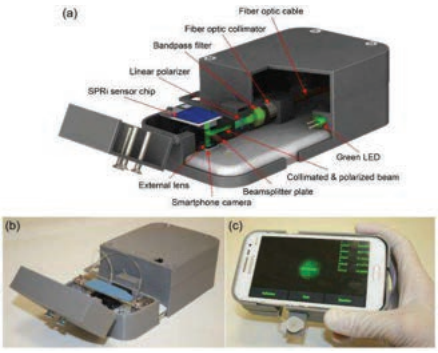


[1] D. J. You, T. S. Park, and J.-Y. Yoon, "Cell-phone-based measurement of TSH using Mie scatter optimized lateral flow assays," *Biosens. Bioelectron.*, vol. 40, no. 1, pp. 180–185, Feb. 2013.



[2] S. Feng, D. Tseng, D. Di Carlo, O. B. Garner, and A. Ozcan, "High-throughput and automated diagnosis of antimicrobial resistance using a cost-effective cellphone-based micro-plate reader," *Sci. Rep.*, vol. 6, no. November, pp. 1–9, 2016.



[3] H. Guner et al., "A smartphone based surface plasmon resonance imaging (SPRi) platform for on-site biodetection," Sensors Actuators B Chem., vol. 239, pp. 571–577, Feb. 2017.

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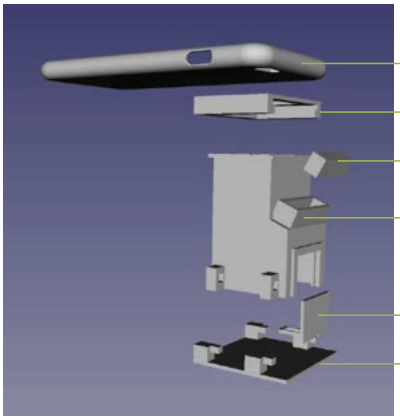
Solution:

Smartphone modulated colorimetric reader (SMCR)



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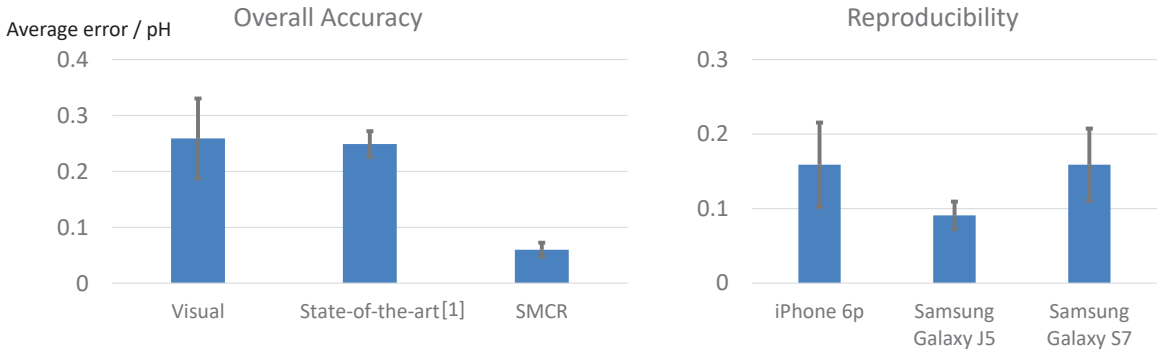

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[1] S. D. Kim, Y. Koo, and Y. Yun, "A smartphone-based automatic measurement method for colorimetric pH detection using a color adaptation algorithm," Sensors (Switzerland), vol. 17, no. 7, 2017.

Challenge 2

Colour interference due to coloured solutions, e.g. red wine

★★☆☆☆ **Don't work at all on red wines, because the wine distorts the color.**
21 January 2018 - **Published on Amazon.com**
Verified Purchase
Obvious once I stopped to think about it, but not mentioned in the item notes that I saw.

4 people found this helpful.

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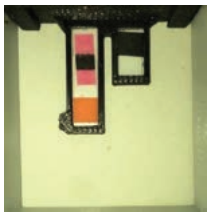
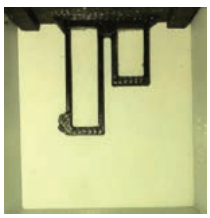
Solution:

Colour subtraction algorithm

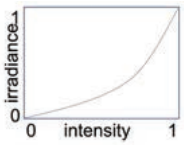


Test image



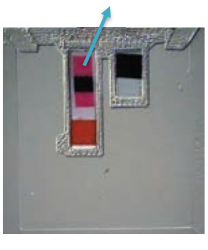
Background image

Illumination correction



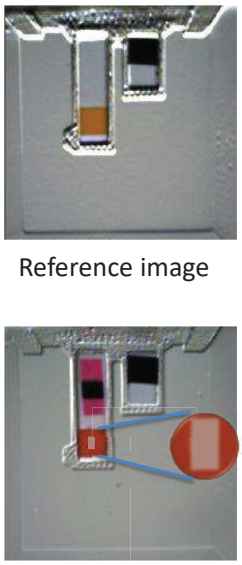
Camera response function

Solution colour catcher



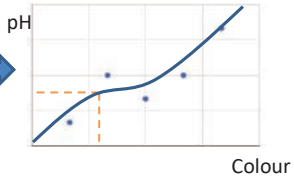
Illumination corrected image

Colour subtraction



Colour subtracted pH test strip

Reference image



Predicting

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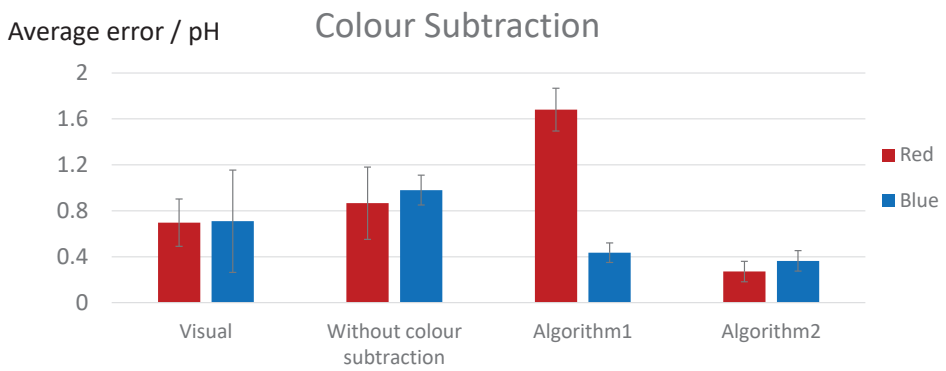

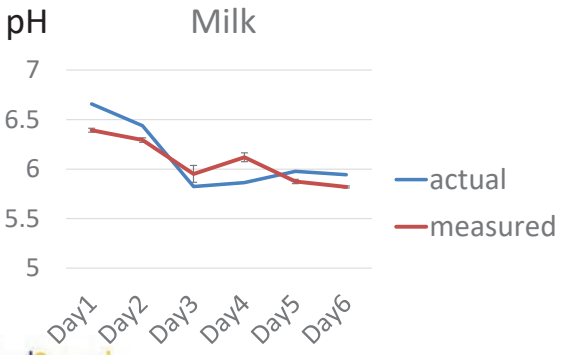

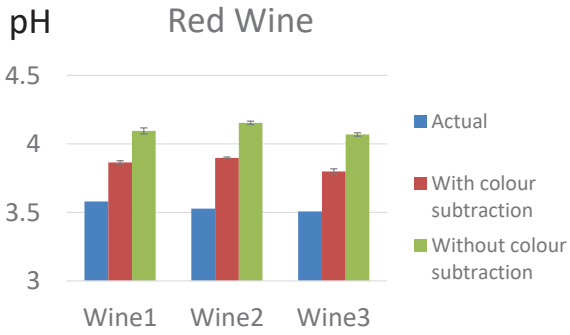

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Challenge 3:

Varied camera colour performance across different smartphone models

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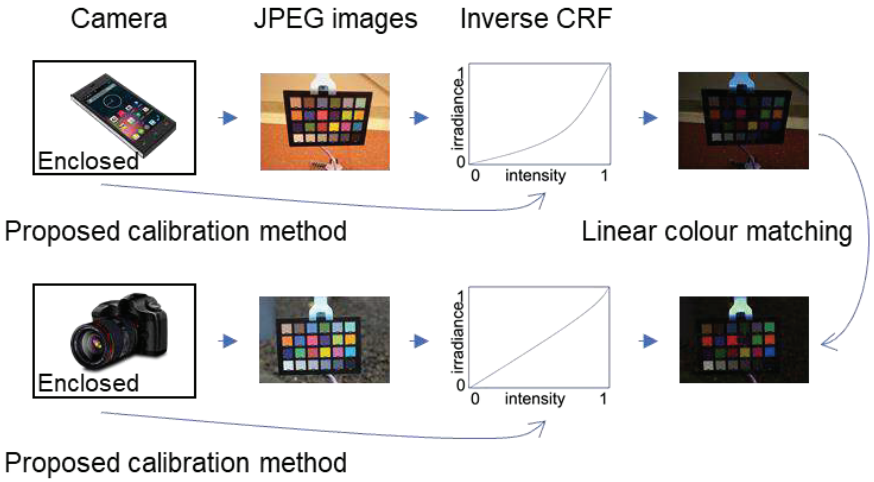
Solution:

Camera colour correction



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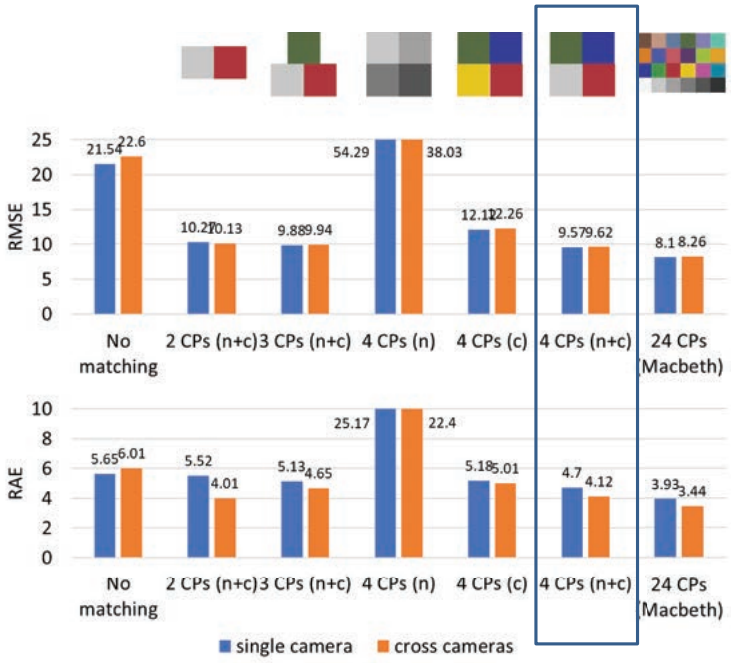

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Challenge 4:

- A tremendous amount of food analytical data is potentially going to be generated.
- Common data storage practice in traditional databases is prone to data tampering which could lead to fraud.
- Current one-to-one on-chain off-chain data storage scheme is inefficient in terms of data volume taken.

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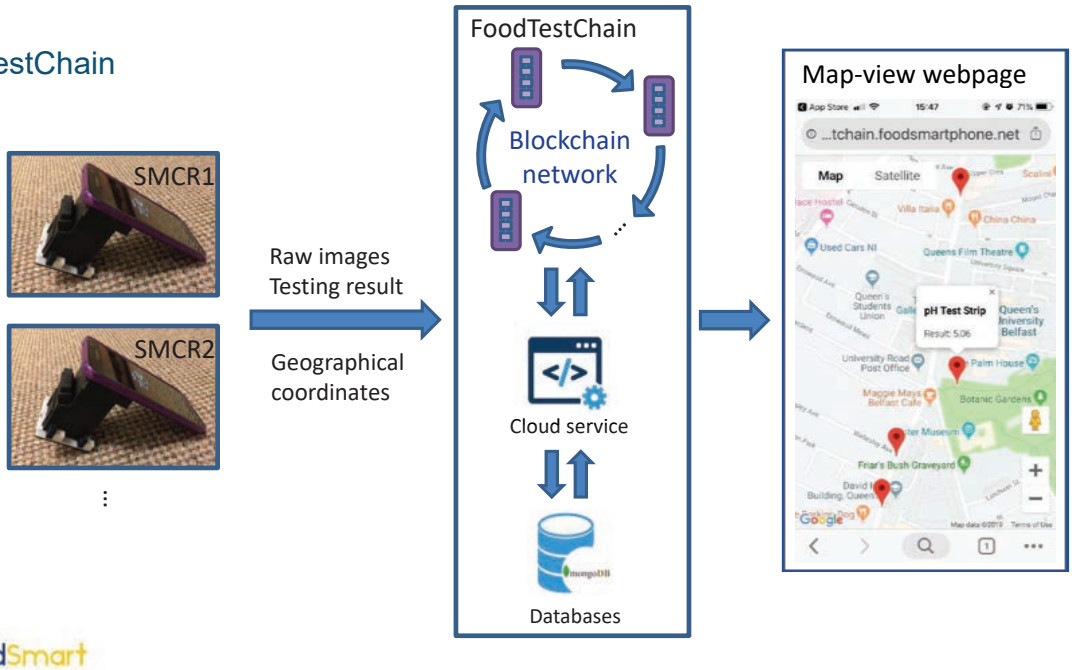
Solution:

FoodTestChain



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Thank you for your attention!

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

Analytical microsystems for monitoring food production processes and quality

César Fernández Sánchez

Grupo de Transductores Químicos

Instituto de Microelectrónica de Barcelona, IMB-CNM (CSIC)

25th November, 2020

Instituto de Microelectrónica de Barcelona

Research and development in fundamental and applied micro - and nanotechnologies

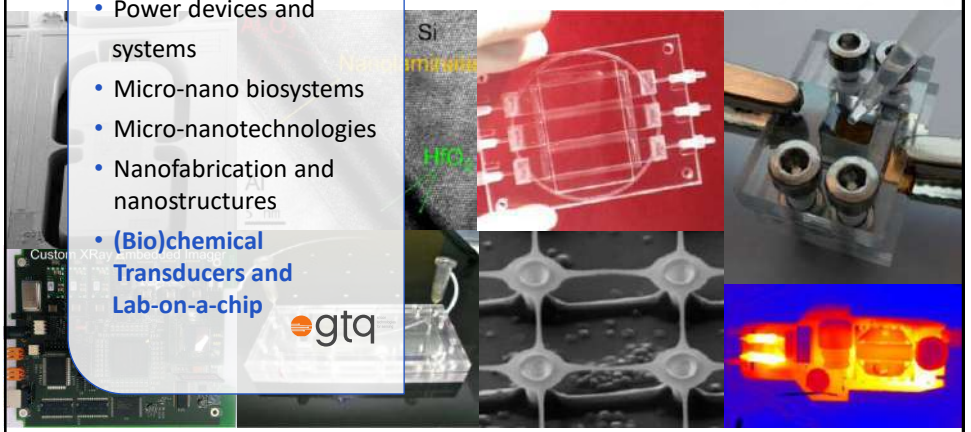
Located at Campus of the Universitat Autónoma de Barcelona

Belongs to the Spanish Research Council (CSIC; www.csic.es)

Instituto de Microelectrónica de Barcelona

Research Lines

- Power devices and systems
- Micro-nano biosystems
- Micro-nanotechnologies
- Nanofabrication and nanostructures
- **(Bio)chemical Transducers and Lab-on-a-chip**



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Instituto de Microelectrónica de Barcelona

Clean Room

MICRO nano FABS

- 1.500 m^2 Class
- 100-10,000 Clean Room
- 2.5 µm standard CMOS line

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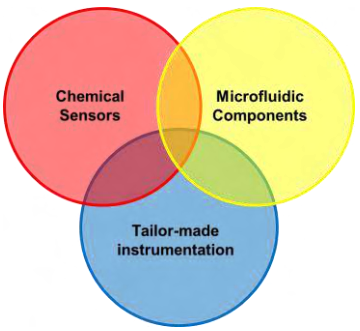
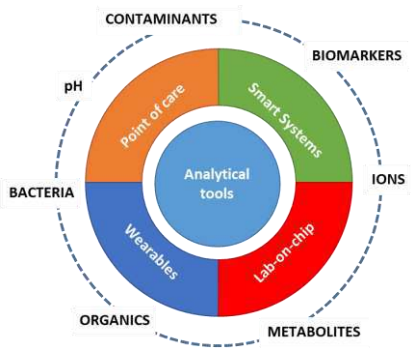
GTQ Mission

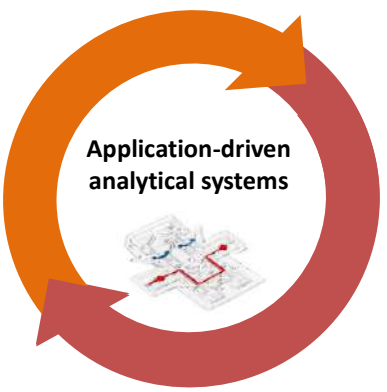
R&D&I ***ad-hoc* analytical tools** for the measurement of (bio)chemical parameters in liquids





GTQ Vision

Market solutions to analytical needs



Address the requirements of different sectors: Environment, **Food**, Health.

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GTQ standards

tecnio catalonia
Fins a 31/12/2023
ACCIÓ
Generalitat de Catalunya

- The GTQ is a member of TECNIO since 2008, a network created by Catalonian Government of the main R+D+I groups and technology transfer agents of Catalonia
- TECNIO is a mark of quality that credits that an institution meets certain requirements that guarantee the quality of services, innovation and the use of leading technologies

GTQ awarded with ISO 9001:2015 certification by IQNet (AENOR)

CERTIFIED IQNet MANAGEMENT SYSTEM
AENOR ER Empresa Registrada ISO 9001 ER-0143/2018

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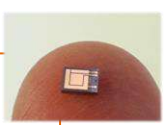
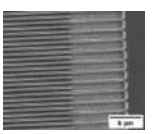
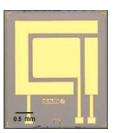
Technological approaches

Electrochemical transducers based on thin-film electrodes

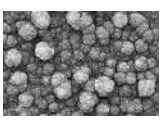
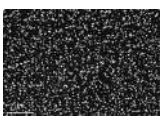
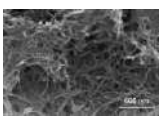
Silicon standard technologies



- ✓ Device size
- ✓ Small sample volumes (biological samples)
- ✓ "Batch" production process
- ✓ Integration of the required electronics
- ✓ Fabrication reproducibility and yield

Functionalization

Sensor packaging adapted to the application

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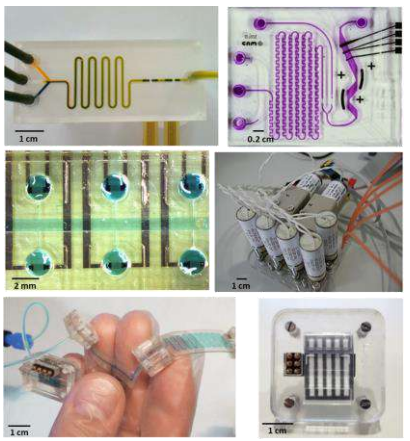
Compact fluidic devices

- ✓ Integration and automation of all the steps of an analytical process (sampling, conditioning, measure)
- ✓ Rapid quantitative analysis
- ✓ Multiparametric
- ✓ Low reagent consumption and waste production
- ✓ In field applications



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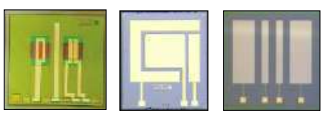
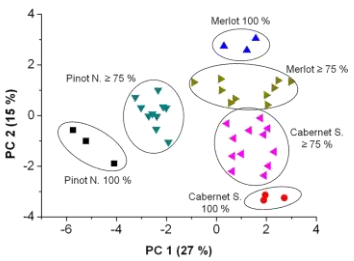
Electronic tongue: Multisensor system

Analysis of wines, beverages and water: Organoleptic evaluation
Detection of frauds

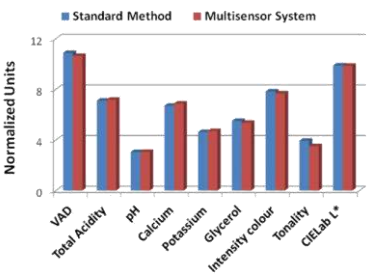
- **Potentiometric: ISFETs**
- **Amperometric:** Metal and carbon-based microelectrodes
- **Conductimetric**



Classification approach



Quantification approach



P. Giménez-Gómez, et al. *Sensors* 2016, 16, 1796
M. Gutiérrez-Capitán, et al. *Sensors.* 2019, 19, 1435

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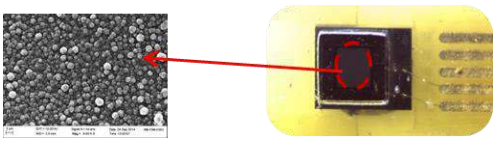
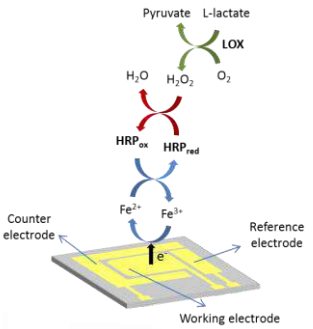
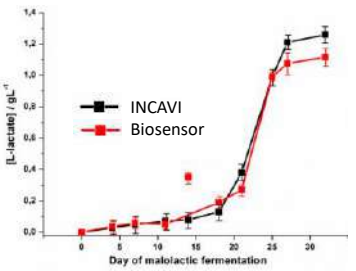
Biosensors for the detection of L-lactic and L-malic acids in wine samples



Thin-film enzyme biosensor with polypyrrole membrane
Working stability: **40 days lifetime**





P. Giménez-Gómez *et al. Anal. Chim. Acta* 2016, *905*, 126-133
P. Giménez-Gómez *et al. Anal. Chim. Acta* 2017, *954*, 105-113

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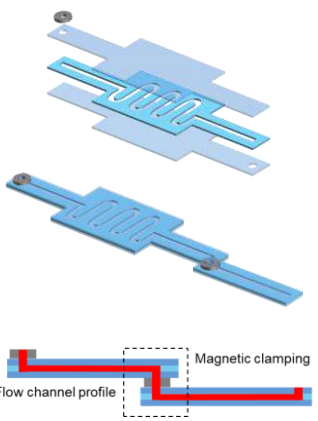
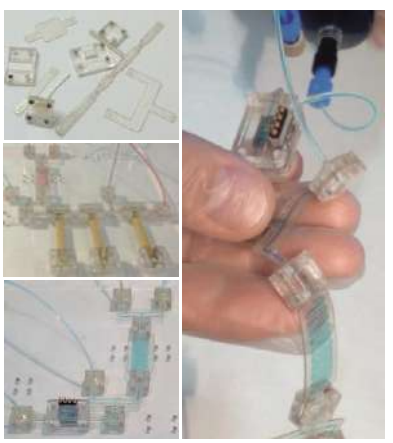
Modular lab-on-chip electrochemical sensors

- Reconfigurable lab-on-chip devices based on flexible transparent microfluidic modules
- Rapid 2D arrangement using magnetic clamping connections
- Reversible integration of electrochemical sensors
- Multiparametric





P. Giménez-Gómez, *et al. ACS Omega* 2019, 4, 6192−6198
P. Giménez-Gómez *et al. ACS Sensors* 2019, 4, 3156-3165

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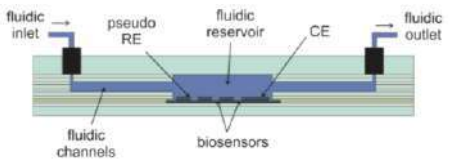
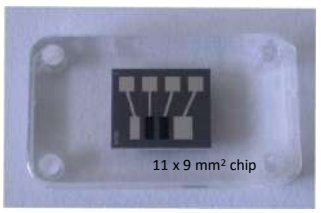
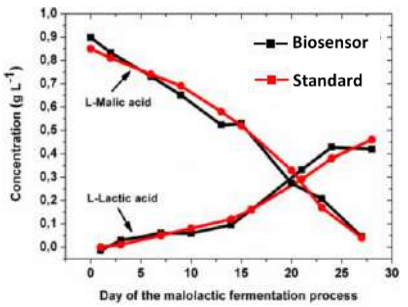
Biosensors for monitoring malolactic fermentation in red wines

Portable device integrating two on-chip biosensors for L-lactic and L-malic acids







P. Giménez-Gómez *et al*. *Sci. Reports* **2020,** 10:19404

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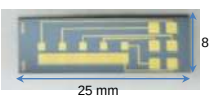
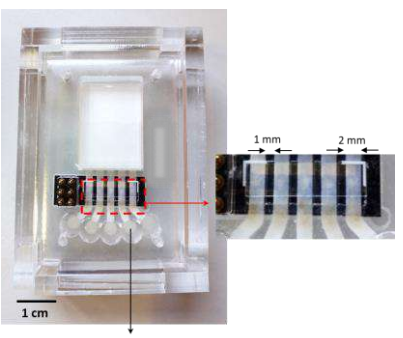
Electrochemical array and paper fluidics

- Electrochemical cell array and paper fluidic component
- Simple assembly
- Multiplexed
- Low-power instrumentation
- Very low cost-per-analysis
- On-site application at the point-of-need





Sample addition – 5 µL

Patent filing: C. Fernández-Sánchez, *et al.*, Application no. EP20382721.7

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Acknowledgements

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cnm Centro Nacional de Microelectrónica IMB

MICRO nano FABS

CSIC Consejo Superior de Investigaciones Científicas

Gobierno de España · Ministerio de Ciencia, Innovación y Universidades

HORIZON 2020

AGAUR Agència de Gestió d'Ajuts Universitaris i de Recerca

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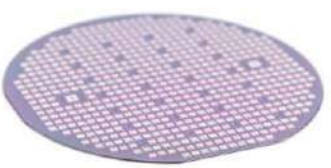
THANK YOU FOR YOUR ATTENTION!

Contact: César Fernández Sánchez
Cesar.fernandez@csic.es

Grupo de Transductores Químicos
Instituto de Microelectrónica de Barcelona
IMB-CNM (CSIC)
http://gtq.imb-cnm.csic.es



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Electrochemical immunosensors for the detection of pesticides in different food matrices

ESR 8 - Klaudia Kopper

Nanobiotechnology for Diagnostics research group (Nb4D) – IQAC-CSIC, Barcelona, Spain

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

Outline

- Introduction and Objective
- Experimental results
  - Evaluation of immunoreagents
  - Biofunctionalization of transducers
    - Electrode printing and characterization
    - Immunosensor development
    - Matrix effect studies and sample validation
- Summary and future prospects

Are you concerned about the safety of your food?

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3

The use of pesticides

- Globally, around 3 million tons of **pesticides** are applied annually, in the European Union (EU), there are almost 500 active substances approved for use in pesticides (EUROSTAT, 2018).

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4

Pesticide residues in food

Protection of crops against insects, weeds, funghi etc.

Increase yields during growth stage

Residues in soil and water (developing countries)

Potentially toxic to humans (acute and chronic health effects)

Regular monitoring of residues in food and environment

Maximume residue limits by WHO

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Our aim: to provide safe and reliable food for everyone

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6

Pesticide detection

- Traditionally HPLC/GC-MS (high sensitivity and accuracy, but also disadvantages…)
- Rapid, reliable and simple on-site screening methods are necessary as complementary detection techniques

- Our proposal:

→ Smartphone-based food analysis

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7

Why Smartphone-based analysis?

Simple, cost-effective, citizen science ready, available on-site

Powerful processors and memories, high resolution screens

Widely accessible, 3 billion smartphone users in 2020

Powerful data trans-mission capabilities: WiFi, USB, Bluetooth

Rich set of built-in sensors (e.g.: camera, microphone)

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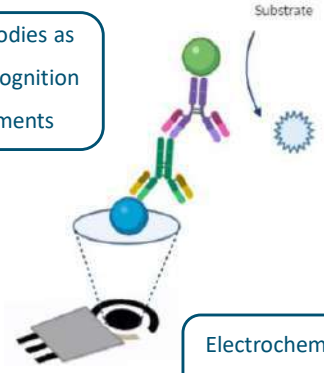
8

Proposed immunosensor design

Pesticide detection in different food matrices

Antibodies as biorecognition elements



Electrochemical transduction

Smartphone-based readout system

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Outline

- Introduction and Objective
- Experimental results
  - Evaluation of immunoreagents
  - Biofunctionalization of transducers
    - Electrode printing and characterization
    - Immunosensor development
    - Matrix effect studies and sample validation
- Summary and future prospects

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Selection of target pesticides

Herbicides

- To kill unwanted plants or to reduce growth of weed
- Glyphosate, **Triazines**, **Diquats**…

Insecticides

- To kill insects in all stages of growth (egg, larva, insect)
- DDT, **Bromopropylate Chlorpyrifos**…

European Union Pesticides Database

Regulation (EC) No 396/2005
MRL for cereals: 0.01-0.5 mg/kg

European Commission

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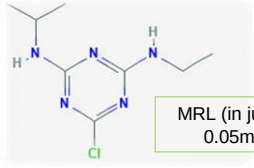
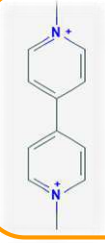
Target pesticides

Atrazine

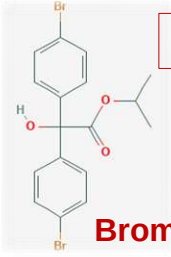


MRL (in juices) = 0.05mg/kg

Paraquat

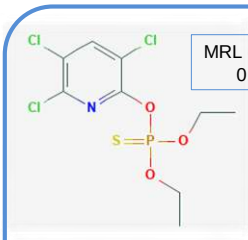


MRL (in juices) = 0.02mg/kg



MRL (in juices) = 0.01mg/kg

Bromopropylate



MRL (in wheat) = 0.5mg/kg

Chlorpyrifos

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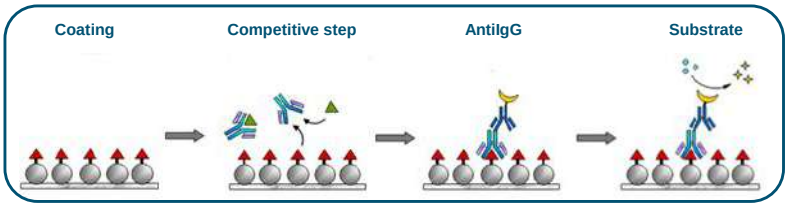
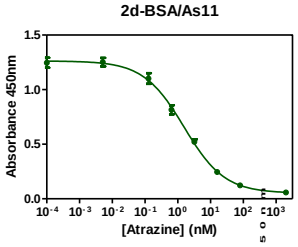
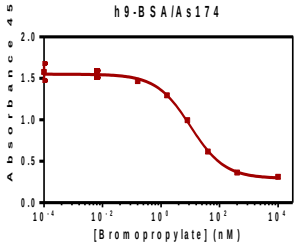
Ref.: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN

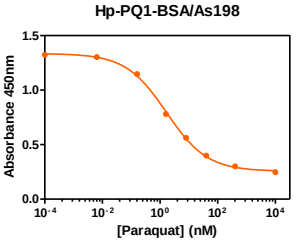
Evaluation of immunoreagents

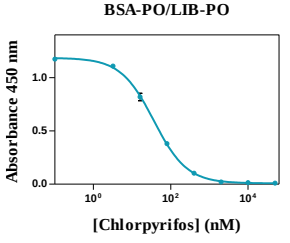
- Indirect competitive ELISA











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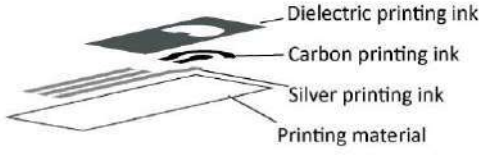
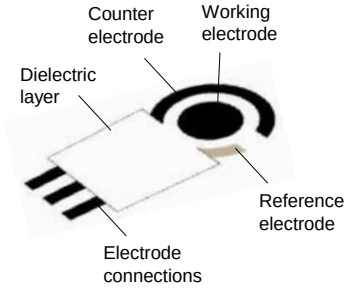
Electrode printing

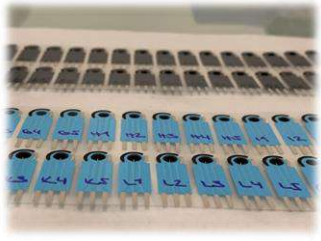
Four different graphite based electrodes were printed



- WEs were modified with different concentrations of the **nanomaterial** Carbon Black (CB).

- in 2 different ways:
  1. Modifying the ink directly during the printing process
  2. By drop-casting





- 2 types of **reference electrode**
  1. Ag/AgCl 80:20
  2. Ag/AgCl 60:40

- 2 types of **Dielectric** (insulator):
  1. Grey Dielectric (standard)
  2. Blue Dielectric (more water impermeability)

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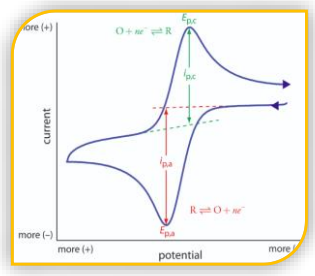
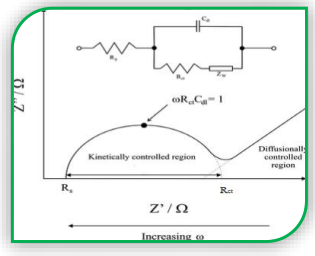
Electrode characterization

- The evaluation was based on the electrocatalytic effect of the electrode's surface on the redox couple (Ferro/Ferricyanide)
- **Cyclic Voltammetry (CV):**
  - Delta V
  - Peak height
- **Electrochemical Impedance Spectroscopy (EIS):**
  - $R_{ct} – R_s$ (by Randles circuit fit)
- According to these parameters the following electrode design was chosen:
  - Grey Dielectric
  - Ag/AgCl 60:40
  - Drop-casted CB (15μg)





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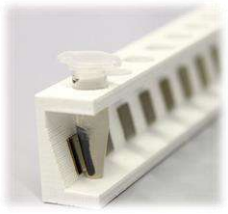
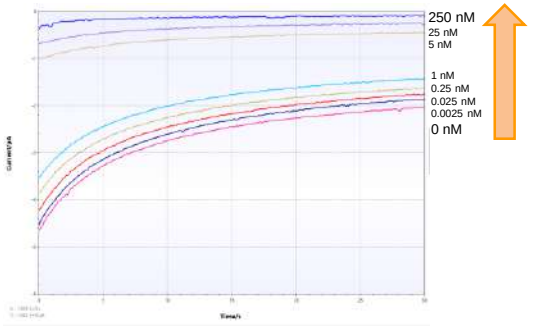
Immunosensor development

- **1st approach:**
  - Using Magnetic beads as a platform for the Immunoassay and the electrode only as a transducer
  - HRP-labelled secondary antibody (the redox couple Hydroquinone- Benzoquinone as substrate)
  - Chronoamperometry as electrochemical detection technique





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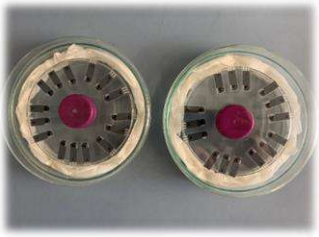
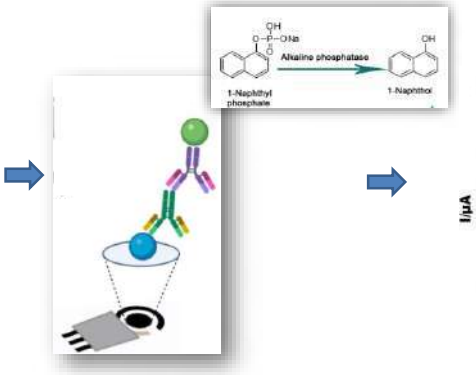
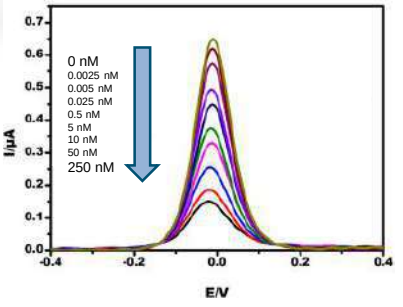
Immunosensor development

- **2nd approach:**
  - Using the electrode surface as a platform for the Immunoassay
  - Alkalyne Phosphatase-labelled secondary antibody (substrate: 1-Naphthyl phosphate)
  - Differential Pulse voltammetry (DPV) as electrochemical detection technique







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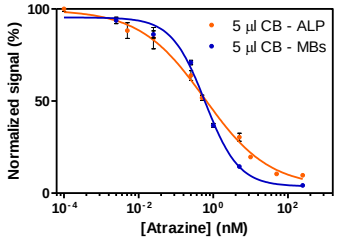
Immunosensor development
→ comparing the two approaches

Comparison of the ALP approach with the MBs



	5 µl CB - ALP	5 µl CB - MBs
IC50 (nM) (ppb)	0.75±0.20 0.162	0.59±0.06 0.127
LOD (nM) (ppb)	0.03±0.01 0.006	0.03±0.01 0.006
WR (nM) ppb	(0.02±0.00)-(11.83±1.72) (0.004)-(2.551)	(0.12±0.03)-(2.99±0.35) (0.026)-(0.645)

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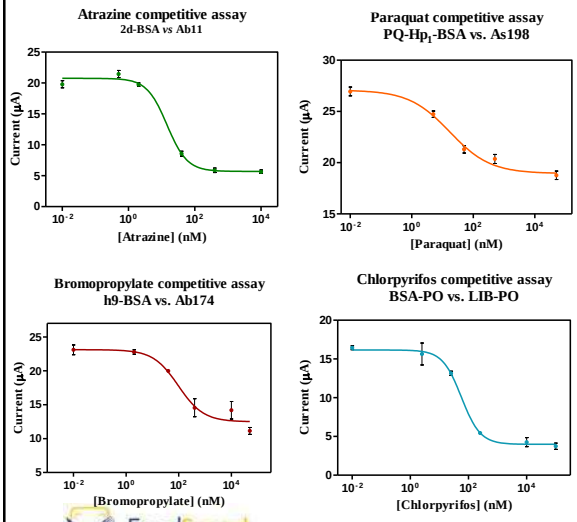
Immunosensor development
→ new electrode design



	Atrazine	Bromo-propylate	Paraquat	Chlorpyrifos
IC50 (nM) (ppb)	14.48±0.10 3.12	96.42±0.23 54.82	15.57±0.17 4.00	56.66±0.08 19.86
LOD (nM) (ppb)	2.09±0.23 0.45	10.08±0.96 4.32	0.95±0.10 0.24	3.22±0.30 9.17
WR (nM) (ppb)	(4.63)-(37.23) (1.00)-(8.03)	(24.92)-(1104.73) (10.67)-(472.92)	(2.58)-(106.62) (0.66)-(27.42)	(18.72)-(170.31) (6.56)-(59.71)
MRL (ppb)	50	10	20	50

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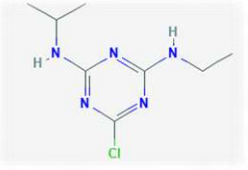
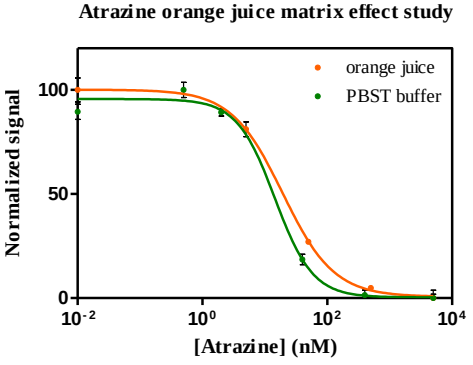
Atrazine immunosensor
→ matrix effect studies





	PBST buffer	Orange juice (1:5 dilution)
IC50 (nM) (ppb)	14.48±0.10 3.12	19.34±0.07 4.17
LOD (nM) (ppb)	2.09±0.23 0.45	2.47±0.38 0.53
WR (nM) (ppb)	(4.63)-(37.23) (1.00)-(8.03)	(5.28)-(74.17) (1.14)-(16.00)

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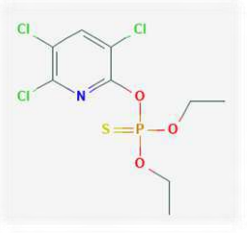
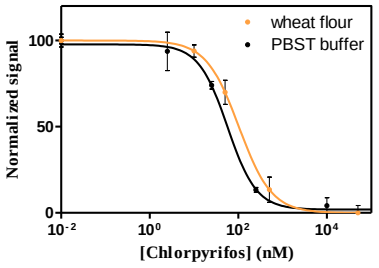
Chlorpyrifos immunosensor → matrix effect studies



Chlorpyrifos wheat flour matrix effect study



	PBST buffer	Wheat flour (1:20 dilution)
IC50 (nM) (ppb)	56.66±0.08 19.86	102.68±1.36 35.99
LOD (nM) (ppb)	9.17±0.30 3.22	15.82±1.55 5.55
WR (nM) (ppb)	(18.72)-(170.31) (6.56)-(59.71)	(31.55)-(333.80) (11.06)-(117.03)

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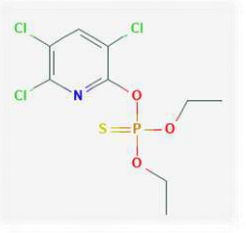
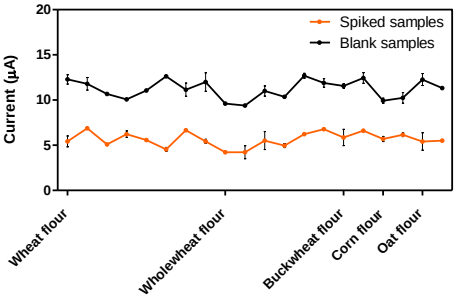
Chlorpyrifos immunosensor → sample validation



- 20 blank and 20 spiked (at the Screening target concentration - half of the MRL for Chlorpyrifos) samples were analyzed
- **Analysed samples:**
  - Wheat flour and whole wheat flour samples from Barilla
  - Buckwheat flour samples
  - Oat flour samples
  - Corn flour samples

Chlorpyrifos sample validation



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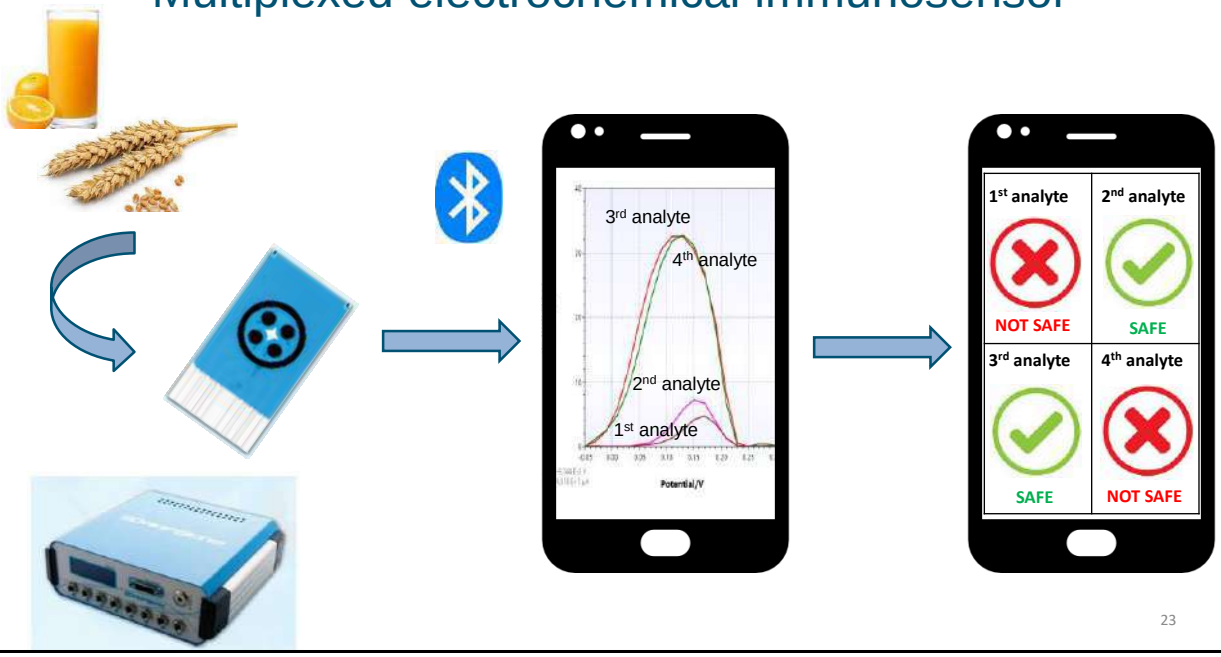
Multiplexed electrochemical immunosensor



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Outline

- Introduction and Objective
- Experimental results
  - Evaluation of immunoreagents
  - Biofunctionalization of transducers
    - Electrode printing and characterization
    - Immunosensor development
    - Matrix effect studies and sample validation
- Summary and future prospects

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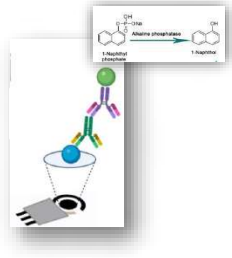
Summary and future prospects

- Immunoreagents evaluated by ELISA
- Printing, modification and characterization of screen printed electrodes
- Smartphone-connected electrochemical immunosensors developed for the detection of pesticides in cereals and fruit juices



- This test is thought out to be used by farmers and inspectors (e.g. at borders) for at-line measurements
- It is easy to learn and affordable (~10€/4 analytes)

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Acknowledgements

Nb4D

iQAC

CSIC
Consejo Superior de Investigaciones Científicas

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SMART TECH for FOOD

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Thank you very much for your attention!

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 720325

FoodSmartphone

Smartphone-based electrochemical analyser for on-site testing of aflatoxin B1 in cereals

Safiye Jafari
ETHZ/CSEM

1

What are aflatoxins?

Liver Cancer

EU limit for aflatoxin B1 in corn [1] : 2 µg/Kg

[1] COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006, "setting maximum levels for certain contaminants in foodstuffs"

2

The aflatoxin testing issue

Rapid screening is needed

Lab-based analysis by an expert

Moldy apricots picture sent by a consumer

Image by Michal Jarmoluk from Pixabay

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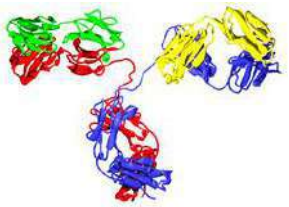
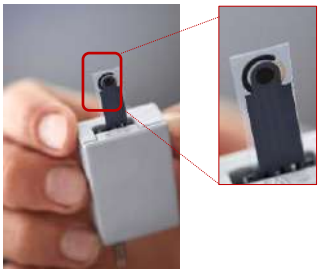
Our solution:

- Smartphone-based electrochemical biosensor

Biorecognition	Transducer	Read-out
Antibody or aptamer	Screen-printed electrode	Phone potentiostat





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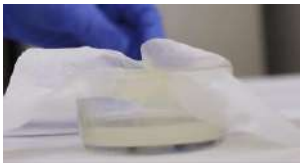
Extraction and sample preparation:

Simple extraction procedure:

- Only 5 minutes
- No need for clean up
- Ethanol as extraction solvent





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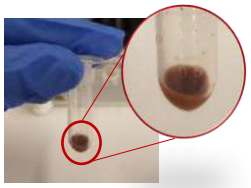
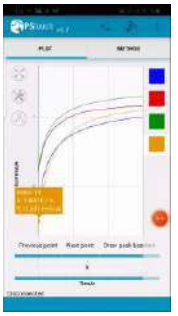
5

Enzyme Linked Immuno-Magnetic Electrochemical assay (ELIME) set up:

Immunoassay

Measurement





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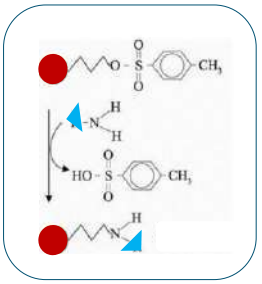
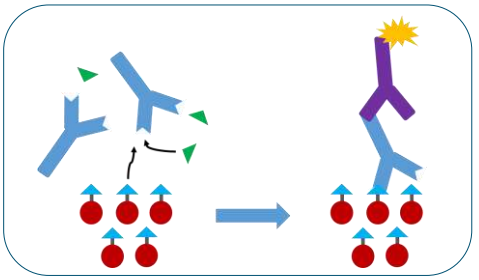
ELIME: working principle

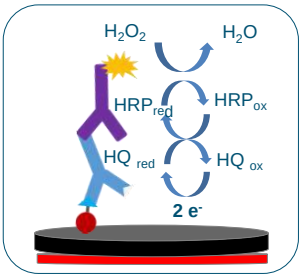
Magnetic beads conjugation

Indirect competitive Immunoassay

Electrochemical measurement







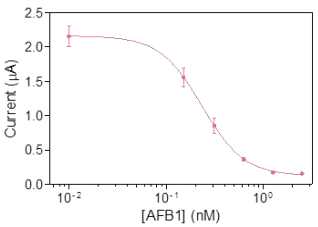
Magnetic beads · Antigen · Analyte · Horseradish Peroxidase (HRP) · H_2O_2 Hydrogen peroxide · HQ Hydroquinone

Primary antibody · Secondary antibody · Electrode with magnet

7

Aflatoxin B1 testing with ELIME assay

Measurement in buffer



IC50 (nM)	0.23
LOD (nM)	0.078
Working Range (nM)	0.116-0.446
RSD	<13%
R square	0.987

Real sample analysis

Spiked	Measured	Recovery %
0.1 nM	0.120 nM	120.0
0.2 nM	0.135 nM	67.5
0.3 nM	0.306 nM	102.0

Contaminated	Measured	Recovery %
0.26 nM	0.190 nM	73
0.24 nM	0.168 nM	70

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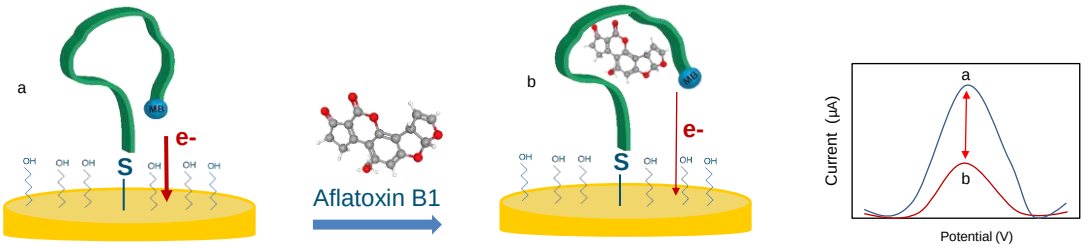
Aptasensor working principle:



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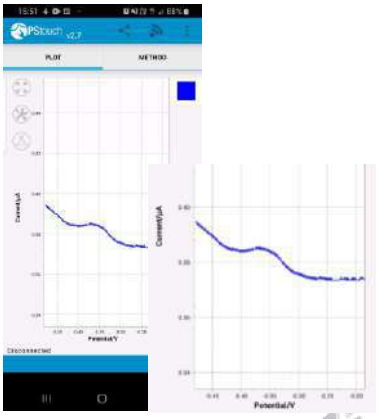
Aptasensor on gold wire electrode: set up

Electrodes

Cell

Potentiostat

Measurement on the phone



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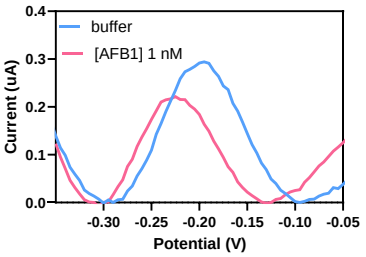
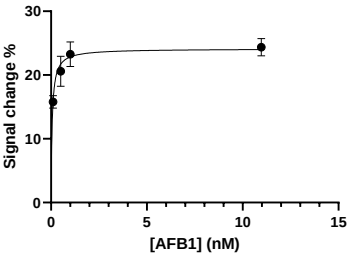
Aptasensor on the gold wire electrode:

Measurement in buffer



The max signal change = 25 %



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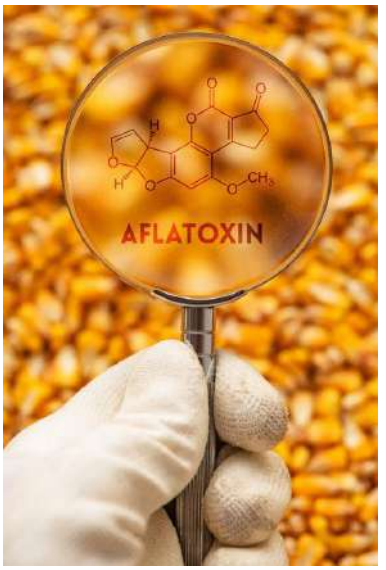
csem

11

Summary

- Portable, disposable &rapid
- Specific detection of aflatoxin B1 in corn
- Measurement performed on Smartphone



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12

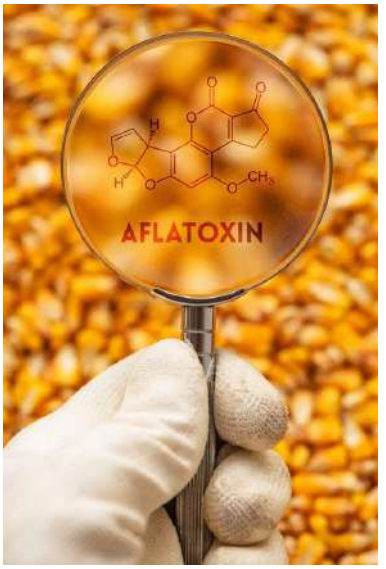
Special thanks to:

Prof. Dr. Shana J. Sturla, ETH Zürich

Dr. Silvia Generelli, CSEM

Dr. Davide Migliorelli, CSEM

Dr. Loïc Burr, CSEM



13

13

Project partners

FoodSmartphone comprises 7 Training Sites (3 universities, 3 research centres, 1 innovation SME), plus 2 Partner Organisations (1 global food industry and 1 diagnostics SME). The consortium has been built upon highly complementary disciplines: (bio)analytical chemists, biologists, physicists, micro-engineers, mathematicians and food chemists will work together on the joint supra-disciplinary goal.

csem WAGENINGEN UNIVERSITY & RESEARCH LINKÖPING UNIVERSITY Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

AQUAMARIJN Micro Filtration B.V.

FoodSmartphone.eu UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE QUEEN'S UNIVERSITY BELFAST IGFS Barilla

14

14

NANOBIOTECHNOLOGY FOR DIAGNOSTICS

Nb4D

PRESENCE OF PESTICIDE RESIDUES IN FRUITS AND VEGETABLES IN EU.

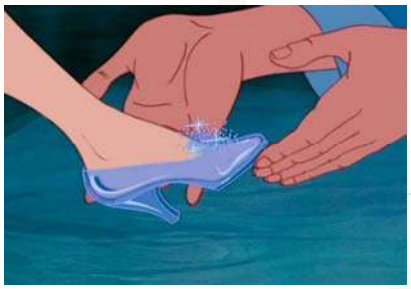
AN ANALYTICAL PERSPECTIVE

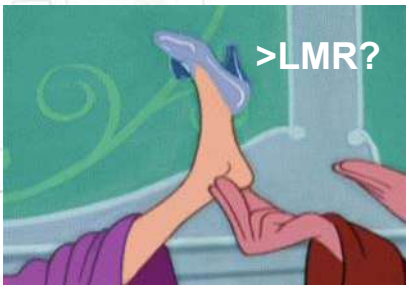
European Commission

EURL-FV

AMADEO R. FERNÁNDEZ-ALBA

RESIDUE



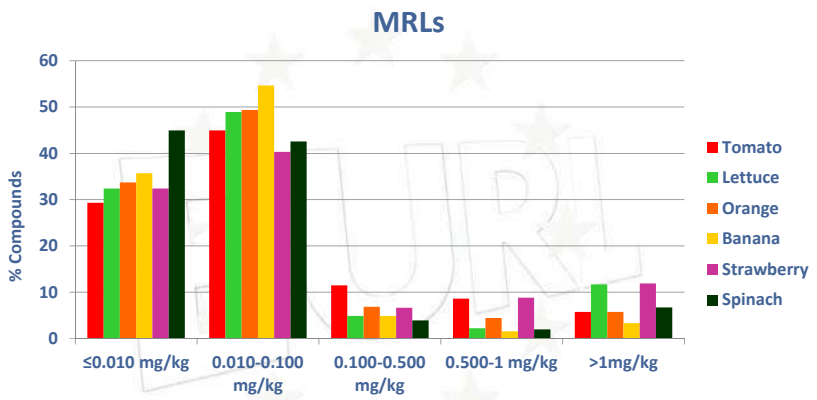


Pesticide/product combinations to be monitored in/on products of plant origin 2021-2023 TARGET LIST

2,4-D	Clothianidin	Etoxazole	Formetanate	Myclobutanil	Spinosad
2-Phenylphenol	Cyazofamid	Famoxadone	**Fosetyl-Al**	Omethoate	Spinetoram
Abamectin	Cyflufenamid	Fenamidone	Fosthiazate	Oxadixyl	Spirodiclofen
Acephate	Cyfluthrin	Fenamiphos	**Glyphosate**	Oxamyl	Spiromesifen
Acetamiprid	Cymoxanil	Fenarimol	**Glufosinate ammonium**	Oxydemeton-methyl	Spiroxamine
Acrinathrin	Cypermethrin	Fenazaquin	**Haloxyfop including haloxyfop-P**	Paclobutrazole	Spirotetramat
Aldicarb	Cyproconazole	Fenbuconazole	Hexaconazole	Parathion methyl	Tau-Fluvalinate
Aldrin and dieldrin	Cyprodinil	**Fenbutatin oxide**	Hexythiazox	Penconazole	Tebuconazole
Ametoctradin	Cyromazine	Fenhexamid	Imazalil	Pencycuron	Tebufenozide
Azinphos-methyl	Deltamethrin	Fenitrothion	Imidacloprid	Pendimethalin	Tebufenpyrad
Azoxystrobin	Diazinon	Fenoxycarb	Indoxacarb	Permethrin	Teflubenzuron
Bifenthrin	Dichlorvos	Fenpropathrin	Iprodione	Phosmet	Tefluthrin
Biphenyl	Dicloran	Fenpropidin	Iprovalicarb	Pirimicarb	Terbuthylazine
Bitertanol	Dicofol	Fenpropimorph	Isocarbophos	Pirimiphos-methyl	Tetraconazole
Boscalid	Diethofencarb	Fenpyrazamine	Isoprothiolane	**Prochloraz**	Tetradifon
Bromide ion	Difenoconazole	Fenpyroximate	Kresoxim-methyl	Procymidone	Thiabendazole
Bromopropylate	Diflubenzuron	Fenthion	Lambda-cyhalothrin	Profenofos	Thiacloprid
Bupirimate	Dimethoate	Fenvalerate	Linuron	Propamocarb	Thiamethoxam
Buprofezin	Dimethomorph	Fipronil	Lufenuron	Propargite	Thiophanate-methyl
Captan	Diniconazole	Flonicamid	Malathion	Propiconazole	Tolclofos-methyl
Carbaryl	Diphenylamine	**Fluazifop-P**	Mandipropamid	Propyzamide	Triadimefon
Carbendazim and benomyl	Dithianon	Flubendiamide	Mepanipyrim	Proquinazid	Triadimenol
Carbofuran	Dithiocarbamates	Fludioxonil	**Mepiquat**	Prosulfocarb	Thiodicarb
Chlorantraniliprole	Dodine	Flufenoxuron	Metalaxyl and **metalaxyl-M**	Prothioconazole	Triazophos
Chlorfenapyr	Emamectin benzoate B1a (emamectin)	Fluopicolide	Methamidophos	Pymetrozine	Tricyclazole
Chlormequat	Endosulfan	Fluopyram	Methidathion	Pyraclostrobin	Trifloxystrobin
Chlorothalonil	Epoxiconazole	Fluquinconazole	Methiocarb	Pyridaben	Triflumuron
Chlorpropham	Ethephon	Flusilazole	Methomyl	Pyridalyl	Vinclozolin
Chlorpyrifos	Ethion	Flutriafol	Methoxyfenozide	Pyrimethanil	
Chlorpyrifos-methyl	Ethirimol	Fluxapyroxad	Metrafenone	Pyriproxyfen	
Clofentezine	Etofenprox	**Folpet**	Monocrotophos	Quinoxyfen	

COMMISSION IMPLEMENTING REGULATION (EU) 2020/585 of 27 April 2020

concerning a coordinated multiannual control programme of the Union for 2021, 2022 and 2023 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin



Number of pesticides under Regulation (EC) No 396/2005:around 1300

Total number of compounds with MRL: ~460

European Reference Laboratory - FV

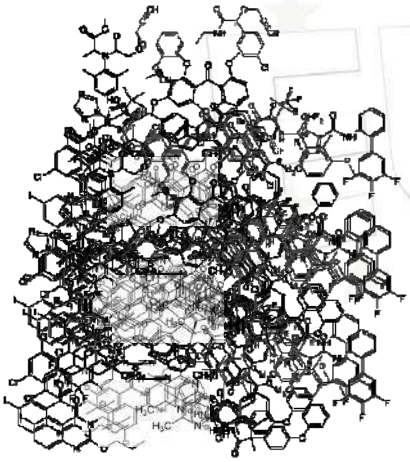
LC-QqQ-MS/MS and GC-QqQ-MS/MS

THEORETICAL IMPLEMENTATION

PRACTICAL IMPLEMENTATION

HUNDREDS OF COMPOUNDS

LC-ESI

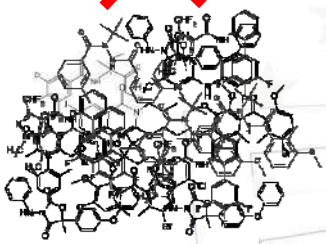


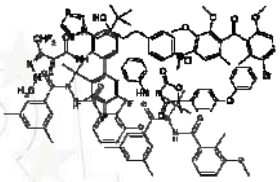
GC-EI

~~TARGET LIST~~

TARGET LIST

EXTRACTION



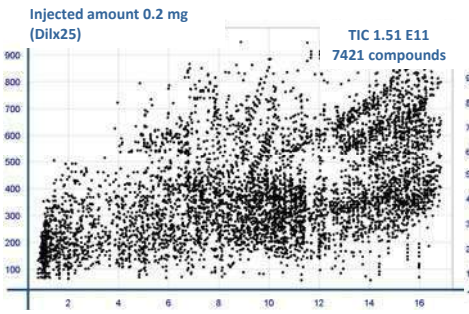


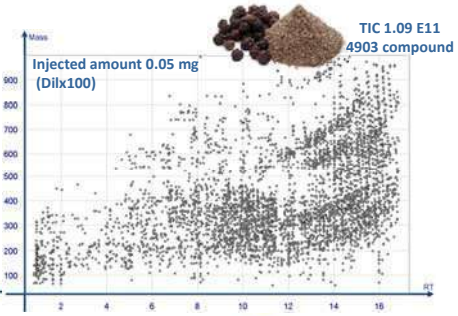
IS THE SCOPE ENOUGH?

Many commodities

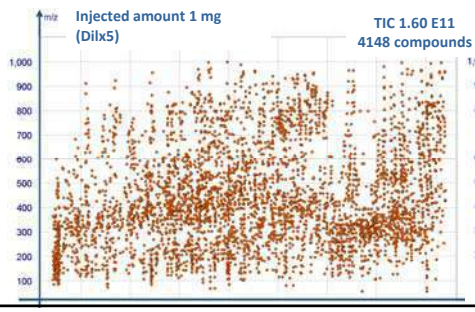
Co-extracted matrix components of Black Pepper

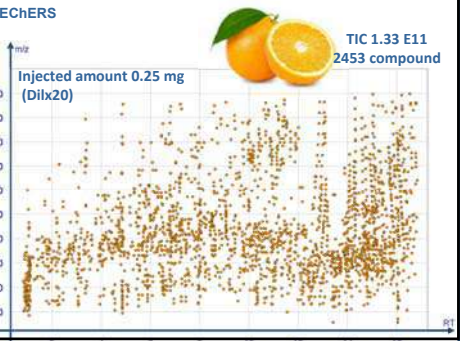
LC-TOF-MS, Citrate QuEChERS+EMR clean-up





Co-extracted matrix components of Orange LC-TOF-MS, Citrate QuEChERS





MANY LABORATORIES

NRLs = 31

OfLs = 134

EU GUIDELINES AND PROFICIENCY TESTS

SAMPLE PREPARATION

INSTRUMENTATION

CHROMATOGRAPHY

MASS SPECTROMETRY

EU GUIDELINES AND PROFICIENCY TESTS

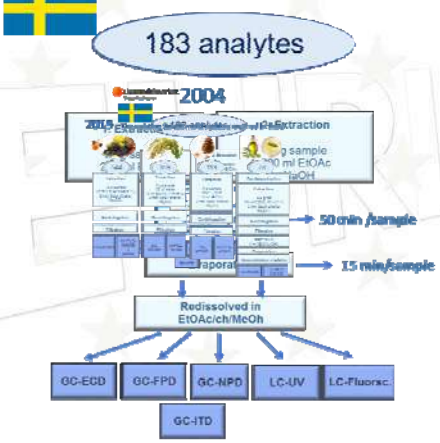
PROFICIENCY TESTS

2004–2019

GUIDANCE DOCUMENTS

SAMPLE PREPARATION

Livsmedelsverket 2004



10 g/ml (150 g)

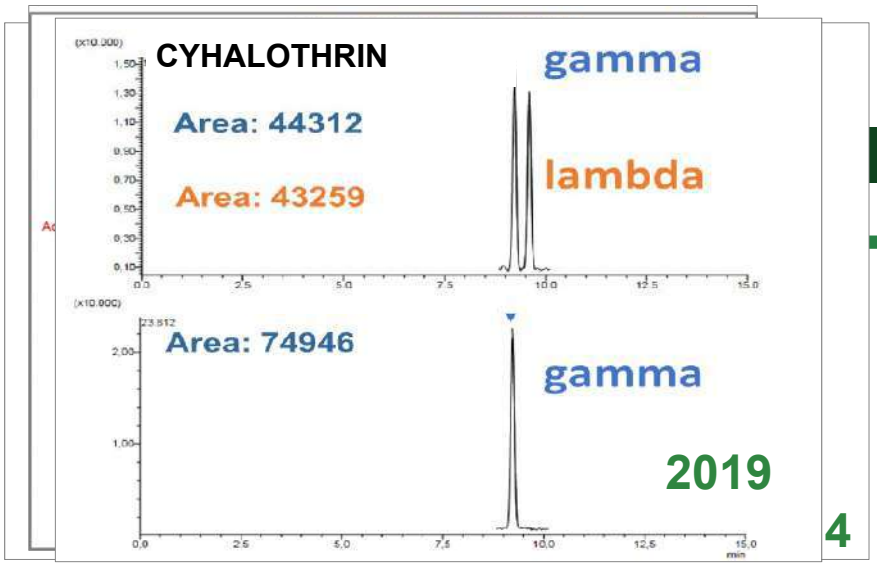
0.5 g/ml (10 g)

INSTRUMENTATION

CHROMATOGRAPHY

MASS SPECTROMETRY

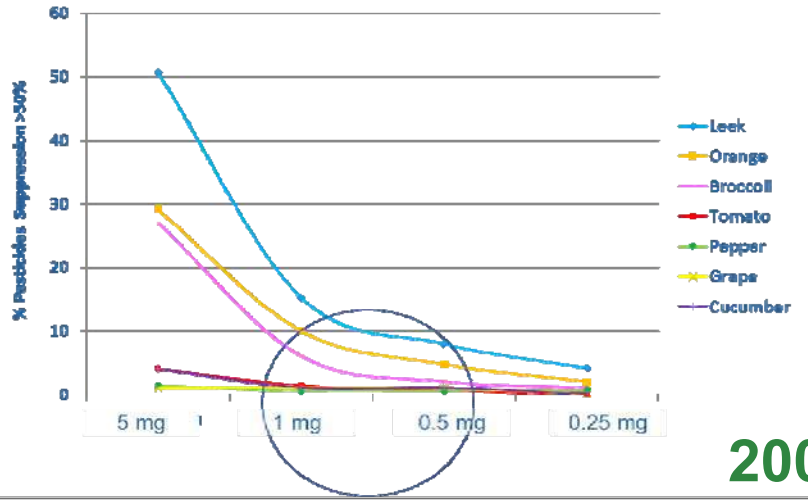
CHROMATOGRAPHY



MASS SPECTROMETRY

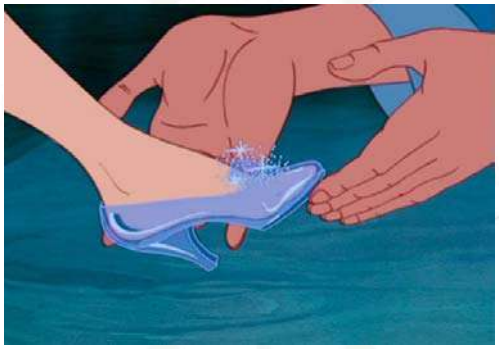
LC-TOF-MS

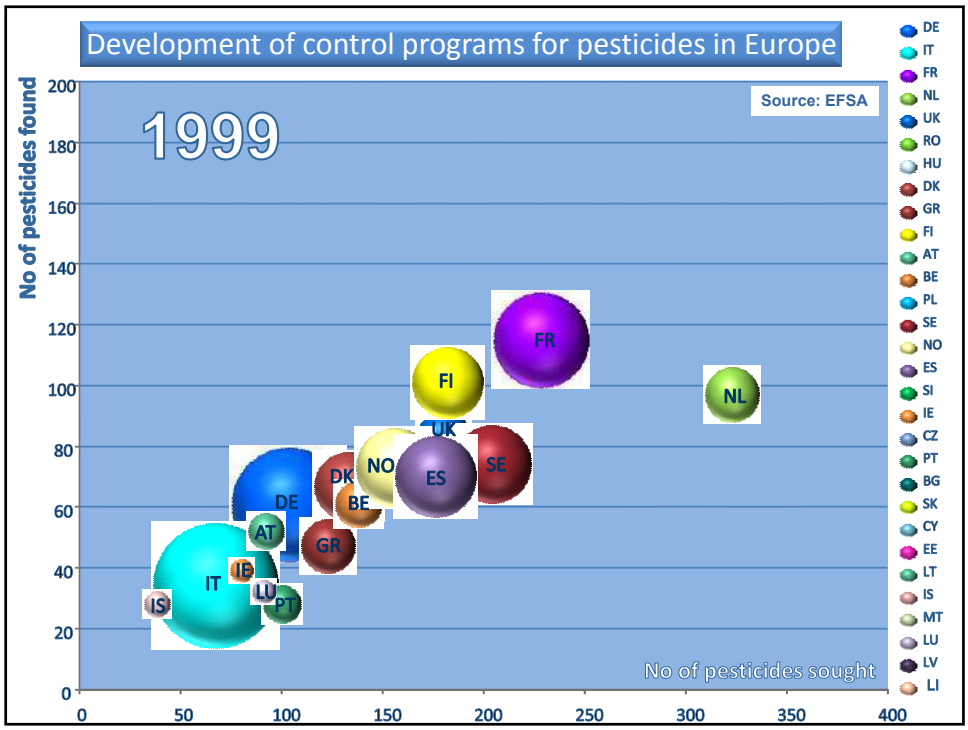
EFFECT OF DILUTION IN "EASY-MEDIUM" COMPLEX MATRICES

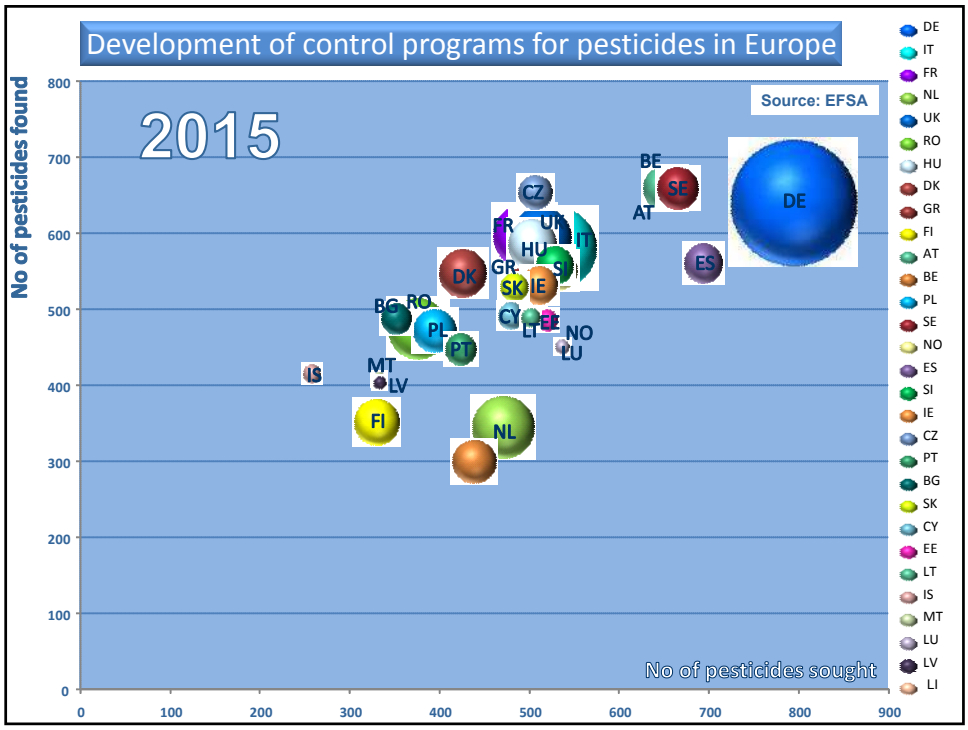


2008

WE NEED IDENTIFY RESIDUES WITHOUT KNOWING THE Rt?




Development of control programs for pesticides in Europe
1999
Source: EFSA
No of pesticides found
No of pesticides sought
0
20
40
60
80
100
120
140
160
180
200
0
50
100
150
200
250
300
350
400
DE
IT
FR
NL
UK
RO
HU
DK
GR
FI
AT
BE
PL
SE
NO
ES
SI
IE
CZ
PT
BG
SK
CY
EE
LT
IS
MT
LU
LV
LI


Development of control programs for pesticides in Europe
2015
Source: EFSA
No of pesticides found
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0
100
200
300
400
500
600
700
800
0
100
200
300
400
500
600
700
800
900
DE
IT
FR
NL
UK
RO
HU
DK
GR
FI
AT
BE
PL
SE
NO
ES
SI
IE
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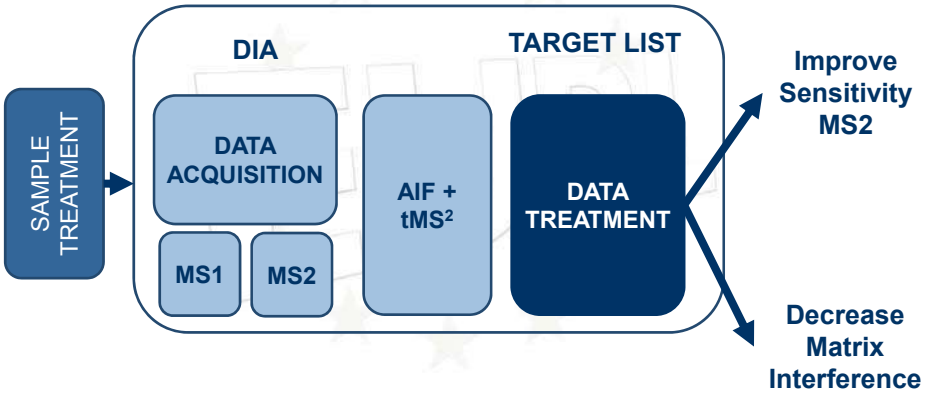
LU
LV
LI

IDENTIFICATION

FS + AIF (MS2) + Target MS2 (tMS2)

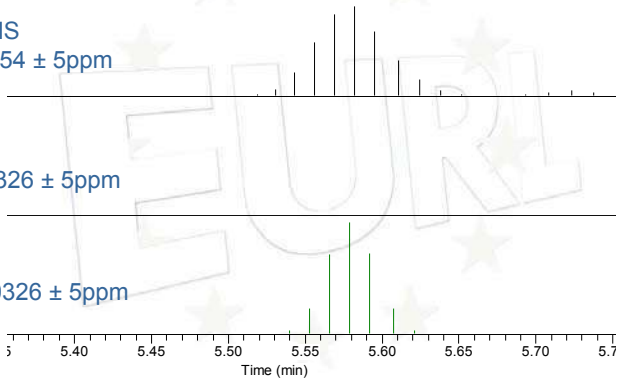


Simazine 0.01 mg/kg in orange

Full scan MS
m/z 202.0854 ± 5ppm

AIF MS2
m/z 132.0326 ± 5ppm

tMS2
m/z 132.0326 ± 5ppm



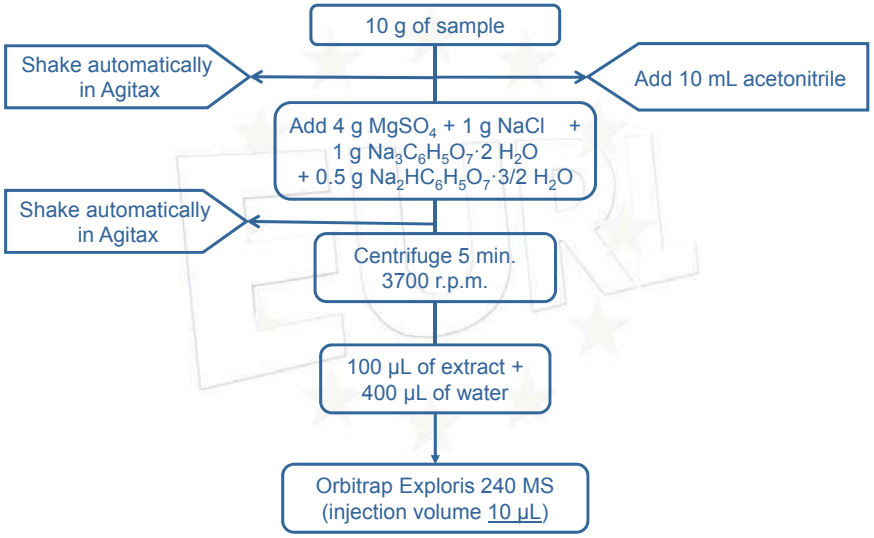
246 Compounds

Acephate	Chlorpyrifos	Fenamidone	Iprovalicarb	Paraoxon methyl	Quinoxyfen
Acetamiprid	Chromafenozide	Fenamiphos	Isocarbophos	Penconazole	Quizalofop-ethyl
Alachlor	Clofentezine	Fenamiphos-sulfone	Isoprocarb	Pencycuron	Rotenone
Albendazole	Clomazone	Fenamiphos-sulfoxide	Isoprothiolane	Pendimethalin	Simazine
Aldicarb	Clothianidin	Fenarimol	Isoproturon	Penflufen	Spinosyn A
Aldicarb-sulfone	Coumaphos	Fenazaquin	Isoxaflutole	Penthiopyrad	Spinosyn D
Aldicarb-sulfoxide	Cyazofamid	Fenbendazole	Kresoxim-methyl	Permethrin, trans-	Spinosyn J
Ametoctradin	Cyflufenamid	Fenbuconazole	Lenacil	Phenthoate	Spinosyn L
Anilofos	Cyhalofop-butyl	Fenhexamid	Linuron	Phosalone	Spirodiclofen
Atrazine	Cymoxanil	Fenobucarb	Malaoxon	Phosmet	Spiromesifen
Avermectin B1a	Cyproconazole	Fenoxycarb	Malathion	Phoxim	Spirotetramat
Azinphos-ethyl	Cyprodinil	Fenpropathrin	Mandipropamid	Pirimicarb	Spiroxamine
Azinphos-methyl	Cyromazine	Fenpropidin	Mepanipyrim	Pirimicarb, desmethyl-	Sulfoxaflor
Azoxystrobin	DEET	Fenpropimorph	Metalaxyl	Pirimiphos-methyl	Tebuconazole
BAC10	Demeton-S-methyl	Fenpyrazamine	Metamitron	Prochloraz	Tebufenozide
BAC8	Demeton-S-Methyl-Sulfone	Fenpyroximate	Metconazole	Profenofos	Tebufenpyrad
Benalaxyl	Demeton-S-methylsulfoxide	Fenthion	Methamidophos	Promecarb	Terbuthylazine
Bendiocarb	Diazinon	Fenthion-sulfone	Methidathion	Prometryn	Terbuthylazine-desethyl
Bifenazate	Dichlorvos	Fenthion-sulfoxide	Methiocarb	Propamocarb	Terbutryn
Bifenthrin	Dicrotophos	Fenuron	Methiocarb-sulfone	Propaquizafop	Tetraconazole
Bitertanol	Diethofencarb	Fipronili_POS	Methiocarb-sulfoxide	Propargite	Thiabendazole
Boscalid	Difenoconazole	Flazasulfuron	Methomyl	Propazine	Thiacloprid
Bromacil	Difenoxuron	Flonicamid	Methoxyfenozide	Propiconazole	Thiamethoxam
Bromuconazole	Diflubenzuron	Fluacrypyrim	Metobromuron	Propoxur	Thiobencarb
BTS_44595	Dimethoate	Fluazifop	Metolachlor	Propyzamide	Thiodicarb
BTS_44596	Dimethomorph	Flufenacet	Metolcarb	Proquinazid	Thiophanate-methyl
BTS-40348	Dimethylvinphos, Z-	Flufenoxuron	Metrafenone	Prosulfocarb	Tolfenpyrad
Bupirimate	Diniconazole	Fluometuron	Monocrotophos	Prothioconazole	Triadimefon
Buprofezin	Diuron	Fluopicolide	Monolinuron	Pymetrozine	Triadimenol
Butoxycarboxim	Dodine	Fluopyram	Monuron	Pyraclostrobin	Triallate
Carbaryl	Edifenphos	Flusilazole	Myclobutanil	Pyrethrini	Triazophos
Carbendazim	Emamectin B1a	Fluxapyroxad	Neburon	Pyrethrinii	Trichlorfon
Carbofuran	Epoxiconazole	Formetanate	Nitenpyram	Pyridaben	Triclocarban
Carbofuran, 3OH-	Ethiofencarb	Fosthiazate	Novaluron	Pyridalyl	Tricyclazole
Chlorantraniliprole	Ethion	Haloxyfop	Omethoate	Pyridaphenthion	Trifloxystrobin
Chlorbromuron	Ethiprole	Hexaconazole	Oxadiargyl	Pyridate	Triflumizole
Chlorfenvinphos, B-	Ethirimol	Hexaflumuron	Oxadixyl	Pyrimethanil	Triflumuron
Chlorfluazuron	Ethoprophos	Hexythiazox	Oxamyl	Pyriofenone	Triticonazole
Chloridazon	Etofenprox	Imazalil	Oxasulfuron	Pyriproxyfen	Tritosulfuron
Chlorotoluron	Etoxazole	Imidacloprid	Oxfendazole	Quinalphos	XMC
Chloroxuron	Famoxadone	Indoxacarb	Paclobutrazol	Quinoclamine	Zoxamide

246 target Pesticides – 63 Acquired with tMS2

Acetamiprid	Fenuron	Pendimethalin
Aldicarb-sulfoxide	Flonicamid	Permethrin, trans-
Avermectin B1a	Fluazifop	Phoxim
Bendiocarb	Fluometuron	Propargite
Bitertanol	Hexaflumuron	Propoxur
BTS-40348	Iprovalicarb	Propyzamide
Carbaryl	Isocarbophos	Prosulfocarb
Chlorfluazuron	Isoprocarb	Prothioconazole
Chloridazon	Isoxaflutole	Pymetrozine
Clothianidin	Linuron	Pyrethrin
Cymoxanil	Metamitron	Quinoclamine
Demeton-S-methyl	Methiocarb	Simazine
Diethofencarb	Methiocarb-sulfone	Sulfoxaflor
Dodine	Methiocarb-sulfoxide	Tebufenozide
Ethiofencarb	Methomyl	Triadimefon
Ethirimol	Methoxyfenozide	Triadimenol
Famoxadone	Metolcarb	Trichlorfon
Fenhexamid	Nitenpyram	Triclocarban
Fenpropathrin	Oxadiargyl	Triflumizole
Fenpropidin	Oxamyl	Tritosulfuron
Fenpropimorph	Paraoxon methyl	XMC

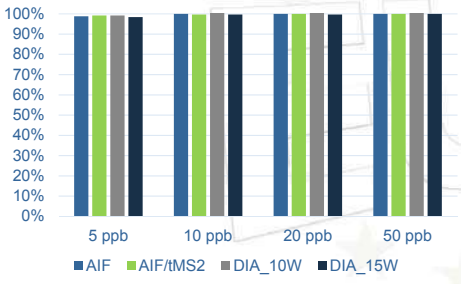
QuEChERS Extraction Method



Bell Pepper

246 pesticides

DETECTED



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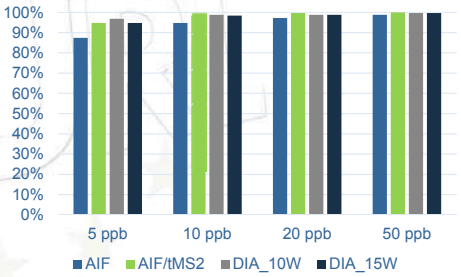
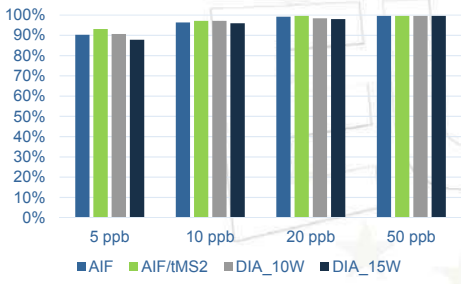


Identified in full compliance with the EU SANTE guideline criteria

Onion

246 pesticides

DETECTED



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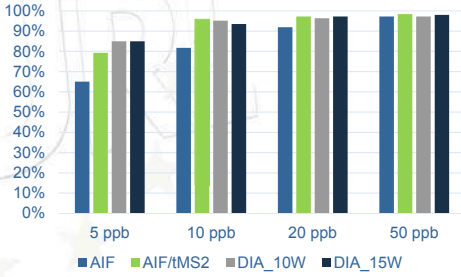
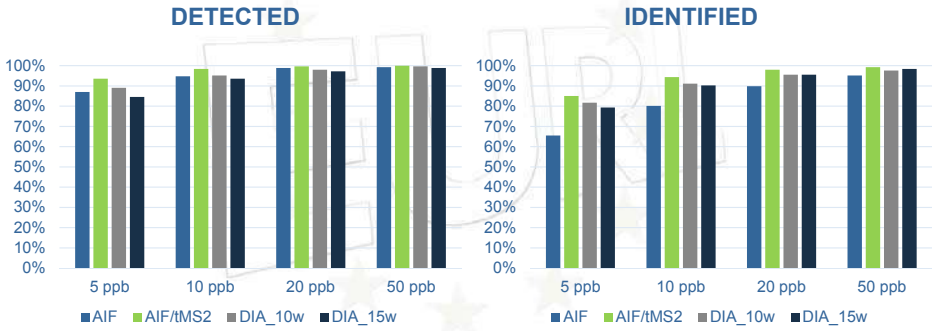


Identified in full compliance with the EU SANTE guideline criteria

Orange

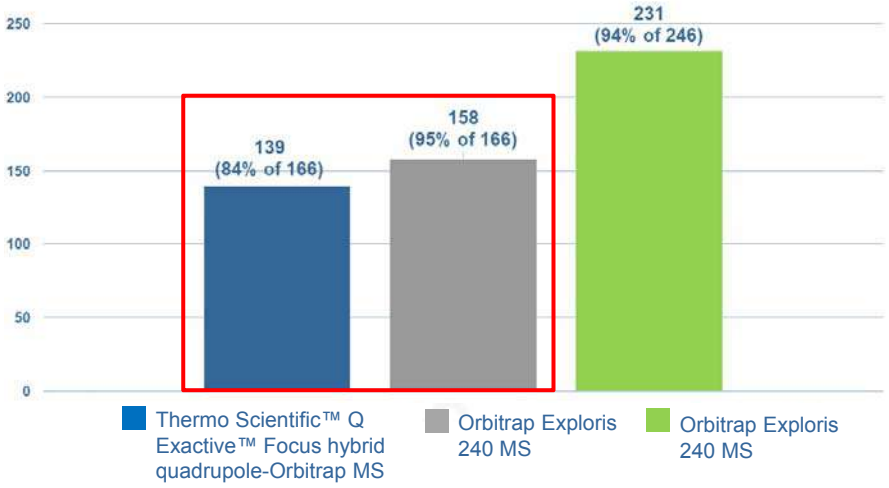
246 pesticides



Identified in full compliance with the EU SANTE guideline criteria

Increasing the scope

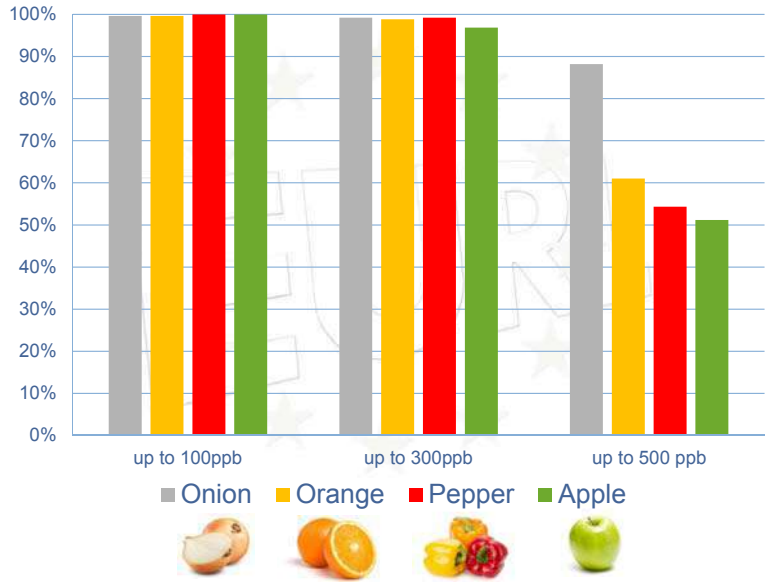
Identified pesticides/10ppb in Orange



Acquistion modes: QE Focus (FS/AIF), Orbitrap Exploris 240 MS (FS/AIF/tMS2)

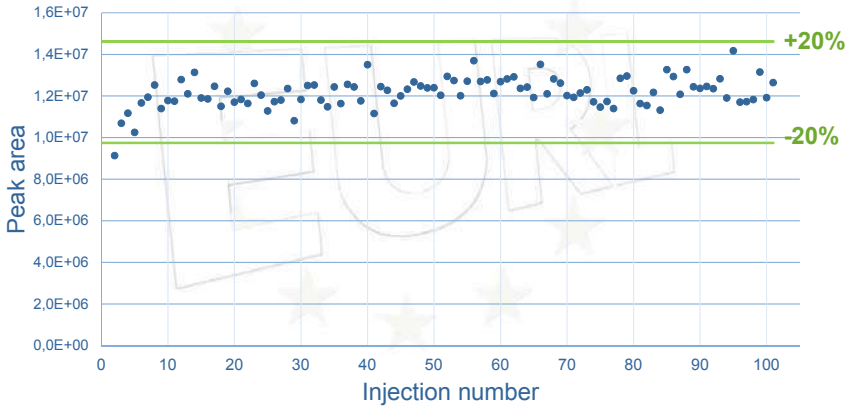
Quantitation

Linearity



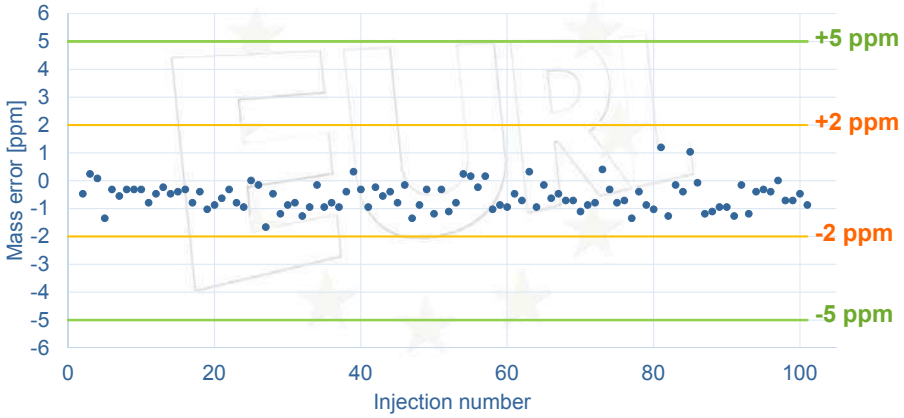
ROBUSTNESS

0.01 mg/kg of carbendazim in orange



0.01 mg/kg of carbendazim in orange

Mass Error

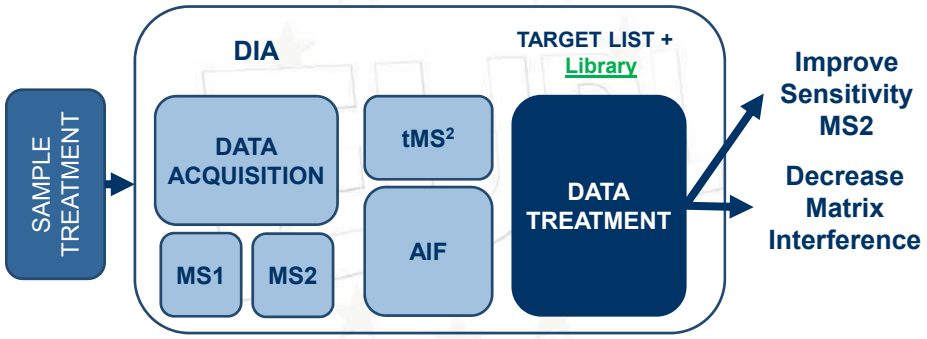


Thermo Scientific EASY-IC™ (internal calibration) ion source

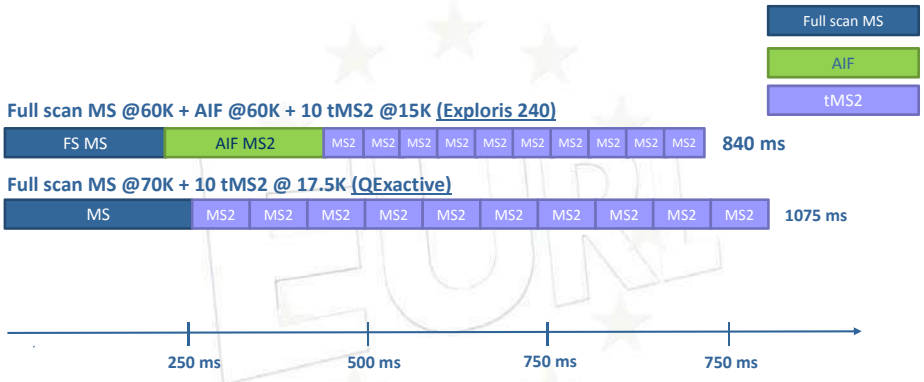
European Reference Laboratory - FV

SCREENING

Screening using a Target List & Database



Comparison of cycle times



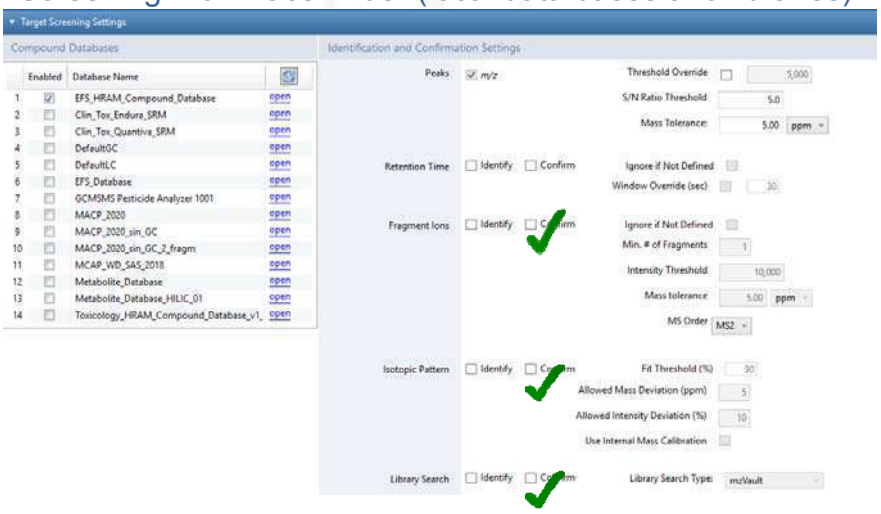
Orbitrap Exploris 240 MS

Vs

Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ MS systems (Classic & Plus)

Screening

Screening with TraceFinder (local data bases and libraries)



SAMPLE	MATRIX	TARGET/QUANTITATIVE	SCREENING
001	Kiwi		
002	Kiwi		
003	Kiwi		
004	Kiwi	Phosmet	
005	Kiwi		
006	Kiwi		
007	Kiwi		
008	Kiwi		Forchlorfenuron
009	Kiwi	Boscalid	Forchlorfenuron
010	Onion		Beauvericin; Penicillic-Acid
011	Onion		
012	Onion		
013	Onion		
014	Onion		Beauvericin
015	Onion		Beauvericin_M+NH4; Penicillic-Acid
016	Onion		
017	Onion		
018	Onion		
019	Onion		
020	Onion		
021	Onion		
022	Onion		Beauvericin
023	Onion	Target/Quantitative	
024	Onion		
025	Onion		Beauvericin
026	Orange	Imazalil	
027	Orange	Acetamiprid; Fenpyroximate; Pyrimethanil	Acetamiprid-metabolite-IM-2-1
028	Orange	Pyrimethanil; Thiabendazole;Imazalil	
029	Orange	Acetamiprid; Pyrimethanil; Propiconazole; Imazalil	
030	Orange	Imazalil	
031	Orange	Imidacloprid; Pyriproxyfen	Imidacloprid,desnitro; Imidacloprid,desnitro-olefin
032	Orange	Pyriproxyfen; Imidacloprid	Imidacloprid,desnitro; Imidacloprid,urea
033	Orange	Acetamiprid; Imazalil	
034	Orange	Acetamiprid; Pyriproxyfen	Acetamiprid-metabolite-IM-2-1
035	Orange	Hexythiazox; Pyriproxyfen; Imazalil	
036	Orange	Thiabendazole; Propiconazole; Imazalil	
037	Orange	Acetamiprid; Pyrimethanil; Imazalil	
038	Orange	Acetamiprid	
039	Orange		
040	Orange	Acetamiprid; Fenpyroximate	
041	Orange		
042	Orange	Acetamiprid; Etofenprox; Phosmet; Imazalil	
043	Orange	Acetamiprid; Imazalil	
044	Orange	Thiabendazole; Pyrimethanil; Imazalil	Imidacloprid,desnitro; Thiabendazole,5OH
045	Orange	Pyriproxyfen; Imazalil	
046	Orange		
047	Orange		Imidacloprid,desnitro
048	Pineapple		Beauvericin_M+NH4
049	Raisins	Famoxadone; Fluxapyroxad; Indoxacarb; Mandipropamid; Metalaxyl; Methoxyfenozide; Metrafenone; Penconazole; Proquinazid; Pyrimethanil; Sulfoxaflor; Tebuconazole; Zoxamide	
050	Strawberry	Boscalid; Fluopyram	

CONCLUSIONS

- Conservative analytical methods cannot cover the expectations in food control for pesticide residue analysis. Therefore innovative approaches and technologies are necessary and running permanently.
- Important difficulties are presented consequence of the large number of pesticide residues, commodities. High sensitivity is a key factor to overcome frequent problems encountered.
- Harmonization of official laboratory results based on performing regular proficiency tests, combined with common Quality Control issues are necessary.
- Advanced mass spectrometry platforms in special HRMS coupled to different chromatographic modes are very promising for the future for increasing the scope of analysis in a very effective way without loosing in the strict quantitative control.

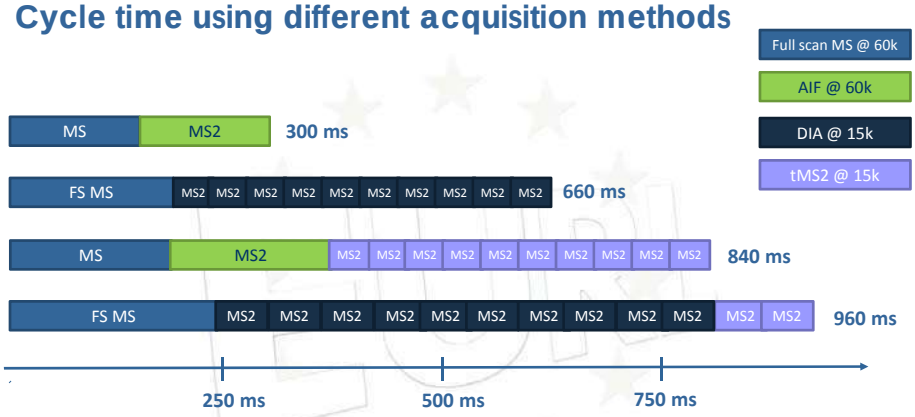
European Reference Laboratory - FV

EURL-FV

Thank you for your kind attention!

www. eurl-pesticides.eu

Cycle time using different acquisition methods



Observed cycle time

- FS/AIF – 300 ms
- FS/DIA – 660 ms
- FS/AIF/tMS2 – 840 ms
- FS/DIA/tMS2 – 960 ms

SMART TECH for FOOD (ST4F)
November 25th 2020

Pharmaceuticals and other emerging contaminants in European seafood samples

Damià Barceló, Ethel Eljarrat, Diana Alvarez-Muñoz, Sara Rodriguez-Mozaz & Antonio Marques

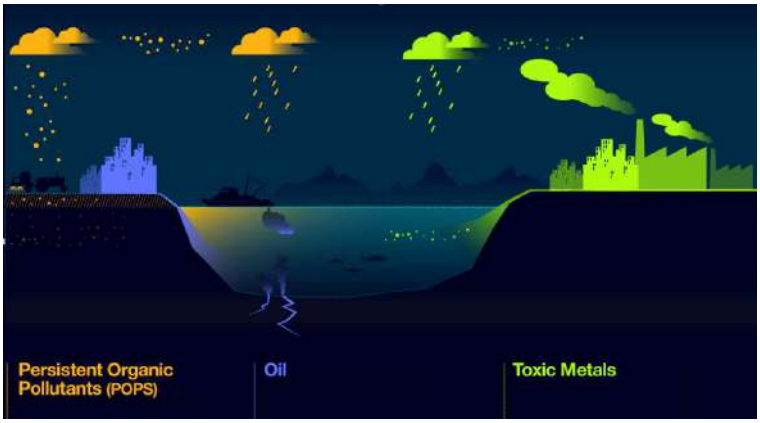
1

Summary

2

Introduction

Marine ecosystems, in particular coastal areas, are severely stressed in many parts of the world as a result of overpopulation, intense coastal development, urbanization, spiralling resource use, pollution, and the spread of invasive species.



3

D. Alvarez-Muñoz et al., Environ. Research. 2015, 143:56–64.

Case Study I.
Bioaccumulation of PhACS, EDCs and FR in seafood of European Hotspots

D. Álvarez-Muñoz[a], S.Rodríguez-Mozaz[a], A.L.Maulvault[b], A.Tediosi[c], M. Fernández-Tejedor[d], F.VandenHeuvel[e], M.Kotterman[f], A.Marques[b], D.Barceló[a,g]

[a]ICRA, Girona, Spain
[b]IPMA, Lisbon, Portugal
[c]AIFORIA, Italy
[d]IRTA, Tarragona, Spain
[e]HORTIMARE, Netherlands
[f]IMARES, Wageningen, Netherlands
[g]IDAEA-CSIC Barcelona, Spain

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PhACS, EDCs and FR in seafood of European Hotspots

Pharmaceutical and personnel care products

Endocrine disruptors

Environmental contaminants in seafood

Marine litter

Toxic metals

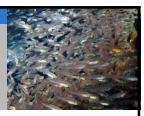
Biotoxins

Brominated flame retardants

ECsafeSEAFOOD: FP7- KBBE.2012.2.4-01 (2013-2017)
Assessment of food safety issues related to priority contaminants present in seafood as a result of environmental contamination and evaluate their impact on public health

5

PhACS, EDCs and FR in seafood of European Hotspots



"Everything contains chemicals"

- **Pharmaceuticals (PhACs)**
- **Endocrine Disrupting Compounds (EDCs)**
- **Brominated Flame Retardants (BFRs)**

Contaminants of Emerging Concern
Large volumes of production

Population exposed

Directly: through the use of essential products in daily life

Indirectly: due to incomplete removal after use these compounds can reach the aquatic environment, potentially being accumulated in organisms that later on can be eaten.

SEAFOOD

6

PhACS, EDCs and FR in seafood of European Hotspots

Sampling sites

7

PhACS, EDCs and FR in seafood of European Hotspots

Monitoring campaign in seafood from Hot spots in Europe

Sampling Species

•Macroalgae
(20 organisms)

Saccharina latissima

Laminaria digitata

•Bivalve
(50 organisms)

Mytilus spp

Chamalea gallina

Crassostrea gigas

•Fish
(25 organisms)

Liza aurata

Platichthys flesus

➢**Pool => Grinded => homogenized => freeze-dried => kept at -20ºC until its analysis.**

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3. PhACs in BIOTA. Target Analysis

Analytical Method

MOLLUSKS

PLE Extraction

SPE Purification

Analysis by UHPLC-MS/MS

- 500 mg of freeze-dried sample placed in PLE cells
- Extraction applied in 3 cycles of 5 min, with a Methanol/water (1:2) as solvent. 50ºC
- Extracts were diluted to 100 ml and purified by SPE Polymeric Sorbent
- Eluates in MeOH collected and evaporated
- Final extracts analyzed by UHPLC/QqLIT

Alvarez-Muñoz et al. Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves and fish from coastal areas in Europe. ***Environmental Research*** *143 (2015) 56–64*

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3. PhACs in BIOTA. Target Analysis

Analytical Method

Therapeutic family	Compound	Precursor ion	RT (min)	Internal standard
Antibiotics	Ronidazole	201 [M+H]+	1.28	Ronidazole-d3
	Metronidazole	172 [M+H]+	1.31	Ronidazole-d3
	Dimetridazole	142 [M+H]+	1.53	Ronidazole-d3
	Sulfamethoxazole	254 [M+H]+	2.02	Sulfamethoxazole- d4
(Metabolite)	N-acetyl-sulfamethoxazole	296 [M+H]+	2.43	Sulfamethoxazole- d4
	Azythromycin	749 [M+H]+	2.88	Azithromycin-d3
	Erythromycin	734 [M+H]+	3.46	Erythromycin-N,N13C2
Psychiatric drugs	Venlafaxine	278 [M+H]+	2.81	Venlafaxine-d6
(Metabolite)	O-demethyl-venlafaxine	264 [M+H]+	2.19	Venlafaxine-d6
	Carbamazepine	237 [M+H]+	3.26	Carbamazepine-d10
(Metabolite)	10,11-Epoxycarbamazepine	253 [M+H]+	2.79	Carbamazepine-d10
(Metabolite)	2-Hydroxycarbamazepine	253 [M+H]+	2.77	Carbamazepine-d10
	Citalopram	325 [M+H]+	2.96	Citalopram-d4
	Alprazolam	309 [M+H]+	3.50	Diazepam-d5
Analgesics/anti-inflammatories	Codeine	300 [M+H]+	1.41	Carbamazepine-d10
	Phenazone	189 [M+H]+	2.12	Phenazone-d3
	Propyphenazone	231 [M+H]+	3.27	Phenazone-d3
	Piroxicam	330 [M-H]-	1.02	Meloxicam-d3
Tranquilizer	Azaperone	328 [M+H]+	2.50	Azaperone-d4

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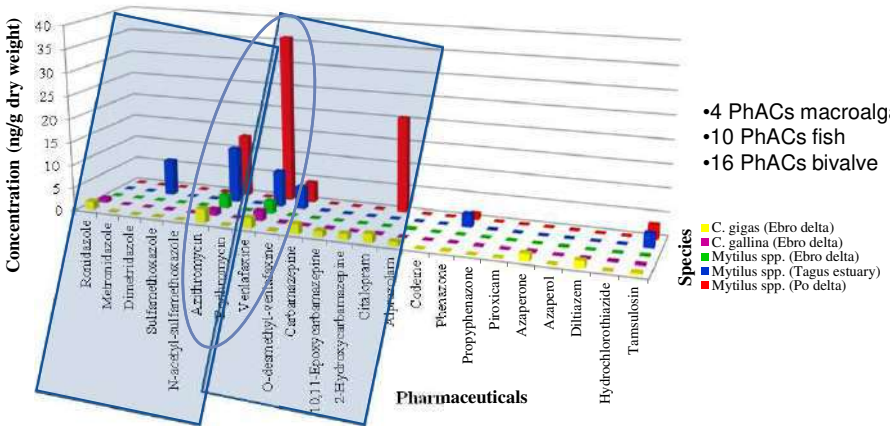
PhACS, EDCs and FR in seafood of European Hotspots

Monitoring campaign in seafood from Hot spots in Europe



- 4 PhACs macroalgae
- 10 PhACs fish
- 16 PhACs bivalve

➢ **Azithromycin and velanfaxine present in all analysed samples**

Alvarez-Muñoz et al. Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves and fish from coastal areas in Europe. ***Environmental Research*** *143 (2015) 56–64*

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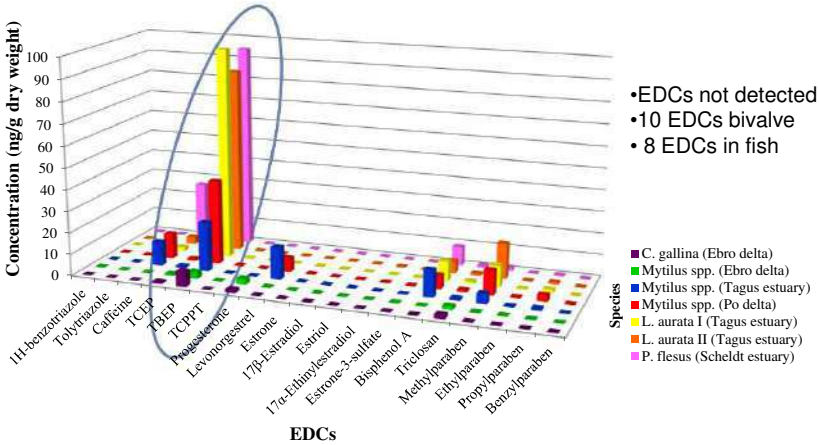
PhACS, EDCs and FR in seafood of European Hotspots

Monitoring campaign in seafood from Hot spots in Europe



- EDCs not detected macroalgae
- 10 EDCs bivalve
- 8 EDCs in fish

➢ **Tris(2-butoxyethyl) phosphate (TBEP) present in all analysed sam**

Alvarez-Muñoz et al. Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves and fish from coastal areas in Europe. ***Environmental Research*** *143 (2015) 56–64*

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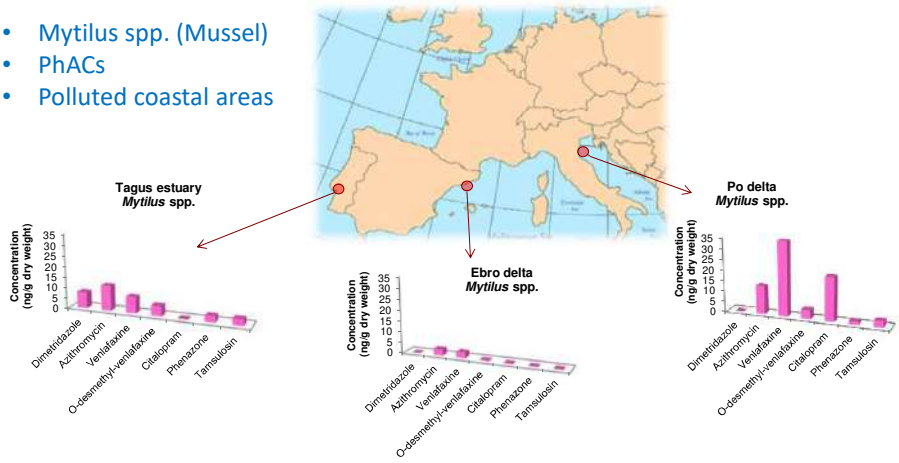
PhACS, EDCs and FR in seafood of European Hotspots

Monitoring campaign in seafood from Hot spots in Europe

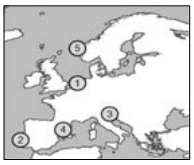
- Mytilus spp. (Mussel)
- PhACs
- Polluted coastal areas



13

PhACS, EDCs and FR in seafood of European Hotspots

Monitoring campaign in seafood from Hot spots in Europe



Hotspot locations: (1) Western Scheldt, (2) Tagus estuary, (3) Po delta and (4) Ebro delta

	S1	S2	S2	S2	S3	S4	S4	Freq. of det. (%)
	Flounder	Mullet	Mullet	Mussel	Mussel	Mussel	Clam	
BDE-28	n. d.	8.42	n. d.	35.0	n. d.	n. d.	n. d.	22
BDE-47	39.9	115	48.7	420	15.2	n. d.	n. d.	56
BDE-100	n. d.	107	33.4	410	n. d.	n. d.	n. d.	33
BDE-99	n. d.	61.0	n. d.	507	n. d.	n. d.	n. d.	22
BDE-154	n. d.	59.4	n. d.	262	n. d.	n. d.	n. d.	22
BDE-153	n. d.	n. d.	n. d.	246	n. d.	n. d.	n. d.	11
BDE-209	n. d.	n. d.	n. d.	367	n. d.	56.1	n. d.	22
∑PBDEs (lw)	**39.9**	**350**	**82.1**	**2,246**	**15.2**	**56.1**	**n. d.**	**67**
∑PBDEs (ww)	**0.46**	**3.91**	**1.42**	**6.04**	**0.21**	**0.69**	**n. d.**	**67**
γ-HBCD	n. d.	n. d.	n. d.	n. d.	13.8	n. d.	n. d.	11
HBB	n. d.	n. d.	n. d.	338	n. d.	n. d.	n. d.	11
PBEB	n. d.	3.70	n. d.	13.9	n. d.	n. d.	n. d.	22
DBDPE	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	22
Dec 602	n. d.	n. q.	n. q.	n. q.	n. d.	n. q.	n. d.	44

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Conclusions

- Occurrence of PhACs and EDCs in highly consumed and/or ecologically relevant seafood species from hot spots in Europe
- The highest levels**: psychiatric drug** venlafaxine and **antibiotic** azithromycin in mussel samples and **TBEP** in fish.
- Levels of contaminants in mussels allows pointing out most contaminated spot: Po Delta > Tagus Estuary > Ebro Delta
- PBDEs were found in 67% of the samples with total levels of 39.9-2,246 ng/g lw and 0.46-6.04 ng/g ww.
- PBDEs/non-PBDE-FRs ratios were 1.10-94.8, showing that PBDEs are still more abundant than the Emerging BFRs. This trend is expected to be inverted in the years to come.
- The samples from the **Tagus estuary** present higher PBDE levels than the samples from the rest of the hotspots. This estuary is located in a more industrialized and populated region than the other hotspots.

15

D. Alvarez-Muñoz et al., Environ. Inter. 2018, 119: 570-581

O. Aznar-Alemany et al., Food and Chem. Toxicol. 2017, 104: 35-47

Case Study II

Human exposure to contaminated seafood

D. Álvarez-Muñoz[a b], Oscar Aznar-Alemany [b], S.Rodríguez-Mozaz[a] , E. Eljarrat [b], A.Marques[c], D.Barceló[a,b] et al.

[a]ICRA, Girona, Spain
[b]IDAEA-CSIC Barcelona, Spain
[c]IPMA, Lisbon, Portugal
[d]ILVO, Oostende, Belgium
[e]IRTA, Tarragona, Spain
[f]AIFORIA, Italy
[g]HORTIMARE, Netherlands
[h]REQUIMTE, Porto, Portugal
[i]IMARES, Wageningen, Netherlands
[j]Universitat Rovira i Virgili, Tarragona, Spain
[k]IGhent University, Belgium
[l]Technical University of Denmark, Soeborg

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Human exposure to contaminated seafood

Monitoring campaign in seafood from Hot spots in Europe

Results

Based on the frequency of detection and levels found:
- **8 EDCs** out of 20 analysed selected for further analysis in commercial samples
- **10 PhACs** out of 20 analysed selected for further analysis in commercial samples

Compound type	Family	Prioritization proposed
PhACs	Analgesics/anti-inflammatories	**Diclofenac**
	Antibiotics	**Azithromycin**
	β-Blockers	**Metroprolol, carazolol**
	Diuretic	**Hydrochlorothiazide**
	Prostatic hyperplasia	**Tamsulosin**
	Psychiatric drugs	**Venlafaxine, citalopram, CBZ, diazepam**
EDCs	Stimulants	**Caffeine**
	Flame retardants	**TCEP, TBPE**
	Plasticizers	**Bisphenol A**
	Antibacterials	**Triclosan**
	Preservatives	**Methylparaben, ethylparaben, propylparaben**

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Human exposure to contaminated seafood

Monitoring campaign in seafood from Hot spots in Europe

Monitoring campaign in commercial seafood in Europe

- **Sampling survey**: autumn 2014 (1st round) and spring 2015 (2nd round).
- **11 European countries**: Portugal, Spain, Italy, Greece, The Netherlands, Scotland, Denmark, Norway, Belgium, France and Ireland.
- **12 high consumed seafood types**: mackerel, tuna, cod, perch, pangasius, sole, seabream, plaice, salmon, mussels, shrimp and brown crab.
- **European markets** although some of them were imported from other locations.
- The specimens were of similar size and they satisfied the legal requirements for **human consumption**.

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Human exposure to contaminated seafood

Monitoring campaign in commercial seafood in Europe

- •Fish (25 organisms)
- •Bivalves (50 organisms)
- • Crustaceans (25 crab, 50 shrimp)

→ Fillets

→ Edible meat

1) Pool RAW
2) Pool COOKED by steaming (15min, 105ºC)

✓**Grinded**
✓**Homogenized**
✓ **Frozen**
✓ **Freeze-dried**
✓**Kept at -20ºC until analysis**

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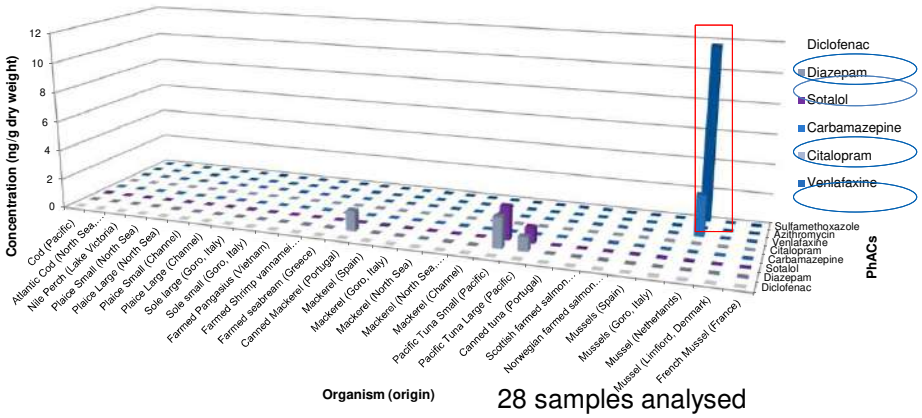
Human exposure to contaminated seafood

Monitoring campaign in commercial seafood in Europe

Results

➢PhACs levels in raw samples from the 1st Round



28 samples analysed
9 samples with at least 1 PhAC detec
4 samples with quantifiable levels

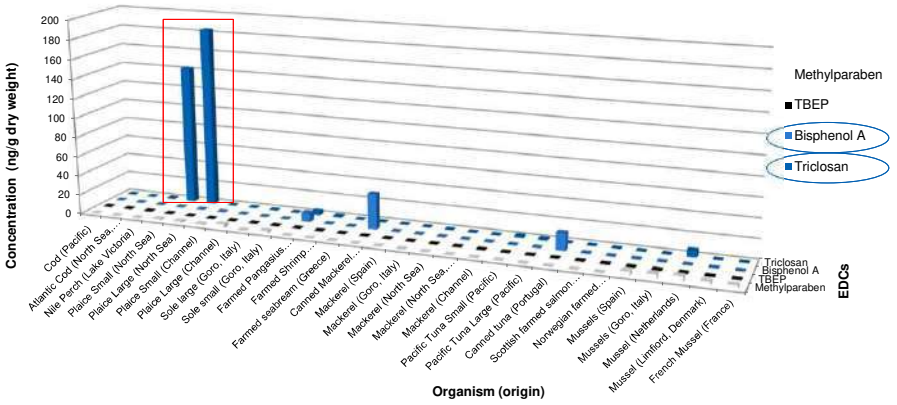
20

Human exposure to contaminated seafood

Monitoring campaign in commercial seafood in Europe

Results

➢EDCs levels in raw samples from the 1st Round



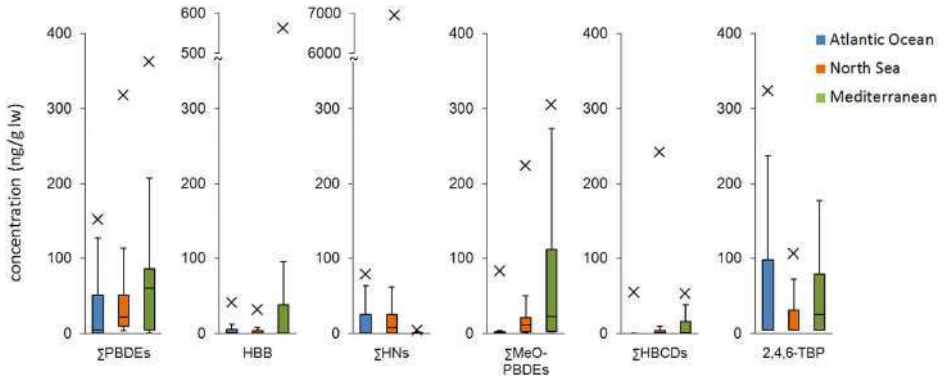
28 samples analysed
In all samples at least 1 EDC detected
17 samples with EDCs levels quantifiable

21

Human exposure to contaminated seafood

HALOGENATED FLAME RETARDANTS

Box plots for concentrations in raw samples from different locations (ng/g lw)



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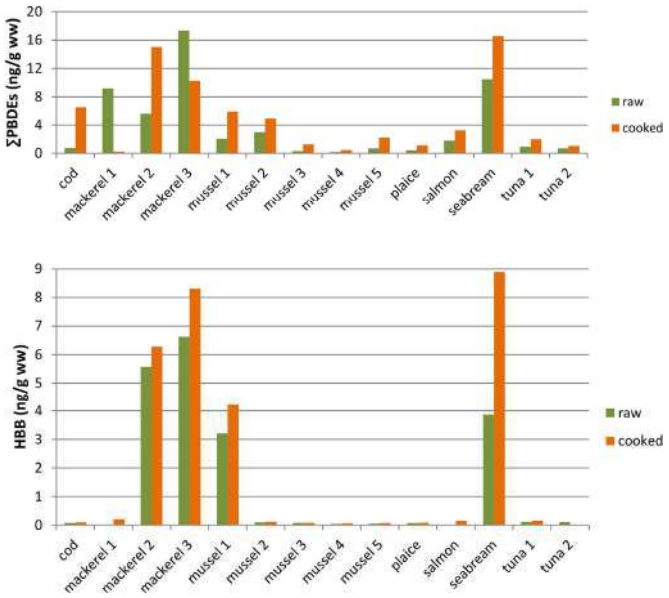
Human exposure to contaminated seafood

Effect of COOKING



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Human Risk Assessmen. Food safety

Water Contamination Sources

SEAFOOD

Pollutants Concentration

bisphenol A
methylparaben
triclosan
BDE-47, BDE-99,
TBBPA, HBCD

Calculated human diet exposure
µg/kg bw/week

Acceptable Daily Intake (ADI) *
µg/kg bw/week

* ECHA; EFSA; FAO; WHO

Human Health Risk Assessment (ERA)

- Highest human exposure: Portugal and Spain
- Human Risk: None of the contaminants
  BUT
  BDE-99?

Alvarez-Muñoz et al. ***Environment International*** *119 (2018) 570–581*
Aznar-Alemany et al. ***Food and Chemical Toxicology*** *104 (2017) 35-47*

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Conclusions

➢In the majority of the **commercial seafood:**

- **PhACs** below quantification limits.
- **EDCs** at 0.28 to 183.8 ng/g dry weight.
- **HFRs** were found in 90.5% of the seafood samples at nd-356 ng/g lw (nd-41.1 ng/g ww). **Mussels** and **seabreams** showed the highest occurrences and concentrations.

➢**Human health risk assessment**:

- Highest EDCs intake via seafood consumption in Spain
- EDCs Intake < guidance values no health risk via seafood consumption
- Other dietary and non-dietary sources of exposure increase exposure but no potential health risk to European consumers
- no health risk related to the exposure to BFRs via seafood consumption. However, a refined risk assessment for BDE-99 is of interest in the future.

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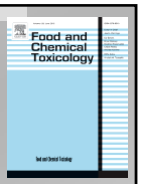
Alves, R.N. et al. Food and Chemical Toxicology, 104, (2017) 69-78



Case Study III

bioaccessibility of emerging pollutants in raw and cooked seafood

D. Álvarez-Muñoz[a,b], Oscar Aznar-Alemany[b], S.Rodriguez-Mozaz[a], E. Eljarrat[b], A.Marques[c], D.Barceló[a,b] et al.

[a]ICRA, Girona, Spain
[b]IDAEA-CSIC Barcelona, Spain
[c]IPMA, Lisbon, Portugal
[d]ILVO, Oostende, Belgium
[e]IRTA, Tarragona, Spain
[f]AIFORIA, Italy
[g]HORTIMARE, Netherlands
[h]REQUIMTE, Porto, Portugal
[i]IMARES, Wageningen, Netherlands
[j]Universitat Rovira i Virgili, Tarragona, Spain
[k]Ghent University, Belgium
[l]Technical University of Denmark, Soeborg

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Conclusions

- In the majority of the **commercial seafood:**
  - **PhACs** below quantification limits.
  - **EDCs** at 0.28 to 183.8 ng/g dry weight.
  - **HFRs** were found in 90.5% of the seafood samples at nd-356 ng/g lw (nd-41.1 ng/g ww). **Mussels** and **seabreams** showed the highest occurrences and concentrations.
- **Cooking by steaming:**
  - no effect on **PhACs** levels,
  - increased the levels of **EDCs**.
  - the cooking process concentrated **PBDEs** and **HBB**.
- **Human health risk assessment**:
  - Highest EDCs intake via seafood consumption in Spain
  - EDCs Intake < guidance values no health risk via seafood consumption
  - Other dietary and non-dietary sources of exposure increase exposure but no potential health risk to European consumers
  - no health risk related to the exposure to BFRs via seafood consumption. However, a refined risk assessment for BDE-99 is of interest in the future.

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Acknowledgements

- 7th EU-FP (**ECsafeSEAFOOD** project; GA nº 311820) and the European Regional Development Fund (FEDER)
- **ECsafeSEAFOOD** FP7/2007-2013
- **CERCA** program
- **Ramon y Cajal** program (RYC-2014-16707)
- Economy and Knowledge Department of the Catalan Government (Consolidated Research Group **ICRA-ENV 2017 SGR 1124**)

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Thanks for your attention!

NATIONAL GEOGRAPHIC
Art: Oliver Uberti. Photo: Rebecca Hale

SMART TECH for FOOD (ST4F)
November 25th 2020

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FoodSmartphone

Analysis through Foodsmartphone - mass spectrometry detection

"Direct analysis of lateral flow immunoassays for deoxynivalenol using electrospray ionization mass spectrometry"

Ariadni Geballa-Koukoula
WFSR

ariadni.geballakoukoula@wur.nl
@AriadniGk

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

1

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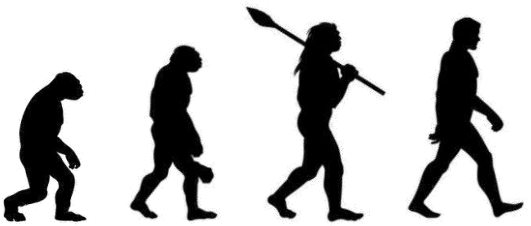

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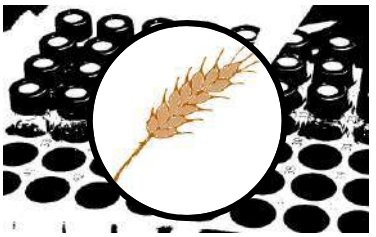
3

4

Current EU Food Safety Control Strategy





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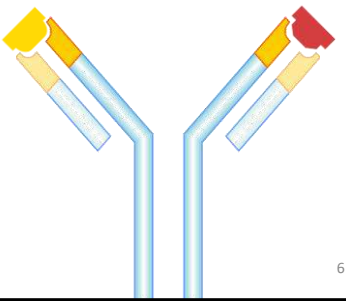
Screening Assays
Immunoassays







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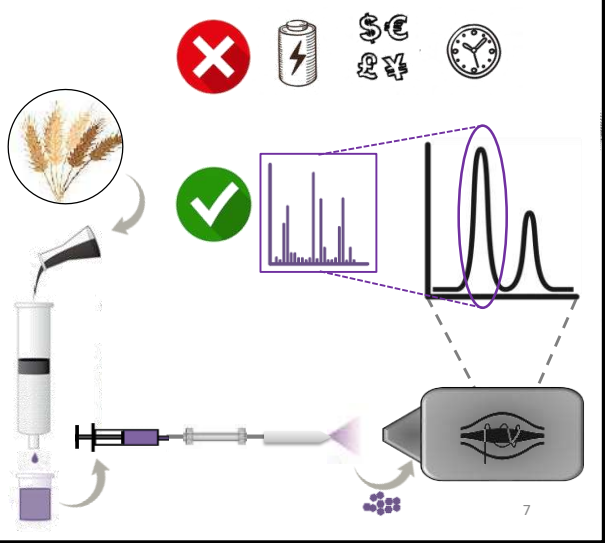
Confirmatory Analysis

Liquid/Gas Chromatography – Mass Spectrometry



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Official Screening Analysis

Official Confirmatory Analysis

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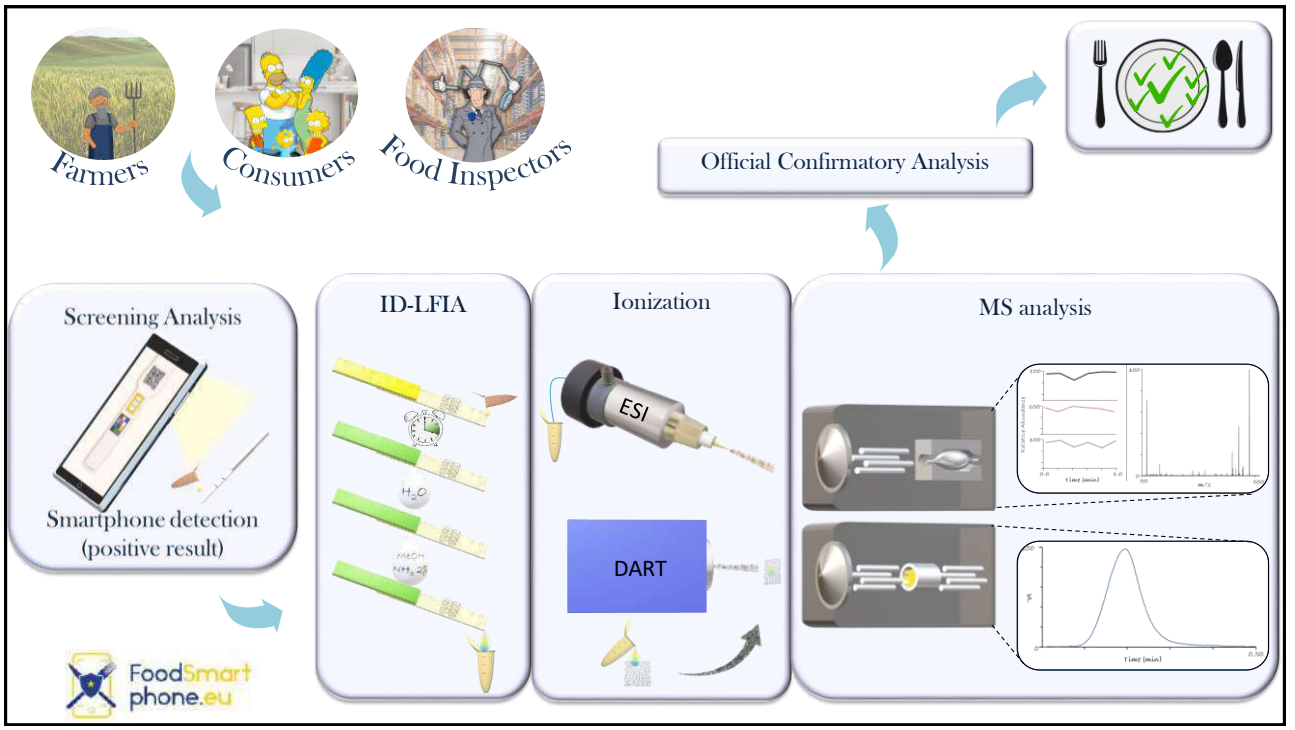
Farmers
Consumers
Food Inspectors
Official Confirmatory Analysis
Screening Analysis
Smartphone detection
(positive result)
FoodSmart
phone.eu
9

9


Farmers
Consumers
Food Inspectors
Official Confirmatory Analysis
Screening Analysis
Smartphone detection
(positive result)
ID-LFIA
Ionization
ESI
DART
MS analysis
FoodSmart
phone.eu

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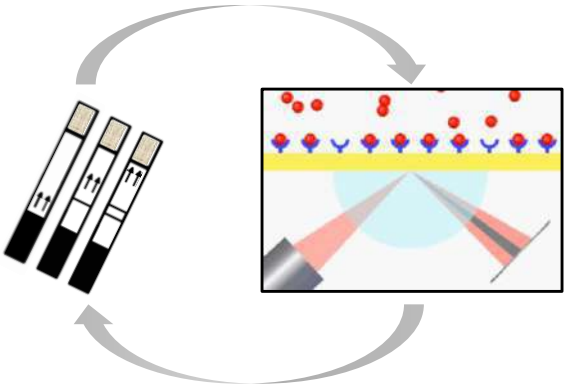
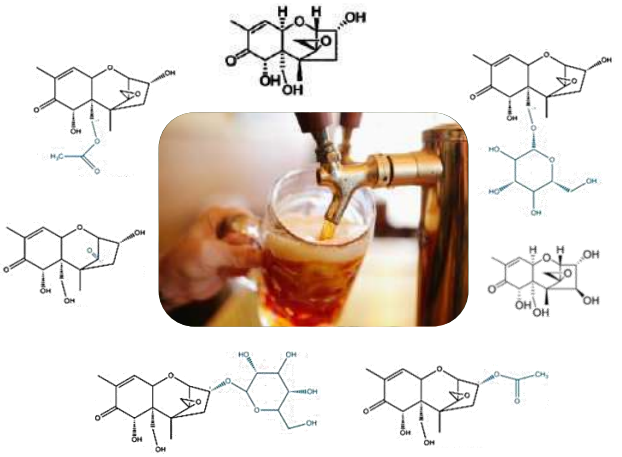
Model system

Lateral Flow Immunoassay



Deoxynivalenol (DON)



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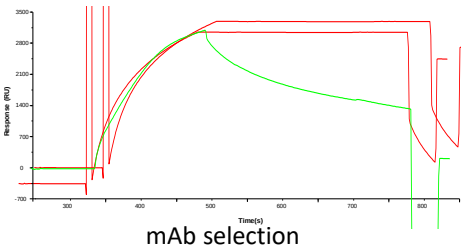
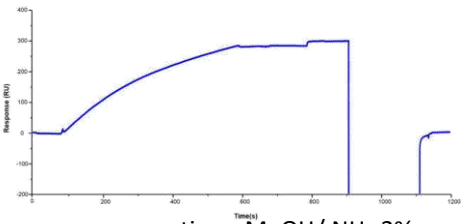
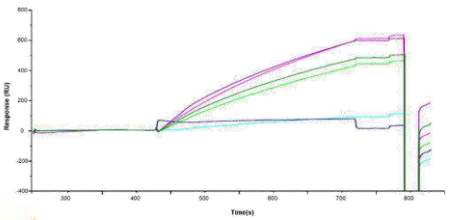
Surface Plasmon Resonance (SPR)

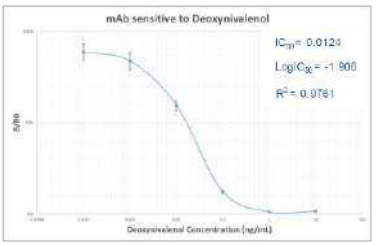


mAb selection



regeneration - MeOH/ NH_3 2%





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Competitive inhibition method

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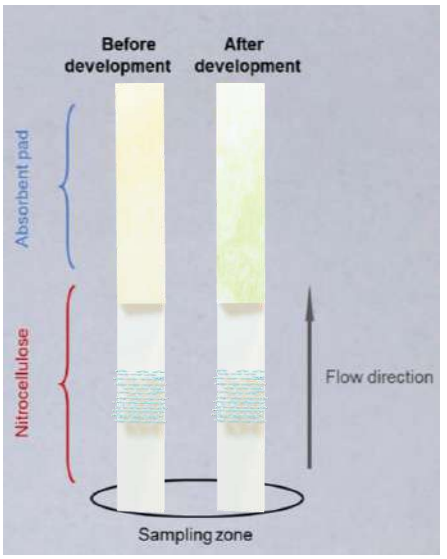
ID-LFIA
Development



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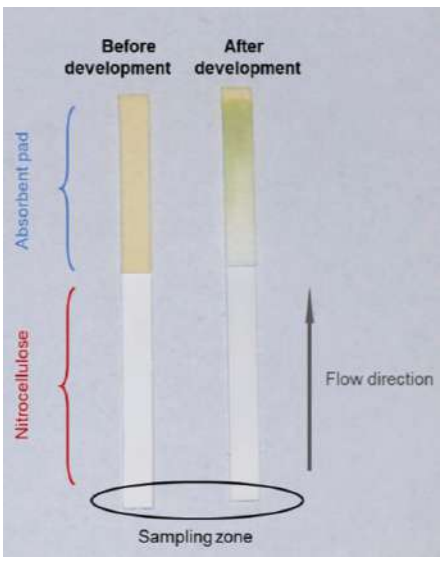
ID-LFIA
Development



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Mass Spectrometric Identification

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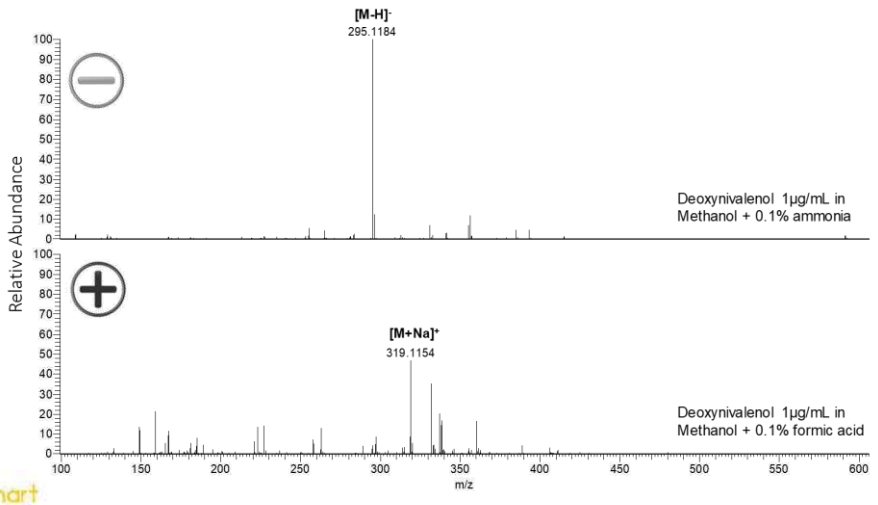
Mass Spectrometric Identification

Ion suppression



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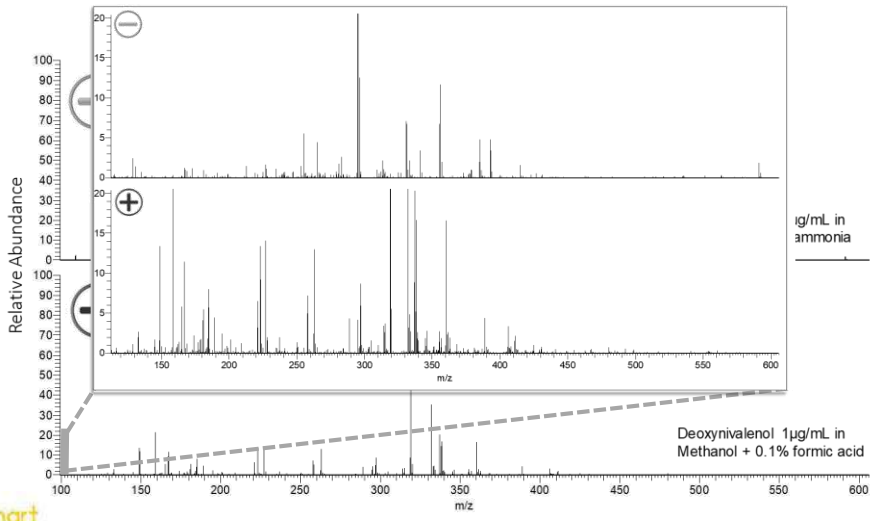
Mass Spectrometric Identification

Ion suppression



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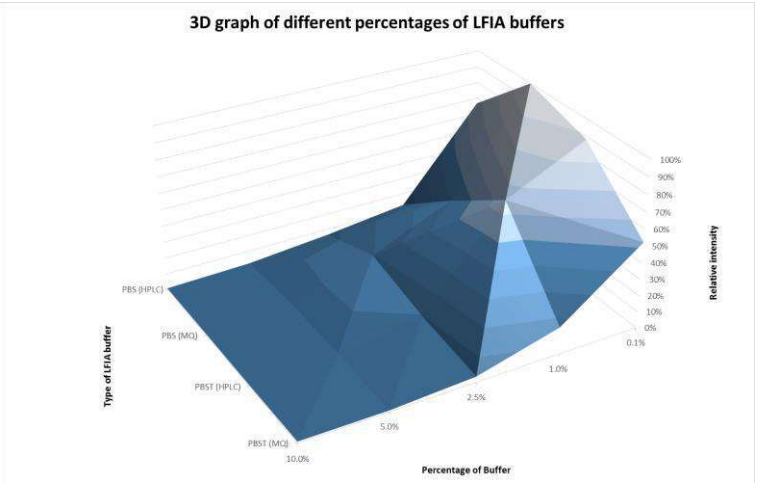
Mass Spectrometric Identification

Ion suppression



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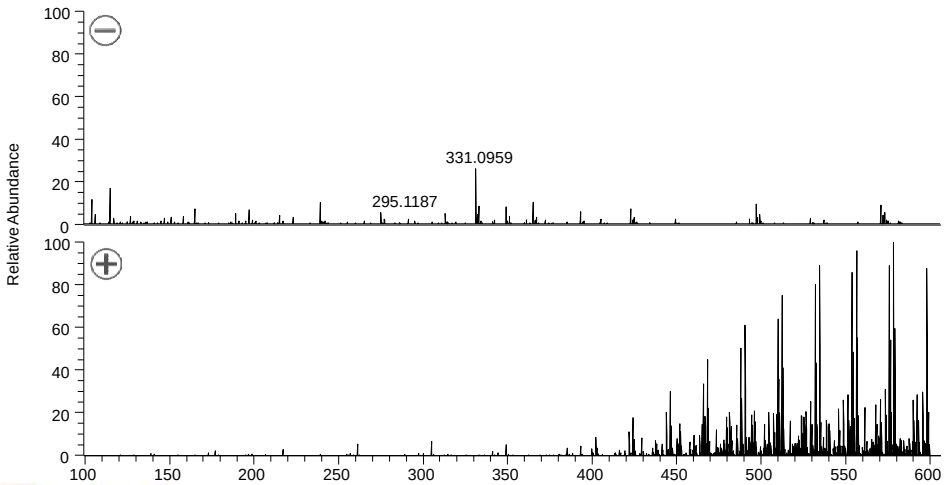
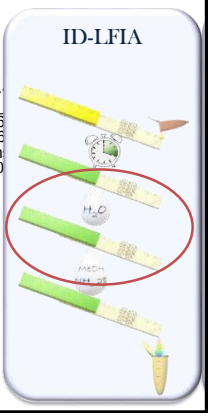
Mass Spectrometric Identification

Ion suppression





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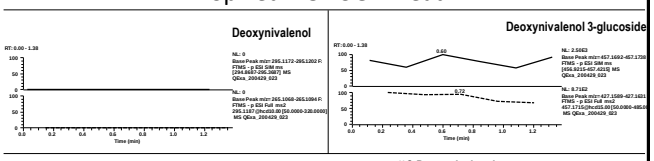
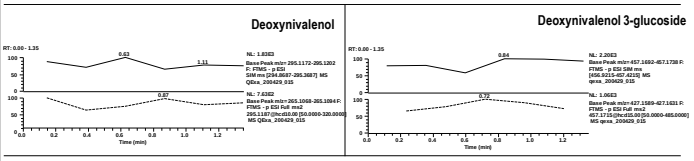
Mass Spectrometric Identification

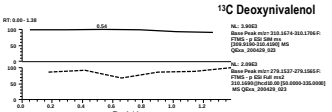
ID-LFIA-ESI-Q-Orbitrap MS

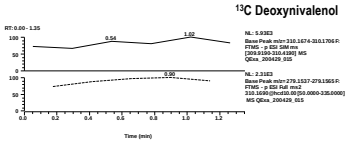
Spiked DON3G wheat



Incurred beer UCT







Results from ID-LFIA-Q-Orbitrap MS analysis.					
Sample	mean absolute intensity of peak height ± SD				LFIA screening result (mg/kg, mean ± SD)
	DON		DON3G		
m/z	295.1187	265.1081	457.1715	427.1610	
Blank wheat	-	-	-	-	< 0.50 (± 0.00)
Spiked DON3G wheat	-	-	2230 (± 30)	983 (± 77)	2.64 (± 0.00)
Incurred beer UCT	3360 (±220)	745 (±34)	3900 (± 140)	820 (± 2)	> 5.50 (± 0.00)

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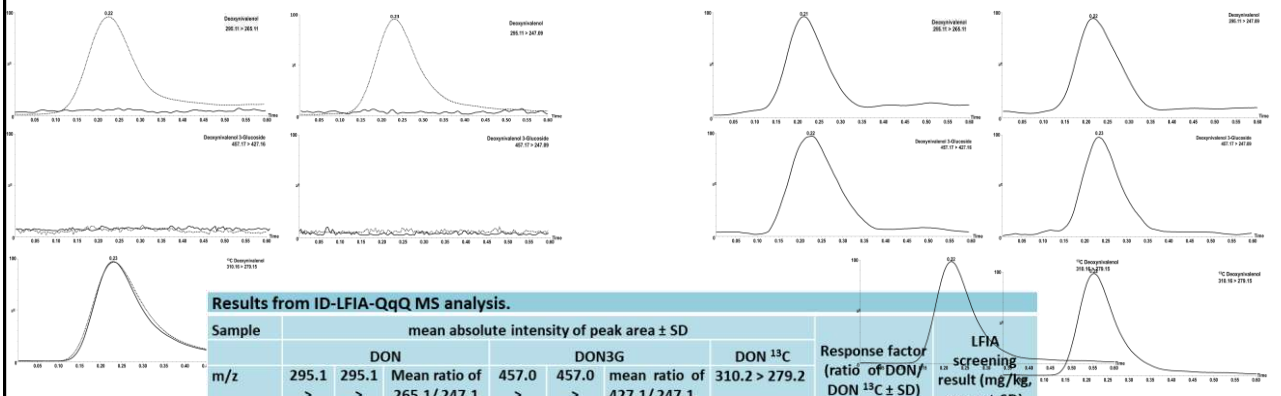
Mass Spectrometric Identification

ID-LFIA-ESI-QqQ-MS/MS

Spiked DON wheat

Incurred beer UCT



Results from ID-LFIA-QqQ MS analysis.

Sample	mean absolute intensity of peak area ± SD							Response factor (ratio of DON/ DON ^{13}C ± SD)	LFIA screening result (mg/kg, mean ± SD)
	DON			DON3G			DON ^{13}C		
m/z	295.1 > 265.1	295.1 > 247.1	Mean ratio of 265.1/ 247.1	457.0 > 427.0	457.0 > 247.1	mean ratio of 427.1/ 247.1	310.2 > 279.2		
Spiked DON Wheat	9364 (±272)	2902 (±17)	3.2 (±0.07)	-	-		62020 (±2915)	0.16 (±0.011)	3.95 (±0.10)
Incurred beer UCT	3504 (±219)	1078 (±57)	3.3 (±0.05)	410 (±6.2)	400 (±12.3)	1.0 (±0.025)	21139 (±801)	0.16 (±0.005)	>5.5 (±0.00)

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Conclusions

Sample on-site following a suspect screening result, and send to lab for confirmation

Analytical and Bioanalytical Chemistry (2020) 412:7547–7558
https://doi.org/10.1007/s00216-020-02890-4

RESEARCH PAPER

Direct analysis of lateral flow immunoassays for deoxynivalenol using electrospray ionization mass spectrometry

Ariadni Geballa-Koukoula[1] · Arjen Gerssen[1] · Michel W. F. Nielen[1,2]

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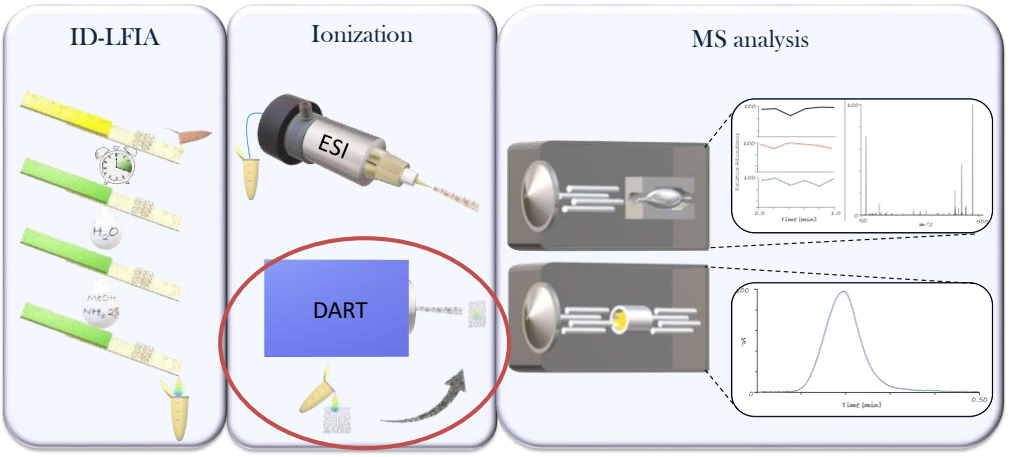
Future plan

- ID-LFIA **DART**-QqQ-MS/MS – optimization and validation.



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Michel W.F. Nielen
Arjen Gerssen
Yao Zhou
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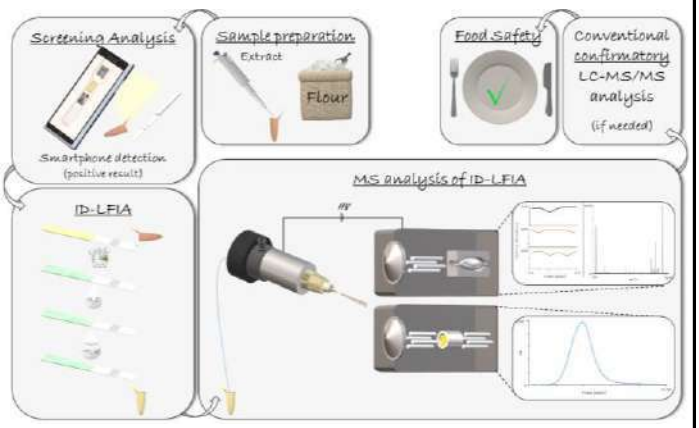
Adil Bouslim



QUEEN'S UNIVERSITY BELFAST
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100years
MARIE CURIE ACTIONS
UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE
CSIC
ZEU
LINKÖPING UNIVERSITY
AQUAMARIJN
csem
Barilla
The Italian Food Company. Since 1877.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

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Laboratory of Public Health Agency of Barcelona

Antoni Rúbies

Laboratori Agència Salut Pública Barcelona

Barcelona, November 26th, 2020

C S B Consorci Sanitari de Barcelona

Agència de Salut Pública

Index

- **Laboratory ASPB:** our origin
- **Laboratory ASPB:** who we are, what we do
- **Legislative framework:** ISO/IEC 17205 Accreditation, Quality assurance and regulations
- **Future of food control?**

Our origin

Barcelona's townhall:

Municipality Institute for Public health

Municipality Laboratory

Generalitat de Catalunya (autonomous government):

Health Dep.: Public Health Directory

Laboratory services:

Laboratory in Barcelona

2003: Barcelona Public Health Agency was created

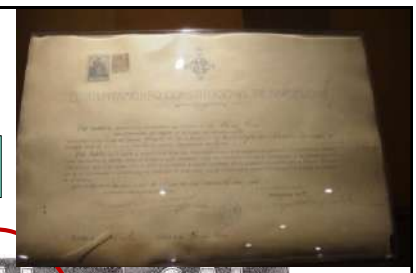
2 laboratories were merged

Laboratory ASPB

Municipality Laboratory:

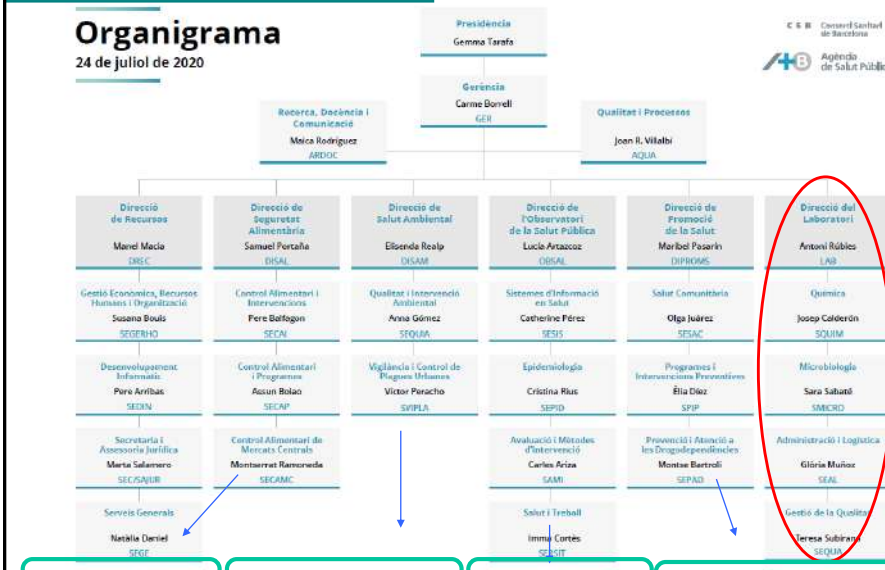
created in 1887

1918

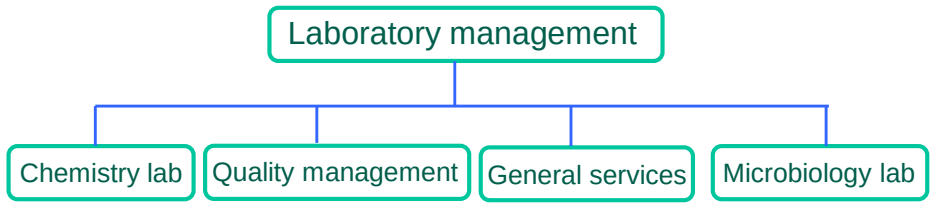


1926

Organisation Chart ASPB



Organisation chart in Public Health Laboratory



Chemistry lab	Quality management	General services	Microbiology lab
•11 A1: Chemistry degree •14 C1: Technical assistant •6 C2: Laboratory assitant	•2 A1: Biochem, Pharm. degree •2 C1 Admintrative staff	•2 A1: Chemistry degree •5 C1 Admintrative staff •2 Admin. support	•6 A1: Biology, Biochem. degree •10 C1: Technical assistant •3 C2: Laboratory assistant
Total: 31 people	Total: 4 people	Total: 9 people	Total: 19 people

Global figures:

- 22 A1: Degree (Chemistry, Biology, Biochemistry, Pharmaceutical degree) (32%)
- 42 C1/C2: Technical and laboratory assistents, administration support. (65%)
- General Management: 2 people

Total: 65 people

Chemistry Lab organisation

Instrumental orgànic analysis	Instrumental inorgànic analysis/ food analysis	Environmental Analysis & Logistic
6 A1: Chemistry degree 6 C1: technical assitant	3 A1 : Chemistry degree 6 C1: technical assitant	1 A1 : Chemistry degree 2 C1: technical assitant

Logistic

6 Laboratory assitant

•**Cooperation agreements:**

- University (UB, UAB, IQS for master/ pHD development), CSIC (Spanish Research Council), ACSA
- Association/ institutions: Celiac association, ...

Participation in research projects:

- FIS, CIBER, II Sant Pau...
- European Projects (Nicotine analysis) , ..

Instrumentation

Environmental analysis: HPLC-DAD, GC/MS, GC-QQQ, GC-HMRS
Organic analysis: LC-DAD/FLD, LC-MS/MS, LC-HRMS
Inorganic analysis: IC, ICP-MS

Microbiology Lab organisation

Food and clinical analysis	Water analysis
1 A1: Biochemistry degree 3 C1 technical assitant	2 A1: Biology degree 5 C1 technical assitant

Instrumental areas:
PCR and Pulsed Field

2 A1: Biology degree
2 C1 technical assitant

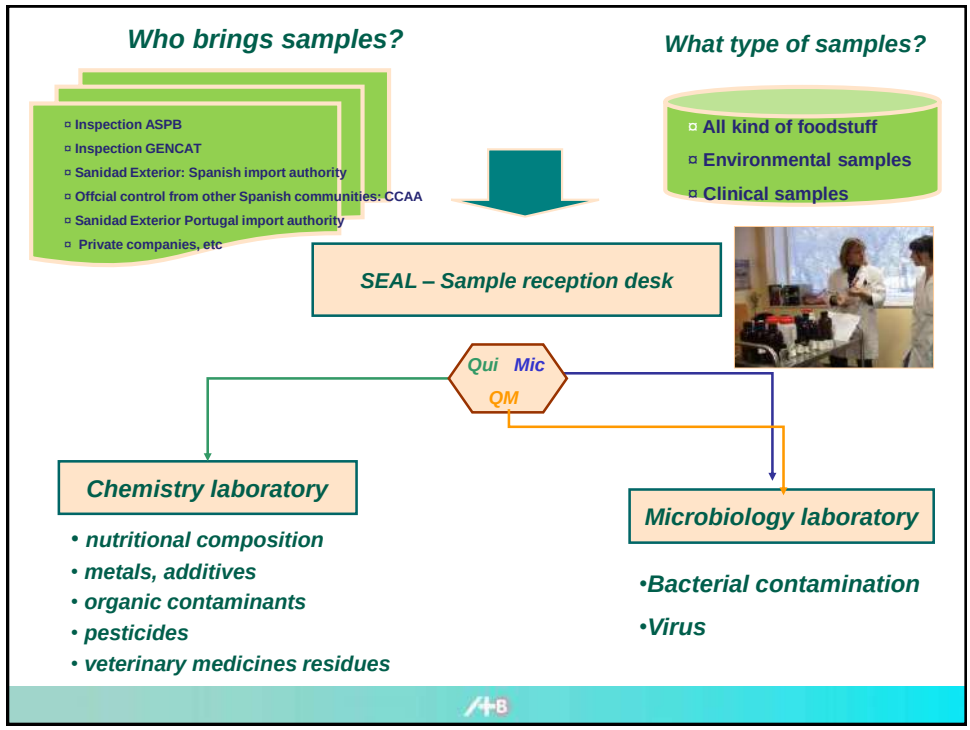
• **Cooperation agreements:** University (UB, UAB, for master/ pHD development)

• **Participation in research projects:** Hospitals and University (Hospital Clínic), UB (norovirus)

Real time PCR

DNA sequencer

Pulsed field



Official control Programs:

- **ASPB (Barcelona's townhall):**
  - IQSA programme: all type of food stuff consumed in town
- **Generalitat Catalunya: Catalunya area:**
  - SIVAL: Regional food stuff commodities
  - PIR: Veterinary residues medicines program (UE)

- **Other administrations:**
  - Spanish regions: Andalusia, Extremadura, Canary Islands, Basqueland
  - Health Ministry:
    - Sanitat exterior, EU alerts (RASFF System)
    - ASPB Laboratory: Control of meat products exports to Eurasia Union Eurasia)
  - ASPB Laboratory: Chinese authority recognition for foodstuff exports control to China.
  - Portuguese authorities: foodstuff imports control (PIF)

Some examples of food outbreaks:

Internal trade problems (inside UE)

CO in tunfish

3rd countries imports

Micotoxins, pesticides, metals,...

Alerts: (RASFF)

Clopidol in meat, Fipronil in eggs,....

Institutional support

Nicarbazin in pigeon blood in BCN,...

Additional problem:

Unusual analytes, urgent resultats and always under accreditation!

Laboratory's activity 2019:

Oralims software controlling activity in the last 5 years:

2019: Samples Invoicing Parameters

Summary 2019:

Analyzed samples: 35,333

Analyzed parameters: 535.243

Indicadors: http://192.1.1.240/Reports/Pages

Data treatment application for:

- Samples per customer,
- Samples per procedure,
- Activity per Chemistry/ Microbiology lab,
- Activity per sample type,
- Proficiency test activity, ...

Any	Clase	Tipus de Client	Núm. Mostres	% Anual	Núm. Paràmetres	Import	Dies
2020	1 Agència Salut Pública Barcelona/Ajuntament BCN	Total Clase	2.567	45,90%	27.078	495.068,00	36
	2 GENCAT i altres organismes públics no facturables	Total Clase	2.339	41,83%	56.602	678.639,60	14
	3 GENCAT i altres organismes públics facturables	Total Clase	25	0,45%	61	1.941,60	8
	5 Ministeri Sanitat	Total Clase	179	3,20%	1.453	19.249,20	5
	6 Administracions públiques de l'Estat i l'estranger	Total Clase	40	0,72%	2.857	7.801,80	13
	7 Empreses i particulars	Total Clase	187	3,34%	473	25.430,00	9
	8 Laboratori ASPB	Total Clase	232	4,15%	2.839	33.363,80	16
	O OBSOLET	Total Clase	23	0,41%	343	11.250,00	12
	Total Any		5.592	100%	91.706	1.272.744,00	23
Total			5.592		91.706	1.272.744,00	23

Online Catalogue:

http://ofertalab.aspb.cat:

Customers can extract information about: procedures, type of matrices, analytes, instrumental technique, public prices, *and get their own budget*

Portalweb:

Where registered customers can dowload their results bulletin

http://lablims.aspb.cat:8080/BulletinBoardWeb/

Analytical challenges: what does foodstuff mean?

Complex matrices:

All type of physical and chemical situations:

- state: solid, liquid,...
- composition: oily, salty, aqueous, protein content, ...

Complex analytes:

All type of situations:

- low concentrations, interactions, interferences (isobaric...)

Environmental control: 25% of LASPB activity

Air:

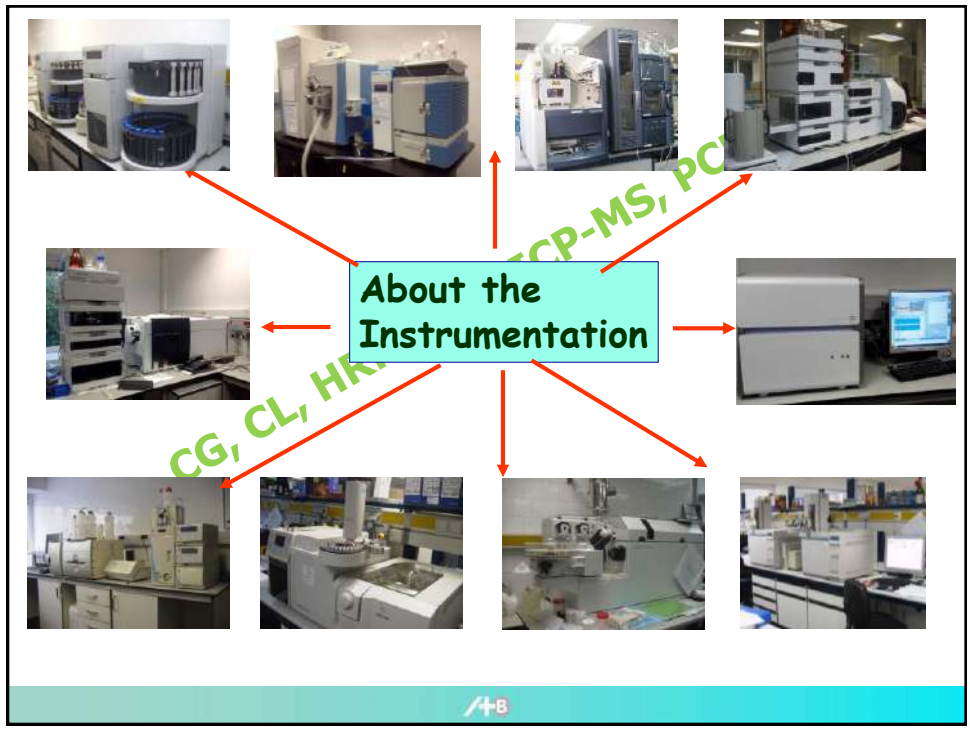
Particulate material: PM10, PM 2,5 Metals, PAHs

Organic volatile compounds and contaminants: VOCs, BTEX (benzene, toluene,....)

Water:

Control plans for drinking water in Catalunya

Programs for specific contaminants (Ebre, Flix,...)



Public Health Laboratories: (625/2017/EU)

Duties:

- High throughput (method automatization) for control
- Analytical methods suitable for very low concentrations.
- Updated Regulation compliance.
- Results following ISO17025 accreditation (mandatory)
- Ability to give quick response/results to the inspection body
- Ability to identify, **confirm and quantify** contaminants in food and evironmental samples

- **High investment capacity**
  - Updated Instrumentation, laboratory facilities,...
  - Very skilled staff, and high degree of specialization

ISO/IEC 17025 accreditation

Accreditation ISO/IEC 17025: mandatory following 625/2017/EU

- **Accreditet by** ENAC (Spanish Accreditation Body) since 2000
- **2019:** 140 accredited procedures
  - 105 Chemistry Lab (SQ)
  - 35 Microbiology Lab (Smic)
- **Flexible scope of accreditation following NT 18 i 19 (by ENAC):**

-NT18: LEBA: 6 groups/ famílies (SQ): 76 procedures

1 group/family (Smic): 2 norovirus procedures

-NT19: LPE, pesticides in foodstuff.

- **Technical List (Anexo técnico):** list of procedures

ISO/IEC 17025 Accreditation

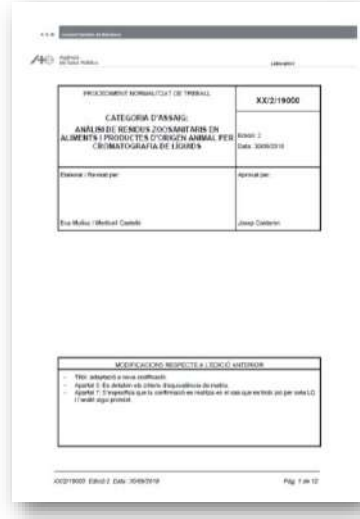
Accreditation: Verification of technical criteria accomplishment for all analytical procedures in our laboratory. Flexible scope following NT-18 and NT-19 by ENAC

LEBA (NT18):

- 6 categories in Chemistry:
  - Natural Toxins
  - Veterinary Drug Residues
  - Metals
  - Ions by Ionic Chromatography
  - Additives
  - Organic contaminants
- 1 category in Microbiology:
  - Norovirus by PCR

LPE (NT19): pesticide analysis

Each category has its own management procedure

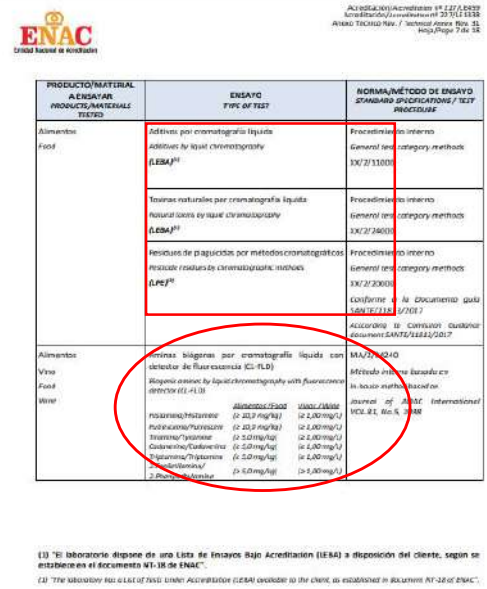


XX:/2/11000: additives **XX:/2/19000: Vet drug residues**

Technical annex: ASPB LE /459 – LE/1338
Flexible and permament scope of accreditation

Analytical categories management

Permanent scope of accreditation



Example of a Category:

Natural toxins in food commodities by HPLC

- **Toxinas naturales por cromatografía líquida en alimentos**

Procedimiento general: XX/2/24000

Procedimientos analíticos:

MA/2/24204 : **Aflatoxina M1** por cromatografía de líquidos y detector de espectrofotometría de fluorescencia (LC-FLD)

MA/2/24200: **Aflatoxinas B y G** por cromatografía de líquidos y detector de espectrofotometría de fluorescencia (LC-FLD)

MA/2/24600: **Alcaloides tropánicos** por cromatografía de líquidos y detector de espectrometría de masas-masas (LC-MS-MS)

MA/2/24550: **Biotoxinas marinas lipofílicas** por cromatografía de líquidos y detector de masas de alta resolución (LC-HRMS)

MA/2/24500: **Biotoxinas marinas: ácido domoico- ASP** por cromatografía de líquidos y detector de espectrofotometría ultravioleta-visible "diodearray" (LC-DAD)

MA/2/24400: **Micotoxinas del Fusarium** por cromatografía de líquidos y detector de espectrometría de masas-masas (LC-MS-MS)

MA/2/24213: **Ocratoxina A** por cromatografía de líquidos y detector de espectrofotometría de fluorescencia (LC-FLD)

MA/2/24260: **Patulina** por cromatografía de líquidos y detector de espectrofotometría ultravioleta-visible "diodearray" (LC-DAD)

In our analytical activity, legal regulations concerns:

a) Accomplishment of legislated limits:
   - Maximum Regulated Limits (MRLs)
   - Parametric values (PV)/ maximum permitted levels

b) Quality parameters of analytical method

c) Availability of analytical instrumentation

c) Analytical results must fulfil legal requirements

d) Validations: new concepts (CCα, CCβ, …)

Mandatory legal documents: Directives, decisions, regulations,…

Not mandatory: Guídelines, technical notes**...**

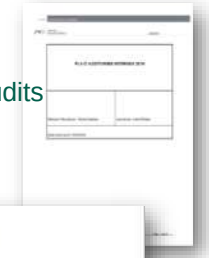
Accomplishment of 625/2017/EU Regulation

Samples:
- Official prospective samples (x1)
- Official regulatory samples (x3)
- Private sector samples

Method validation:
- Internal methods are fully validated
- ISO/17025: methods are checked daily

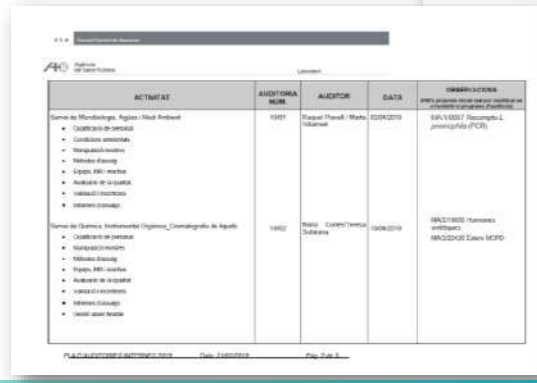
Quality control:
- Participation in interlaboratory exercices
- Internal quality controls: blank samples, spiked samples, reference samples, etc

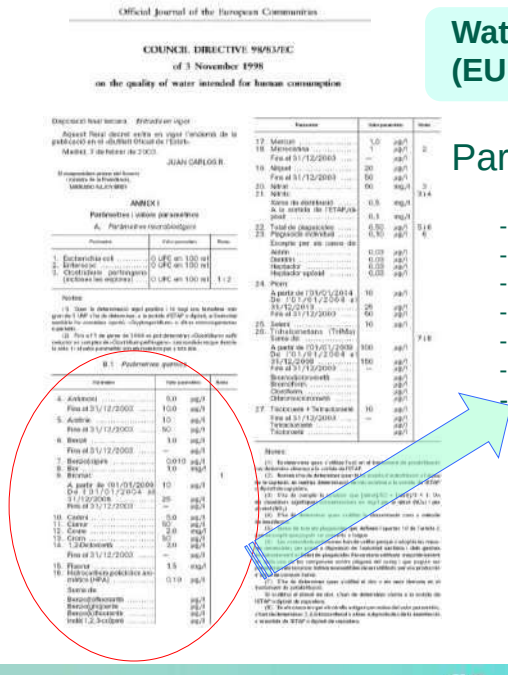
Accomplishment of 625/2017/EU Regulation



Audits:
- Regular internal audits: 2019 plan included 6 internal audits
- External audit: every 18 months by ENAC
  ➡ (last external audit: december 2019)

Personal staff:
- Internal qualification
- External qualification




Official Journal of the European Communities

COUNCIL DIRECTIVE 98/83/EC
of 3 November 1998
on the quality of water intended for human consumption

Water for human consumption (EU Directive 98/83)

Parametric values:

- 0,01 µg/L benzo-a-pyrene
- THMs (sum): 100 µg/L
- Aluminium: 200 µg/L
- Arsenic: 10 µg/L
- Nitrates: 50 mg/L
- Pesticides (sum): 0.5 µg/L
- …

Over 50 parameters to control

Continuous update: Directive 1787/2015 (updating Directive 98/83/UE, on Water Quality for human consumption)

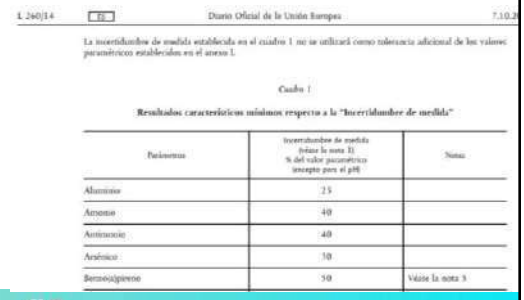
- Sampling: Flexible sampling, based on risk

- Quality Control: ISO/IEC 17025 for validation of analytical methods,

e.g.:

- Introduction of LOQ (not LD) < 30% PV
- Uncertainty estimation:

starting 2019


L 260/14 ES Diario Oficial de la Unión Europea 7.10.2

Cuadro 1

Resultados característicos mínimos respecto a la "Incertidumbre de medida"

Parámetros	Incertidumbre de medida (véase la nota 1) % del valor paramétrico (excepto para el pH)	Notas
Aluminio	25	
Arsénico	40	
Antimonio	40	
Arsénico	10	
Benzo(a)pireno	50	Véase la nota 3

Commission Decision 2002/657/CE implementing 96/23 Directive on veterinary medicine residues in foodstuff of animal origin:

And some examples of concerning:

- Analytical Instrumentation
- Method Validation
- Requirements for analytical methods: use of internal standards, etc.

- Through a points system: Mass spectrometry is mandatory for forbidden substances!
- New concepts for validation: CCα and CCβ, MRPL, MMPR

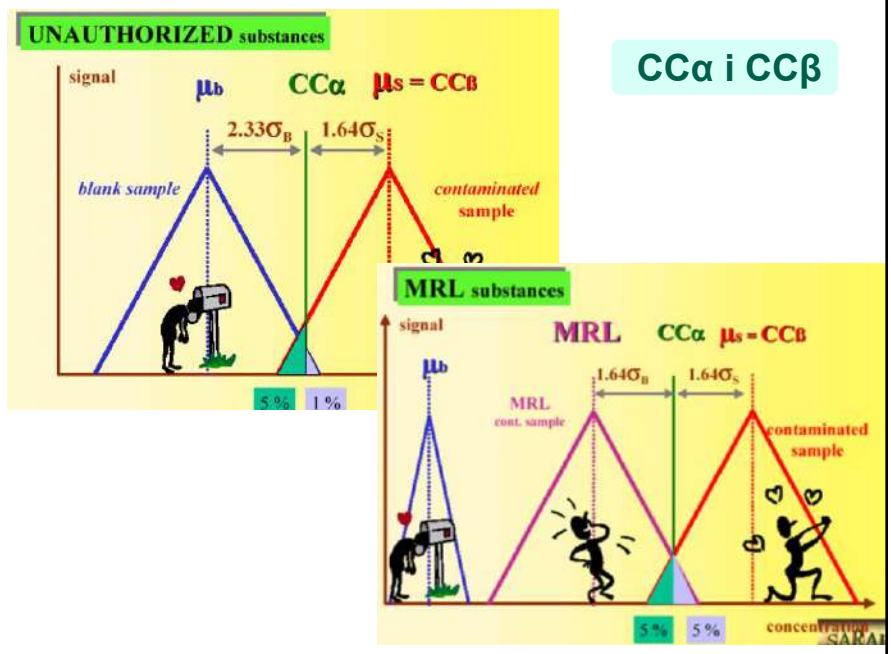
But…657/2002 Decision is outdatet in some aspects:

Use of LC-HRMS:

- point attribution,
- confirmation criteria in HRMS
- etc…


COMISIÓN

CCα i CCβ



Published MRPL → Reference Values (2019)

Chloramphenicol = 0.3 µg/kg — Decisió 2003/181

Malachite Green + leuco metabolite = 2 µg/kg — Decisió 2004/25/CE

Metabolites of nitrofuranes = 1 µg/kg — Decisió 2003/181

Example Laboratory ASPB:

Chloramphenicol: CCα = 0.06 µg/kg LQ = 0.2 µg/kg

1871/2019 Regulation

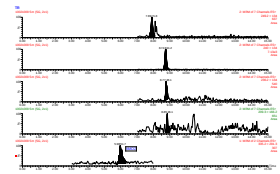
Sustancia	Valor de referencia (µg/kg)	Otras disposiciones
Cloranfenicol	0,15	
Verde de malaquita	0,5	0,5 µg/kg para la suma de verde de malaquita y verde de leucomalaquita
Nitrofuranos y sus metabolitos	0,5 (1)	0,5 µg/kg para cada uno de los metabolitos furazolidona (AOZ o 3-amino-2-oxazolidinona), furaltadona (AMOZ o 3-amino-5-metilmorfolino-2-oxazolidinona), nitrofurantoína (AHD o 1-aminohidantoína), nitrofurazona (SEM o semicarbazida) y nifursol (MDSH o hidracida del ácido 3,5-dinitrosalicílico)

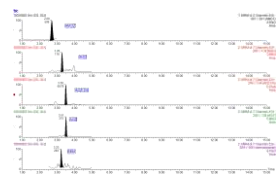
+ CRL GUIDANCE (09/2020): Minimum Method Performance Requirements **(MMPR)** on Hormones, β-agonists, Thyreostats, Nitroimidazoles, etc…

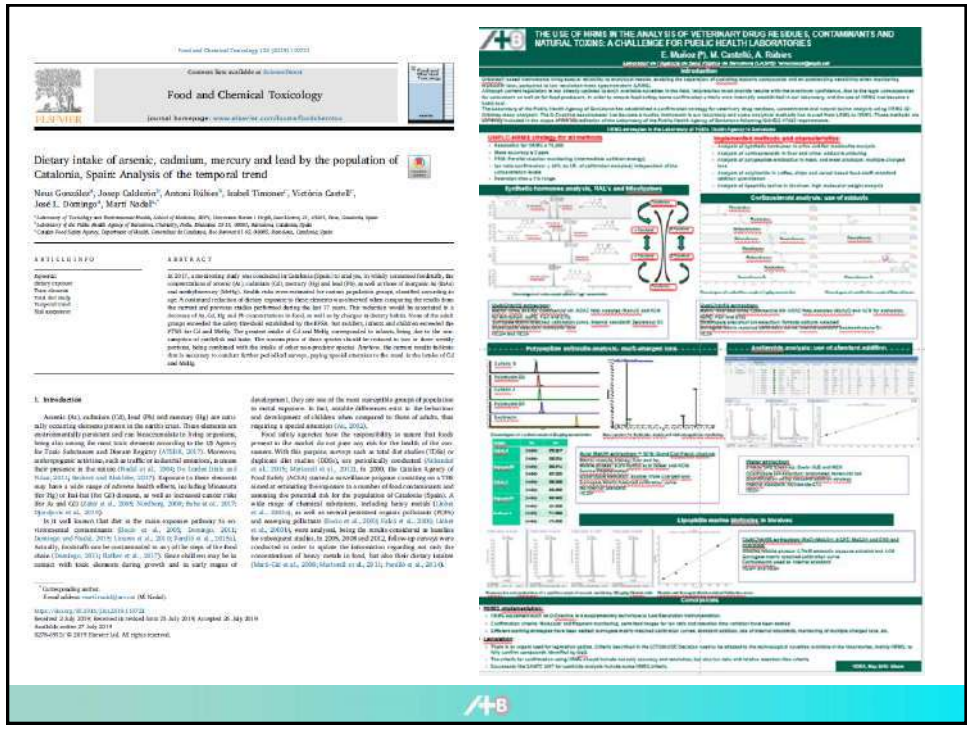
Future of food control?

Continuous updating:

- Use of new technology, LC-HRMS and GC-HRMS, UPLC, etc…
- Extraction automation
- Update on emerging contaminants
- Use of flexible scope of analysis
- Avaliability of updated legislation





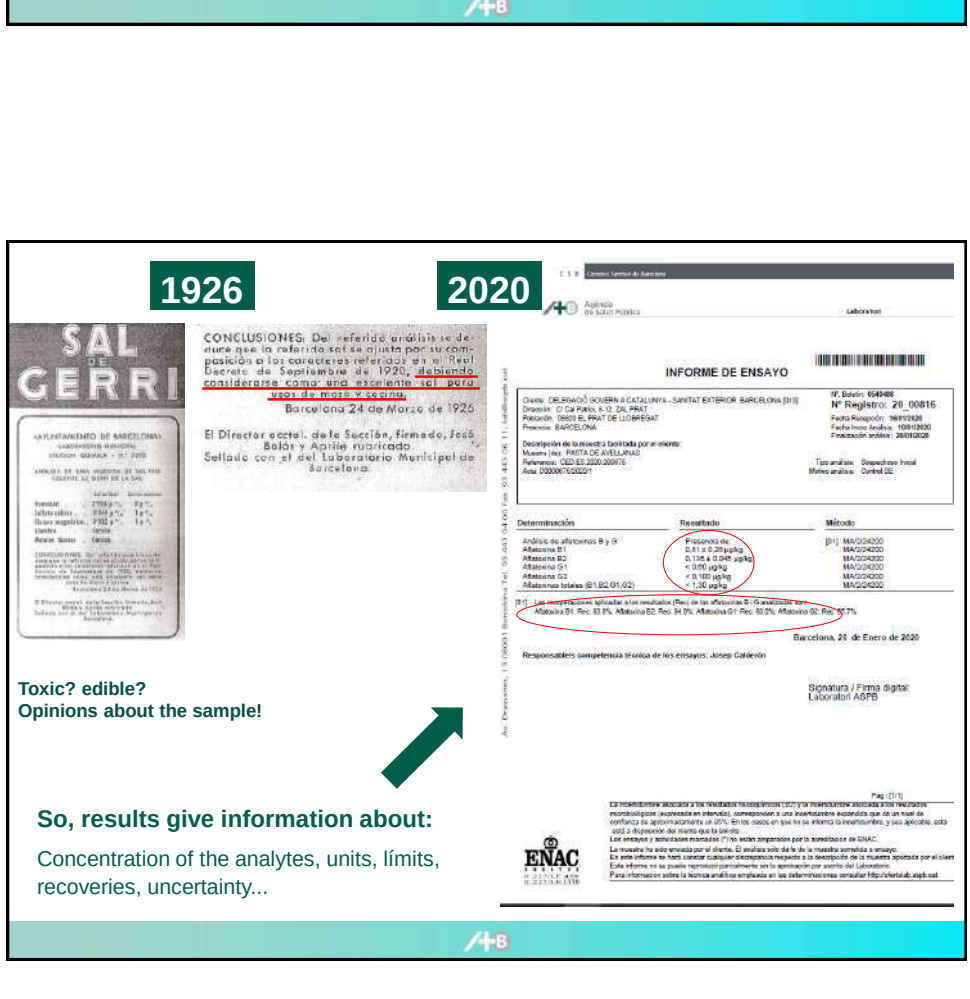


Food and Chemical Toxicology

Dietary intake of arsenic, cadmium, mercury and lead by the population of Catalonia, Spain: Analysis of the temporal trend

THE USE OF HRMS IN THE ANALYSIS OF VETERINARY DRUG RESIDUES, CONTAMINANTS AND NATURAL TOXINS: A CHALLENGE FOR PUBLIC HEALTH LABORATORIES

E. Muñoz (*), M. Castelló, A. Rúbies



1926

CONCLUSIONES: Del referido análisis se deduce que la referida sal se ajusta por su composición a los caracteres referidos en el Real Decreto de Septiembre de 1920, debiendo considerarse como una excelente sal para usos de mesa y cocina.

Barcelona 24 de Marzo de 1926

El Director acctal. de la Sección, firmado, José Balós y Aprile rubricado.
Sellado con el del Laboratorio Municipal de Barcelona.

2020

INFORME DE ENSAYO

Nº Registro: 20_00816

Determinación	Resultado	Método
Análisis de aflatoxinas B y G	Presencia de:	[01] MA/Q/24200
Aflatoxina B1	0,81 ± 0,35 µg/kg	MA/Q/24200
Aflatoxina B2	0,135 ± 0,048 µg/kg	MA/Q/24200
Aflatoxina G1	< 0,60 µg/kg	MA/Q/24200
Aflatoxina G2	< 0,160 µg/kg	MA/Q/24200
Aflatoxinas totales (B1,B2,G1,G2)	< 1,30 µg/kg	MA/Q/24200

[01] Las incertidumbres aplicadas a los resultados (Rec) de las aflatoxinas B y G analizadas son:
Aflatoxina B1: Rec. 83.9%, Aflatoxina B2: Rec. 84.9%, Aflatoxina G1: Rec. 83.5%, Aflatoxina G2: Rec. 83.7%

Barcelona, 20 de Enero de 2020

Responsables competencia técnica de los ensayos: Josep Galdeiro

Signatura / Firma digital: Laboratori ASPB

Toxic? edible?
Opinions about the sample!

So, results give information about:

Concentration of the analytes, units, límits, recoveries, uncertainty...

Thanks for your attention!

Laboratori ASPB

lab@aspb.cat

CSB Consorci Sanitari de Barcelona

Agència de Salut Pública

www.aspb.cat

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European Commission

Directorate-General for
Health & Food Safety

Official control of contaminants in the food chain: challenges and opportunities for innovative analytical techniques

Frans Verstraete

Health and Food Safety

European Commission

Ensuring a high level of human health protection

- Food placed on the market has to be safe
- Maximum levels established at EU level for contaminants in food and feed to ensure a high level of human health and animal health protection
- Level of a contaminant in food
  - > maximum level: "not safe" , cannot be placed on the market/has to be withdrawn from the market
  - ≤ maximum level: "safe"
- Ensuring a high level of human health and animal health protection
  - Strict maximum levels: important
  - **Effective enforcement: more important**

Health and Food Safety

Effective enforcement - compliance testing

Controls must be performed

- Risk based → (highest) probability of non-compliance with established standards combined with "horizon scanning" to detect/identify new hazards or new hazard/matrix combinations (emerging risks)
- High frequency
- At all stages of the production and distribution. Most appropriate stage
  - In function of stage where contamination occurs
  - In function of objective of control (close to production/close to consumer)
- Most appropriate matrix (prohibited substances)

3

Effective enforcement - compliance testing

Official controls are compliance testing of lots, batches, consignments of feed and food

For effective enforcement it is important to have a representative sampling and a correct sample preparation and reliable method of analysis

Sample has to be taken in a way that it is representative for the lot, batch

→ Sample not compliant → lot not compliant → lot cannot be marketed → has to be safely disposed of

→ False positives/false negatives: negative economic consequences/ negative consequences for consumer protection

4

Official Control Regulation (EU) 2017/625 ("OCR")

- The legal basis for an integrated, and thus more efficient, system of official controls along the agri-food chain.
- Contains, *inter alia,*
  - specific provisions as regards methods of sampling and analysis to be used for official controls

Official Control Regulation Provisions on sampling and analysis

- To ensure the reliability and consistency of official controls and other official activities across the Union, the methods used for sampling and for laboratory analyses, tests and diagnoses have to meet scientific standards, satisfy the specific analytical, testing and diagnostic need of the laboratory concerned, and have to offer sound and reliable analytical, test and diagnostic results.
- Clear rules are established for the choice of the method to be used for official control

Official Control Regulation "Methods cascade"

1. Methods used for sampling and for laboratory analyses, tests and diagnoses during official controls and other official activities shall comply with Union rules **establishing those methods or the performance criteria for those methods**.

2. In the absence of the Union rules and in the context of official controls and other official activities, official **laboratories shall use one of the following methods** according to the suitability for their specific analytical, testing and diagnostic needs:

Official Control Regulation "Methods cascade"

(a) available methods complying with relevant internationally recognised rules or protocols (including those that the European Committee for Standardisation (CEN)) has accepted; or

relevant methods developed or recommended by the **European Union reference laboratories** and validated in accordance with internationally accepted scientific protocols;

Official Control Regulation "Methods cascade"

(b) in the absence of the suitable rules or protocols, as referred to in point (a), methods which comply with relevant rules established at national level, or, if no such rules exist, relevant methods developed or recommended by **national reference laboratories** and validated in accordance with internationally accepted scientific protocols; or

relevant methods developed and validated with inter or intra-laboratory methods validation studies in accordance with internationally accepted scientific protocols

9

Official Control Regulation "Methods cascade"

Where laboratory analyses, tests or diagnoses **are urgently needed** and none of the methods referred to before exists, the relevant national reference laboratory or, if no such national reference laboratory exists, any other official control laboratory may use other methods until the validation of an appropriate method in accordance with internationally accepted scientific protocols.

10

Official Control Regulation sampling and analysis

Legal rules can be established on

(a) the methods to be used for sampling and for laboratory analyses, tests and diagnoses;

(b) performance criteria, analysis, test or diagnosis parameters, measurement uncertainty and procedures for the validation of those methods;

(c) the interpretation of analytical, testing and diagnostic results.

Contaminants - Sampling procedures

- Sampling procedures are established in EU legislation for the control of contaminants
- Adequate sampling procedure is of crucial importance for estimating lot average levels in particular in case contaminants are heterogeneously distributed throughout a lot and is therefore an essential component in the development of any regulatory level
- Sampling procedures have an exporter's risk /producer's risk against importer's risk/ consumer's risk: EU sampling procedures are
  - practicable
  - minimise the consumer's risk without rendering trade impossible

Contaminants - Methods of analysis

- No specific methods of analysis established in EU legislation
- Establishment of performance criteria with which methods of analysis used for the control have to comply with
  - laboratories can use the analytical method most appropriate for their facilities and can make use of technological progress and newest technologies
  - includes parameters such as limit of quantification, repeatability, coefficient of variation, reproducibility recovery, for various matrices and regulatory levels

13

Enforcement approach: Screening method combined with confirmatory method

- Making use of screening methods is to select those samples with increased levels (suspect to be non-compliant with the regulatory level)
- Screening methods shall ensure cost-effective high sample-throughput.
- Have to have a (very) low incidence on false-compliant results.
- Providing a yes/no-decision over the possible exceedance of the regulatory level or provide a semi-quantitative level

14

Enforcement approach: Screening method combined with confirmatory method

- Samples suspected to be non-compliant through a screening method must be determined or confirmed by a confirmatory method
- Confirmatory methods allow the unequivocal identification and quantification of contaminant present in a sample.
- EU legislation provides criteria for the screening method (e.g. low rate of false negative results).

15

Innovative analytical approaches Challenges for regulation

- Development of innovative analytical methodologies/ approaches
  - **Multi-analyte methods**
  - **More sensitive methods**
  - **Rapid, reliable screening methods and approaches**
  - **Portable, smartphone based food analysis**
  - **…**

→ Is without any doubt of importance to further increase the safety of our food chain

- But regulation (maximum levels) with effective enforcement (compliance testing) also important
- Synergy or not ?

Smartphone based analytical applications - opportunities and challenges

- Laboratory → field /factory/shop
- Can be used by everyone: official inspectors, feed and food business operators, consumers
- Immediate result/outcome
- To which extend outcome / result usable/useful for official compliance testing?
- In any case very important and a challenge is to ensure effective follow-up of "signals" (of non-compliance / unsafe) → information flow, data sharing , interpretation of results …

Examples

- Presence of lead, chromium and mercury in counterfeit curcuma /turmeric from India →
  - very high levels of lead (2020 ppm), chromium (503 ppm), mercury (6 ppm)
  - mixture of paprika (ca. 65%) and rice starch (ca.25%) with the illegal use of leadchromate as colour.

- Mineral oil in infant formula → press attention → mothers concerned → if they would be able to check absence with smartphone →a reassurance

Analytical challenges for regulating contaminants in feed and food

Recent experience in regulating contaminants in food:

- Availability/application of a robust method of analysis, (validated and compliant with the performance criteria), to be used for enforcement/official control/compliance testing which can be routinely applied is in certain cases an issue and an important factor in delaying the establishment of regulatory provisions needed to be taken to ensure the safety of feed and food

Analytical challenges for regulating contaminants in feed and food

Maximum/regulatory level is the sum of different compounds ("straightforward sums")

- Currently regulated: aflatoxin total (sum of B1, B2,G1 and G2), fumonisins (sum of FB1 and FB2), polycyclic aromatic hydrocarbons (PAH) – PAH4: benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene), NDL-PCBs (sum of ICES-6 PCBs)
- Foreseen: (more complex from an analytical point of view): ergot alkaloids (sum of 12 ergot alkaloids), pyrrolizidine alkaloids (sum of 21 compounds → 35 compounds because of the problem of co-elution), deoxynivalenol and modified forms

Analytical challenges for regulating contaminants in feed and food

Maximum/regulatory level is the sum of different compounds with different potency (Toxic Equivalency factor (TEF) / Relative Potency Factor (RPF)

- Currently regulated: sum of dioxins and dioxin-like PCBs (7 PCDD, 10 PCDF and 12 DL-PCBs**)**
- Possibly to be regulated in the future: sum of zearalenone and metabolites (with different RPF ranging from 1 to 60)

Analytical challenges for regulating contaminants in feed and food

Maximum/regulatory level has to be set at low level because of toxicological characteristics of the contaminant --> analytical challenge sensitivity of the routine method of analysis

- regulated : tropane alkaloids (atropine and scopolamine) in baby foods, lead in baby foods
- to be regulated: mineral oil aromatic hydrocarbons in infant formula

Other contaminants with analytical challenges for possible regulation

- Micro- and nanoplastics
- Polyfluoroalkyl Substances (PFAS)
- Brominated flame retardants (BFRs) (HBCDDs, TBBPA, Brominated phenols, PBDEs, emerging and novel BFRs)
- Methylmercury
- Quinolizidine alkaloids
- Glycoalkaloids
- Furans

Conclusions – Guaranteeing a high level of feed and food safety Opportunities and challenges

For guaranteeing a high level fo food safety very important that through research **new analytical innovative methodologies /approaches**, multi-analyte methods, screening methodologies, smartphone-based methods become available

Opportunities:

- → enables a high frequency of testing
- → enables horizon scanning (new hazards / new hazard/matrix combinations)
- → from laboratory → field, shop, factory
- → immediate result
- → accessible

Conclusions – Guaranteeing a high level of feed and food safety Opportunities and challenges

For guaranteeing a high level fo food safety very important that through research **new analytical innovative methodologies /approaches**, multi-analyte methods, screening methodologies, smartphone-based methods become available

Challenges:

- → how to use the results in compliance testing
- → how to ensure accurate follow-up to « signals »
- → result/outcome interpretation, information flow, information sharing, …

Health and Food Safety

Conclusions – Guaranteeing a high level of feed and food safety Opportunities and challenges

but also very important:

- Ensure timely availability of robust methods of analysis that can be routinely and widely applied for enforcement/control/compliance testing compliant with performance criteria, validated)

How to turn challenges into opportunities?

How to ensure synergy ?

How to bridge that gap ?

Health and Food Safety

Thank you for your attention !

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Health and Food Safety

European food safety legislation with respect to veterinary drugs and the role of rapid tests in its implementation

Dr. Wim Reybroeck
ILVO
25/11/2020

ILVO

Flanders research institute for agriculture, fisheries and food

ILVO

EUROPEAN FOOD SAFETY LEGISLATION

Aim: to assure a high level of Food Safety
to apply an integrated approach from farm to fork

Implementation: involves several actions:

- to assure **effective control systems** and evaluate **compliance with EU standards**
- to manage international relations with non-EU countries
- to manage relations with EFSA and ensure science-based risk management

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EU VMP RESIDUE LEGISLATION

To achieve a high level of health protection for the consumer
→ risk analysis procedure that is based on sound scientific evaluation and takes into account other factors such as the feasibility of control

Specific legislation on residues of veterinary medicinal products (VMPs) used in food producing animals:

- scientific evaluation before respective VMPs are authorized
- establishment of maximum residue limits (MRLs)
- in some cases the use of substances is prohibited

ILVO

EU VMP RESIDUE LEGISLATION

Food-producing animals may be treated with veterinary medicines to prevent or cure disease
→ possibility of residues in the food

The levels of residues in food should not harm the consumer.

<u>Obligations:</u>

• EU countries must implement **residue monitoring plans** to detect the illegal use or misuse of authorized veterinary medicines in food producing animals and investigate the reasons for residue violations

• Non-EU countries exporting to the EU must implement a residue monitoring plan which guarantees an equivalent level of food safety

ILVO

EU VMP RESIDUE LEGISLATION:STANDARDS

Responsibility of EMA for assessing Maximum Residue Limits (MRLs) for VMPs marketed in the EU

Regulation (EC) No 470/2009 of the European Parliament and of the Council laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin
Off. J. Eur. Union 2009 L152: 11-22.

Commission Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin

Table 1: Allowed substances: MRL, (temporary MRL) or no MRL required
Table 2: Prohibited substances (zero tolerance → mRPL)

Off. J. Eur. Union 2010 L15: 1-72 (and amendments)

ILVO

REGULATION (EU) 2017/625 – OFFICIAL CONTROLS

- REGULATION (EU) 2017/625 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, **amending** Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and **repealing** Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation).

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EU VMP RESIDUE LEGISLATION: MONITORING

Member States must monitor food of animal origin for the presence of residues.

- Decision 97/747/EC - Additional sampling frequencies for milk, eggs, honey, rabbits and game meat
- Decision 98/179/EC - Rules for taking official samples and accreditation requirements for official laboratories
- Regulation (EU) 2019/1871 - Reference points for action for non-allowed pharmacologically active substances present in food of animal origin and repealing Decision 2005/34/EC
- Decision 2002/657/EC - Rules for the validation of analytical methods used in the residue monitoring plan.
- Guidelines for the validation of screening methods for residues of veterinary medicines (20/01/2020)

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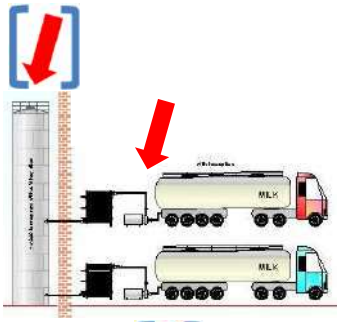
EU VMP RESIDUE LEGISLATION: OTHER

- Directive 96/22/EC: Banned the use of certain substances in food producing animals
- Directive 2001/82/EC: Established the rules for the authorisation and use of veterinary medicinal products
- Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin
- Directive 2004/41/EC repealing certain Directives concerning food hygiene and health conditions for the production and placing on the market of certain products of animal origin intended for human consumption and amending Council Directives 89/662/EEC and 92/118/EEC and Council Decision 95/408/EC

ILVO

DAIRY INDUSTRY: MILK FLOW



Sampling

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INHIBITORS, ANTIBIOTICS AND SULFONAMIDES IN MILK – SHARED RESPONSABILITIES WITHIN AN INTEGRATED DETECTION SYSTEM

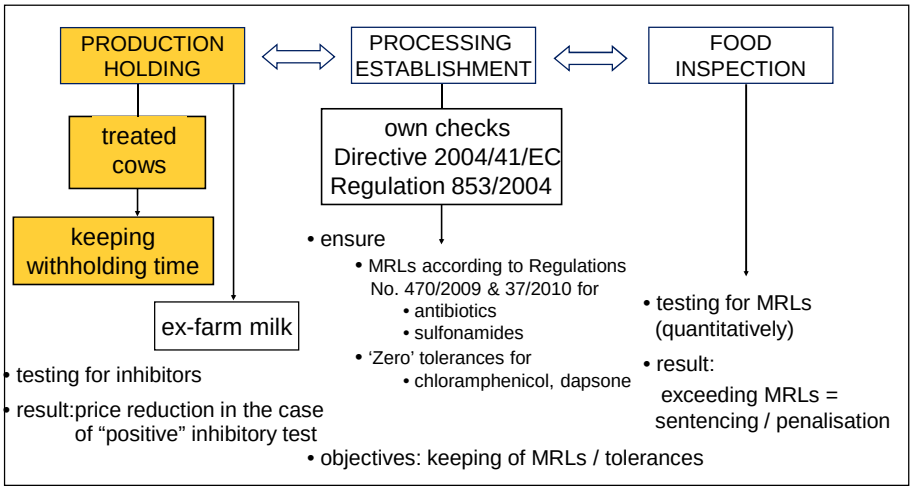
PRODUCTION HOLDING ⟺ PROCESSING ESTABLISHMENT ⟺ FOOD INSPECTION

- PRODUCTION HOLDING
  - treated cows
    - keeping withholding time
  - ex-farm milk
    - testing for inhibitors
    - result: price reduction in the case of "positive" inhibitory test
- PROCESSING ESTABLISHMENT
  - own checks Directive 2004/41/EC Regulation 853/2004
    - ensure
      - MRLs according to Regulations No. 470/2009 & 37/2010 for
        - antibiotics
        - sulfonamides
      - 'Zero' tolerances for
        - chloramphenicol, dapsone
    - objectives: keeping of MRLs / tolerances
- FOOD INSPECTION
  - testing for MRLs (quantitatively)
  - result: exceeding MRLs = sentencing / penalisation

(after HEESCHEN & SUHREN, 1996)

ILVO

INHIBITORS, ANTIBIOTICS AND SULFONAMIDES IN MILK – SHARED RESPONSABILITIES WITHIN AN INTEGRATED DETECTION SYSTEM

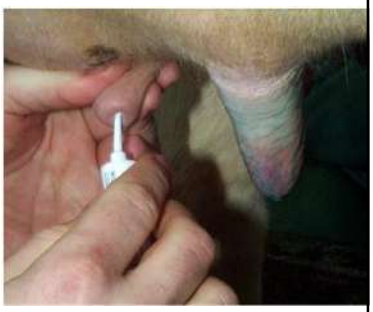


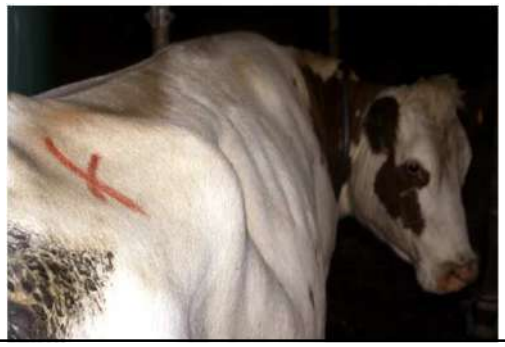
(after HEESCHEN & SUHREN, 1996)

ILVO

DAIRY FARM: RESPONSIBILITY OF VETERINARIAN AND DAIRY FARMER

- correct diagnosis
- prescription of VMP if medication is needed
- correct use of registered VMPs
- good marking of treated cows
- respecting the withdrawal time





ILVO

TESTING AT THE DAIRY FARM

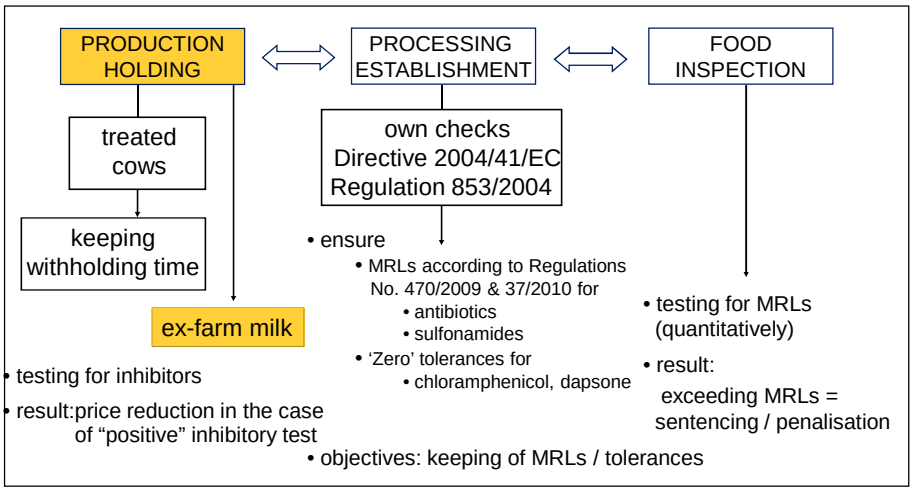
- testing on voluntary basis
  - milk of treated cow at the end of the withdrawal period
  - mostly broad-spectrum microbial inhibitor test in ampoule format

  - Delvotest T mini, Delvotest SP-NT mini
  - Charm Cowside II, Eclipse Farm 3G with e-reader,…
  - Test4 All (Eclipse 4G = COMET incubator, Bluetooth connection with smartphone

- experiments with rapid tests

ILVO

INHIBITORS, ANTIBIOTICS AND SULFONAMIDES IN MILK – SHARED RESPONSABILITIES WITHIN AN INTEGRATED DETECTION SYSTEM



(after HEESCHEN & SUHREN, 1996)

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EX-FARM MILK: LEGAL CONTROL MEASURES

Regulation (EC) No 853/2004: a representative number of raw milk samples, collected from milk production holdings taken by random sampling, must be checked on the presence of antibiotic residues for that the raw milk placed on the market is not containing residues in a quantity >MRL

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TESTING OF EX-FARM MILK SAMPLES

Sampling before or during the collection of farm milk

Samples analysed in a milk control station on antimicrobials:
- each sample (BE, NL,…) or less
- screening with a microbiological test
- post-screening with a microbiological test and/or a receptor assay
- some countries start using LC-MS/MS analysis

Result: - penalty for the farmer
- withdrawal of collection of the next production (in some cases)

⇒ penalization level not in line with MRL: e.g. benzylpenicillin (MRL=4 ppb)
BE: between 3 and 4 µg/kg DE: from 4 µg/kg on
NL: from 2.2 µg/kg on FR: from 1.5 – 2 µg/kg on
No clear requirements set in the European legislation.
Belgium: the tests should be in line with FASFC acceptance criteria

Results are only known after the milk has been processed

2019 Belgium: 1,008,091 samples; 281 positive = 0.028%

16

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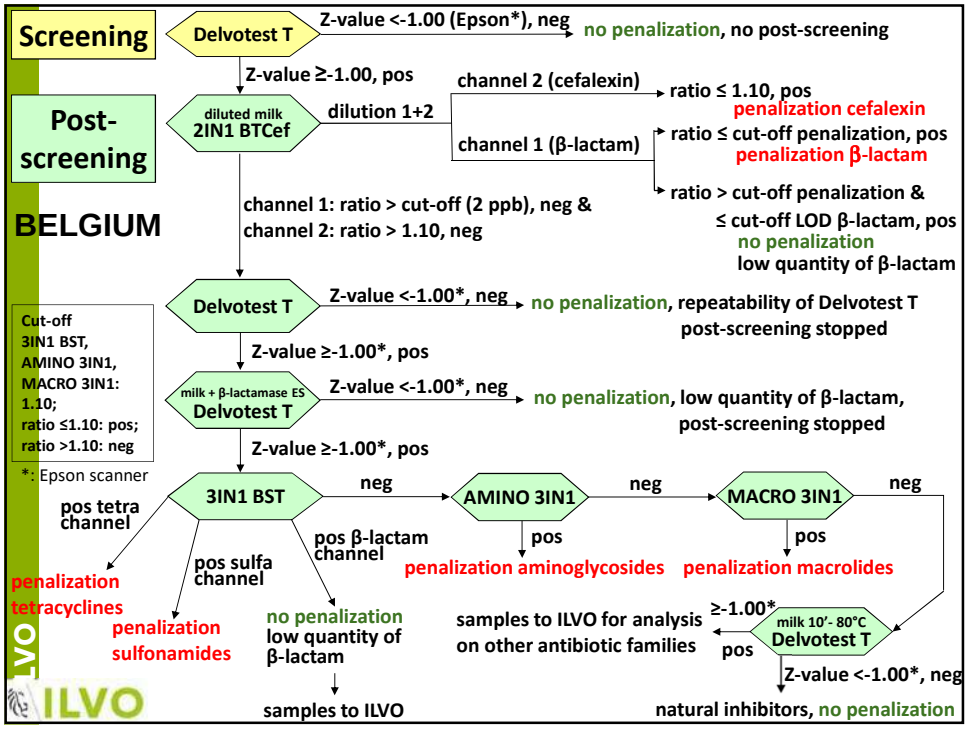
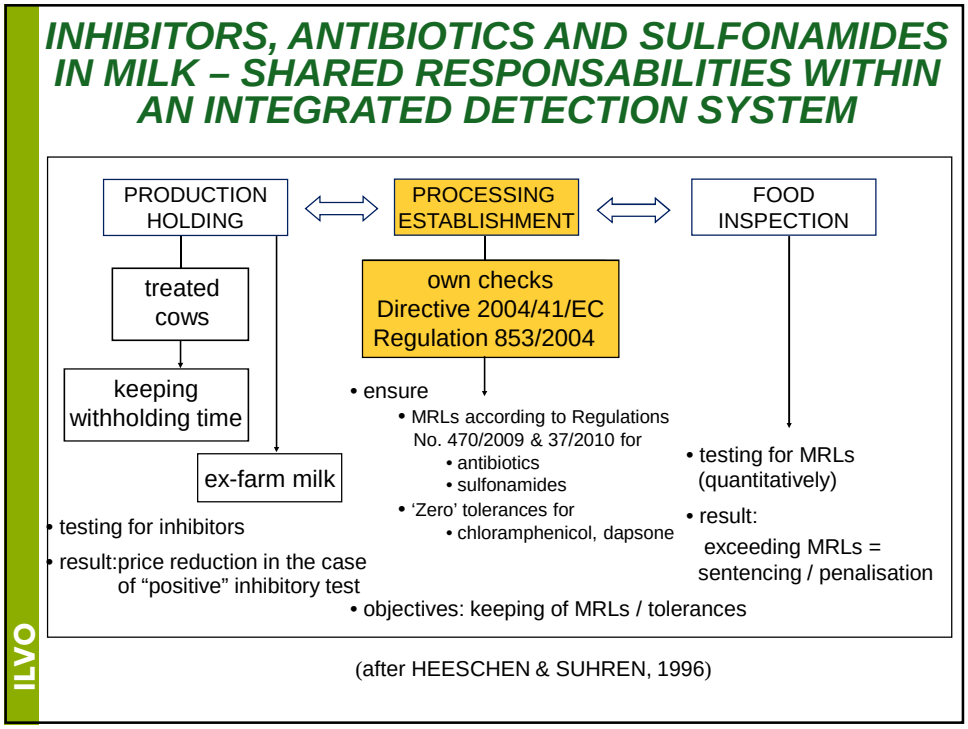

Screening
Post-screening
BELGIUM
Delvotest T
Z-value <-1.00 (Epson*), neg
no penalization, no post-screening
Z-value ≥-1.00, pos
diluted milk
2IN1 BTCef
dilution 1+2
channel 2 (cefalexin)
ratio ≤ 1.10, pos
penalization cefalexin
channel 1 (β-lactam)
ratio ≤ cut-off penalization, pos
penalization β-lactam
ratio > cut-off penalization &
≤ cut-off LOD β-lactam, pos
no penalization
low quantity of β-lactam
channel 1: ratio > cut-off (2 ppb), neg &
channel 2: ratio > 1.10, neg
Delvotest T
Z-value <-1.00*, neg
no penalization, repeatability of Delvotest T post-screening stopped
Z-value ≥-1.00*, pos
milk + β-lactamase ES
Delvotest T
Z-value <-1.00*, neg
no penalization, low quantity of β-lactam, post-screening stopped
Z-value ≥-1.00*, pos
Cut-off
3IN1 BST,
AMINO 3IN1,
MACRO 3IN1:
1.10;
ratio ≤1.10: pos;
ratio >1.10: neg
*: Epson scanner
3IN1 BST
neg
AMINO 3IN1
neg
MACRO 3IN1
neg
pos tetra channel
pos β-lactam channel
pos sulfa channel
pos
pos
penalization aminoglycosides
penalization macrolides
penalization tetracyclines
penalization sulfonamides
no penalization
low quantity of β-lactam
samples to ILVO
samples to ILVO for analysis on other antibiotic families
≥-1.00*
pos
milk 10'- 80°C
Delvotest T
Z-value <-1.00*, neg
natural inhibitors, no penalization
ILVO
ILVO


INHIBITORS, ANTIBIOTICS AND SULFONAMIDES IN MILK – SHARED RESPONSABILITIES WITHIN AN INTEGRATED DETECTION SYSTEM
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• testing for inhibitors
• result:price reduction in the case of "positive" inhibitory test
• objectives: keeping of MRLs / tolerances
(after HEESCHEN & SUHREN, 1996)
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DAIRY PLANT: LEGAL CONTROL MEASURES

Hygiene provisions formulated in Regulation (EC) 852/2004: food business operators have to comply with appropriate Community and national legislative provisions, related to the control of hazards in primary production and associated operations, including measures to control contamination arising from veterinary medicinal products.

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TESTING AT THE DAIRY PLANT

Dairies have to perform autocontrol programmes (Regulation (EC) No 853/2004).

Regulation (EC) No 853/2004: food business operators in the dairy sector are not allowed to place on the market raw milk containing levels of antibiotic residues >MRL.

⇒ In practice: performance of **rapid screening tests** on milk Screening for **β-lactam** residues (penicillins and cephalosporins)

Spain: Real Decreto 1728/2007: also tetracyclines (one test per 5 tanker loads)

US: the testing of all incoming shipments of milk for β-lactam antibiotics is mandatory since January 1, 1992.

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TESTING AT THE DAIRY PLANT

Testing of incoming milk at the raw milk reception

- biggest number of antibiotic tests used in whole food industry
- lack of details in the European legislation about the minimal requirements for the (rapid) tests
- BE: criteria set by the Belgian Food Agency (FASFC)

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TESTING AT THE DAIRY PLANT (BELGIUM)

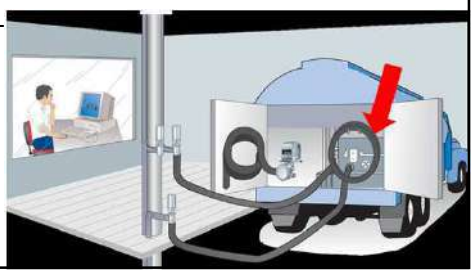
Acceptance criteria set by FASFC

- test must be capable to detect at least 85% (or 12/14) of the marker residues at MRL of the pharmacologically active substances belonging to the β-lactam group, registered in Belgium for use in dairy cattle
- test must be robust and should give max 5% of negative results at CCβ
- test must be validated by the NRL

So far (27/10/2020) 19 tests approved

- test time 1 to 9 minutes
- 2 tests detecting all β-lactams at MRL
- 6 tests missing 1 β-lactam at MRL

http://www.afsca.be/productionanimale/produitsanimaux/circulaires/_documents/20201027_clean_circ-ob_tests_detection_subs_inhibitrices_lait_FR.pdf



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TESTING OF INCOMING MILK

In some countries (US) rapid tests performed at the farm in order to prevent destruction of large volumes (tanker load) of contaminated milk → requests a very short test time

The test result must be known before the milk is accepted
- at the raw milk collection point
- or the dairy farm if the test is performed there

Small production holdings are allowed to test the milk of their collection silo so far they wait on the result before starting with the production.

Rapid tests specifically designed for the market:
- US: safe level: benzylpenicillin at 5 µg/kg and tolerance levels for ampicillin, amoxicillin, cloxacillin at 10 µg/kg, ceftiofur at 100 µg/kg and cephapirin at 20 µg/kg
- Russian Federation: penicillins at 4 µg/kg, tetracyclines at 10 µg/kg, streptomycin at 200 µg/kg and chloramphenicol at 0.3 µg/kg

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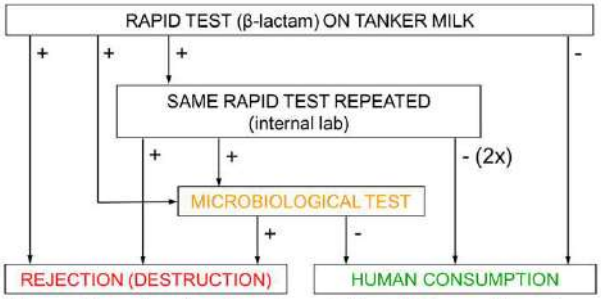
TESTING OF INCOMING MILK

milk showing a positive result of a screening test is deemed to be unsafe
→ contaminated milk must be destructed (animal by-product)
→ farmer can get the invoice for the costs of the milk destruction

Belgium: dairy industry is allowed to use a microbiological inhibitor test to compensate for testing below MRL (cefalosporins)



BE: 2018: 95 positive tanker loads while 296 positive farm milk samples

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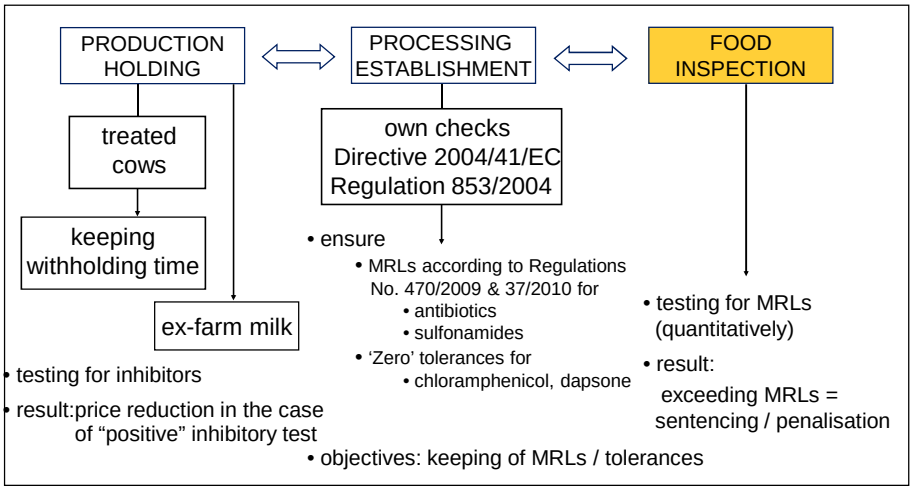
INHIBITORS, ANTIBIOTICS AND SULFONAMIDES IN MILK – SHARED RESPONSABILITIES WITHIN AN INTEGRATED DETECTION SYSTEM



(after HEESCHEN & SUHREN, 1996)

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EUROPEAN SURVEILLANCE PROGRAMMES: minimum sampling by each member state

To ensure safe food from animal origin controlling that MRLs are not violated

Commission Decision 97/747/EC

- beef: 0.4 % (% of slaughtered animals (previous year))
- pigs: 0.05% (% of slaughtered animals (previous year))
- sheep and goats: 0.05% (% of slaughtered animals (previous year))
- horses: determined by the number of identified problems
- poultry: 1 sample per 200 ton year production
  minimum of 100 samples if the year production is > 5000 ton
- aquaculture products: 1 sample per 100 ton year production
- **milk*: 1 sample per 15,000 ton produced milk; minimum of 300 samples**
- chicken eggs: 1 sample per 1000 ton eggs; minimum of 200 samples
- rabbits: first 3000 ton: 10 samples per 300 ton; further 1 sample per 300 ton
- breeded and free game: each minimum 100 samples
- honey: first 3000 ton: 10 samples per 300 ton; further 1 sample per 300 ton

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BE: MONIMILK AND FAVV MONITORING FOR RESIDUES AND CONTAMINANTS

Ad random sampling; limited number of samples

Different types of compounds, including non-authorised products
→ battery of screening tests or a multiplex screening test
(e.g. EXTENSO, 17 families,>100 compounds, 13 min)
→ more and more multiresidue LC-MS/MS analysis

Speed is of less importance, results mainly for data collection

Aim: to monitor as much compounds as possible at MRL (or lower)

Suspect samples: physico-chemical identification and quantification with LC-MS/MS to declare compliant or non-compliant

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OTHER FOOD INDUSTRIES

Use of rapid tests for:

- fast product release e.g. carcasses in slaughter houses
- ante-mortem analysis on non-food matrices

saliva of pigs
antibiotics - ILVO

feathers of poultry
fluoroquinolones - RIKILT

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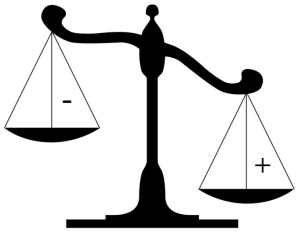
CONCLUSIONS

Screening tests and mainly rapid tests remain very useful and even crucial due to following characteristics:

- easy - cheap - sensitive - fast - high sample throughput
- reliable (false compliance rate of <5 %)
- pro-active



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Thank you!

Wim.Reybroeck@ilvo.vlaanderen.be

Flanders Research Institute for Agriculture, Fisheries and Food
Brusselsesteenweg 370
9090 Melle – Belgium
T + 32 (0)9 272 30 11
F +32 (0)9 272 30 01

t&vbu@ilvo.vlaanderen.be
www.ilvo.vlaanderen.be

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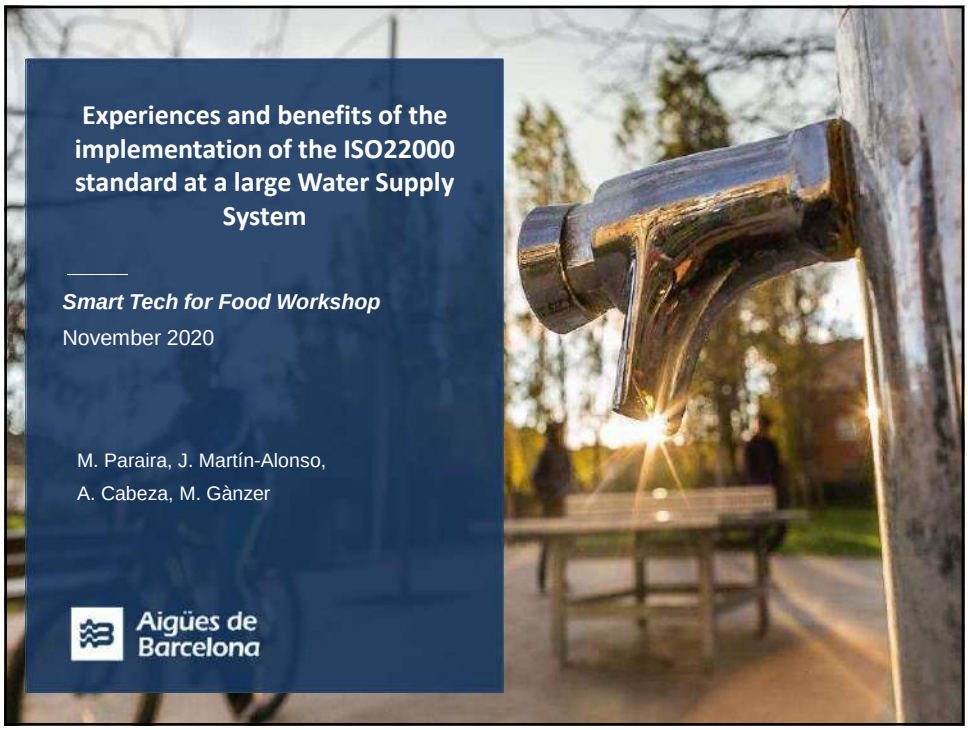
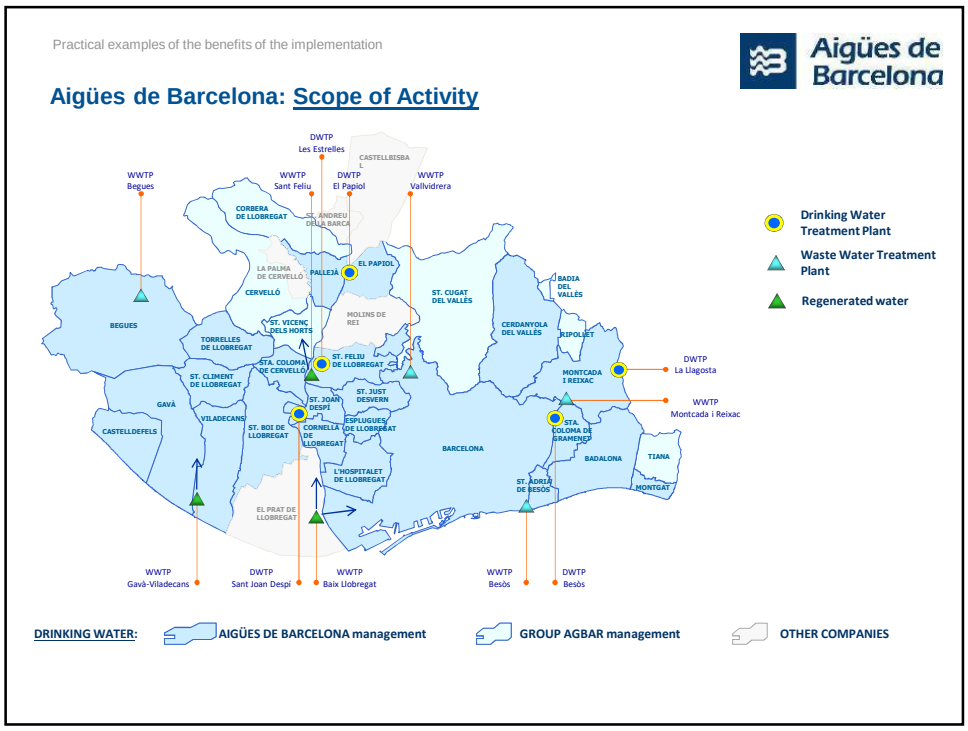

Experiences and benefits of the implementation of the ISO22000 standard at a large Water Supply System
Smart Tech for Food Workshop
November 2020
M. Paraira, J. Martín-Alonso,
A. Cabeza, M. Gànzer
Aigües de Barcelona

1

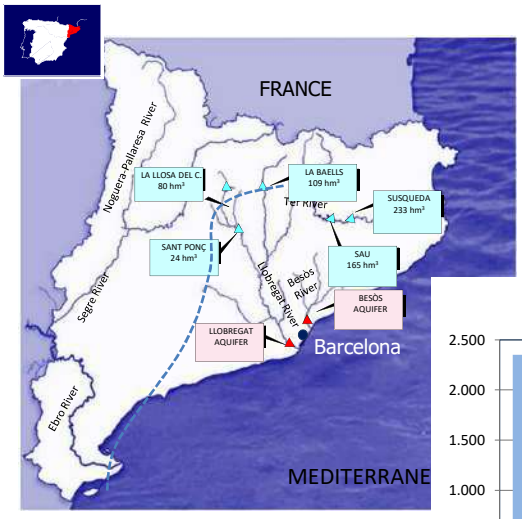

Practical examples of the benefits of the implementation
Aigües de Barcelona
Aigües de Barcelona: Scope of Activity
DWTP Les Estrelles
WWTP Begues
WWTP Sant Feliu
DWTP El Papiol
WWTP Vallvidrera
CASTELLBISBAL
CORBERA DE LLOBREGAT
ST. ANDREU DELLA BARCA
LA PALMA DE CERVELLÓ
PALLEJÀ
EL PAPIOL
CERVELLÓ
ST. CUGAT DEL VALLÈS
BADIA DEL VALLÈS
BEGUES
MOLINS DE REI
ST. VICENÇ DELS HORTS
CERDANYOLA DEL VALLÈS
RIPOLLET
TORRELLES DE LLOBREGAT
STA. COLOMA DE CERVELLÓ
ST. FELIU DE LLOBREGAT
DWTP La Llagosta
MONTCADA I REIXAC
WWTP Montcada i Reixac
ST. CLIMENT DE LLOBREGAT
ST. JUST DESVERN
ST. JOAN DESPÍ
GAVÀ
VILADECANS
ESPLUGUES DE LLOBREGAT
STA. COLOMA DE GRAMENET
CASTELLDEFELS
ST. BOI DE LLOBREGAT
CORNELLÀ DE LLOBREGAT
BARCELONA
BADALONA
TIANA
L'HOSPITALET DE LLOBREGAT
ST. ADRIÀ DE BESÒS
MONTGAT
EL PRAT DE LLOBREGAT
WWTP Gavà-Viladecans
DWTP Sant Joan Despí
WWTP Baix Llobregat
WWTP Besòs
DWTP Besòs
Drinking Water Treatment Plant
Waste Water Treatment Plant
Regenerated water
DRINKING WATER:
AIGÜES DE BARCELONA management
GROUP AGBAR management
OTHER COMPANIES

2

Practical examples of the benefits of the implementation

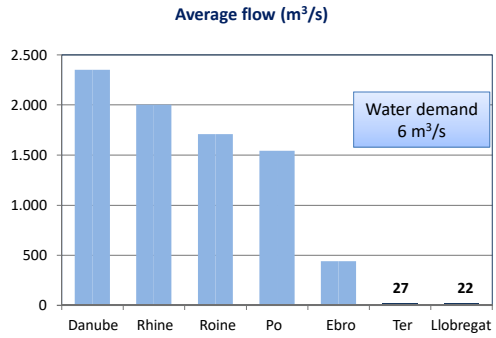
Context: Water Resources Scarcity

Aigües de Barcelona



Water resources for Barcelona's metropolitan area water supply → Surface water from **Llobregat and Ter rivers**. Groundwater as a complement.

Low flow with high flow peaks (Mediterranean climate)

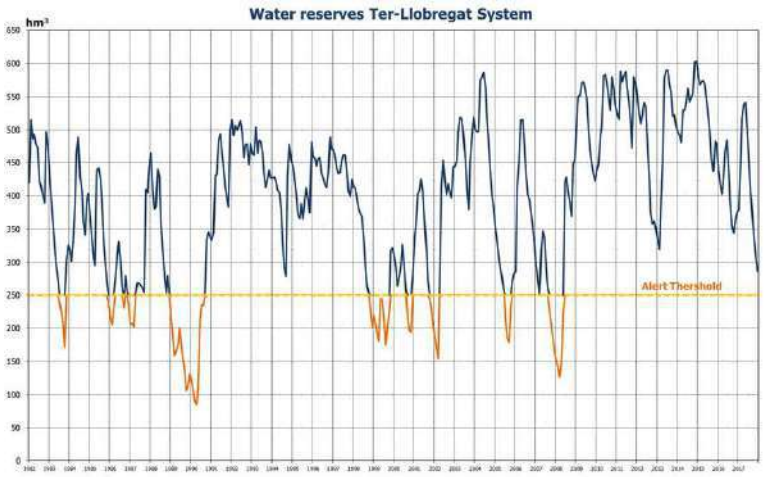


3

Practical examples of the benefits of the implementation

Context: Water Resources Scarcity

Aigües de Barcelona



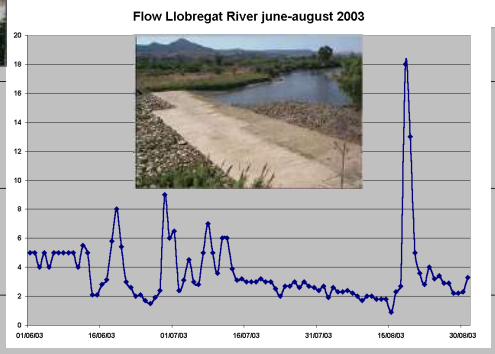
Frequent drought episodes
Over the last 30 years, Ter-Llobregat **lower alert threshold has been reached 20% of the time**

4

Context: Water Resources Scarcity

Aigües de Barcelona

Flow Llobregat River 2003 (m3/s)



And there are more than 2000 industrial activities, almost 1 million people and uncountable number of cattle located upstream

5

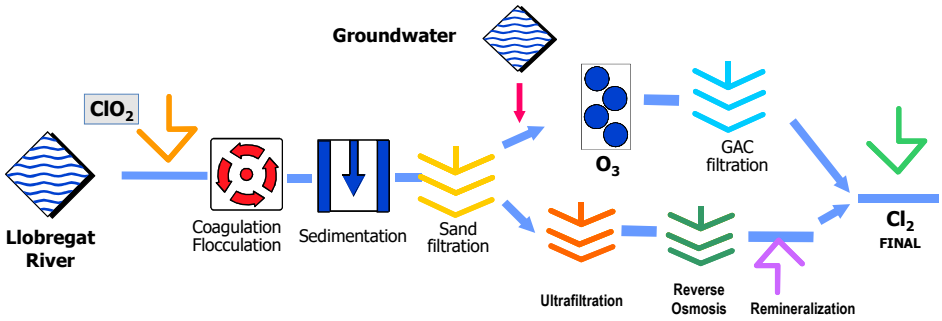
Context: Water Quality Problems

Aigües de Barcelona

Salinization, lack of dilution, industrial & urban contamination, etc.

6

Context: Sant Joan Despí DW Treatment Plant



Because of the quality of the surface water used for drinking water production, the Plant is really complex and control is not always easy

7

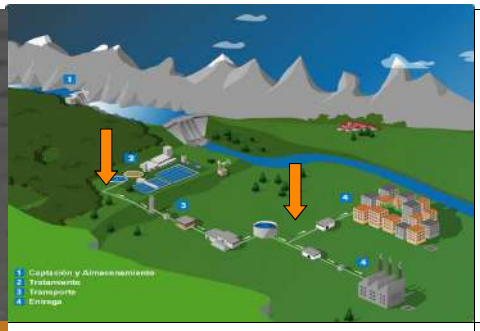
Why to implement a WSP? Our Motivations

- Water resources scarce and polluted.
- Preventive management already implemented, but not formalized.
- Better control of the supplied drinking water, based in preventive concepts.
- To anticipate the compliance of future regulations.
- To prioritize investments related to safe drinking water.
- To integrate the WSP as an ISO 22000 certification with other systems such as ISO 9001, ISO 14001 and OHSAS 18001.
- First water supply in Spain to obtain the ISO 22000 certification.

8

Aigües de Barcelona

Water Quality Monitoring and Control



Classical strategy:

Monitoring of treated water

⇩

Sampling points located at:

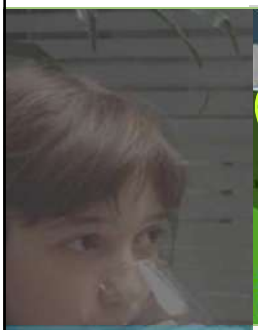
Main inconvenient: it is retrospective

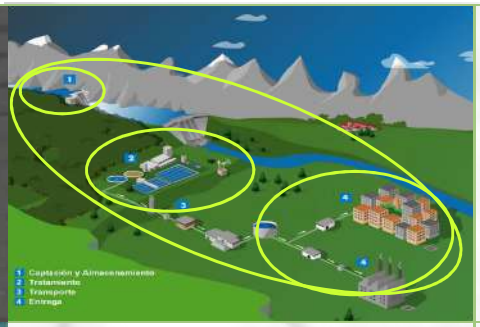
- Final treated water
- Distribution network and consumer's tap

9

Development and Implementation of a Risk Management Plan in a Drinking Water Supply

Aigües de Barcelona





'New' approach:

WHO (2004)

⇩

Water Safety Plans

GLOBAL evaluation and management of sanitary risks

Preventive management

- Resources
- Treatment steps
- Distribution network and tap water

10

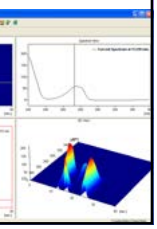
A full set of data is needed for the identification of sanitary risks

Aigües de Barcelona

SANITARY RISK MANAGEMENT IN A DRINKING WATER SUPPLY

Hazard identification

Pharmaceuticals
Pesticides
Endocrine Disruptors
Viruses
Etc…
Disinfection By-Products
Emerging Pathogens
Analytical data / Expert advice
Solvents
Pathogens
Chlorinated solvents
Aromatic hydrocarbons
Radioactivity
Heavy metals



11

Every organization has to identify the hazards and its causes, in order to minimize risks

Aigües de Barcelona

SANITARY RISK MANAGEMENT IN A DRINKING WATER SUPPLY

Hazard causes

Surface water pollution
Groundwater pollution
Vandalism
Pipeline breakage
Leakage in reservoir tanks
Etc…
Lack of maintenance
Deficient cleaning
Low dosage of reagents
Incorrect calibration
High dosage of reagents
Lack of training for the personnel

12

Priorization of the identified risks

Aigües de Barcelona

SANITARY RISK MANAGEMENT IN A DRINKING WATER SUPPLY

DOCUMENTED JUSTIFICATIONS:

- **Severity:** Toxicological studies
- **Likelihood:** Analytical data

SANITARY RISK QUANTIFICATION

Risk Assessment Matrix

ADAPTED BY AGBAR

(Risk factor = Likelihood x Severity)

Gravedad	Probabilidad: 1 Decenal o más	2 Cuatrienal	3 Anual	4 Trimestral	5 Mensual
1	1	2	3	4	5
5	5	10	15	20	25
10	10	20	30	40	50

R ≤ 10	Riesgo bajo
15 ≤ R ≤ 20	Riesgo medio
R ≥ 25	Riesgo elevado

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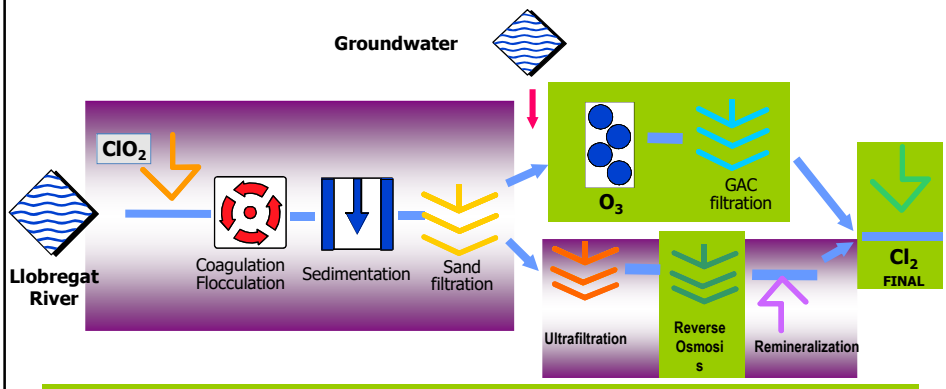
13

Sant Joan Despí Waterworks - CCPs identified

Aigües de Barcelona

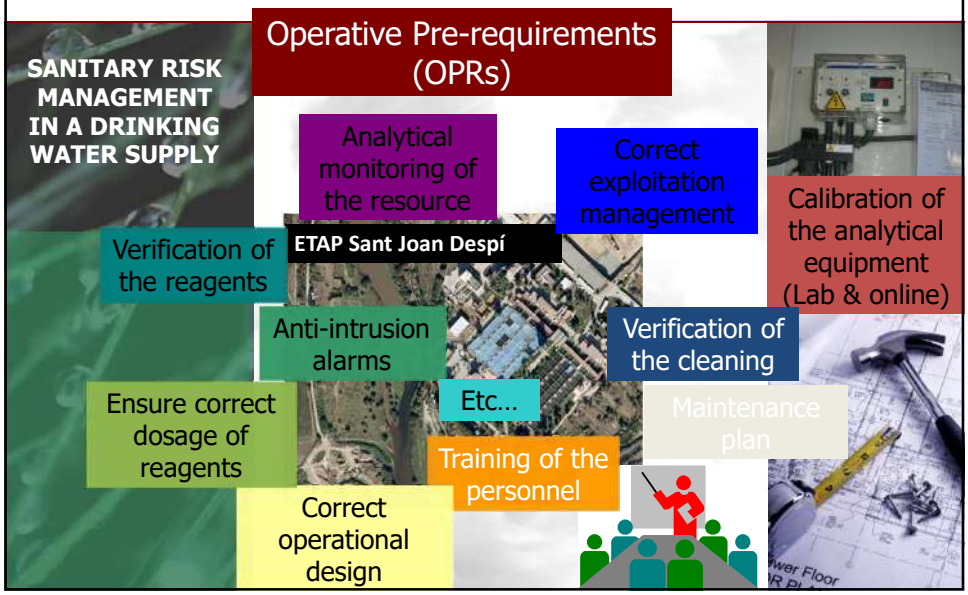


CCPs: Critical Control Points – every one has its own Control Parameter that ensures the correct operation of the unit/barrier

14

The essential forefront: OPRs

Aigües de Barcelona



15

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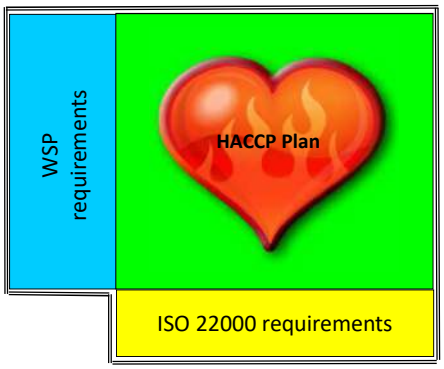
Main difficulties / Challenges

- Adaptation of the ISO 22000 standard (food industry) to the water sector.
- To carry out a correct risk analysis.
- To develop our own decision tree in order to identify correctly the CCPs / OPRs.
- To develop the best HACCP Plan possible based on preventive management.
- To use the same methodology and criteria for waterworks, reservoirs and the distribution network.
- To create a multidisciplinary, motivated and proactive team.

16

Aigües de Barcelona

WSPs in Barcelona's Metropolitan Area: the added value of the ISO 22000 certification



WSP: based on the WHO Guidelines.

ISO 22000: External certification (trustworthiness)

System certified in **2009**, covering the whole Drinking Water Cycle (catchment, treatment, transportation & distribution)

First to be obtained in Spain

17

Aigües de Barcelona

NOW LET'S TAKE A LOOK AT SOME OF THE IMPROVEMENTS AND BENEFITS WE'VE REACHED AFTER MORE THAN A DECADE OF THE ISO 22000 CERTIFICATION

18

Practical examples of the benefits of the implementation

Improvements after ISO22000 certification:

On-line sensors

Aigües de Barcelona

Gradual deployment over the years of a netowrk of **on-line quality analyzers** in the whole DWS system: switch from traditional lab. analysis (corrective actions) to on-line analyzers (preventive control):

- Around **200 on-line analyzers** in the **DN**
- More than **100 on-line analyzers** in the **DWTP**
- Connected to SCADA and LIMS (24x7)

19

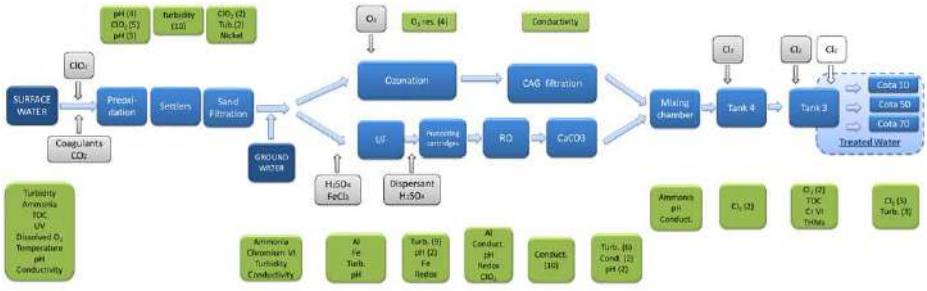
Practical examples of the benefits of the implementation

Improvements after ISO22000 certification:

On-line sensors (DWTP)

Aigües de Barcelona

The Sant Joan Despí Waterworks is very complex, and the risk-based approach for its management has been very useful to improve the quality of the produced drinking water



PRETREATMENT **OZONATION/GAC & UF/RO** **FINAL DISINFECTION**

20

Practical examples of the benefits of the implementation

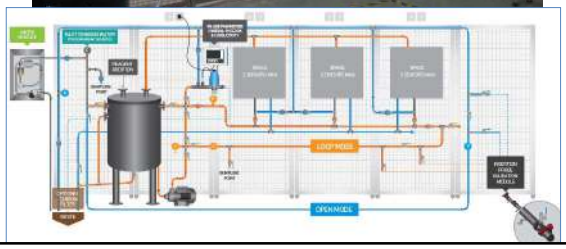
Improvements after ISO22000 certification:
On-line sensors

Aigües de Barcelona

The first Platform in Spain for the **Validation of WQ on-line sensors**

Aigües de Barcelona

CETAQUA CENTRO TECNOLÓGICO DEL AGUA

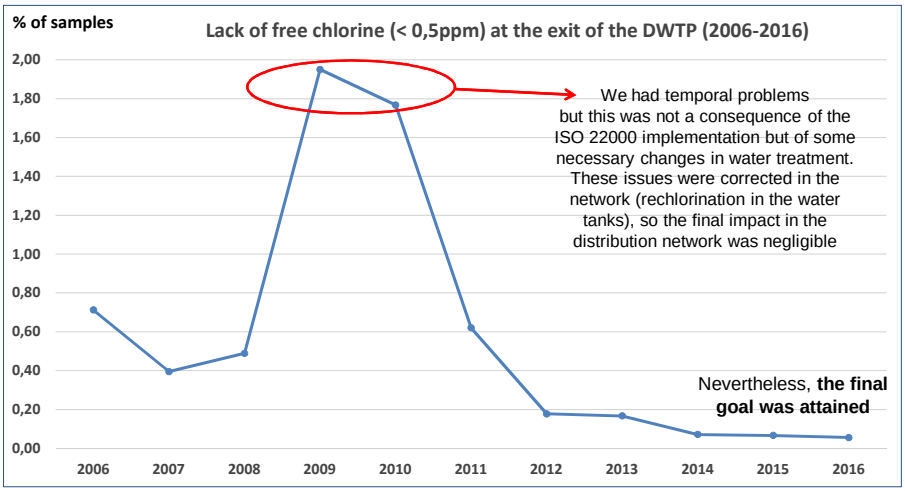


21

Practical examples of the benefits of the implementation

Improvements after ISO22000 certification:
Quality Incidents

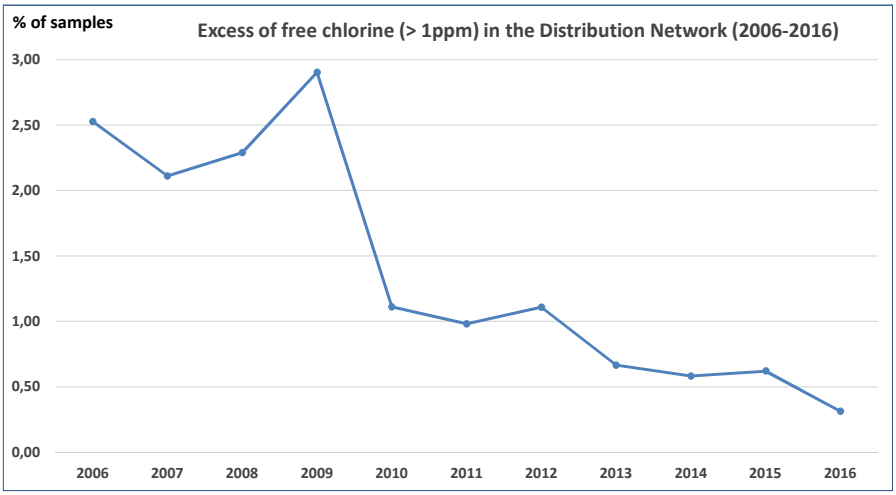
Aigües de Barcelona



22

Practical examples of the benefits of the implementation

Improvements after ISO22000 certification:
Quality Incidents



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Practical examples of the benefits of the implementation

Improvements after ISO22000 certification:
Reduction of legal WQ Breaches

REGULATORY NONCOMPLIANCES (European Directive *98/83/CE*)

Parameter	Before ISO 22000 (2008)	After ISO 22000 (2019)
Trihalomethanes	24	0
Total coliforms	7	0
Clostridium perfringens	5	1

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Practical examples of the benefits of the implementation

Aigües de Barcelona

Improvements after ISO22000 certification:
Optimization of THMs levels

TRIHALOMETHANES	Quality Incidents (values > 100 µg/l)		Average Values (µg/l)		
	Before ISO 22000 (2008)	After ISO 22000 (2019)	Before ISO 22000 (2008)	After ISO 22000 (2019)	Reduction (%)
DWTP	68	0	96,9	13,5	86
Distribution Network	116	0	96,0	44,8	53

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Practical examples of the benefits of the implementation

Aigües de Barcelona

Improvements after ISO22000 certification:
General levels of WQ Compliance (Indicators)

Indicator	2008	2019
Microbiological overall compliance	ND	99,8%
Physico-chemical overall compliance	ND	100%
Water disinfection (DN)	97,7%	99,9%

Thousands of samples analyzed !

26

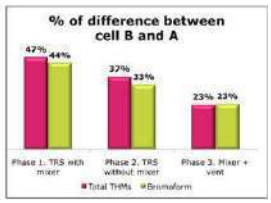
Practical examples of the benefits of the implementation

Improvements after ISO22000 certification:
Specific Investments

Aigües de Barcelona

ISO 22000 has helped to prioritize investments focused on water quality improvements, such as:

- Multiple treatment enhancements
- On-line analyzers (broad network)
- Information systems
- ***Trihalometane Removal Systems (TRS)***. 'Satellite' systems for critical points of the DN.



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SUMMARY: WHAT HAS CHANGED SINCE THE IMPLEMENTATION OF ISO 22000 ?

- We now work as a TEAM and everybody BELIEVES in the System
- Water Quality is a PRIORITY and is considered BEYOND DEPT's BORDERS
- We assure CONTINOUS compliance
- We perform investments focusing on RISKS' MINIMIZATION
- Main focus not only on compliance but also on **SAFETY**
- SECURITY elements included (prevention of attacks)
- Preventive ON-LINE CONTROL
- Not surprisingly, **IT DOES WORK !**

SYSTEM CERTIFICATION ISO 22000 SGS

And now…WHAT'S NEXT?

28

A CHALLENGE AHEAD: WSP & SSPs

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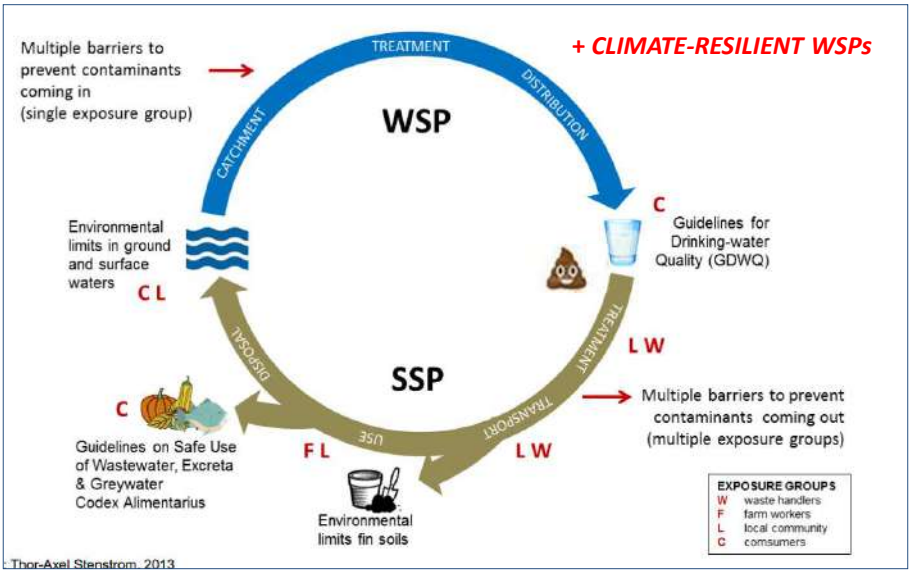

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Aigües de Barcelona

<u>One goal</u>: development of a management system based on risk assessment criteria that integrates the whole water cycle

To achieve this objective we have to work together: scientists, government, users, operators, citizens..

30

26 November 2020

Contribution of science and technology to efficient food risk assessment in the EU

Iñaki Eguileor

Management Board member

Trusted science for safe food

European Food Safety Authority

Contribution of science and technology to efficient food risk assessment in the EU

1. Food risk assessment in the EU and EFSA.

2. How science and technology contribute to regulatory science.

3. Some hits and challenges from 2002 to 2020.

4. The new EFSA regulation and the future.

1. Food risk assessment in the EU and EFSA

1.a Creation of the EUROPEAN FOOD SAFETY AUTHORITY (EFSA)

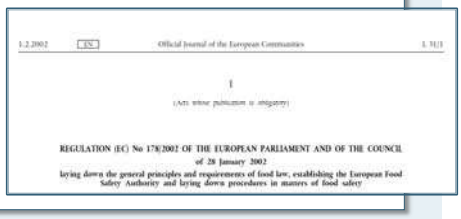
Food crises in the late 1990s (BSE, dioxins)

Until 2002 the EC fully responsible of food risk analysis. Risk assessment provided by scientific committees.

General Food Law (178/2002) created a European food safety system

Separated:

- Responsibility for risk assessment (science)
- Responsibility for risk management (policy)


1.2.2002 EN Official Journal of the European Communities L 31/1

I

(Acts whose publication is obligatory)

REGULATION (EC) No 178/2002 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 28 January 2002
laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety

1. Food risk assessment in the EU and EFSA

1.a Creation of the EUROPEAN FOOD SAFETY AUTHORITY (EFSA)

EFSA established as source of:

- scientific advice
- scientific and technical support

for the Community's legislation and policies on food and feed safety.

and communication on risks associated with the food chain.

EFSA

Risk assessment and communication.

European Commission, European Parliament and Member States

Risk management (policy, management and enforcement).

1. Food risk assessment in the EU and EFSA

1.b Governance of EFSA

Management Board (MB)

14 members Mandated to act in the public interest

Wide range of expertise related to the food chain but do NOT represent a government, organisation or sector

Selection:

- open call for expressions of interest
- shortlist by EC, consulting the European Parliament and appointed by the Council of the EU

+ **1 EC representative**

Ensures that the Authority functions effectively and efficiently, delivers its mandate as defined in its founding Regulation and meets the expectations of European and national institutions, stakeholders and the public.

Audit Committee Deal with audit needs ensuring that all audit services providers (IAS, ECA, and external) recommendations are taken into account and receive appropriate follow-up. It provides advice on financial, reporting and monitoring issues.

1. Food risk assessment in the EU and EFSA

1.b Organization of EFSA

Operational management

Executive Director (ED) Appointed by EFSA's MB

Legal representative of the Authority, responsible for operational matters, staffing issues and drawing up work programme.

Management Team under responsibility of the ED collaborates in the day-to-day operation of EFSA.

EFSA's experts

Scientific Committee

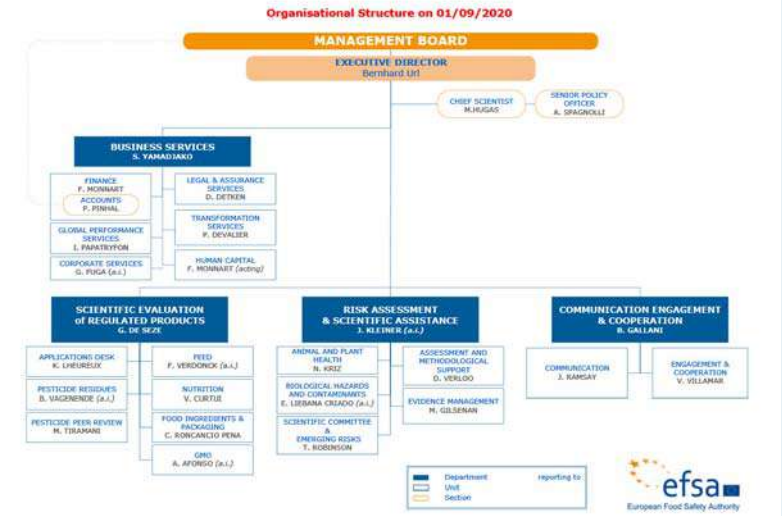
Senior scientists, with experience of work within scientific bodies, covering all disciplines across EFSA's areas of responsibility.

Panels

Scientists from across Europe with expertise in: *risk assessment, modelling, toxicology, microbiology and pathology, epidemiology, animal production, analytical chemistry, food and feed processing, exposure assessment, statistics, …*

1. Food risk assessment in the EU and EFSA

1.b Organization of EFSA



1. Food risk assessment in the EU and EFSA

1.b Organization of EFSA

Advisory Forum

Representatives national food safety authorities. Advise EFSA on scientific matters and to identify emerging risks.

Focal Points

Interface between EFSA and national food safety authorities, research institutes and other stakeholders.

Stakeholder Forum

Provide strategic input to EFSA's work plans and future priorities on an annual basis.

1. Food risk assessment in the EU and EFSA

1.c EFSA work

Most of EFSA's work is undertaken in response to requests for scientific advice from policymakers/risk managers of:

European Commission, European Parliament, EU Member States and own initiative.

Priorities agreed with the European Commission and other partners, taking into account available resources.

Grants & Procurements

EFSA regularly awards grants or subsidies for projects and activities that contribute to EFSA's mission in the areas of data collection, preparatory work for scientific opinions, other scientific and technical assistance.

Article 36 Competent organizations designated by MS

Calls for scientific and technical support

EFSA is establishing a list of scientists to assist its units in carrying out the preparatory work for scientific outputs.

2. How science and technology contribute to regulatory science

2.a Expert´s contribution

Scientific Committee

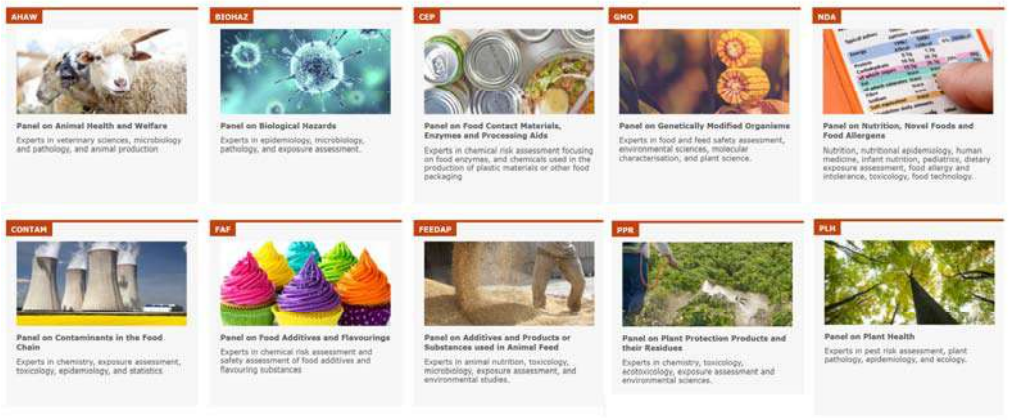
- Develops harmonised risk assessment methodologies on scientific matters of a horizontal nature.
- Provides general co-ordination to ensure consistency in the scientific opinions prepared by EFSA's Scientific Panels.
- Provides strategic scientific advice to EFSA's management.

2. How science and technology contribute to regulatory science

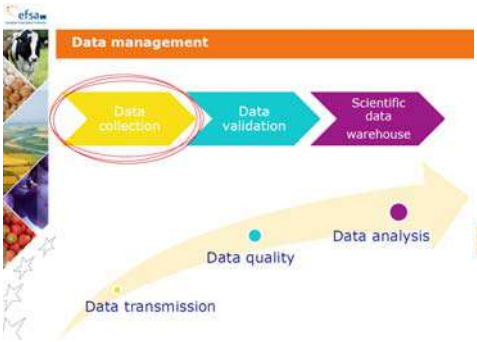
2.a Expert´s contribution

Panels

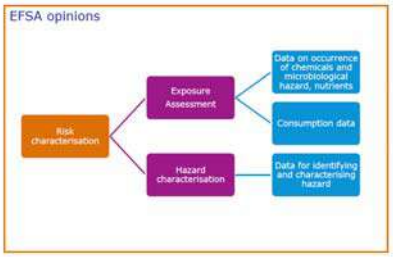
The Panels provide scientific advice in their areas of responsibility.



2.b The flow of information for decision. Public data collection



EFSA Risk assessment



Risk characterization

2. How science and technology contribute to regulatory science

2.c **Uncertainty analysis and communication in food risk**

Uncertainty analysis:

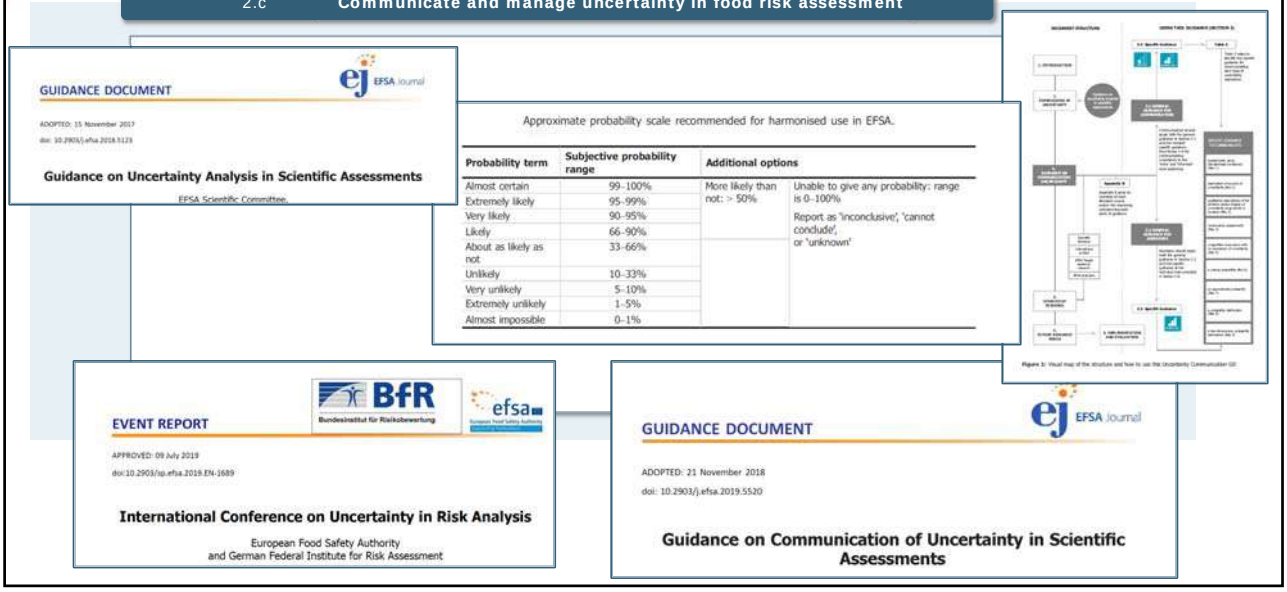
The process of **identifying limitations** in scientific knowledge and **evaluating their implications** for scientific conclusions.

The nature and context of each assessment and the degree of uncertainty present affects the form and extent of uncertainty analysis and how the conclusions should be reported.

EFSA provides a concise guidance on how to identify which options for uncertainty analysis are appropriate in each assessment, and how to apply them.

2. How science and technology contribute to regulatory science

2.c **Communicate and manage uncertainty in food risk assessment**



3. Some hits and challenges from 2002 to 2020

3.a Harmonization and Innovative tools in RA

Foodex 2 Harmonised classification of the food consumption data. Basic for exposure assessment

OpenFoodTox 2.0 Database with summary on identification of chemicals, hazard identification and characterisation.

Guides Qualified presumption of safety of microorganism, Threshold of toxicological concern, Uncertainty, …

Pesticide evaluation Tools: Simulation models and scenarios, Calculation model Pesticide Residue Intake, Assessment of exposure of operators, workers, residents and bystanders, …

Modern methodologies and tools for hazard assessment In vitro systems, in silico tools and OMICs technologies.

Data base IT Challenge

3. Some challenges and hits from 2002 to 2020

3.b Challenges

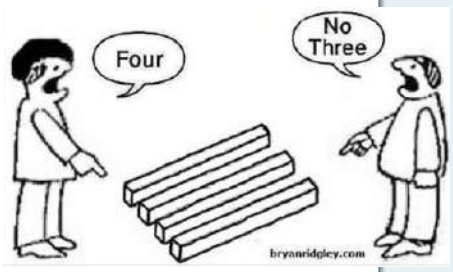
Cumulative risk assessment Multiple exposures

Divergent opinions

Glyphosate

Risk - benefit Fish consumption

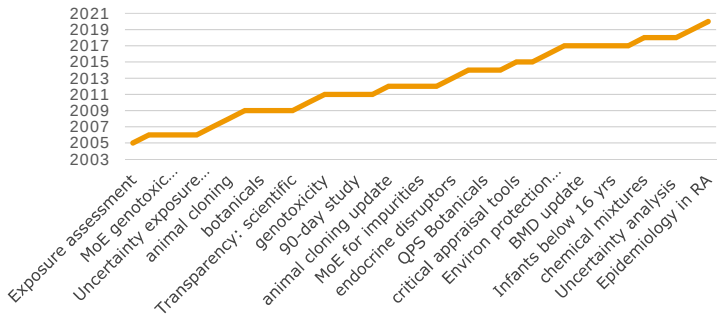
Increase in requests, tasks. Regulated products.



3. Some challenges and hits from 2002 to 2020

3.b HITS

Scientific Committee activities 2003- 2020



55 Opinions (Art 29)
11 Scientific reports (Art 31)
12 Data collection (Art 33)
24 Public Consultations
38 Procurements

3. Some challenges and hits from 2002 to 2020

3.c Collaboration among EU agencies

Collaboration among EU agencies (ECHA / MEA / ECDC)

Not only for better resource management (IT, processes, ...)

Experience and knowledge sharing

Sustainability "One substance - one assessment"

ECHA EUROPEAN CHEMICALS AGENCY

In support of the EU chemicals strategy for sustainability: One substance – one assessment

August 2020

In December 2019, the Commission published its European Green Deal, announcing a **chemicals strategy for sustainability**.

The Commission will look at how to simplify and strengthen the legal framework and review how to use the EU's agencies and scientific bodies better to move towards **'one substance – one assessment'**.

EFSA and ECHA have drafted a joint position paper around the idea of one substance - one assessment for chemicals. The paper provides an analysis of the current situation and proposes solutions that support simplification, cost savings and improved regulatory predictability.

4. The new EFSA regulation and the future

4.a Transparency, sustainability ... and glyphosate

EC Fitness Check of General Food Law (GFL) 2018.

Some Shortcomings identified:

National differences in the implementation and enforcement of EU legislative framework.

Need to protect the reputation of EFSA's work:

Risk assessment in authorisation dossiers. Perception certain lack of transparency and independence as EFSA is bound by food legislation *(strict confidentiality rules and primarily base assessment on industry studies)*.

Risk communication. More effectiveness to maintain consumers' trust and acceptability of EFSA's scientific work by the general public.

Need to improve capacity of EFSA to maintain a high level of scientific expertise and to fully engage all MS in scientific cooperation.

Lengthy authorisation procedures in some sectors (e.g. feed additives, plant protection, food improvement agents, novel foods, health claims) slow down the market entry process.

4. The new EFSA regulation and the future

4.b New Regulation ... new challenges

6.9.2019 EN Official Journal of the European Union L 231/1

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(Legislative acts)

REGULATIONS

REGULATION (EU) 2019/1381 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 20 June 2019
on the transparency and sustainability of the EU risk assessment in the food chain and amending Regulations (EC) No 178/2002, (EC) No 1829/2003, (EC) No 1831/2003, (EC) No 2065/2003, (EC) No 1935/2004, (EC) No 1331/2008, (EC) No 1107/2009, (EU) 2015/2283 and Directive 2001/18/EC

4. The new EFSA regulation and the future

Transparency & Confidentiality

- Establishment of register of studies and the proactive publication of studies' results
- Centralised confidentiality checks
- Clarification of interplay between public access to document and Aarhus Regulations

Engagement & Risk Communication

- Inclusive risk communication (EC, MSs).
- Improve coordination between risk assessors and risk managers to ensure a better communication to stakeholders and the general public.

4. The new EFSA regulation and the future

Scientific value

- More active role of Member States in building EFSA's pool of experts
- Strengthened support to applicants and small/medium enterprises
- Improved assurance of industry studies underlying EFSA scientific outputs

Governance

Organisational governance As in EU Agencies

- Fixed & mandatory quota for MS, EU Parliament, stakeholders

Scientific governance

- Stronger emphasis on experts' active involvement in research
- Enhanced flexibility for EFSA to organise its scientific production (i.e. staff/MSs)

4. New EFSA regulation and the future

4.c The Future

Holistic knowledge. Risk - benefit assessment (socio-economic and sustainable)

Transdisciplinary expertise. Challenges of recruiting scientists

Data sharing and reuse

Collaboration and sustainability

New technologies. New way of working


EDITORIAL

APPROVED: 14 June 2019
doi: 10.2903/j.efsa.2019.e170622

Food Safety Regulatory Research Needs 2030

European Food Safety Authority (EFSA),
Stef Bronzwaer, Georges Kass, Tobin Robinson, José Tarazona, Hans Verhagen, Didier Verloo, Domagoj Vrbos and Marta Hugas

4. New EFSA regulation and the future

4.c The Future

- **Deliver trustworthy scientific assessment and communication of risks from farm to fork.**
- **Build partnerships for the scientific advice of the future.**
- **Empower people and inspire a culture to realise EFSA's strategy.**

Thank you for your attention!

Keep safe!

www.efsa.europa.eu

Anna Jofré
Food Safety Programme
Food Industry Area
Monells (Girona)

Predictive microbiology for the in silico simulation of the microbial behaviour. Applications in Food Quality and Safety

Generalitat de Catalunya
Government of Catalonia

PREDICTIVE MICROBIOLOGY

It is a discipline that wants to **describe** and **predict** the **response** of microorganisms to the environmental factors

Behaviour

Growth (increase)
Inactivation (reduction)
Production of metabolites (e.g. toxins)

- over time or their probability -

QUANTITATIVELY

Quantitative microbial ecology

How do we predict?

$$\sqrt{\mu_{max}} = a_1 \cdot (T - T_{min}) \cdot \sqrt{(pH - pH_{min}) \cdot (a_w - a_{w\,min}) \cdot (NO_{2\,max} - NO_2)}$$

$$ts = \frac{\log N_s - \log N_0}{\log 2} \cdot g$$

$$\mu_{max}(T, pH) = \mu_{opt} \cdot \varsigma(T) \cdot \Psi(pH)$$

$$Log N_t = \frac{d}{a + e \cdot [-B(t - M)]}$$

donde,

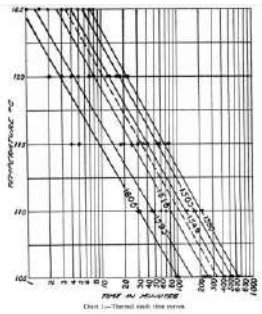
$$\varsigma(T) = \begin{cases} T < T_{min}, & 0 \\ T_{min} < T < T_{max}, & \frac{(T - T_{min})(T - T_{max})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{min})(T_{opt} + T_{min} - 2T)]} \\ T > T_{max}, & 0 \end{cases}$$

$$\text{logit}(P) = b_0 + b_1 \cdot \ln(T - T_{min}) + b_2 \cdot \ln(pH - pH_{min}) + b_3 \cdot \ln(a_w - a_{w\,min}) + b_4 \cdot (NO_{2\,max} - NO_2)$$

$$Log(S) = -k(T) \cdot t = \frac{t}{D}$$

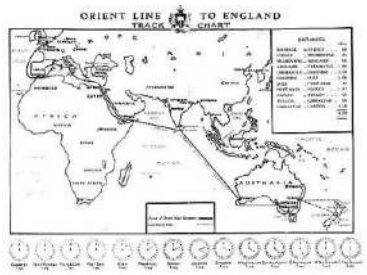
Thermal inactivation model

Bigelow (1921) The logarithmic nature of thermal death curves



Growth models

Scott (1937) *The growth of microorganisms on ox muscle. I. The influence of temperature. J Counc Sci Ind Res Aus.*



"Predictive Microbiology"

Roberts & Jarvis (1983)

IRTA

Extrinsic factors
-Processing (thermal treatment, high pressure processing, etc.)
-Temperature
-Packaging
-Humidity
...

Intrinsic factors
-pH
-A_w
-Redox potential
-Antimicrobials
-Background microbiota
...

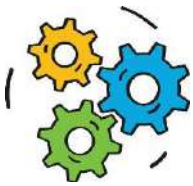
Environmental factors

MICROBIAL RESPONSE

Reproducible
Quantifiable
Predictable

5

IRTA



input → MATHEMATICAL MODEL → **output**

T, pH, a_w, …

growth rate
inactivation rate
probability of growth/toxin formation

How is the data obtained/generated?
How are the mathematical models build?

IRTA

Sample preparation





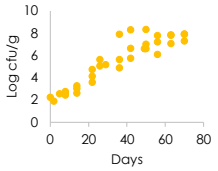

challenge test

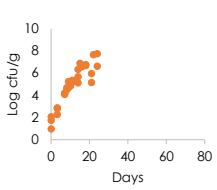
Experimental data generation

4°C

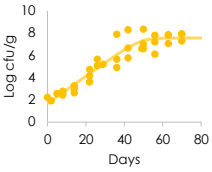
storage & periodical enumeration

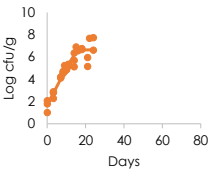
7°C





Modelling





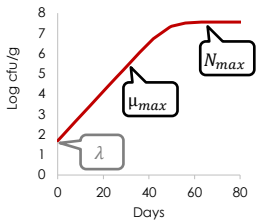
IRTA

Primary models

Aim to describe the population density against time under constant conditions to estimate the growth/inactivation kinetic parameters

Logistic growth model

$$\mathrm{Log}(N_t) = \mathrm{Log}\left(\frac{N_{max}}{1+\left(\frac{N_{max}}{N_0}-1\right)*\left(\exp\left(-\mu_{max}*(t-\lambda)\right)\right)}\right)$$

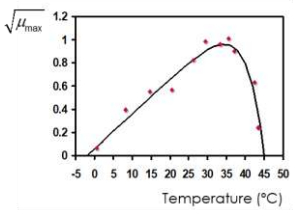


Secondary models

Describe the effect of environmental conditions on the values of the parameters of the primary models

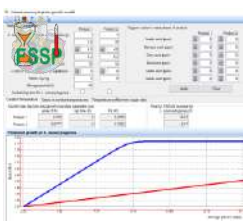
Square root model

$$\sqrt{\mu_{max}} = a_1 \cdot (T - T_{min})$$



Terciary models

user friendly interfaces



Predictive microbiology is a cost-effective, reliable and recognized methodology

product & process characterization

historical data

Scientific literature

predictive models ⟷ **Lab tests**

! Domain Validation

Application of predictive microbiology to the food industry

SUPPORT

Legal requirements

*PRP, **HACCP**, **GMP***

Client requirements

BRC, ISO 22000, IFS, etc.

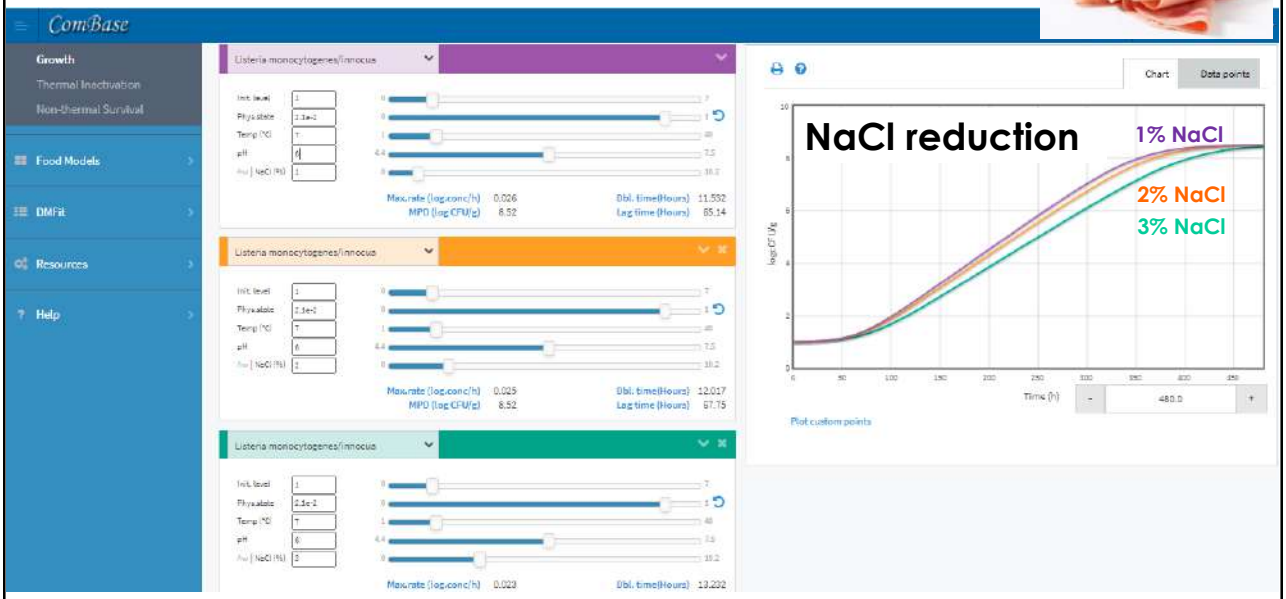
DESIGN
ReDESIGN

ensure Food Safety through **intrinsic & extrinsic factors**

Validation

- process
- shelf life

Simulation of the growth of *Listeria monocytogenes* in cooked ham **in different scenarios**:



www.combase.cc

Salmonella in fermented meat products

Home Made! model

Microbiological criteria for *Salmonella* in dry-fermented sausages

AIM of the study:

To provide a risk management tool assisting the design of a feasible and cost-effective control measure contributing to ensure the accomplishment of zero-tolerance policies

corrective storage

Fuet is a very appreciated fermented sausage highly appreciated by consumers

RTE product
Regulation (EC) 2073/2005
No detection in 25 g

Challenge test

to evaluate the behaviuor of *Salmonella* in *fuet*

Inoculation of *Salmonella*
6 Log cfu/g
3-strain mix

meat bater
meat ingredients
additives
(starter culture)

Fermentation & ripening
3 different processes

a_w (final product)
0.88
0.90
0.93

Storage
(3 months)

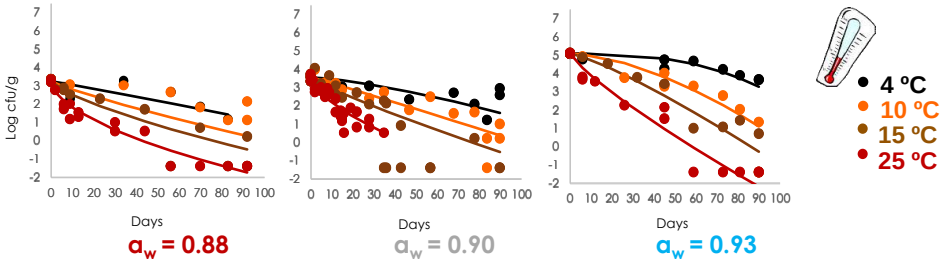
4 ºC
10 ºC
15 ºC
25 ºC

Enumeration
CHROMAagar
Salmonella plus

Modelling

Challenge test

to evaluate the behaviuor of *Salmonella* in *fuet*



a_w = 0.88 a_w = 0.90 a_w = 0.93

VIABILITY
Bactericidal against *Salmonella*

LETHALITY
- a_w
- Temperature

R Studio

$$Log(N) = Log(N_0) - \left(\frac{time}{\delta}\right)^p$$

Time to reduce 1 log the concentration of *Salmonella*

Shape of the inactivation curve

Serra-Castelló et al. (2019)

Lethality treatment

QUARANTINE

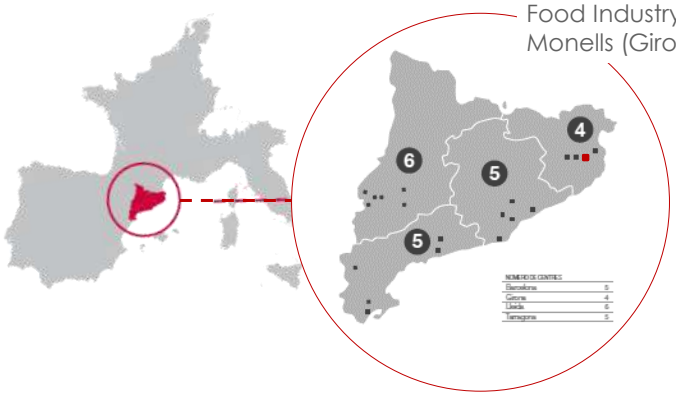
a_w	Temperature (ºC)	Days to inactivate *Salmonella* by **1 log**
0.88	10	25
	15	15
	25	**5** ✓
0.93	10	38
	15	22
	25	**8** ✓

Developed model to predict the inactivation of *Salmonella* in fuet

risk management tool to design a corrective storage and establish a risk minimization strategy to enhance *Salmonella* reduction in dry-fermented sausages

IRTA

Food Safety Programme
Food Industry Area
Monells (Girona)



Teresa Aymerich
Yolanda Beltrán
Fatma Boukid
Sara Bover-Cid
Massimo Castellari
Maria Hortós
Núria Ferrer
Anna Jofré
Belén Martín
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Susana Rubiño
Gerard Sabeña
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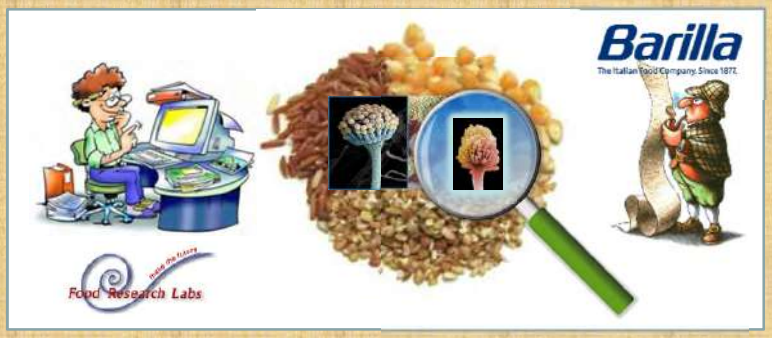
anna.jofre@irta.cat

"Industrial Food Safety Management of Mycotoxin Issues: the example of Deoxynivalenol"

Michele Suman, Ph.D
Food Safety & Authenticity Research Manager
Research, Development & Quality
Phone: +39-0521/262332
e-mail: michele.suman@barilla.com

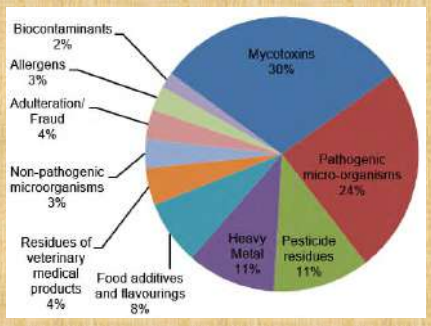


The control of Mycotoxins in Raw Materials: why it is so important for food industry?

…because it is devoted to guarantee the safety of the consumers from potential risks

The control of Mycotoxins in Raw Materials: why it is so important for food industry?

…because it is the main responsible of food safety alerts



Introduction & Context

From field… to table (more than 20% of the crops affected)

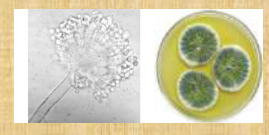

Harvest


Storage


Growth


Food

Mycotoxins: a worldwide problem



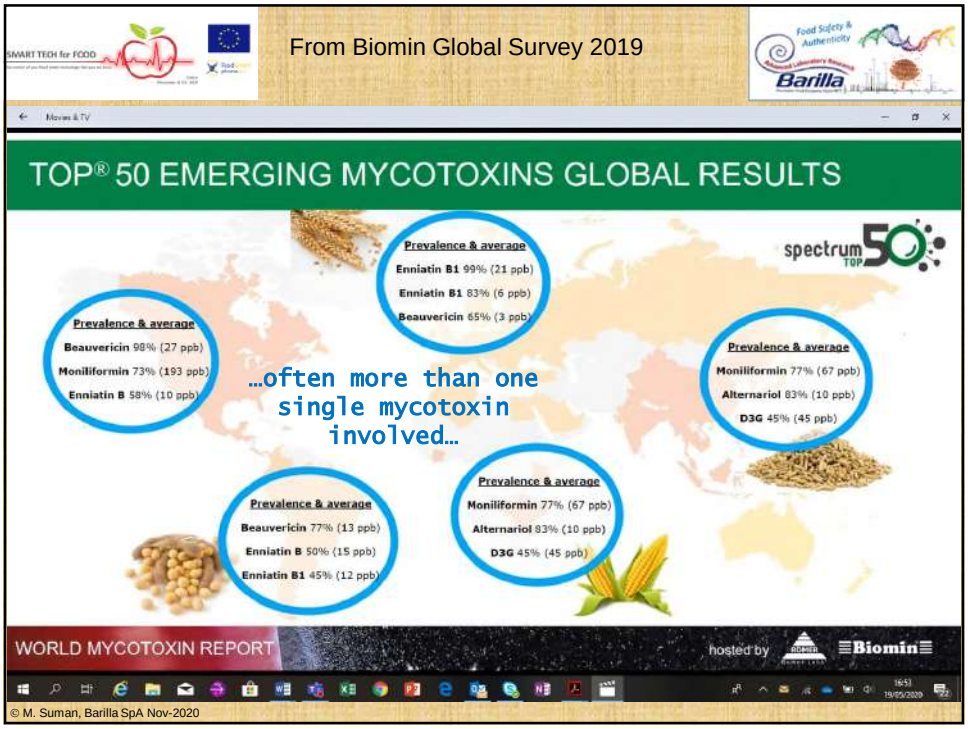
Generally produced by species within Fusarium, Aspergillus & Penicillium

Economic losses estimated at billions of dollars

Regulatory levels to be respected

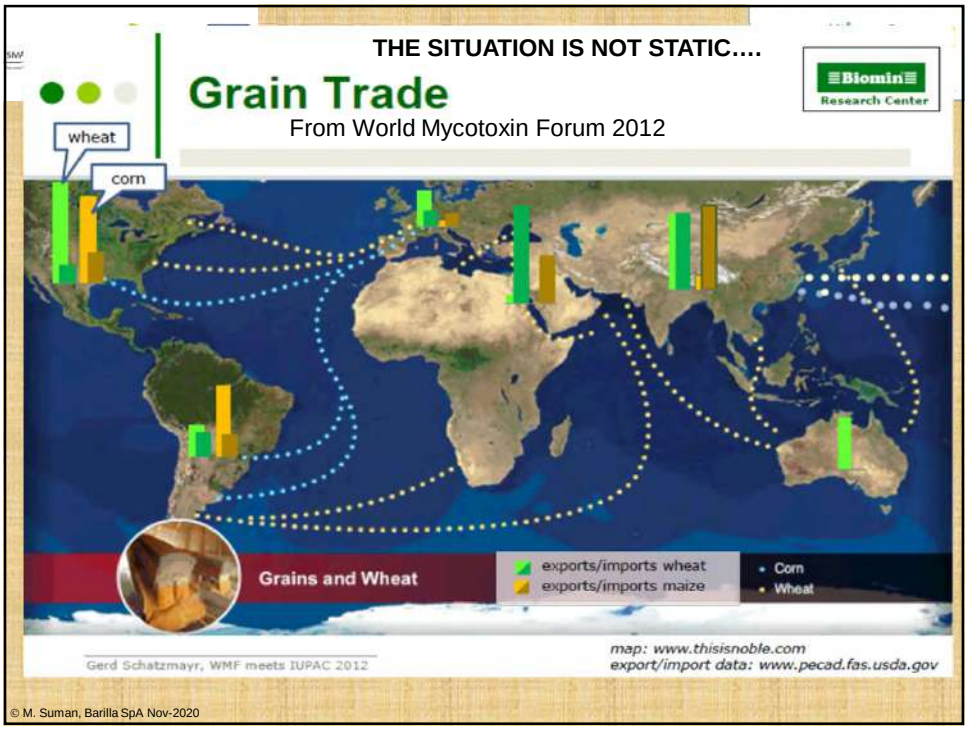
Toxicological risk for humans and animals

From Biomin Global Survey 2019



© M. Suman, Barilla SpA Nov-2020

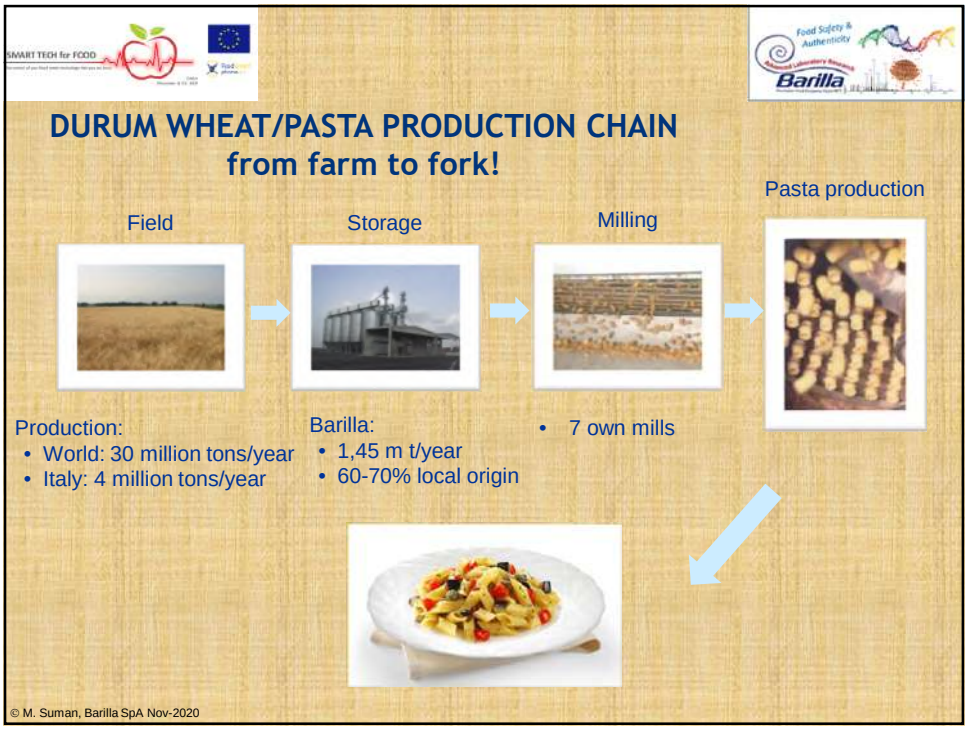
THE SITUATION IS NOT STATIC….



From World Mycotoxin Forum 2012

© M. Suman, Barilla SpA Nov-2020

From World Mycotoxin Forum 2014



Risk Management Strategy

Pre-harvest phase (April-May) → *Harvest phase* (June – July-August) → *New campaign* (November – March)

Final Area Risk Outcome

- DON risk estimation modelling
- Geographical risk evaluation
- Revision of procedures & protocols

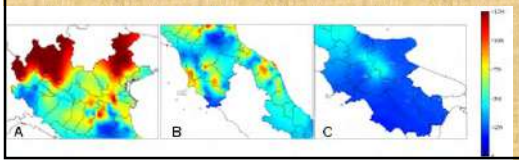
High Risk:
- 100% lots analyzed
- only approved lots

Medium Risk:
- Confirmation through rapid analytical mapping

- Official sampling and confirmatory analysis in external accredited labs;
- Wheat lots database

Low Risk:
- Monitoring plan

- Rapid Tests o Mill Plants
- What lots management
- HACCP official controls

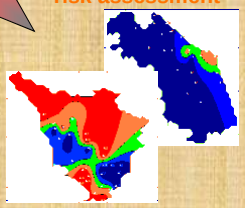


RISK ASSESSMENT MODEL DELPHI

Meteorology

DON risk assessment model

DON geographic risk assessment



Agronomic Management

Plant phenology model

Soil Characterization

Durum wheat campaign – kind permission from Marco Silvestri (Barilla)

An example from wheat harvesting campaigns 2014
diffuse DON presence but with a low risk level

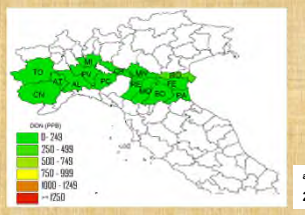
DURUM WHEAT

Micotossine	N. campioni analizzati	N. campioni positivi[a] (%)	Intervallo (µg/kg)	Media campioni positivi (µg/kg)	media (µg/kg)	N. campioni > limite UE
DON	61	45 (74)	35-1936	148	109	1
ZEA	61	1 (2)	-	10	-	-
T-2+HT-2	61	13 (21)	13-57	26	-	-

[a] No. campioni positivi: $DON_{(\mu g/kg)}$ > LOQ. (30 µg/kg); $ZEA_{(\mu g/kg)}$ > LOQ. (10 µg/kg); HT-2/T-$2_{(\mu g/kg)}$ > LOQ (10 e 10 µg/kg)

COMMON WHEAT

- **DON diffused; low levels**
- **1 positive ZEA**
- **T2+HT2: 15% positives**



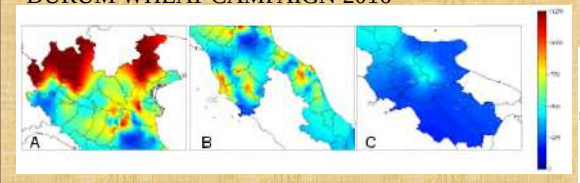
Micotossine	N. campioni analizzati	N. campioni positivi[a] (%)	Intervallo (µg/kg)	Media campioni positivi (µg/kg)
DON	41	18 (44)	30-605	101
ZEA	41	1 (6)	10	10
T-2+HT-2	41	6 (15)	21-90	38

[a] No. campioni positivi: $DON_{(\mu g/kg)}$ > LOQ. (30 µg/kg); $ZEA_{(\mu g/kg)}$ > LOQ. (10 µg/kg); HT-2/T-$2_{(\mu g/kg)}$ > LOQ (10 e 10 µg/kg)

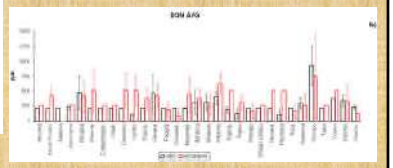
Fusarium & DON Risk Modelling

- **Neural Network Model developed with CNR_IBIMET;**
- **Weekly update**

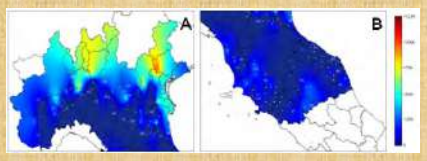
DURUM WHEAT CAMPAIGN 2016

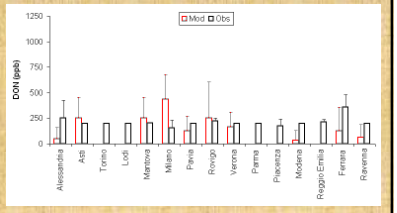


Comparison among observed values (OBS-black) and simulated/predicted ones (MODNEW-red)



COMMON WHEAT CAMPAIGN 2017





Analytical Strategies – Sampling…

COMMISSION REGULATION (EC) No 1881/2006
of 19 December 2006
setting maximum levels for certain contaminants in foodstuffs
(Text with EEA relevance)

COMMISSION REGULATION (EC) No 1126/2007
of 28 September 2007
amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards Fusarium toxins in maize and maize products
(Text with EEA relevance)

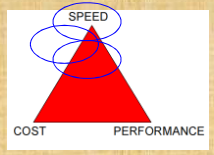
COMMISSION REGULATION (EC) No 401/2006
of 23 February 2006
laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs
(Text with EEA relevance)

EU Legislative Requirements

Sampling protocol projects

	Official controls (CE/401/2006)	IWA Seattle	CEN pr24333.2	AFNOR XP V03-777
Project leader	DGSANCO	ADCS, ICC, AACC, ANSI	CEN TC338 (DE, FR, UK)	AFNOR
Publication	23/02/2006	Mid 2009 ?	Summer 2009	June 2008
Scope	Regulated mycotoxins	technological and safety criteria,	technological and safety criteria	technological and safety criteria
Number of samples	-	Project based on pr24333.2	pr24333.2 (uncertainty: 8%)	Less than pr24333.2 (uncertainty: 15%)
Statistical model	NO	YES for technological and safety criteria NO for GMO	YES for technological and safety criteria	YES for technological and safety criteria
Products	Food products	All grains and derived products	Cereals and derived products	Cereals and derived products
Transport	Road, Railway, Cargo	Road, Railway, Cargo	Road, Railway, Cargo	Road, Railway, Cargo

ANALYTICAL TECHNIQUES ON MYCOTOXINS ISSUE



Rapid Screening solutions

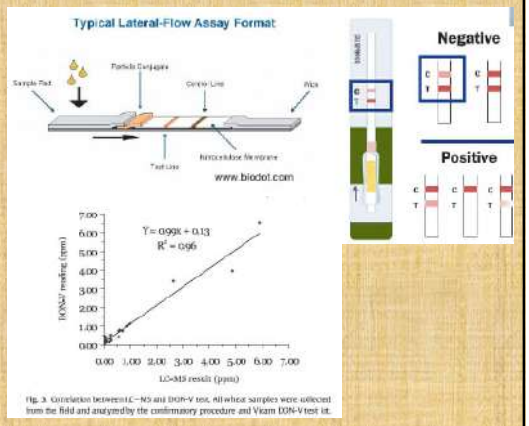
Rapid Screening Solutions: focus on Lateral Flow Devices development!

- *antibody-colloidal gold particle complex mixed with sample extract*
- *migration onto a nitrocellulose membrane*
- *mycotoxin-protein conjugate coated on test zone to capture free antibody-colloidal gold*
- *photometric reflectance reader for colour reaction*

- They are studied for having a fast answer "on-site" (attractive also in the places where there is no laboratory setting available) of the contamination
- Possibly portable, cost-effective,…
- No expensive equipment\high trained personnel required

❖ *Analytical determination for "semiquantitative" proposals: yes/no answer at a certain cut off level.*

❖ *Work is going on in the quantitative screening direction…looking for adequate levels of accuracy, repeatability and reproducibility…*



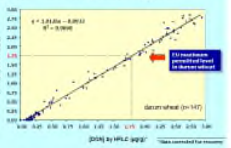
Development and practical application in the cereal food industry of a rapid and quantitative lateral flow immunoassay for deoxynivalenol

J. Liu[a], S. Zanardi[b], S. Powers[a], M. Suman[b,*]

Food Control 26 (2012) 88–91

FPIA – DON in wheat and derivative products

- ✓ applicability: durum wheat, common wheat, semolina, pasta
- ✓ detection limit: 0.08 µg/g
- ✓ accuracy: 98-102%
- ✓ precision: ≤ 4%
- ✓ time of analysis: ≤ 10 min
- ✓ linearity range: 0,1 – 2 µg/g (for concentration > 2 µg/g dilution of extract is required)



Lippolis V., Pascale M., Visconti A., J. Food Prot., 2006, 69, 2712-2719

FP (Fluorescence Polarisation immunoassay format)

The **toxin is marked with a fluorotrope**.

Marked toxin and free toxin (extracted from the sample) compete with antibodies for the specific binding sites.

If the antibody bind marked toxin, the increase of the mass of the complex cause **change of the polarity of the incident radiation**.

The measured change of polarization is therefore inversely proportional to the free toxin concentration in the sample.

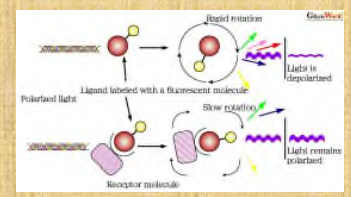
Automated FPIA - DON in wheat and derivative products*

✓ The automated FP system has been developed by assembling a FP reader with an autosampler assisted by a PC through a specific software for data handling.

ADVANTAGES

- Fully automated
- Easy-to-use
- Good precision (<6%)
- Useful and practical alternative to HPLC
- More convenient than HPLC for routine analyses due to higher throughput (15 samples / 2 h vs. 1 sample / 2 h)

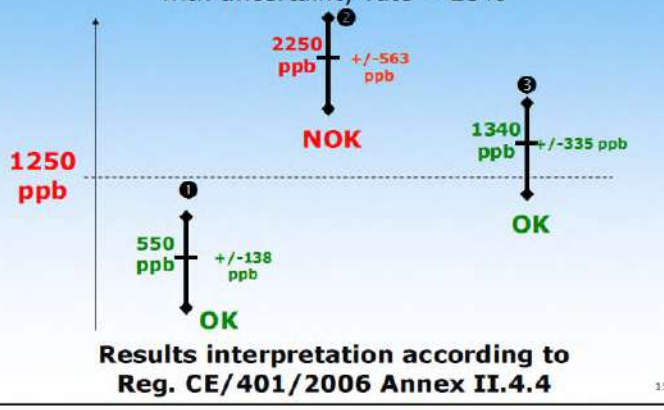
* European Patent Application No. 1882938A2. Visconti A., Pascale M., Lippolis V., Ranieri R., Silvestri M. e D'Alessandro A.



Valenzano, S.; Lippolis, V.; Pascale, M.; De Marco, A.; Maragos C.M.; Suman, M.; Visconti, A. *"Determination of Deoxynivalenol in Wheat Bran and Whole-Wheat Flour by Fluorescence Polarization Immunoassay"* *Food Analytical Methods* **2014**, 7(4), pp. 806-813

Uncertainty about result analysis

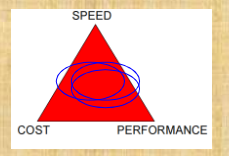
Example : DON results on wheat for food chain with uncertainty rate = 25%



Results interpretation according to Reg. CE/401/2006 Annex II.4.4

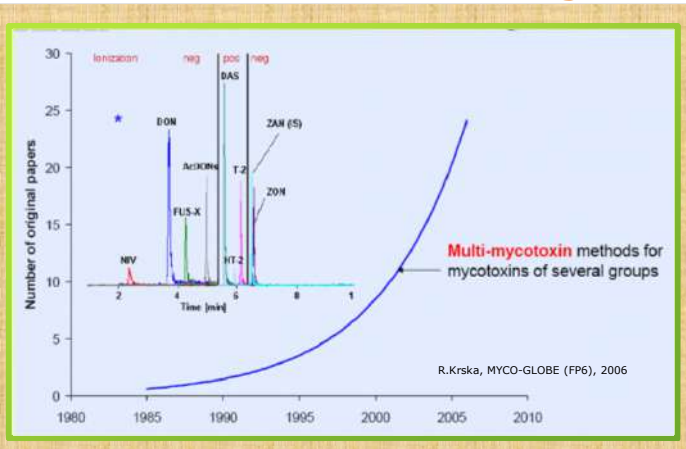
15

ANALYTICAL TECHNIQUES ON MYCOTOXINS ISSUE



Accurate-sensitive solutions

Trend: Multi-micotoxin methods using LC-MS(MS)

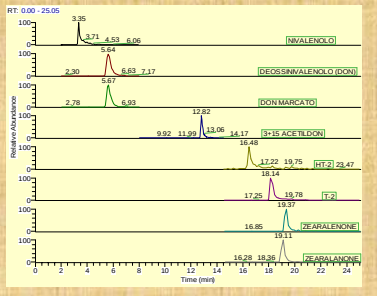


Trichothecenes and ZON: ION TRAP LC/MS^n approach:

- 1. Sampling: *20g*
- 2. Extraction: *100 mL of a mixture of acetonitrile/water (84:16, v/v)*
- 3. Homogenization: *Ultraturrax blending 3 minutes*
- 4. Filtration: *6 mL*
- 5. Addition to a vial containing internal standards: *ZAN + ($^{13}C_{15}$)-DON*
- 6. Evaporation to dryness: *under a nitrogen stream*
- 7. Clean up: *through MycoSep® 226 column.*
- 8. Final preconcentration and LC-MS^n analysis

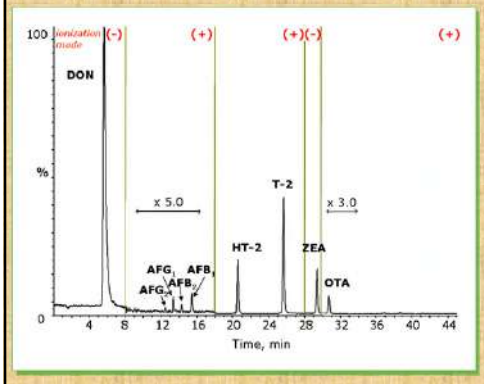
Zearalanone (ZAN), which does not occur in nature, was used as internal standard for quantification of Zearalenone (ZON).
An isotope-labelled ($^{13}C_{15}$)-DON internal standard was used for the determination of the other trychothecenes and in particular for DON to efficiently correct for losses during sample preparation as well as matrix effects and ion-suppression effects in the ESI source.

Mycotoxin	*Parent ion m/z*	*Fragments monitored m/z*	*Normalized Collision Energy %*
[NIV+CH3COO]$^-$	371	281, 311	24
[DON+CH3COO]$^-$	355	295, 265	24
[(^{13}C15)-DON +CH3COO]$^-$	370	310, 290	24
[FUS-X+CH3COO]$^-$	413	353, 187	20
[ADONs+CH3COO]$^-$	397	337, 173	24
[DAS+NH4]$^+$	384	349, 307	20
[HT-2+NH4]$^+$	442	381	24
[T-2+NH4]$^+$	484	423	24
[ZON-H]$^-$	317	299, 273, 203, 161	34
[ZAN-H]$^-$	319	301, 275, 205, 163	34



Source: M. Suman, D. Catellani; ***World Mycotoxins Journal 2008****, Vol-1(3), pp. 255-262.*

SPE\LC-MS/MS to simultaneously quantify AFLA, OTA, DON, ZEA, T2-HT2 in cereal based foods



Electrospray ionization triple quadrupole tandem mass spectrometry (MRM mode) [13C-labelled mycotoxins as IS]

Source: S. Della Gatta, V. Lattanzio, M. Suman, A. Visconti
Rapid Communication in Mass Spectrometry 2011 2011, 25, pp. 1869-1880

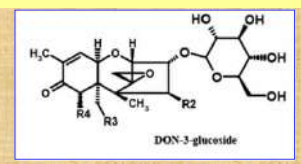
THE ADDED «CONJUGATED OR MASKED MYCOTOXINS» SCENARIO…

- In recent years it emerged that, many structurally related compounds, generated by **plant metabolism**, can co-exist together with the native toxins: for example, plants are able to convert the relatively apolar trichothecenes in more polar derivatives **via conjugation** with sugars, amino acids or sulphate groups, in order to compartmentalize them in vacuoles

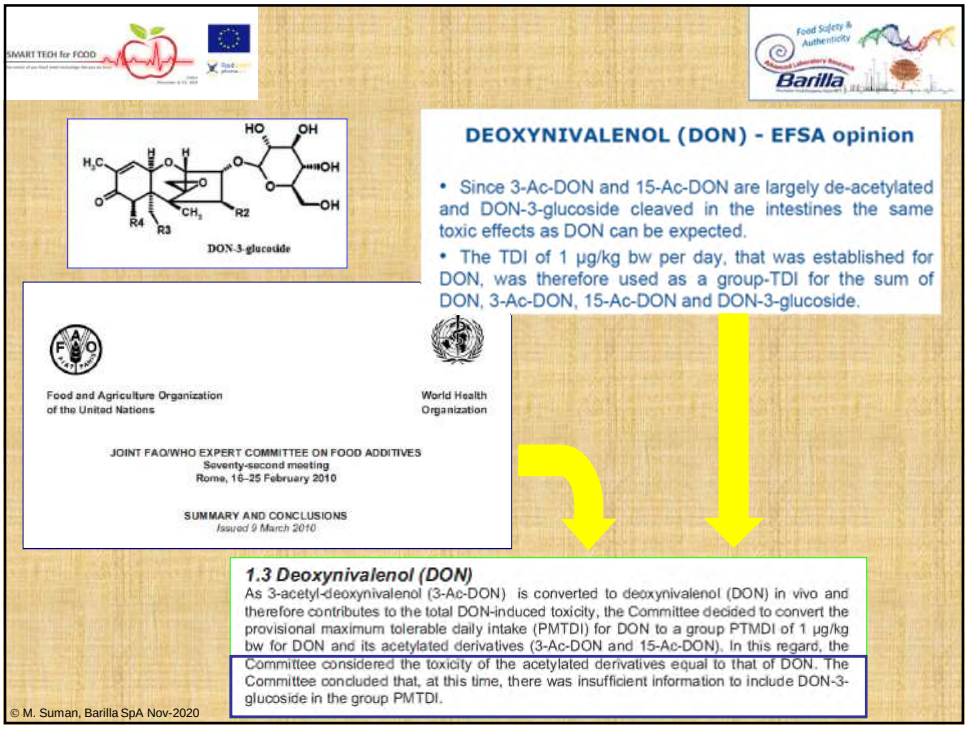
- Food processing, especially heating or fermentation steps, can potentially alter mycotoxins: **mechanical or thermal energy** during the transformation process may cause modification, inducing **reactions with macromolecular components** such as sugars, proteins or lipids as well as **release of the native form**

- **DON-3β-glucopyranoside** (DON-3G) was firstly identified in cereals by comparing the fragmentation pattern with a chemically-synthesized standard. LC-ESI-MS^2 analysis of naturally contaminated wheat and maize samples showed DON-3G to be the major form of masked DON. It was also found in barley, malt and beer and currently the authors are still focusing their work on it.

- Masked mycotoxins are not easy to be extracted\cleaned up. There also a lack of adequate analytical standard up to now. They **can escape routine analytical methods** but they can **potentially be released after ingestion by hydrolysis** in the gastrointestinal tract: preliminary results indicate that DON-3G is resistant to the acid conditions in the stomach of mammals and to most enzymes, while several bacteria in the intestinal tract of humans are able to cleave it into DON.



DON-3-glucoside


DON-3-glucoside
DEOXYNIVALENOL (DON) - EFSA opinion
• Since 3-Ac-DON and 15-Ac-DON are largely de-acetylated and DON-3-glucoside cleaved in the intestines the same toxic effects as DON can be expected.
• The TDI of 1 µg/kg bw per day, that was established for DON, was therefore used as a group-TDI for the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside.
Food and Agriculture Organization of the United Nations
World Health Organization
JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES
Seventy-second meeting
Rome, 16–25 February 2010
SUMMARY AND CONCLUSIONS
Issued 9 March 2010
1.3 Deoxynivalenol (DON)
As 3-acetyl-deoxynivalenol (3-Ac-DON) is converted to deoxynivalenol (DON) in vivo and therefore contributes to the total DON-induced toxicity, the Committee decided to convert the provisional maximum tolerable daily intake (PMTDI) for DON to a group PTMDI of 1 µg/kg bw for DON and its acetylated derivatives (3-Ac-DON and 15-Ac-DON). In this regard, the Committee considered the toxicity of the acetylated derivatives equal to that of DON. The Committee concluded that, at this time, there was insufficient information to include DON-3-glucoside in the group PMTDI.
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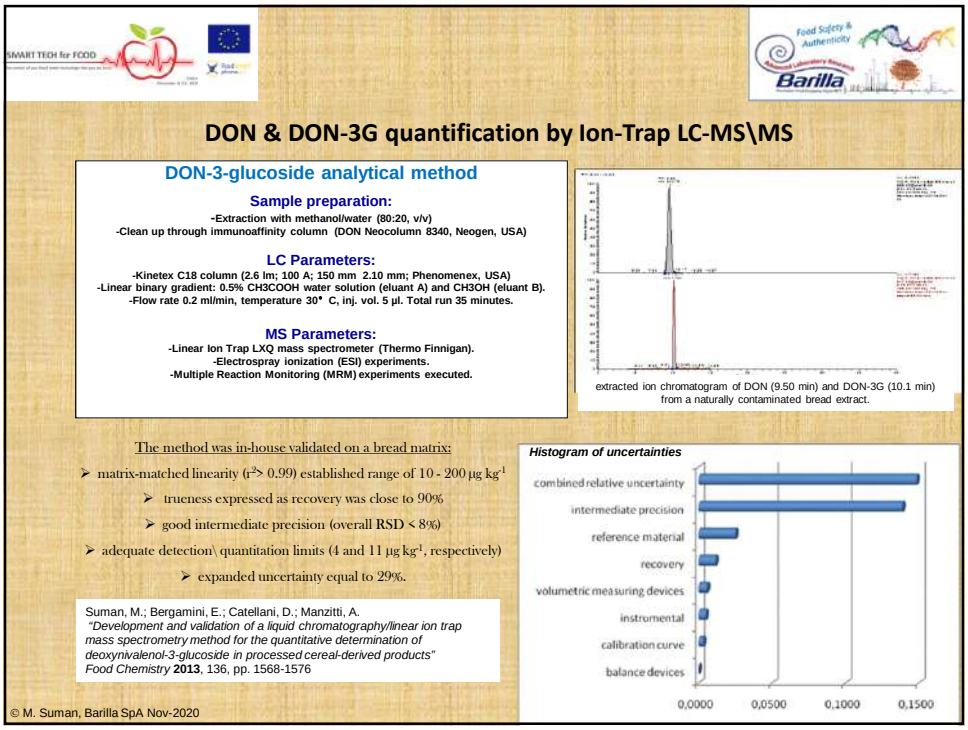
DON & DON-3G quantification by Ion-Trap LC-MS\MS
DON-3-glucoside analytical method
Sample preparation:
-Extraction with methanol/water (80:20, v/v)
-Clean up through immunoaffinity column (DON Neocolumn 8340, Neogen, USA)
LC Parameters:
-Kinetex C18 column (2.6 lm; 100 A; 150 mm 2.10 mm; Phenomenex, USA)
-Linear binary gradient: 0.5% CH3COOH water solution (eluant A) and CH3OH (eluant B).
-Flow rate 0.2 ml/min, temperature 30° C, inj. vol. 5 µl. Total run 35 minutes.
MS Parameters:
-Linear Ion Trap LXQ mass spectrometer (Thermo Finnigan).
-Electrospray ionization (ESI) experiments.
-Multiple Reaction Monitoring (MRM) experiments executed.
extracted ion chromatogram of DON (9.50 min) and DON-3G (10.1 min) from a naturally contaminated bread extract.
The method was in-house validated on a bread matrix:
➢ matrix-matched linearity (r^2> 0.99) established range of 10 - 200 µg kg^{-1}
➢ trueness expressed as recovery was close to 90%
➢ good intermediate precision (overall RSD < 8%)
➢ adequate detection\ quantitation limits (4 and 11 µg kg^{-1}, respectively)
➢ expanded uncertainty equal to 29%.
Suman, M.; Bergamini, E.; Catellani, D.; Manzitti, A.
"Development and validation of a liquid chromatography/linear ion trap mass spectrometry method for the quantitative determination of deoxynivalenol-3-glucoside in processed cereal-derived products"
Food Chemistry 2013, 136, pp. 1568-1576
Histogram of uncertainties
combined relative uncertainty
intermediate precision
reference material
recovery
volumetric measuring devices
instrumental
calibration curve
balance devices
0,0000
0,0500
0,1000
0,1500
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Let's expand HRMS potentialities…

Research article

RAPID COMMUNICATIONS IN MASS SPECTROMETRY

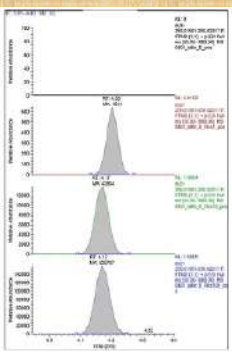
(wileyonlinelibrary.com) DOI: 10.1002/rcm.3074

2013

Targeted screening of pesticides, veterinary drugs and mycotoxins in bakery ingredients and food commodities by liquid chromatography-high-resolution single-stage Orbitrap mass spectrometry†

Emiliano De Dominicis,[a] Italo Commissati[a] and Michele Suman[b]*

Food Additives & Contaminants: Part A, 2015
Vol. 32, No. 10, 1617–1627, http://dx.doi.org/10.1080/19440049.2015.1061703



2015

Quantitative targeted and retrospective data analysis of relevant pesticides, antibiotics and mycotoxins in bakery products by liquid chromatography-single-stage Orbitrap mass spectrometry

Emiliano De Dominicis[a], Italo Commissati[a], Elisa Gritti[a], Dante Catellani[b] and Michele Suman[b]*

The Method Now – Bakery Matrixes Sample Prep Sheet Flow

A simple, generic and fast method for extraction and purification steps has been optimized through a proper adaptation of procedures previously reported in scientific literature (*)

Sample

1st extraction (acidic buffer – pH 4) + mechanical shake

2nd extraction (acidic CH_3CN) + vortex shake

$MgSO_4$ (1st purification L/L) + mechanical shake

Centrifuge

2nd purification D-SPE

(HCOOH-PSA-C18-$MgSO_4$)

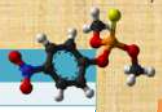
Solvent Change (Dry N_2 30 ° C)

Inj. Exactive Orbitrap LC-MS
(Resolution set: FWHM 50.000)

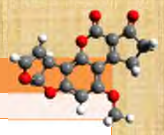
(*) European Standard UNI EN 15662:2009.
S. J. Lehotay, *Journal of AOAC International*, 2007, 90 (2), 485.
R.J.B. Peters et al., *Trends in Analytical Chemistry*, 2010, 29 (11), 1250

Chemical Contaminants Categories

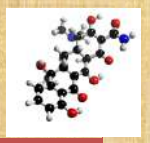
Validated Pesticides, Antibiotics and Mycotoxins



Pesticides
carbendazim
carbaryl
desethylterbuthylazine
simazine
pymetrozine
dodine
metoxuron
prometryn
oxycarboxin
(D,L)-metalaxyl
piperonyl butoxide
azoxystrobin
tebuconazole
pirimiphos methyl
malathion
tricyclazole

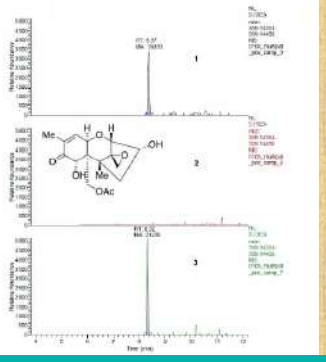


Toxins
aflatoxin B1
aflatoxin B2
aflatoxin G1
aflatoxin G2
ochratoxin A
deoxynivalenol
toxin T2
toxin HT2
zearalenon
fumonisin B1
fumonisin B2
aflatoxin M1

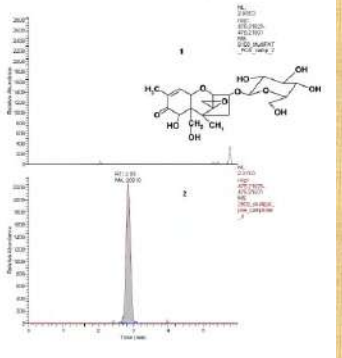


Antibiotics
abamectin
tetracycline
chlortetracycline
oxitetracycline
chloramphenicol
thiabendazole
sulfathiazole
sulfadimethoxine

3-AcDON - Retrospective Data Analysis.
Hypothesis of:
1) presence of 3-AcDON in minicake product (chrom. 1 and 3);
2) not presence of 3-AcDON in minicake product (chrom. 2);



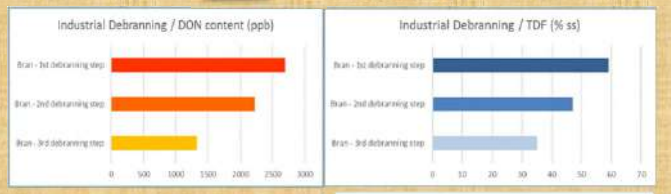
DON-3-glucoside - Retrospective Data Analysis.
Hypothesis of:
1) presence of DON-3-glucoside (ammonium adduct) in minicake product (chrom. 2);
2) not presence of DON-3-glucoside (ammonium adduct) in minicake product;

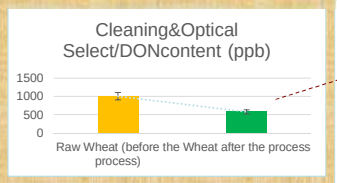


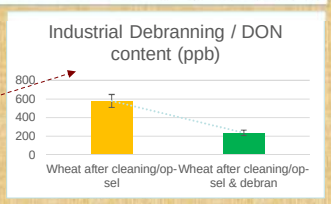
Retrospective Potentialities

The potential presence of further contaminants or potentially hazardous molecules have been evaluated exploiting retrospective investigations.

Post Harvest – Innovative Milling

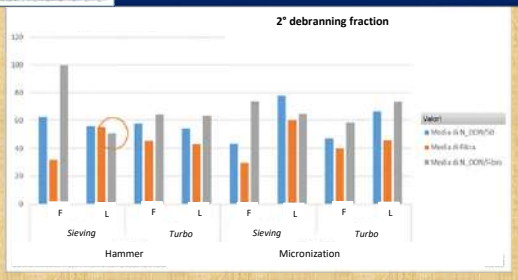






This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 678012

Post Harvest – Innovative Milling



DON (Average values)

Fiber content TDF (Average values)

DON/TDF (Average values)

F→ Small particle size set

L→ Large particle size set

2nd bran fraction (2nd abrasion) is in general the most interesting one because of **less other contaminants concentration** (such as pesticides, heavy metals, etc.) and **contemporaneously interesting organoleptic properties** in terms of "positive wholegrain taste/flavor perception"

Focusing exactly on the 2nd abrasion, even comparing the different separation technologies (Turbo vs. Sieve), the best results are always found in the *Hammer Milling/Large Particle Size set-fraction*.

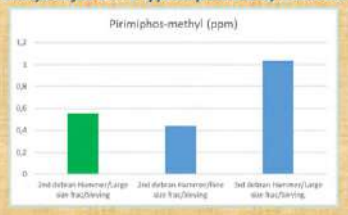
Among the two following applied separation technologies, the best resulted to be the simple *Traditional Sieving*

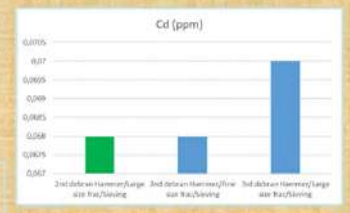
Hammer milling system
Traditional Sieving Sifter

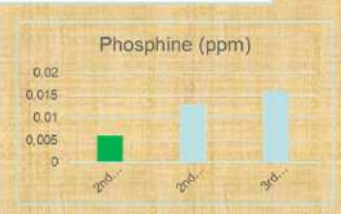
Post Harvest – Innovative Milling

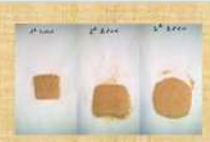
The industrially produced debranned powder raw materials were also *analyzed for some typical pesticides, insect control agents and heavy metal residues.*

Contaminants



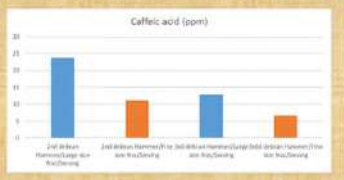


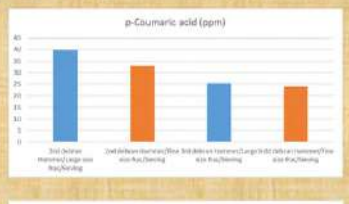


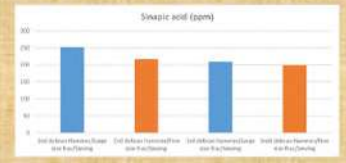


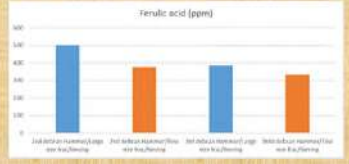
Post Harvest – Innovative Milling

Finally, phenolic acids were measured in the same industrially produced debranned powder raw materials: 2nd debran hammer/large size fractioning/sieving to include the highest content of these antioxidants.









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Post Harvest – Innovative Milling

Innovative milling strategy has led to a **3% higher fiber content and improved fiber/DON ratios in wholegrain pasta**

	DON Deoxynivalenol (µg/kg)	TDF (Total Dietary Fiber) (g/100 g)
Wholegrain std commercial pasta	144	7,3
Novel MyToolBox Pasta	217	10,4

Post Harvest – Food Processing

Develop analytical methodology to elucidate the fate and behavior of DON during baking

1. Prepare bakery products from fortified flour
   - Identify ALL transformation products
   - Develop quantitative method
2. Prepare bakery products from naturally cont. flour under different processing conditions
   - Determine DON degradation during real-life conditions
   - Determine influence of different processing parameter on DON degradation

flour

crackers

Raising agent: yeast
48 h fermentation
High heat load

biscuits

Raising agent: baking soda
Rich dough (fat)
High heat load

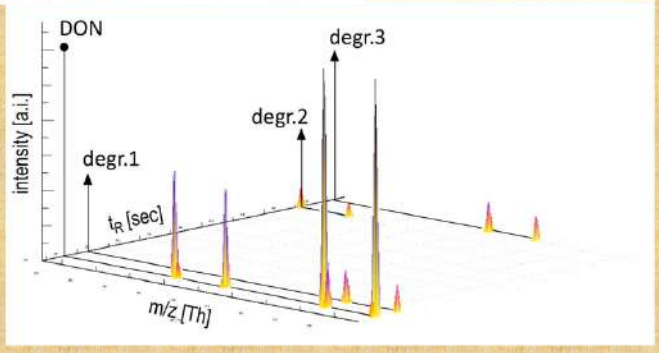
bread

Raising agent: yeast
Low heat load

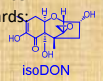
Post Harvest – Food Processing

Food Chemistry

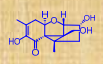
Untargeted LC–MS based ^{13}C labelling provides a full mass balance of deoxynivalenol and its degradation products formed during baking of crackers, biscuits and bread



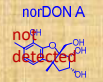
Reference standards:


isoDON


DOM-1


norDON A

not detected


norDON B


norDON C

Post Harvest – Food Processing

Understand the Influence of Processing Parameters on the Mitigation of Deoxynivalenol during Industrial Baking

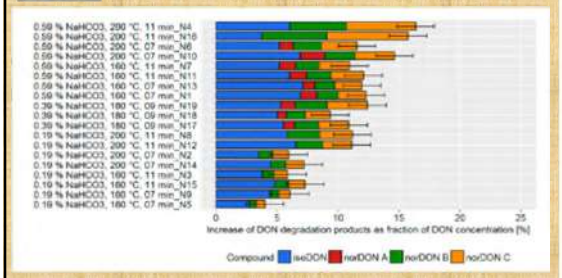
To study the main processing factors affecting the mitigation of DON during industrial baking, **crackers, biscuits, and bread were chosen as representative commodities of the main bakery categories produced with very different technologies.**

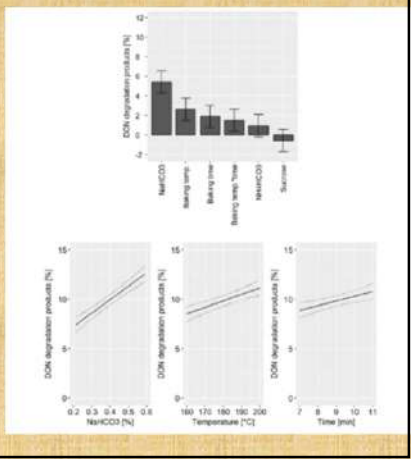
A **Design of Experiments** (DoE) approach was applied to set up the experimental trials. The **main technological parameters monitored were**: *(i) Baking conditions (i.e., time and temperature), (ii) pH modifying agents (NaHCO3, NH4HCO3, and other minor ingredients) and iii) leavening conditions (time, temperature, presence, and type of leavening agents).* **Each factor was modified within a range according to technological feasibility.**

Post Harvest – Food Processing

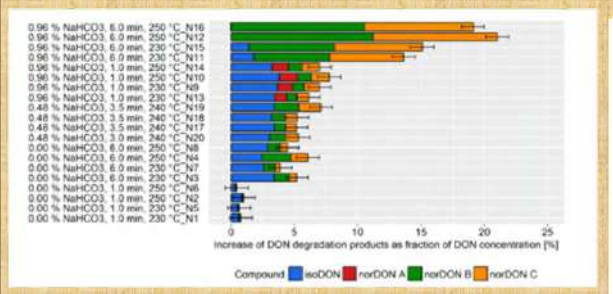
biscuits

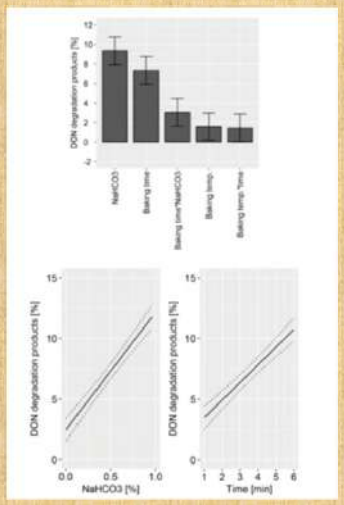
Understand the Influence of Processing Parameters on the Mitigation of Deoxynivalenol during Industrial Baking





Post Harvest – Food Processing





Open Access Article

The Influence of Processing Parameters on the Mitigation of Deoxynivalenol during Industrial Baking

David Stadler [1], Francesca Lambertini [2], Lydia Woelflingseder [3], Heidi Schwartz-Zimmermann [1], Doris Marko [3], Michele Suman [2], Franz Berthiller [1,*] and Rudolf Krska [1,4]

[1] Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad-Lorenz-Str. 20, 3430 Tulln, Austria
[2] Barilla G. R. F.lli SpA, Advanced Laboratory Research, via Mantova 166, 43122 Parma, Italy
[3] Department of Food Chemistry and Toxicology, University of Vienna, Währingerstraße 38, 1090 Vienna, Austria
[4] Institute for Global Food Security, School of Biological Sciences, Queens University Belfast, University Road, Belfast BT7 1NN, Northern Ireland, UK
* Author to whom correspondence should be addressed.

Toxins **2019**, *11*(6), 317. https://doi.org/10.3390/toxins11060317

crackers

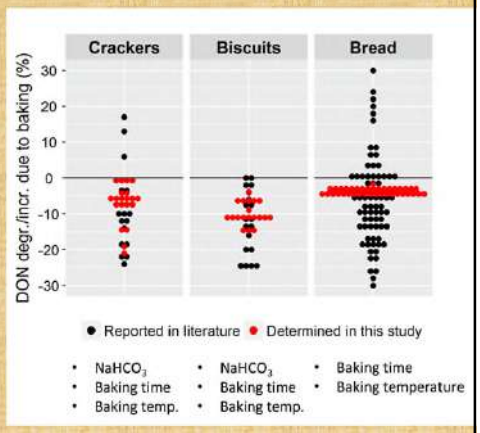
Post Harvest – Food Processing

The pH Value of the Dough

- **DON** has been reported to be **unstable in alkaline solutions**
- pH of the dough is clearly an important processing factor, regulated here primarily by **chemical raising agents**.
- We found that the type of the chemical raising agent as well as its concentration was crucial regarding DON degradation. Whereas the use of **NaHCO3 led to higher DON degradation**, the use of NH4HCO3 did not influence DON concentration. This can be explained by the different pH values of the dough resulting from the different chemical nature of the raising agents.

Baking Conditions

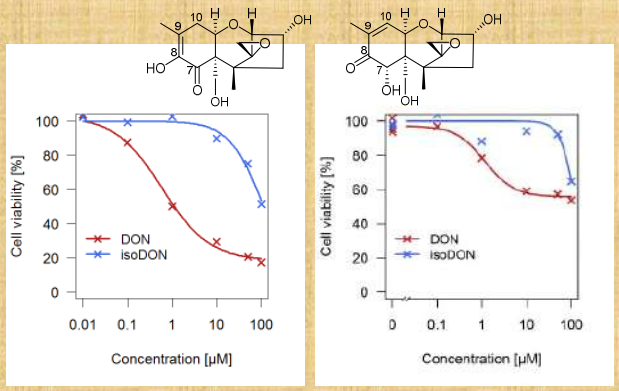
- For all three commodities, the **baking conditions (i.e., temperature and/or time) influenced DON degradation**.
- For the production of crackers and bread, baking time was found to be more important than baking temperature. The contrary was observed for biscuit production. The reason for this observation might relate to **differences in moisture content and surface to volume ratio of the individual baking commodities.**



Post Harvest – Food Processing

Toxicity of the formed degradation products is essential to make a sound conclusion on the potential impact of the baking process and the parameters chosen on the safety of the final food product.

Only when a decrease of a mycotoxin results in the formation of degradation products that are less toxic than the parent mycotoxin, food processing can be considered to have a mycotoxin mitigating effect.



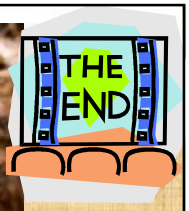
© M. Suman, Barilla SpA Nov-2020

SUMMARIZING…

Integrated System
form field to fork

"Industrial Food Safety Management of Mycotoxin Issues: the example of Deoxynivalenol"

- Prevention
  - Study of fungal populations
  - Modelling *Fusarium* & DON risks
  - Selection *Fusarium*/DON resistance varieties
- Monitoring
  - Risk analysis on contaminants presence
  - Geographical distribution/frequency
- Rapid & Innovative Analytical Methods
  - Multi-direction approach (rapid-confirmatory)
  - Research & Innovation (multi-contaminants check)
- Management / Post Harvest
  - Risk management strategy on contaminants
  - Online programme to handle the various lots
  - Innovative Milling
  - Food Processing Understanding & Mitigation



Thank you

QUEEN'S UNIVERSITY BELFAST | IGFS THE INSTITUTE FOR GLOBAL FOOD SECURITY

Development of smartphone hyphenated colorimetric, plasmonic and electrochemical biosensors for food contaminant detection

FoodSmartphone.eu

by
Jordi Nelis

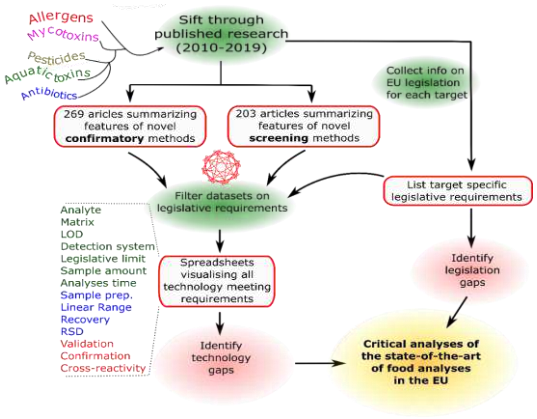
European Commission

Step one: determine PhD Research directions

2

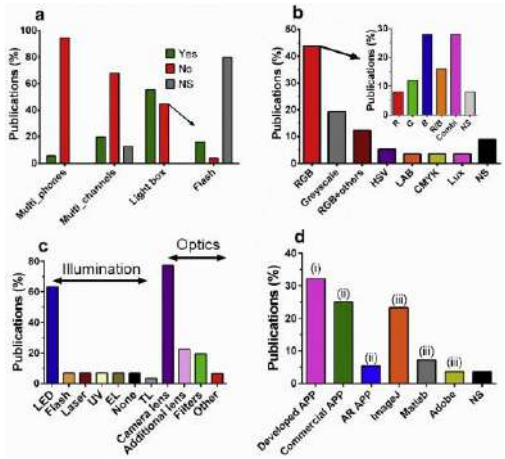
Getting the bearings

Screening and Confirmatory food analyses



Tsagkaris, A; **Nelis JLD,** *et al.,* **TrAC 2019**

Smartphone capabilities

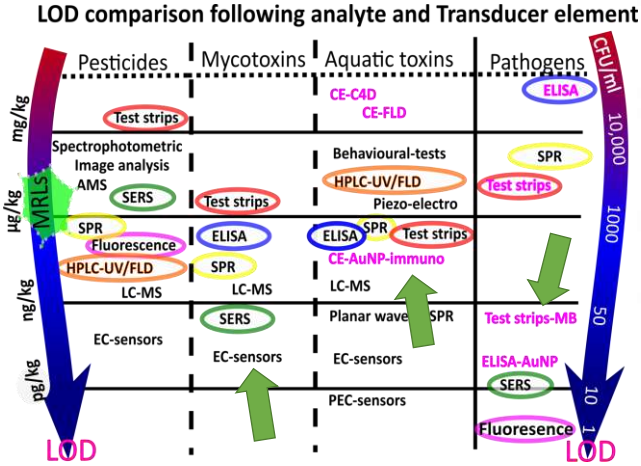


Nelis JLD, Tsagkaris *et al.,* **TrAC 2020**

3

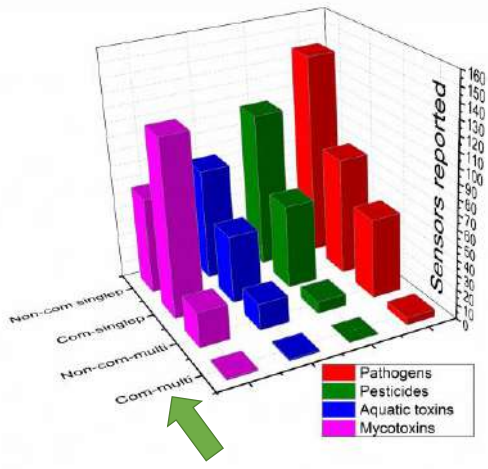
TEST: An biosensor database listing ~1000 biosensors

Transducer capabilities



Nelis *et al.*, *Biosens. Bioelectron. 2019*

Commercial competition



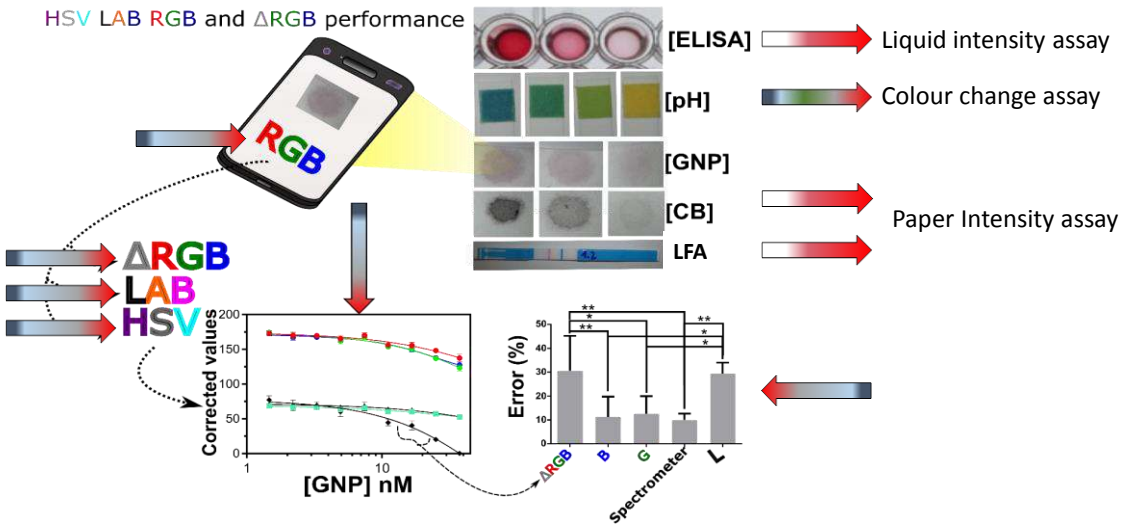
4

PhD Research directions

- Colorimetrics/image analyses
- Electrochemistry
- Plasmonics/Darkfield microscopy

5

Study 1: Which single colour channel works best for which assay?



Nelis *et al.,* The Efficiency of Color Space Channels to Quantify Color and Color Intensity Change in Liquids, pH Strips, and Lateral Flow Assays with Smartphones. **Sensor, 2019**

6

Take home message study 1:

No need to convert to LAB, HSV or D-RGB --> RGB channels work the best (but check all 3)

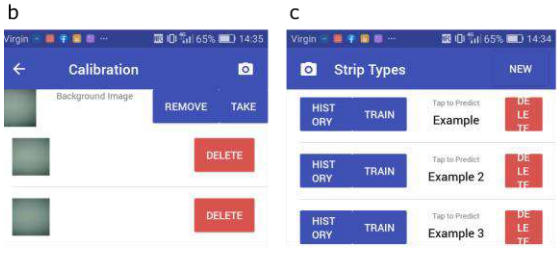
Background correction may make use of a light shielding box superfluous

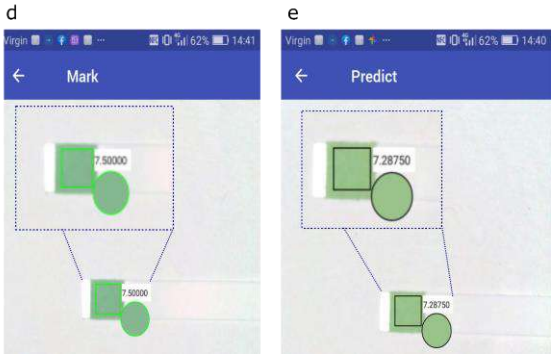
Interphone variation and prediction errors much bigger for intensity as colour-change based assays.

Can we improve intensity based colorimetric sensing with machine learning and channel optimization?

Study 2: Novel channel combinations and app development

- Data Storage and localisation
- Pixel-wise background correction
- Regression by Polynomial cost function

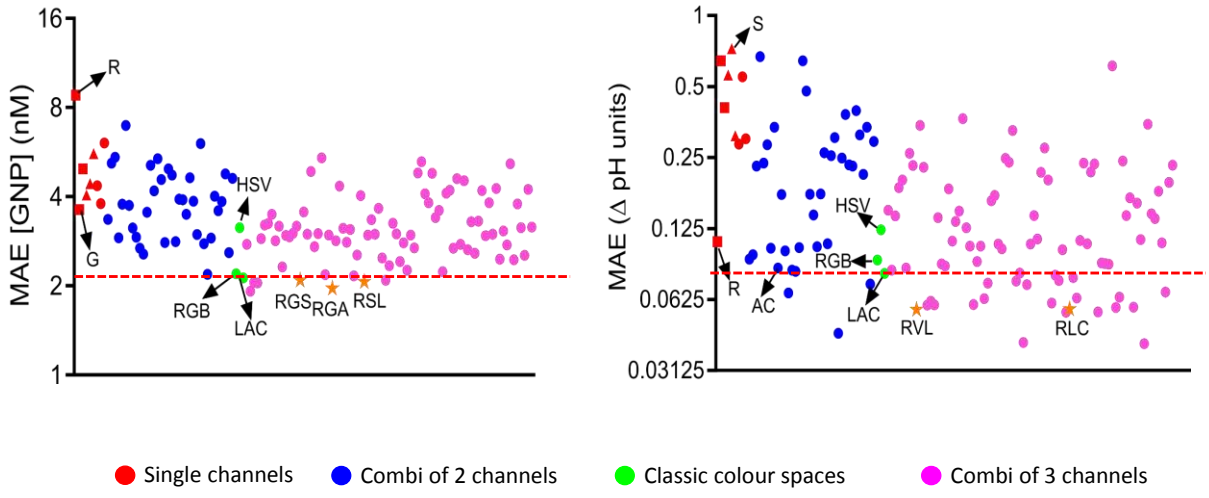




Nelis et al., A randomised combined channel approach for the quantification of colour and intensity based assays with smartphones **(Anal. Chem 2020)**

8

Optimum channel combination



9

Take home message:

Novel combinations reduce prediction error and interphone variations

Smartphone colorimetrics does not have to follow colour space models

Nelis et al., A randomised combined channel approach for the quantification of colour and intensity based assays with smartphones **(Anal. Chem 2020)**

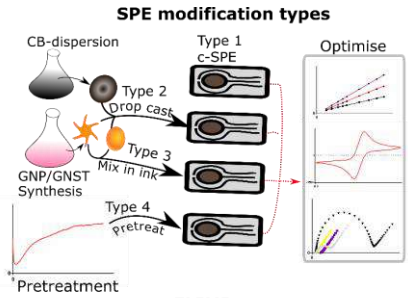
10

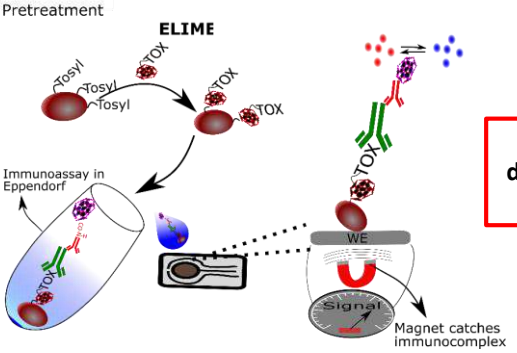
PhD Research directions

- Colorimetrics/image analyses
- Electrochemistry
- Plasmonics/Darkfield microscopy

11

Study 1: Nanomaterials and Electrochemistry



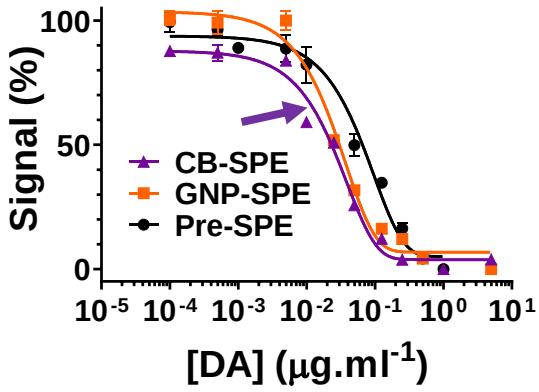


Investigate the potential of carbon black to improve screen printed electrode performance

Develop and validate MB based assay for domoic acid detection in shellfish using carbon black modified electrodes

12

Immuno-assays



Assay	Matrix	LOD (ng/ml)	R^2
Pre-SPE	Buffer	4	0,98
CB-SPE	Buffer	0,4	0,98
CB-SPE	Scallop extract	0,7	0,98
ELISA	Buffer	0,9	0,99

13

Take home message:

Carbon black has great potential for electrochemical sensing


Springer Link

Original Paper | Open Access | Published: 12 February 2020

The benefits of carbon black, gold and magnetic nanomaterials for point-of-harvest electrochemical quantification of domoic acid

Joost L.D. Nelis, Davide Migliorelli, Safiye Jafari, Silvia Generelli, Javier Lou-Franco, J. Pablo Salvador, M. Pilar Marco, Cuong Cao, Christopher T. Elliott & Katrina Campbell

Microchimica Acta 187, Article number: 164 (2020) | Cite this article

Microchimica Acta

Analytical Sciences Based on Micro- and Nanomaterials

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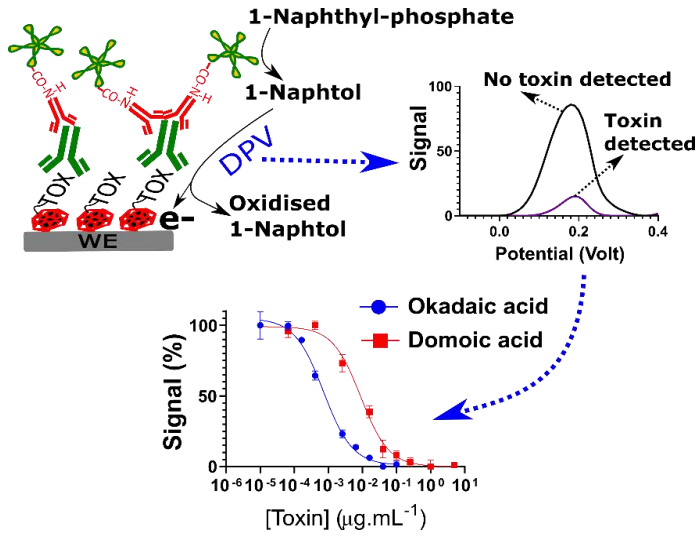
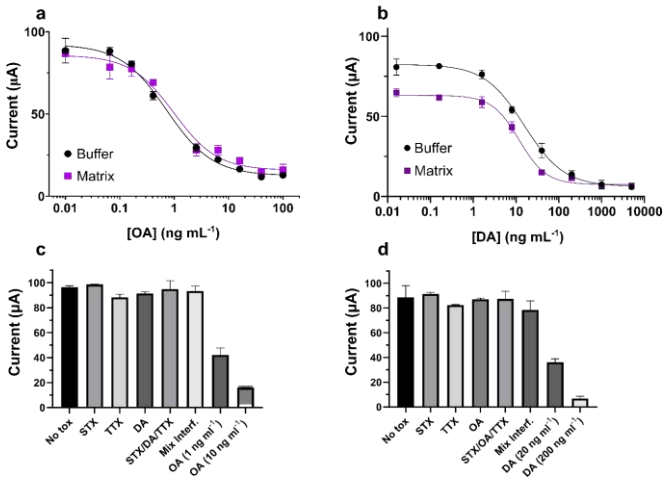
Study 2: Electrochemical assay with multiplexing potential



- Direct immobilisation to facilitate ease of use
- Differential Pulsed voltammetry to allow capacitive current noise reduction

Performance (sensitivity/selectivity/stability) tested for two marine toxins in mussels.

Study 2: Electrochemical assay with multiplexing potential



Assay	Matrix	LOD (ng mL^{-1})	RSD (%)
DA CB-SPE	Buffer	1.7	8.7 ± 9.0
DA CB-SPE	Mussel	**1.9**	5.6 ± 4.1
OA CB-SPE	Buffer	0.15	6.1 ± 4.1
OA CB-SPE	Mussel	**0.18**	7.2 ± 5.2

Nelis et al., Highly sensitive electrochemical detection of the marine toxins okadaic acid and domoic acid with carbon black modified screen printed electrodes, **(Submitted)**

Take home message:

Carbon black SPEs have better stability and longterm performance as normal carbon SPEs.

Good stability, selectivity and reproducibility were obtained with DPV based detection and direct immobilization

Nelis et al., Highly sensitive electrochemical detection of the marine toxins okadaic acid and domoic acid with carbon black modified screen printed electrodes, **(Submitted)**

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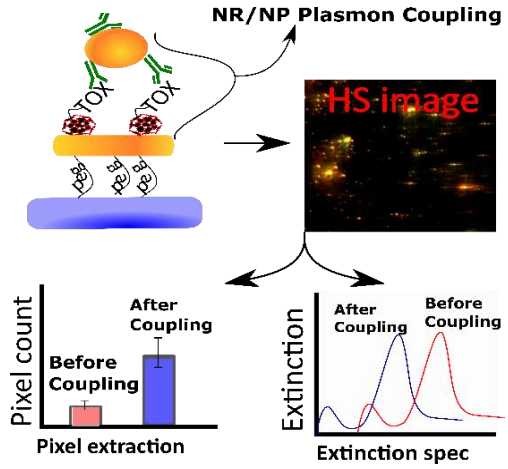
PhD Research directions

- Colorimetrics/image analyses
- Electrochemistry
- Plasmonics/Darkfield microscopy

18

Exploiting plasmon coupling for domoic acid detection

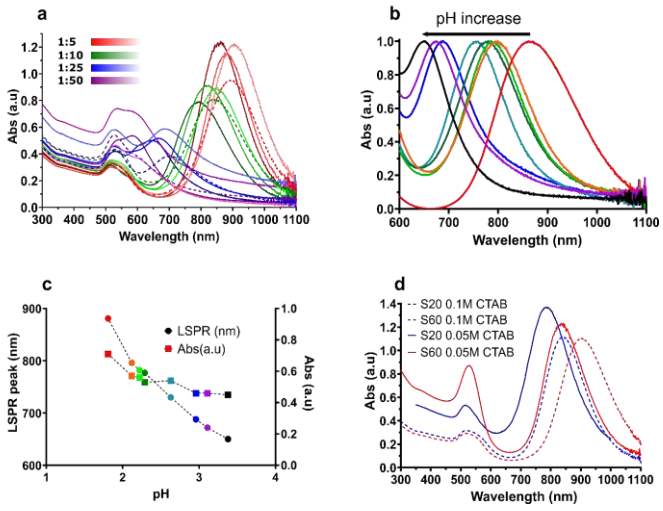


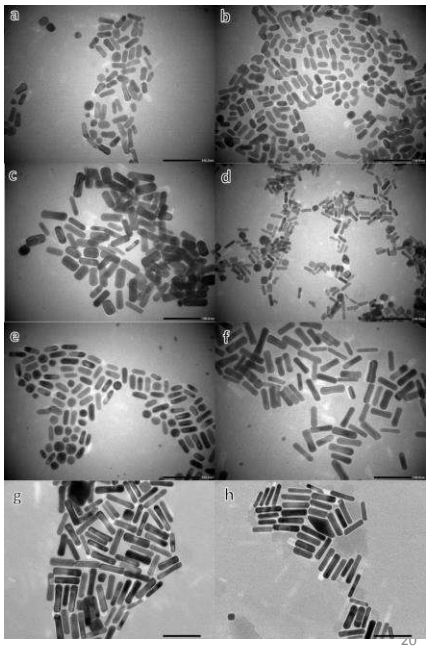
- **Enhance LSPR plasmon shift through plasmon coupling**
- **Establish rapid detection method using darkfield microscopy and pixel extraction**

Nelis et al., A plasmonic biosensor array exploiting plasmon coupling between gold nanorods and spheres for domoic acid detection via two methods **(Submitted).**

19

Optimize NR synthesis





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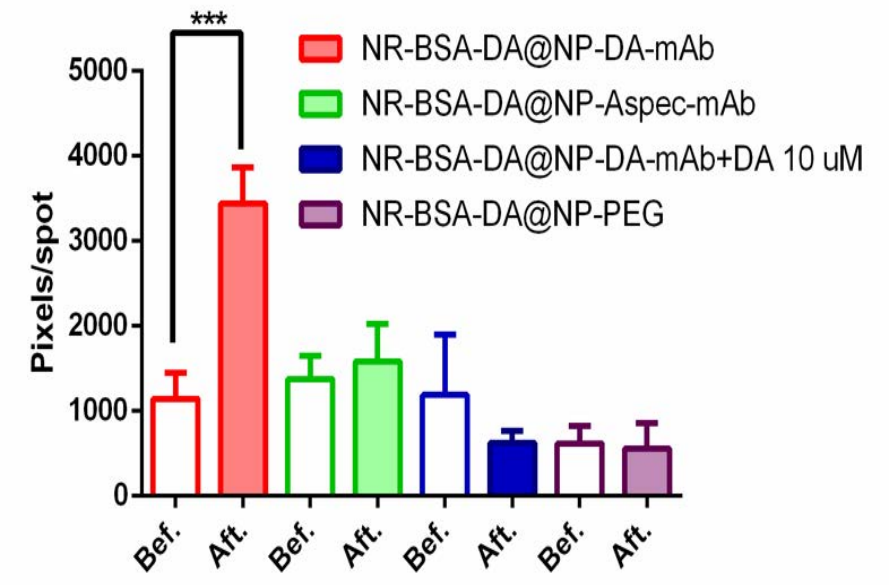
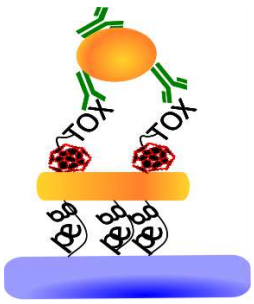
Plasmonic coupling





21

Take home message:

Pixel extraction shows potential for toxin qualification

Next step is developing a smartphone based darkfield microscope

Nelis et al., Electrochemical detection of the marine toxins okadaic acid and domoic acid with carbon black modified screen printed electrodes, **(Submitted)**

22

Final Conclusion

All three approaches showed great potential for smartphone based sensing

Electrochemical detection has the best potential for quantitative detection without Inter-phone variation issues. But assay must be further validated and optimized for end-user use

23

FoodSmartphoners: Thanks to you all for the great collaboration and amazing pHd!!!

24

List of publications

1. **Nelis JLD,** Tsagkaris A, Dillon M, Hajslova J, Elliott CT. Systematic review of smartphone-based optical assays in the food safety field. TrAC Trends Anal Chem https://doi.org/10.1016/j.trac.2020.115934 **(IF 8.43; H-index 142; TC 0)**
2. **Nelis JLD**, Zhao Y, Bura L, Rafferty K, Elliott CT, Campbell K (2020) A randomised combined channel approach for the quantification of colour and intensity based assays with smartphones. Anal. Chem. **(IF 6.35; H-Index 305; TC 1)**
3. **Nelis JLD**, Migliorelli D, Jafari S, Generelli S, Lou-Franco J, Salvador JP, Marco MP, Cao C, Elliott CT, Campbell K (2020) The benefits of carbon black, gold and magnetic nanomaterials for point-of-harvest electrochemical quantification of domoic acid. Microchim Acta 187. **(IF 5.48; H-index 74; TC 1)**
4. **Nelis JLD**, Bura L, Zhao Y, Burkin KM, Rafferty K, Elliott CT, Campbell K (2019) The Efficiency of Color Space Channels to Quantify Color and Color Intensity Change in Liquids, pH Strips, and Lateral Flow Assays with Smartphones. Sensors 19. (**IF 3.03; H-index 132; TC 3**)
5. Zhao Y, Choi SY, **Nelis JLD**, Zhou H, Cao C, Campbell K, Elliott C, Rafferty K Smartphone Modulated Colorimetric Reader with Color Subtraction. Proceedings of the IEEE Sensors 2019 Conference, Montreal, Canada 1–4 **(IF 0.0; H-index 35; TC 1)**
6. Tsagkaris AS, **Nelis JLD**, Ross GMS, Jafari S, Guercetti J, Kopper K, Zhao Y, Rafferty K, Salvador JP, Migliorelli D, Salentijn GIJ, Campbell K, Marco MP, Elliot CT, Nielen MWF, Pulkrabova J, Hajslova J (2019) Critical assessment of recent trends related to screening and confirmatory analytical methods for selected food contaminants and allergens. TrAC Trends Anal Chem 121:115688. **(IF 8.43; H-index 142; TC 9)**
7. **Nelis JLD**, Tsagkaris AS, Zhao Y, Lou-Franco J, Nolan P, Zhou H, Cao C, Rafferty K, Hajslova J, Elliott CT, Campbell K (2019) The end user sensor tree: An end-user friendly sensor database. Biosens Bioelectron 130. **(IF 9.52; H-index 170; TC 9)**
8. **Nelis J**, Elliott C, Campbell K (2018) "The smartphone's guide to the galaxy": In situ analysis in space. Biosensors 8. **(no IF; H-index 25; TC 4)**
9. **Nelis JLD,** Salvador P, Marco MP, Elliott CT, Campbell, K. A plasmonic biosensor array exploiting plasmon coupling between gold nanorods and spheres for domoic acid detection via two methods. **(Under review)**
10. **Nelis JLD**, Migliorelli D, Mühlebach L, Generelli S, Stewart L, Elliott CT, Campbell K. Highly sensitive electrochemical detection of the marine toxins okadaic acid and domoic acid with carbon black modified screen printed electrodes. **(Submitted)**
11. A.S. Tsagkaris**, J.L.D. Nelis,** K. Campbell, C.T. Elliott, J. Pulkarabova a, J. Hajslova. Smartphone and microfluidic systems in medical and food analysis (**Under review)**

25

FOCOS
FOOD CONSULTING... STRATEGICALLY

Portable Food Safety Testing Devices
The Future of Food Safety Testing

Bert Popping
ST4F Virtual Meeting
November 25, 2020

1

The presentation contains **QR codes** that if you scan them with your smartphone, will take you to the publication or websites

2

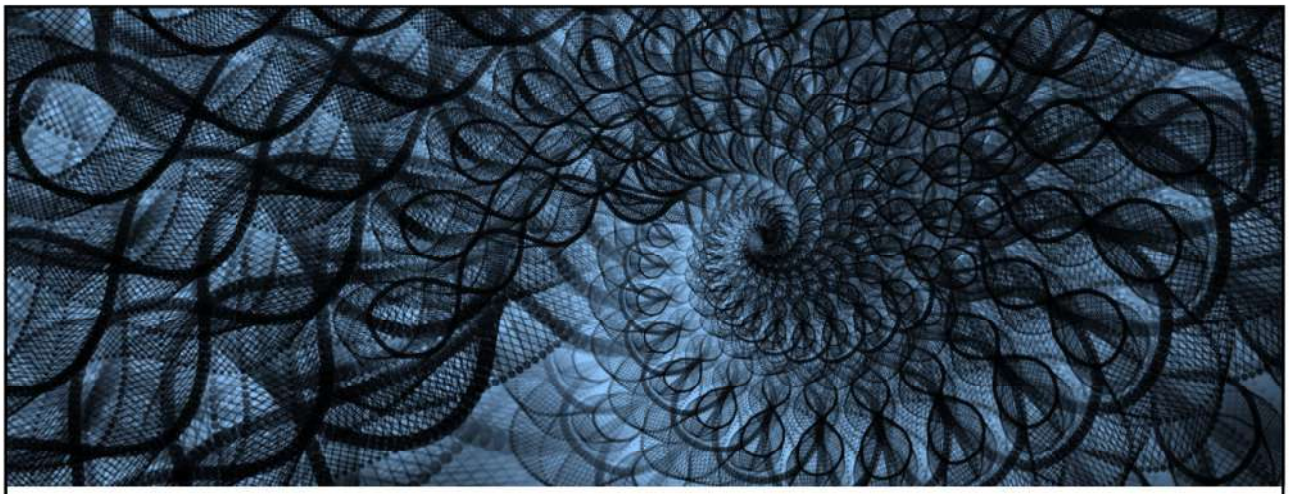
The Challenges Food The Food Industry (and Government Inspectors)

3

4

5

6



Complexity of the Food Supply Chain

7

8

9

Where testing is really needed

Pain Points of food industry when requesting laboratory (food fraud) testing:

- Long TATs
- Cost

10

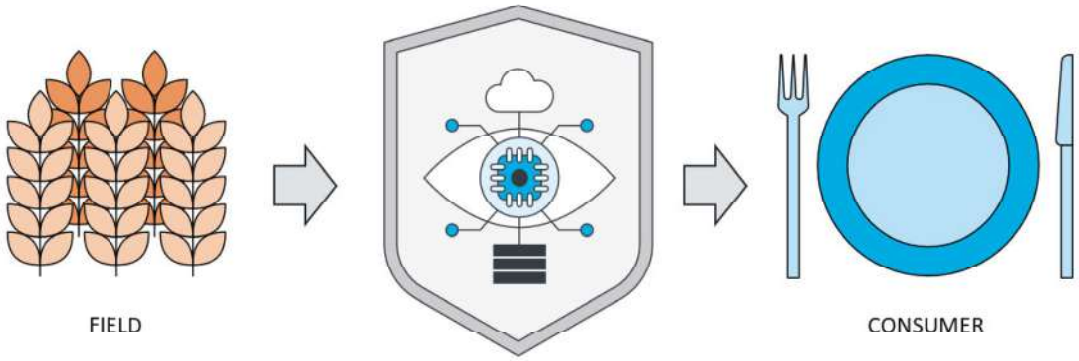

Where testing is
really needed?
250ml
200
150
100
80
60
40
100ml
Bert Popping
© 2020 FOCOS

11


Ideally
On location
Fast
No or very little sample preparation
Raw material Buyer & Quality Control Manager

12

Shifting the First Line of Defense

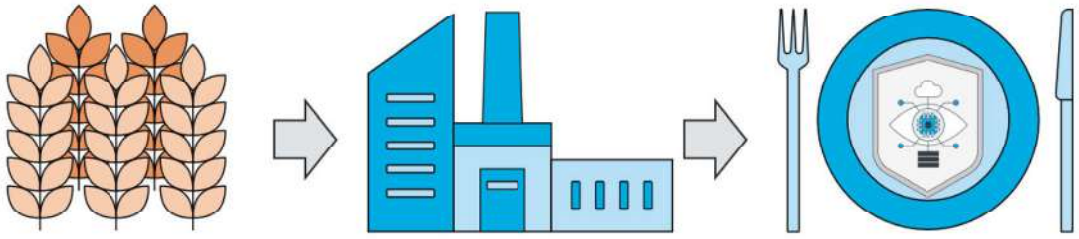


FIRST LINE OF DEFENSE (portable devices)

Bert Popping

13

...and it gets much more complex when



Bert Popping

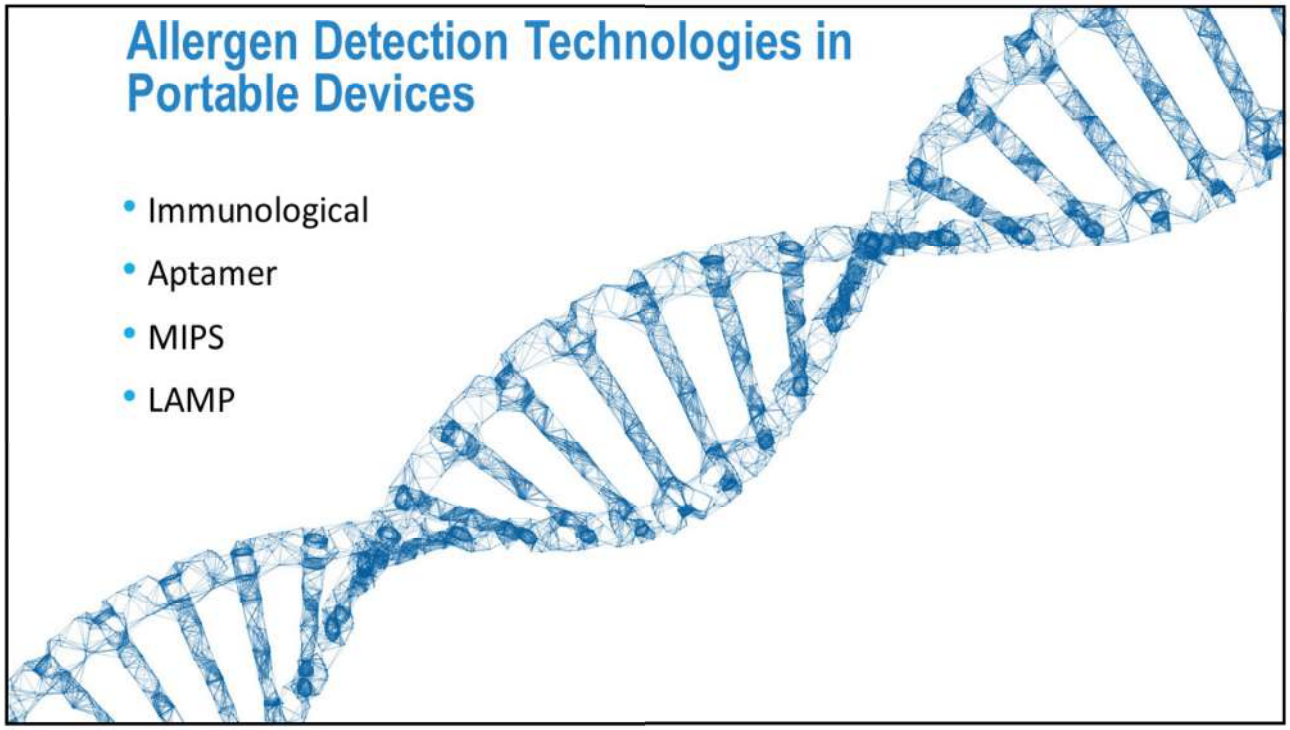
14

Allergen Detection Technologies

15

Allergen Detection Technologies in Portable Devices

- Immunological
- Aptamer
- MIPS
- LAMP



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Validation

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Guidance on Validation of Portable Devices

POPPING ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 101, NO. 1, 2018 185

SPECIAL GUEST EDITOR SECTION: FOOD ALLERGENS NEW METHODS

Stakeholders' Guidance Document for Consumer Analytical Devices with a Focus on Gluten and Food Allergens

BERT POPPING
FOCOS–Food Consulting Strategically, Zum Kälterhaus 6b, 63755 Alzenau, Germany
LAURA ALLRED
Gluten Intolerance Group, 730C Commerce Center Dr, Sebastian, FL 32958
FRANÇOIS BOURDICHON
16 Rue Gaston de Caillavet, 75015 Paris, France
KURT BRUNNER
Romer Laboratories Division Holding GmbH, Technopark 1, 3430 Tuln, Austria
CARMEN DIAZ-AMIGO
FOCOS–Food Consulting Strategically, Zum Kälterhaus 6b, 63755 Alzenau, Germany
PATRICIA GALAN-MALO
Zeulab S.L., C/Bari, 25dedo, 50197 Zaragoza, Spain
MARKUS LACORN
R-Biopharm AG, An der Neuen Bergstrasse 17, 64297 Darmstadt, Germany
JENNIFER NORTH
Beyond Celiac, PO Box 544, Ambler, PA 19002
SALVATORE PARISI
Via Roccazzo 25, 90135 Palermo, Sicily, Italy
ADRIAN ROGERS
Romer Labs UK, Ltd, The Heath Business and Technical Park, Runcorn, Cheshire, WA7 4QX United Kingdom
JENNIFER SEALY-VOYKSNER
ImmunogenX, 1600 Dove St, Suite 300, Newport Beach, CA 92660
TRICIA THOMPSON
Gluten Free, Watchdog LLC, 348 Summer St, Manchester, MA 01944
JUPITER YEUNG
Nestlé Nutrition R&D Centers. Nestlé Development Center Fremont, Inc., Fremont, MI 49412

https://www.focos-food.com/special-section-of-the-journal-of-aoac-on-food-allergens/

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AOAC Food Fraud Task Force

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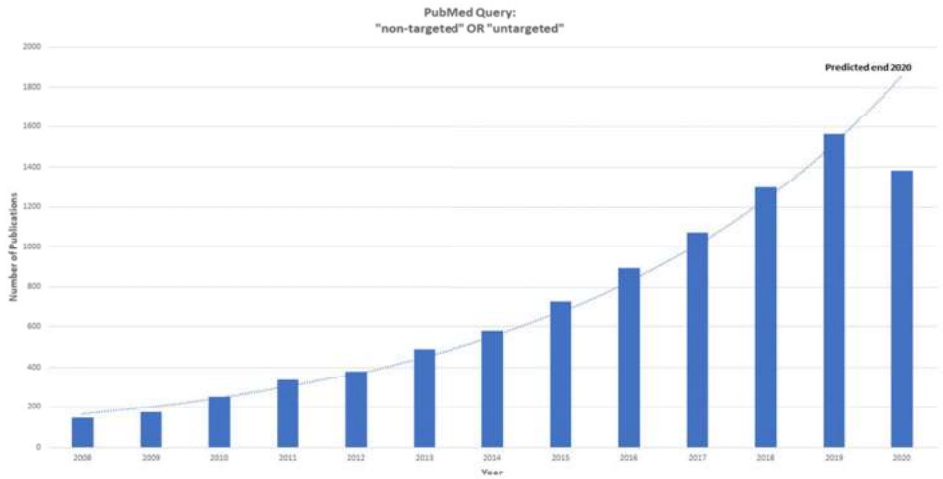
Non-Targeted and Un-Targeted



PubMed Query ('non-targeted' or 'untargeted') on 2020-09-25

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The Challenge for Analysts *Cont'd*

- Which methods are useful?
- Which criteria should I apply to select the best methods from the countless publications?
- Which criteria should a method I develop fulfil?

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Can Standardisation help?

- Yes, but takes a long time, has little flexibility
- To address constantly changing requirements, faster alternatives that provide guidance need to be looked into
- SMPRs

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Appendix F – Guideline to SMPR

Download Appendix F

Appendix F: Guidelines for Standard Method Performance Requirements

Contents

Introduction to Standard Method Performance Requirements

Standard method performance requirements (SMPRs) are a unique and novel concept for the analytical methods community. SMPRs are voluntary consensus standards, developed by stakeholders, that prescribe the minimum analytical performance requirements for classes of analytical methods. In the past, analytical methods were evaluated and the results compared to a "gold standard" method, or if a gold standard method did not exist, then reviewers would decide retrospectively if the analytical performance was acceptable. Frequently, method developers concentrated on the process of evaluating the performance parameters of a method, and rarely set acceptance criteria. However, as the *Eurachem Guide* points out: "... the judgment of method suitability for its intended criteria" documents were prepared for publication in late 2009, but the format of the acceptance criteria documents diverged significantly from one another in basic format. AOAC realized that a guidance document was needed to promote uniformity.

An early version of the SMPR Guidelines were used for a project to define the analytical requirements for endocrine disruptors in potable water. The guidelines proved to be extremely useful in guiding the work of the experts and resulted in uniform SMPRs. Subsequent versions of the SMPR Guidelines were used in the Stakeholder Panel for Infant Formula and Adult Nutritionals (SPIFAN) project with very positive results. The SMPR Guidelines are now published for the first time in the *Journal of AOAC INTERNATIONAL* and *Official Methods of Analysis*.

Users of the guidelines are advised that they are: (*1*) a *guidance* document, not a statute that users must conform to, and (*2*) a "living" document that is regularly updated, so users should check the AOAC website for the latest version before using these guidelines.

The SMPR Guidelines are intended to provide basic information for working groups assigned to prepare SMPRs. The guidelines consist of the standard format of an SMPR, followed by a series of informative tables and annexes.

SMPR Format

The general format for an SMPR is provided in *Annex A*.

Each SMPR is identified by a unique SMPR number consisting of the year followed by a sequential identification number (YYYY.XXX). An SMPR number is assigned when the standard is approved. By convention, the SMPR number indicates the year a standard is approved (as opposed to the year the standard is initiated). For example, SMPR 2010.003 indicates the third SMPR

Bert Popping

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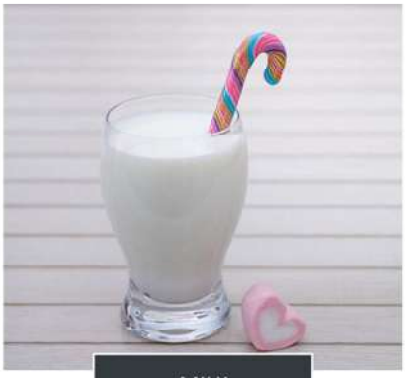
Prioritization of commodities



MILK

HONEY

OLIVE OIL

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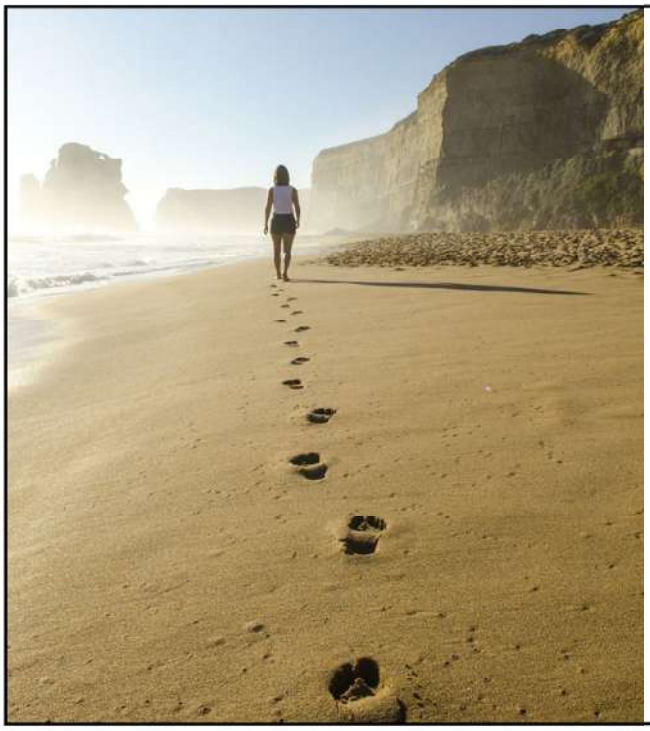

The Next Steps

1. Expansion of matrix groups: herbs & spices, botanicals
2. Expansion of methodology groups: DNA-based technologies

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SMPR Targeted & Non-Targeted Methods

AOAC Food Fraud Task Force Meeting – The Next Steps

By Bert Popping | September 15, 2020

https://www.focos-food.com/aoac-food-fraud-task-force-meeting-the-next-steps/

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J. AOAC Special Section on Portable Food Safety Testing Devices

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A Paradigm Shift: from "Sample to Laboratory" to "Laboratory to Sample"

Journal of AOAC International, 2020, 1–6

SPECIAL GUEST EDITOR SECTION

A Paradigm Shift: From "Sample to Laboratory" to "Laboratory to Sample"

Bert Popping
Guest Editor

Carmen Diaz-Amigo
Guest Editor

https://https://academic.oup.com/jaoac/advance-article-abstract/doi/10.1093/jaoacint/qsaa091/5869038?redirectedFrom=fulltext

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and
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CARMEN DIAZ-AMIGO
carmen.diaz.amigo@focos-food.com

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Our International Collaborators

FOCOS

BERT POPPING
Food Safety • Food Fraud
Food Analysis • Food Contaminants
Food Allergens

CESARE VARALLO
Food Law • Compliance
Food Safety • Food Fraud

FOCOS

CARMEN DIAZ-AMIGO
Fraud Safety • Food Allergens
Visual and Scientific Communication

FSMH.

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Food Microbiology

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www.focos-food.com
info@focos-food.com

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Multiplex analysis of food allergens: the challenge of developing a test

FoodSmart phone.eu

ZEU MAKING TESTING EASIER

INNOVATE SME

Patricia Galán-Malo, PhD.

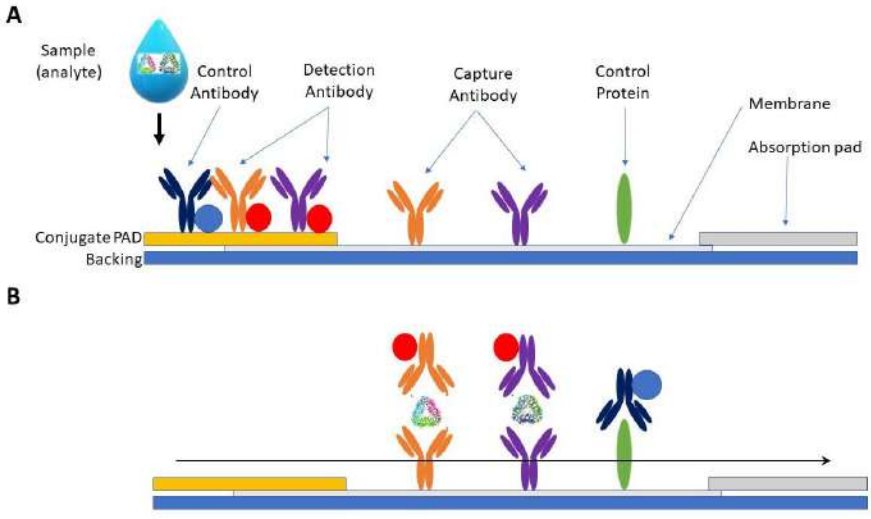
ZEU

INTRODUCTION

- Food allergy patterns depends on age, genetic and environmental factors
- European Union (EU) Regulation Nº1169/2011 → 14 priority allergens
- Each industry has particular risk management requirements
- Each protein has different extraction conditions

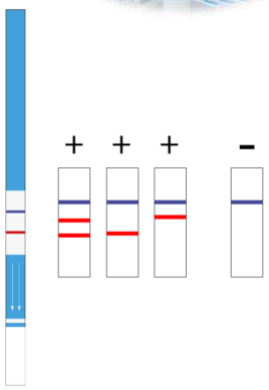
Which allergens include in a multiplex allergen test?

INTRODUCTION



ZEULAB has explored two approaches

- Same allergen: two targets
  - •Proteon Duo Milk
    - Casein
    - β-lactoglobulin
  - •Proteon Duo Soy
    - β-conglycinin
    - Thermal resistant region of glycinin
- Two different food allergens:
  - •Proteon Duo Nuts
    - Cor a 9
    - Pru du 6



Proteon Duo Milk

- Whey products (β –lactoglobulin), milk, caseinate or cheese

- Is there a thermal process implied?
- Detection of some dairy ingredients

Product (ppm)	Whey Protein Concentrate		Sweet whey		Acid whey		NaCas		Milk powder	
	Casein	LGB	Casein	LGB	Casein	LGB	Casein	LGB	Casein	LGB
1	N	N	N	N	N	P	N	N	N	N
5	P	P	N	P	N	P	P	N	P	N
10	P	P	N	P	N	P	P	N	P	P
25	P	P	P	P	N	P	P	N	P	P

Result: N= negative, P= positive

Proteon Duo Soy

- Flour soy, soy protein isolate

Soy proteins (ppm)	Protein isolate 1		Protein isolate 2		Protein isolate 3	
	β-CG	Gly	β-CG	Gly	β-CG	Gly
3	P	P	P	N	N	P
6	P	P	P	N	N	P
12	P	P	P	N	P	P
120	P	P	P	P	P	P

Result: N= negative, P= positive

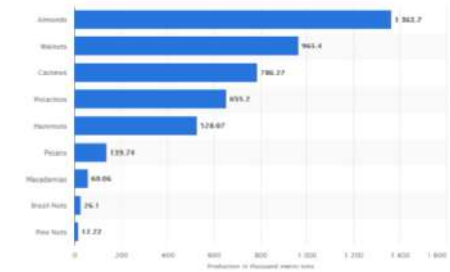
- Industrial processes: thermal , hydrolysis , extrusion process

Soy proteins (ppm)	Tofu		Pate		Soy infant formula 1		Soy infant formula 2	
	β-CG	T-Gly	β-CG	T-Gly	β-CG	T-Gly	β-CG	T-Gly
2	N	P	N	N	N	N	N	N
10	N	P	N	P	N	N	N	P
20	P	P	N	P	N	N	N	P
100	P	P	N	P	N	P	N	P

Proteon Duo Nuts

- Hazelnut food allergy is one of the main causes of food allergy, at least in Europe

- Production of tree nuts worldwide in 2019/2020



https://www.statista.com/statistics/1030790/tree-nut-global-production-by-type/

- Tree nuts food allergy present a high degree of cross-reactivity

Proteon Duo Nuts

Is there a thermal process implied?

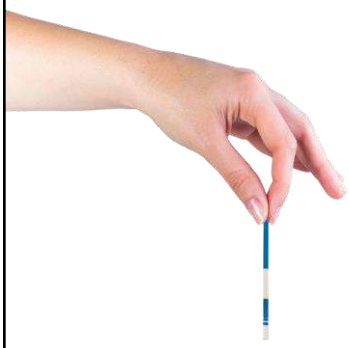
Proteins (ppm)	Almond biscuit				Hazelnut biscuit			
	Unbaked		Baked		Unbaked		Baked	
	Pru du 6	Cor a 9	Pru du 6	Cor a 9	Pru du 6	Cor a 9	Pru du 6	Cor a 9
0,5	N	N	N	N	N	N	N	N
1	P	N	N	N	N	N	N	N
2	P	N	P	N	N	P	N	N
5	P	N	P	N	N	P	N	P
10	P	N	P	N	N	P	N	P

Result: N= negative, P= positive

Milestones in the path of the development

- Selection of targets (Abundance, representativeness, compatibility, thermal stability,…)
- Antibody compatibility in the conjugate PAD and in the nitrocellulose membrane
- Harmonize extraction phase:
  - •Maximum extractability of the target proteins
  - •Blocking matrix effects
  - •Avoid a complex extraction procedure without sensitivity lost

Real-time data is key to make the right decisions

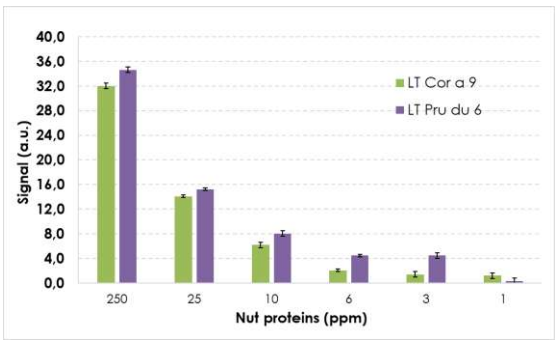


Food industry stakeholders to share test results in real-time. Small portable devices processing automatically ready-to-use tests and controlled by smartphones.

ADAPTABILITY & TEST4ALL
Smart using the lastest technologies

FLEXIBILITY
Each user has a specific need

Anywhere, anytime, anyone



Could we quantify allergens with LFIA test? The next generation…

Thank you to our partners

Universidad Zaragoza
Cátedra AgroBank
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
UNIÓN EUROPEA
FONDO EUROPEO DE DESARROLLO REGIONAL
RTC-2015-3818-2

THANK YOU

info@zeulab.com
+34 976 731 533
www.zeulab.com

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Smart Allergen & Mycotoxin management

The use of smartphone technology in food contaminant management

R-Biopharm AG • An der neuen Bergstraße 17 • 64297 Darmstadt, Germany • E-mail: info@r-biopharm.de • www.r-biopharm.com

Smart Allergen & Mycotoxin management | November 2020

Why we started looking for smartphone based analytical tools….

For mycotoxins you like to take a decision practically in the field….

How to get (mycotoxin) analysis out of a lab environment into the field….

So instead of „bringing the lab (almost) in the field“, we envisioned to go one step beyond and develop a truly mobile analytical tool – use smartphones!

Why use smartphone technology?

First of all: Technology

- Camera quality in most smartphones is very good
- Smartphones are globally and easily available
- Smartphone software can do the mathematics….

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But taking your analysis out of the lab means more than a smartphone…

What else do we need for that – for mycotoxins in this case?

- Good sampling method
- Appropriate sample preparation and sample size
- Easy to use extraction method (preferably no organic solvents)
- Easy to use testing method (out of the lab environment)
- Reliable method (fit for purpose)
- Data sharing (preferably digital)

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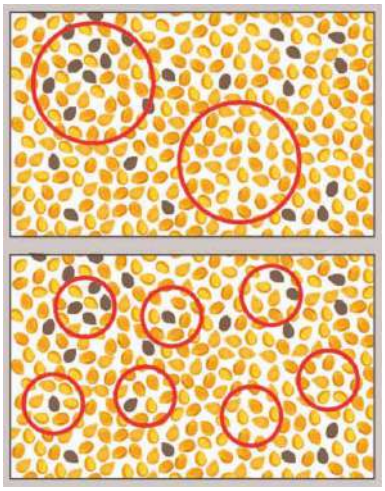
Take mycotoxin analysis out of the laboratory – what do we need?

Good sampling method

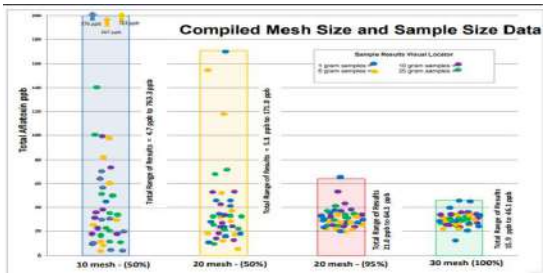


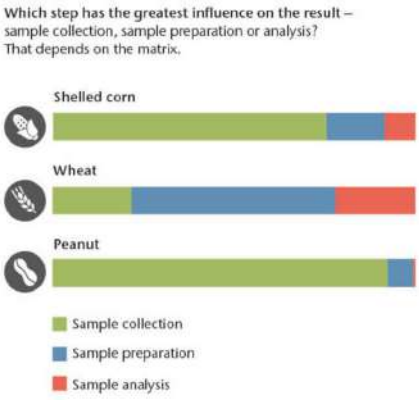
You are often looking for the “needle in the haystack“

Take mycotoxin analysis out of the laboratory – what do we need?

Appropriate sample preparation and sample size:

The particle size and sample size have a large influence on the precision of the results





Take mycotoxin analysis out of the laboratory – what do we need?

Easy to use extraction method:

Water based extraction solvents,
avoid the use of organic solvents

Smart Allergen & Mycotoxin management | November 2020

Take mycotoxin analysis out of the laboratory – what do we need?

Easy to use testing method:

Lateral flow based design,
3 – 5 minutes test implementation time,
quantitative results with smartphone and app!

Take mycotoxin analysis out of the laboratory – what do we need?

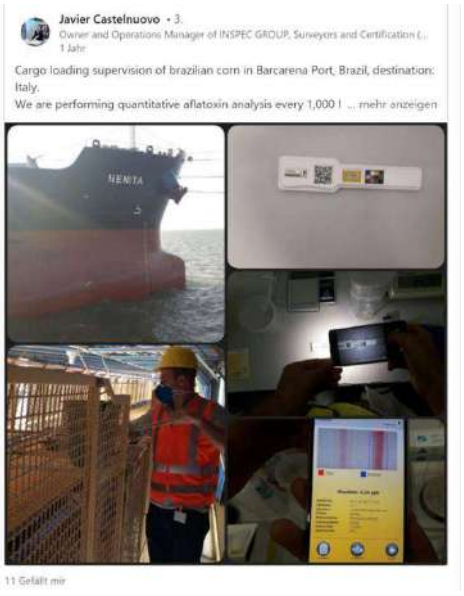
A reliable method – fit for purpose

RIDA®QUICK DON (Art. No. R5908)	Trilogy® reference material (wheat)							
Target value	ND	0.5	0.9	1.6	2.1	3.5	4.5	6.2
Recovery [%]	-	106	93	93	100	95	104	93
	Trilogy® reference material (corn)							
Target value	ND	0.5	1.1	1.9	2.7	3.6	4.8	6.2
Recovery [%]	-	113	104	102	105	100	104	97
RIDA®QUICK Aflatoxin RQS (Art. No. R5205)	Trilogy® reference material (corn)							
Target value	ND	1.7	5.9	14.3	20.2	31.6	50.8	98.7
Recovery [%]	-	-	97	110	104	106	96	82
RIDA®QUICK Aflatoxin RQS ECO (Art. No. R5206)	Trilogy® reference material (corn)							
Target value	ND	1.7	5.9	14.3	20.2	31.6	50.8	98.7
Recovery [%]	-	-	88	84	93	88	100	97
RIDA®QUICK Zearalenon RQS (Art. No. R5504)	Trilogy® reference material (corn)							
Target value	ND	59	88	121	165	267	472	1021
Recovery [%]	-	73	117	121	111	86	82	84
RIDA®QUICK Fumonisin RQS (Art. No. R5606)	Trilogy® reference material (corn)							
Target value	ND	0.6	1.0	2.2	3.2	6.8	9.2	12.5
Recovery [%]	-	92	111	98	94	101	84	-
RIDA®QUICK T-2 / HT-2 RQS (Art. No. R5304)	Oats sample (for spike level see target values)							
Target value	ND	50	100	200	400	600	800	1000
Recovery [%]	-	103	113	96	93	93	92	91

Take mycotoxin analysis out of the laboratory – in reality now…

Found on LinkedIn – cargo loading inspection in Brazil of corn shipment to Italy

This method is currently used for mycotoxin testing on 1000+ locations all around the globe.



The good thing about smartphones is “Connectivity”!

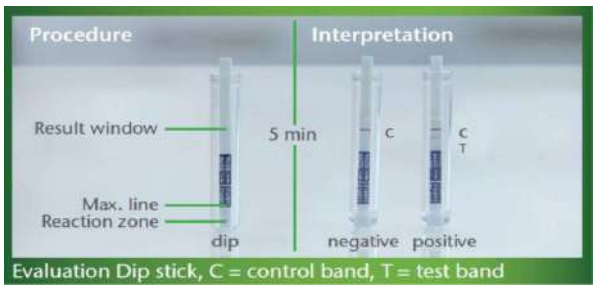
As the central QC manager you can have real-time mycotoxin analytical data from the field!

You could also do quality assurance remotely by sending quality control samples on a regular basis to your „analysts in the field“

This principle may work for various applications

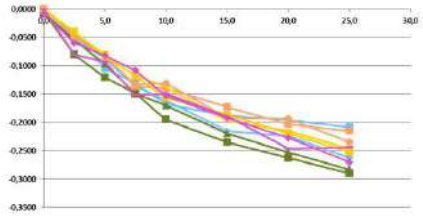
For example milk testing for antibiotics (or AFM1) at the milk collection points

We are working on a mobile application for gluten and allergen testing



Preliminary tests show a proof of principle for a gluten lateral flow test..

Results were generated with the RIDA® SMART APP and RIDA® QUICK Gliadin



Concentration [ppm]	Matrix 1	Matrix 2	Matrix 3	Matrix 4
2,5	101%	109%	139%	74%
5,0	89%	112%	122%	85%
7,5	88%	102%	137%	86%
10,0	89%	102%	123%	81%
15,0	89%	101%	128%	80%
20,0	91%	96%	102%	78%
25,0	91%	103%	114%	76%

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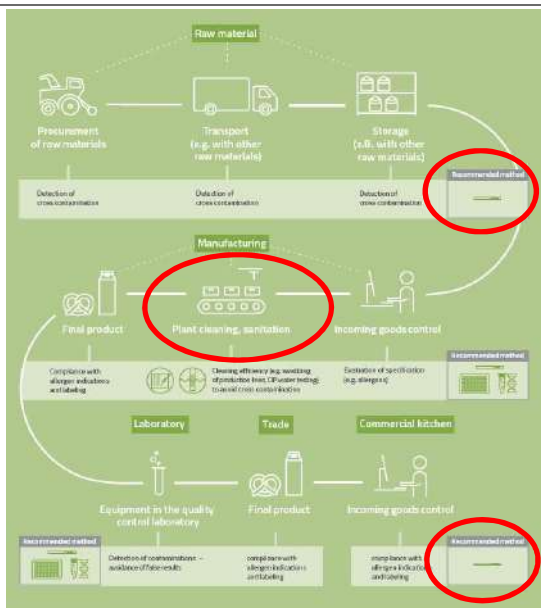
Food allergen (risk) management….

Lateral flow based immunoassays play a very important role in food allergen management in the production chain

Quantitative results are necessary for risk management in e.g. VITAL program

Using a smartphone will not just enable quantitative analysis but also to perform data analysis and calculations!



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Would this be interesting for testing by the consumers themselves?

Yes and no..

The analytical method as such, together with a smartphone based measurement and data processing should work..

But.. What about sampling?

Can you get a representative sample of the food just purchased, in case this is not a liquid?

…?

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What is the true added value of smartphone analysis here?

Besides a „gadget like“ reader for e.g. LFD‘s (or other analytical methods) ☺

Why are smartphone based analytical tools real game changers?

18

What is the true added value of smartphone analysis: Big Data

Combine real-time analytical data from the field with other for agriculture relevant data and share those data

MycoKey App: monitoring mycotoxins in wheat and kernel maize.

http://www.wheatscab.psu.edu/

Smart phone based analytical tools can be true game changers

- Cost efficient analytical tools for quantitative, on-site analysis
- Smartphones come with many high quality features (camera, connectivity, calculating power) in one device
- Various analytical applications possible
- Data can be uploaded and shared online – to make rapid decisions, even from a distance
- Analyze and evaluate data, use calculation models
- Connect analytical data with other product(-ion) data („Mycotoxin / Food Allergen Big Data“)
- ……..

Thank you

for your attention!

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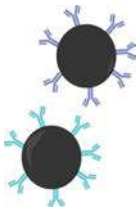

From Sample to Smartphone:
Consumer-Operable Multiplex Allergen Immunodetection

ESR1: Gina Ross

WFSR

26/11/2020

1

Project Aims:

- Simplified allergen detection
- Using inexpensive and optimal immunoreagents
- With interconnectable and portable sample preparation
- And smartphone-based readout

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2

Allergen Testing

GLUTEN SESAME NUTS PEANUTS EGGS

FISH MUSTARD MILK CELERY CRUSTACEAN

SOYA SHELLFISH LUPINS SO_2 SULPHITE

FoodSmart phone.eu

3/16

3

Ross, G.M.S., Bremer, M.G.E.G, Nielen, M.W.F., 2018. Consumer-friendly food allergen detection: moving towards smartp based immunoassays. *Analytical and Bioanalytical Chemistry*. 410, 5353- 5371. doi: 10.1007/s00216-018-0989-7

Rapid

Easy to use

Safe

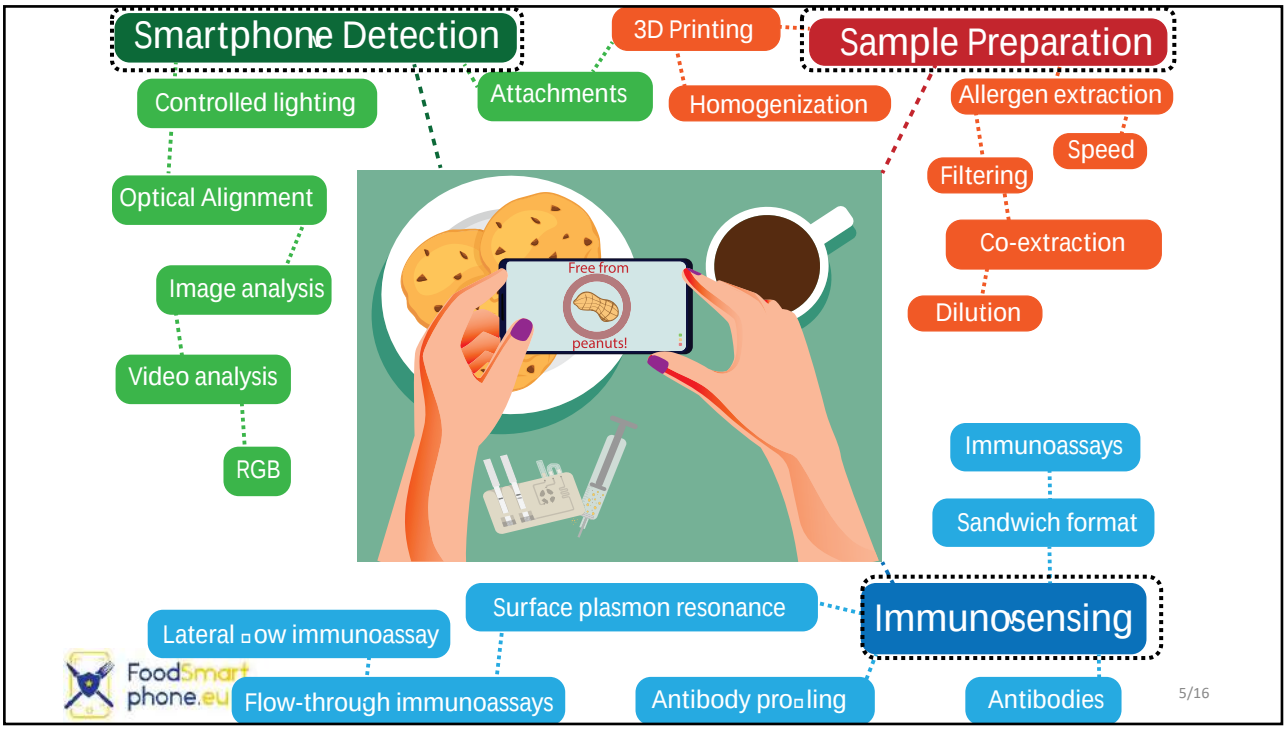
Sensitive

Affordable

Consumer-operable allergen detection

Multiplex

4


Smartphone Detection
Controlled lighting
Optical Alignment
Image analysis
Video analysis
RGB
Attachments
3D Printing
Homogenization
Sample Preparation
Allergen extraction
Speed
Filtering
Co-extraction
Dilution
Free from
peanuts!
Immunoassays
Sandwich format
Immunosensing
Surface plasmon resonance
Lateral flow immunoassay
Flow-through immunoassays
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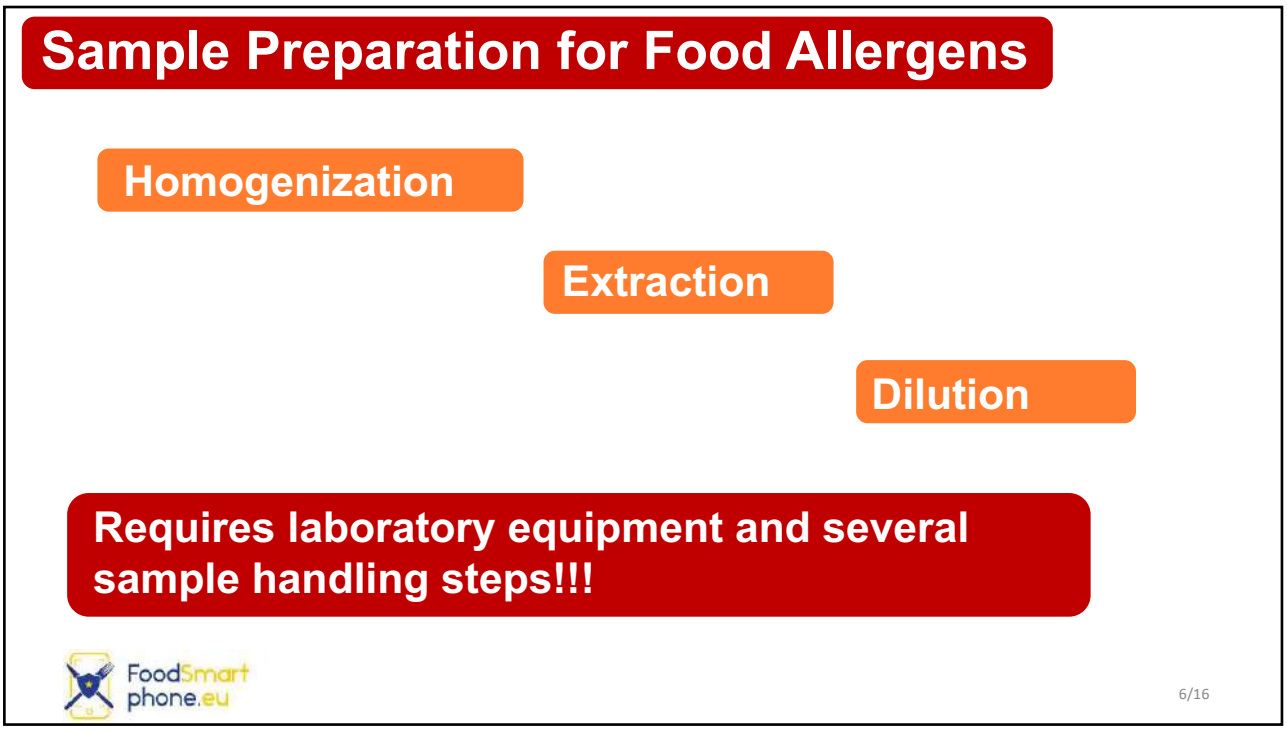
5/16

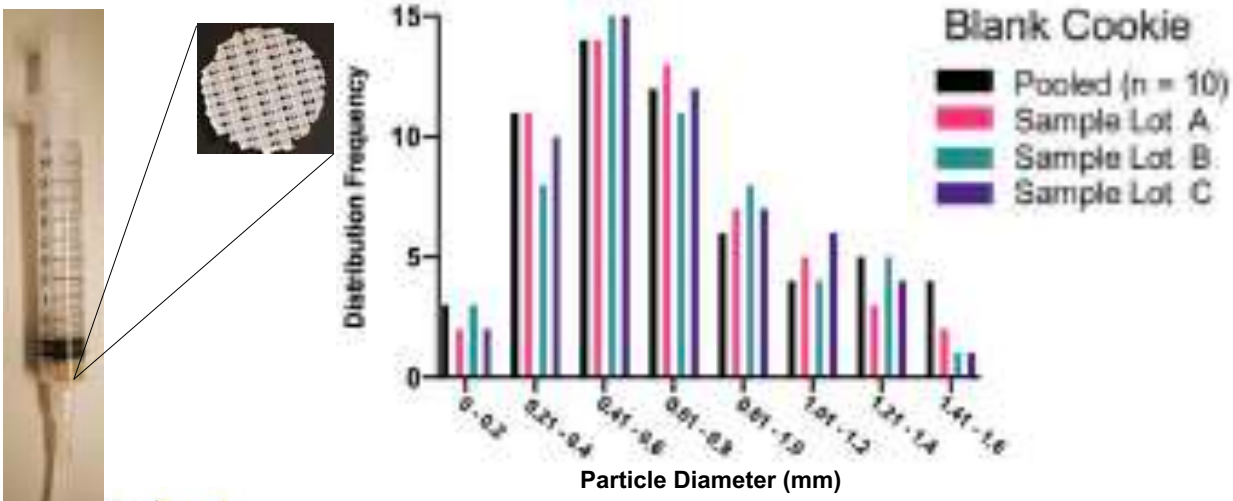
5


Sample Preparation for Food Allergens
Homogenization
Extraction
Dilution
Requires laboratory equipment and several sample handling steps!!!
FoodSmart phone.eu
6/16

6

Portable Sample Preparation: 3D-Printing

Homogenization



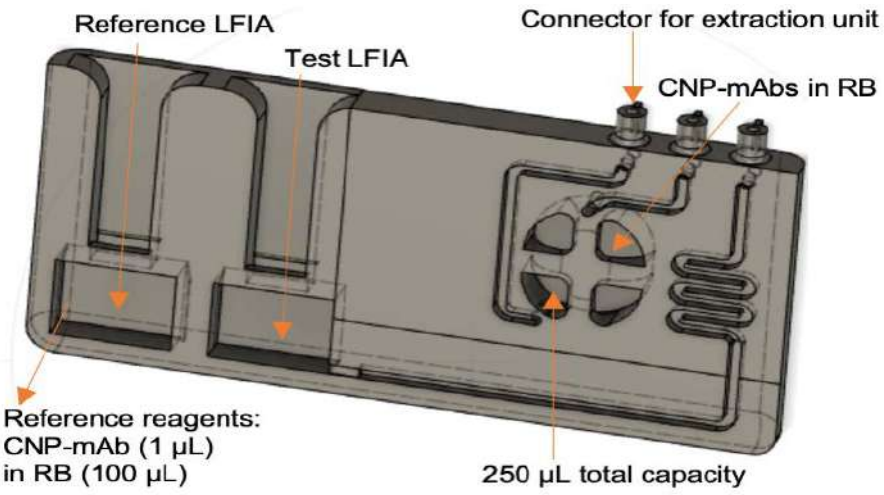
FoodSmart phone.eu

Ross, G.M.S., Filippini, D., Nielen, M.W.F., Salentijn, G.IJ, 2020. Interconnectable solid-liquid protein extraction unit and chip-based dilution for multiplexed consumer immunodiagnostics. *Analytica Chimica Acta.*1140. doi: 10.1016/j.aca.2020.10.018

7/16

7

On-chip Dilution

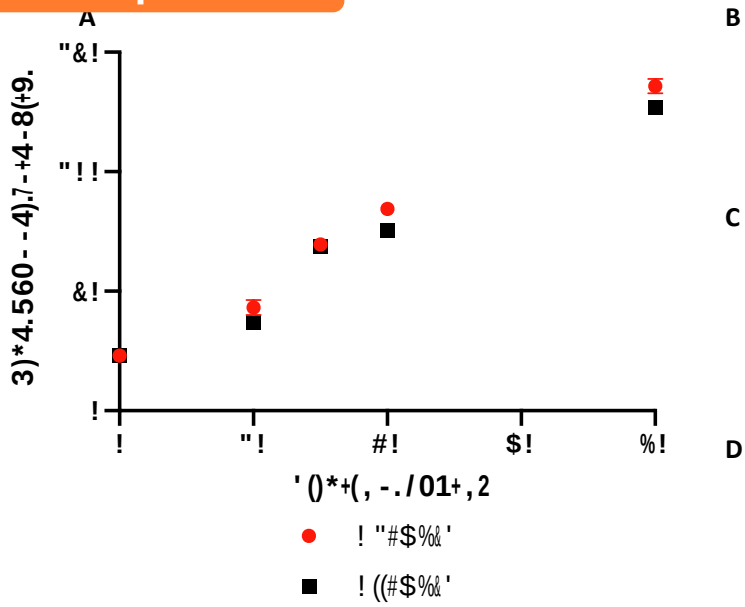


FoodSmart phone.eu

doi: 10.1016/j.aca.2020.10.018 8/16

8

On-chip Dilution



B

Water

C

Manual dilution

Mixing dye with water

D

ULOC dilution

phone.eu

9/16

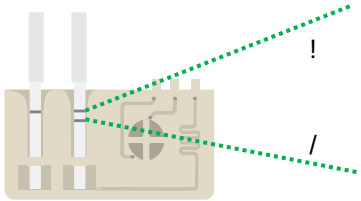
doi: 10.1016/j.aca.2020.10.018

9

Immunosensing

! "#$%#&'(&)"#*+,,-.

! "#$%#&'(&)"#*+,,-.



Singleplex

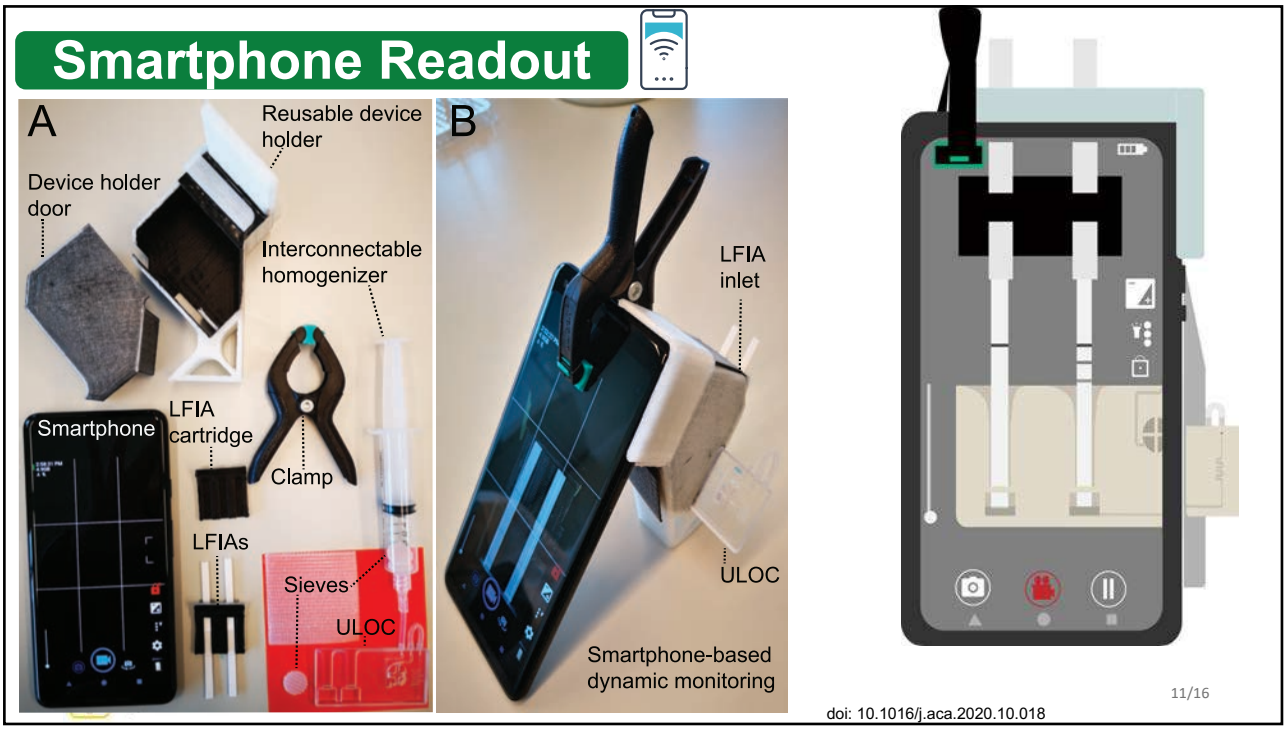
Multiplex

FoodSmartphone.eu

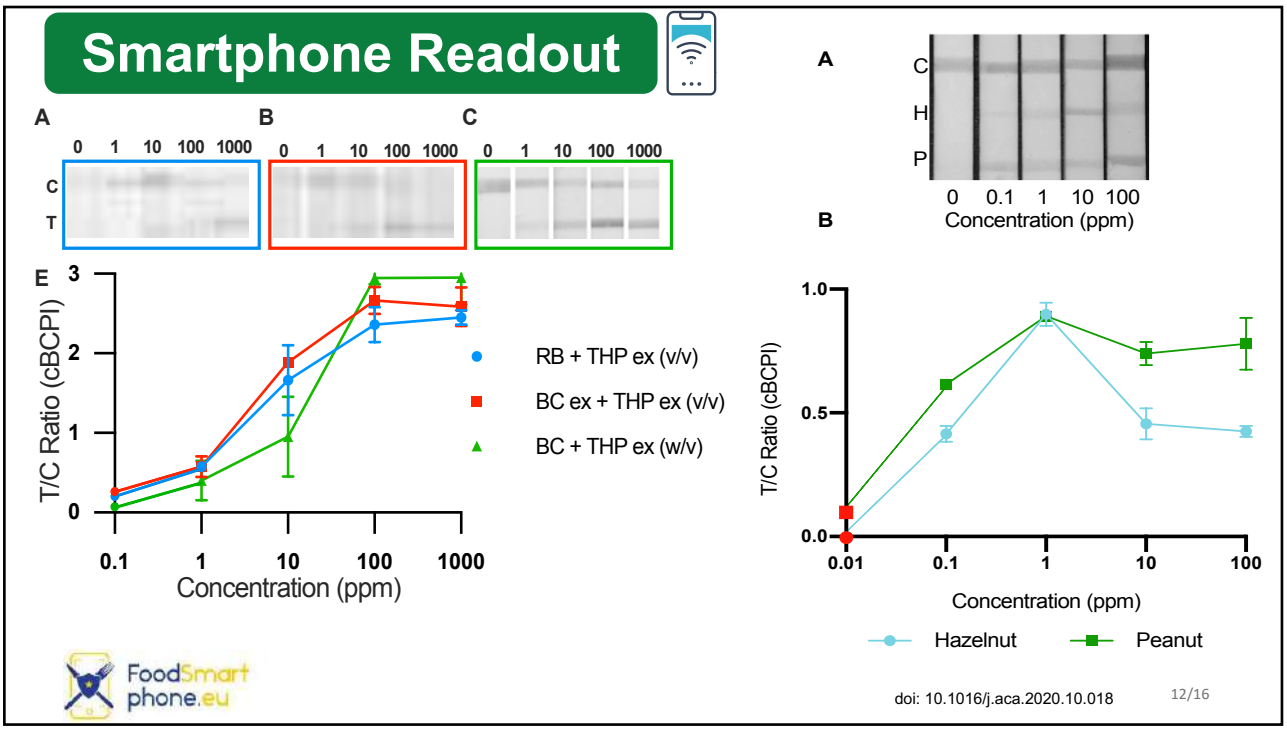
Ross, G.M.S., Filippini, D., Nielen, M.W.F., Salentijn, G.IJ, 2020. Interconnectable solid-liquid protein extraction unit and chip-based dilution for multiplexed consumer immunodiagnostics. *Analytica Chimica Acta*.1140. doi: 10.1016/j.aca.2020.10.018

10/16

10


Smartphone Readout
A
Reusable device holder
Device holder door
Interconnectable homogenizer
Smartphone
LFIA cartridge
Clamp
LFIAs
Sieves
ULOC
B
LFIA inlet
ULOC
Smartphone-based dynamic monitoring
11/16
doi: 10.1016/j.aca.2020.10.018

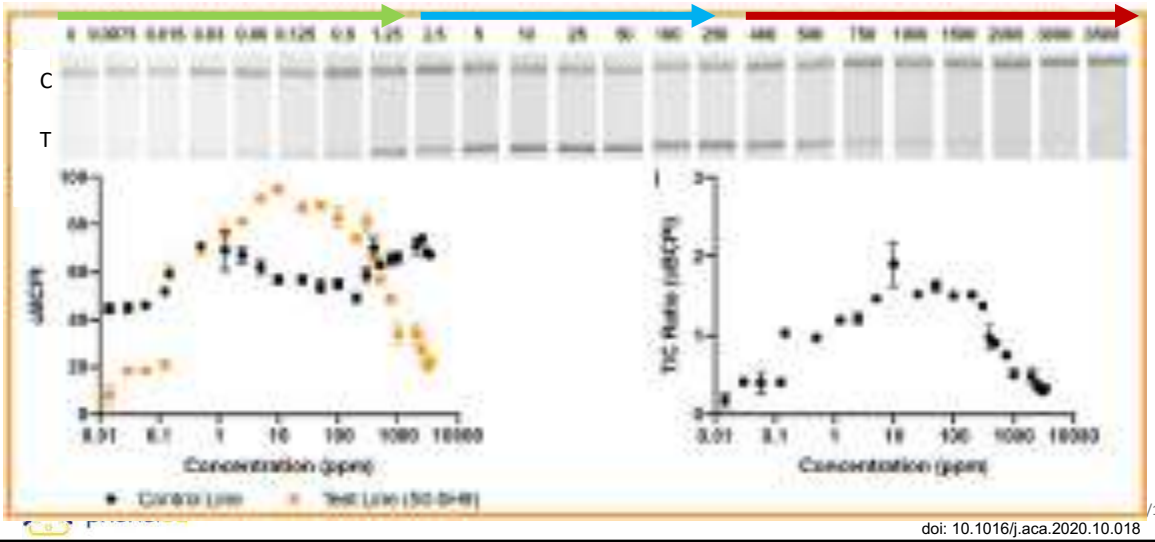
11


Smartphone Readout
A
B
C
0 1 10 100 1000
C
T
E
T/C Ratio (cBCPI)
Concentration (ppm)
RB + THP ex (v/v)
BC ex + THP ex (v/v)
BC + THP ex (w/v)
A
C
H
P
0 0.1 1 10 100
Concentration (ppm)
B
T/C Ratio (cBCPI)
Concentration (ppm)
Hazelnut
Peanut
FoodSmartphone.eu
doi: 10.1016/j.aca.2020.10.018
12/16

12

Smartphone Dynamic Monitoring

- **Antigen concentration clearly influences signal development in sandwich format LFIA**

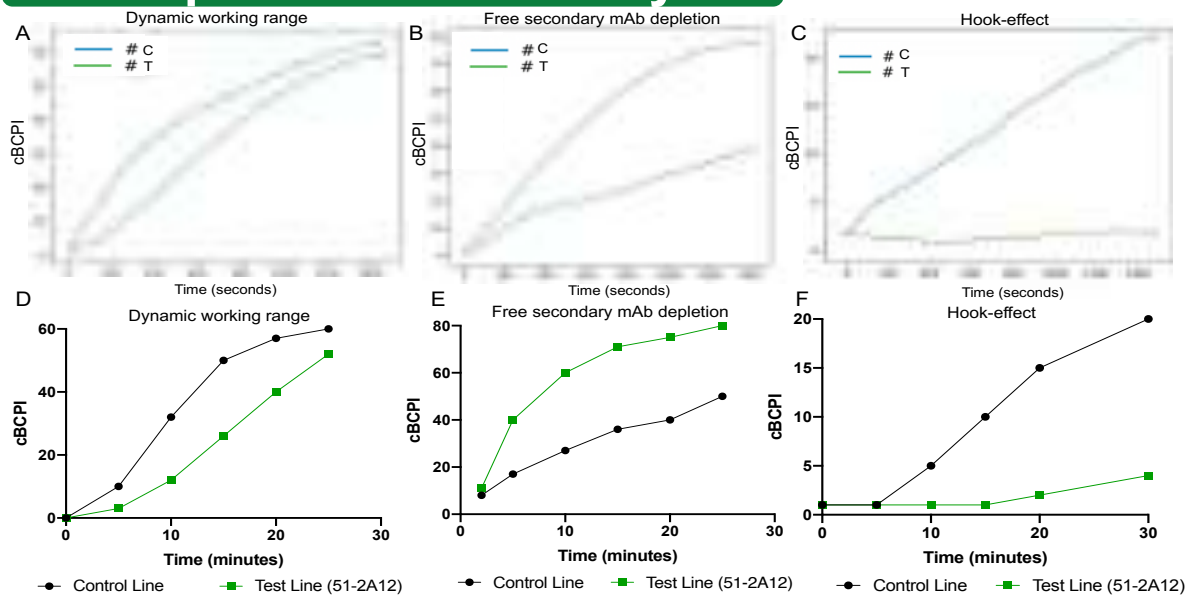


/16

doi: 10.1016/j.aca.2020.10.018

13

Smartphone: Video Analysis



phone.eu

14/16

doi: 10.1016/j.aca.2020.10.018

14

Consumer Allergen Detection Outlook

- **Before FoodSmartphone:** Allergen analysis = lab-based with limited options for consumer-operable allergen detection.
- **Now:** Prototype consumer-operable device for the total analysis of multiple allergens in foods, from sample preparation to smartphone detection.
- **Beyond FoodSmartphone:** Increased options for integrating sample preparation with portable analytical devices for consumers.

FoodSmartphone.eu

15/16

15

Acknowledgements

FoodSmartphone.eu
MARIE CURIE ACTIONS
UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE
Nb4D
LINKÖPING UNIVERSITY
Barilla
AQUAMARIJN
FUTURE TECHNOLOGY, ENGINEERED FOR NOW
QUEEN'S UNIVERSITY BELFAST
WAGENINGEN UNIVERSITY & RESEARCH
csem
ZEU

16/16

16

Think

Food Diagnostics

Differently.

unisensor
DIAGNOSTIC ENGINEERING

EXTENSO:
a new multiplex and connected platform for antibiotics detection in food

ST4F workshop
26th November

Olga Matveeva-Kolm
Extenso Business Unit Leader

1

One step ahead of innovation in food diagnostics for over 20 years

AVAILABLE IN MORE THAN 60 COUNTRIES

13 MIO TESTS PRODUCED ANNUALLY

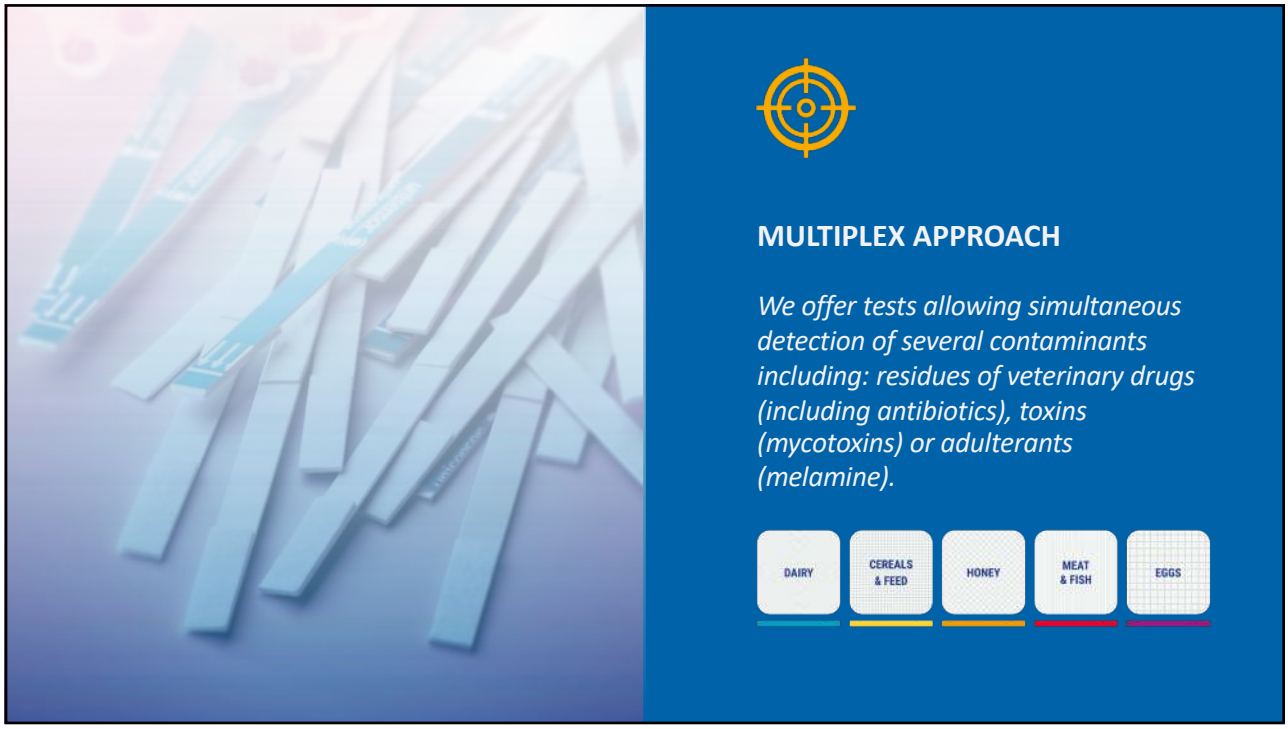
70 EMPLOYEES

CONTINUOUS GROWTH

VALUES
EFFICIENCY
INTEGRITY
TEAM SPIRIT
CUSTOMER ORIENTED

5 PATENTS

2



3

Tailor-made products for the right application

Lateral Flow Tests For Daily Screening

A wide range of tests available for different matrices and allowing multiple detection of contaminants.

Indirect Competitive Immuno-chromatographic Assays

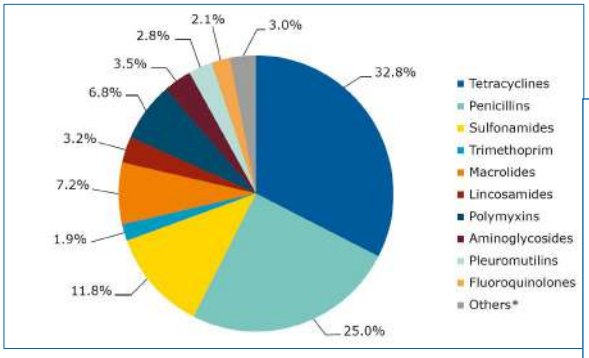
unisensor DIAGNOSTIC ENGINEERING

page 04

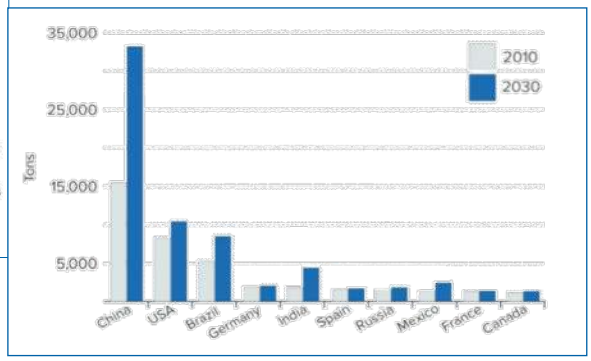
4

Global food safety context

Antibiotic consumption in livestock & sales of veterinary antimicrobials



Sales of veterinary antimicrobial agents in 30 European countries in 2015
7th ESVAC report (EMA/184855/2017- Veterinary Medicines Division)



Antibiotics consumption in livestock in ten countries 2010-2030 (projection)
Van Boeckel et al 2015

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5

Global food safety context

General trends

- The use of **antimicrobials** in production animals **increases the risk of human pathogen resistance** => Human and animal **worldwide healthcare** concern
- **Laws & Regulations become stricter** in terms of number of tests to do, number of contaminants to detect and tolerance /sensitivity (MRLs, MRPLs)
- **Manufacturers & Retailers impose** stricter controls on supplies.
- These controls and their responsibility are more and more transferred to the **producers** and performed by **field operators**
- The digitalization is also growing in the food industries. The **Internet of Things** (IoT) is expected to transform how food companies use and process information…

6

Milk quality is a major concern…

Farmers, producers and laboratories have to meet European and international rules. They must perform even more stricter controls to ensure the quality of their products.

You need breakthrough solutions to detect more contaminants, while allowing you to choose the contaminants you want to be tested.

7

REVOLUTIONARY
MILK ANALYSIS PLATFORM

FROM RISK ANALYSIS TO DAILY SCREENING

extenso
by unisensor

8

EXTENSO is the all-in-one multiplex, fast, easy and automated solution the dairy industry needs to detect the widest range of contaminants in milk. All in the shortest possible time and providing a simple data-processing interface.

BENEFITS

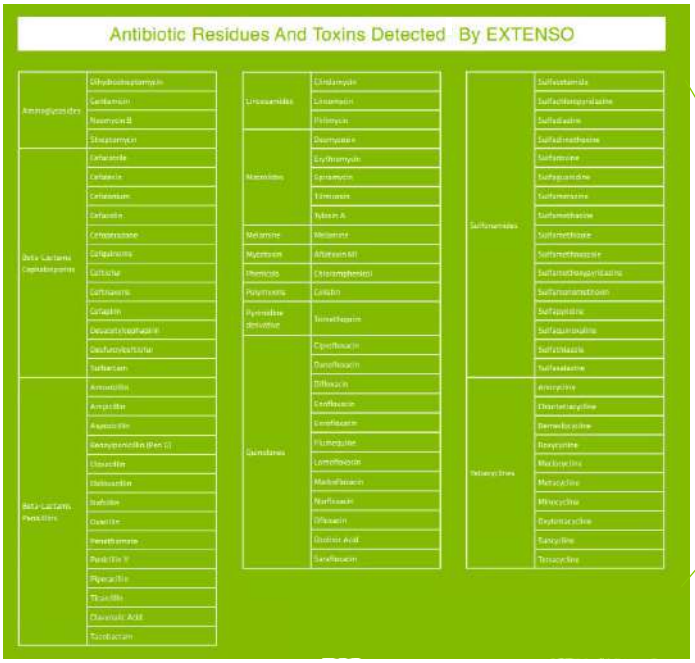
MULTIPLE
Detect more than 100 antibiotic residues and toxins in milk in one single test.

FLEXIBLE
Whether for monitoring or for a release plan, choose any combination of contaminants to test amongst 131071 possibilities.

RAPID
Only 13 minutes to get your results. EXTENSO helps you to take immediate action.

CONNECTED
Remote access, multiple data export options, full traceability and automated actions.

9



Detection of >100 molecules in one qualitative test (POS/NEG)

Discrimination in 17 contaminant groups

page 010

10

Channel	Drug family	Compounds detected
AFLA	mycotoxins	aflatoxin M1 and aflatoxin B1
BETA	β-lactams: penicillins + cefalosporins	amoxicillin, ampicillin, benzylpenicillin, phenoxymethylpenicillin, cloxacillin, nafcillin, dicloxacillin, oxacillin, penethamate, piperacillin, ticarcillin and aspoxicillincefalonium, cefazolin, cefoperazone, cefquinome, ceftiofur, desfuroylceftiofur, cephapirin, desacetylcephapirin, cefacetrile, cefuroxime, ceftriaxone and ceftizoxime
CAP	phenicols	chloramphenicol
CEFA	β-lactams: cefalosporins	cefalexin and cefadroxile
COLI	polymyxins	colistin
ERYTHRO	macrolides	erythromycin A, gamithromycin and roxithromycin
GENTA	aminoglycosides	gentamycin and sisomycin
LINCO	lincosamides	lincomycin, clindamycin and pirlimycin
MELA	melamine and pyrimidine derivatives	Melamine, ammeline, baquiloprim and trimethoprim
NEO	aminoglycosides	neomycin B and apramycin
QUINO	(fluoro)quinolones	danofloxacin, enrofloxacin, ciprofloxacin, marbofloxacin, ofloxacin, difloxacin, enofloxacin, lomefloxacin, flumequine, norfloxacin, pefloxacine orbilfloxacin, orbilfloxacin, oxolinic acid, nalidixic acid and sarafloxacin
SDX	sulfadoxine	sulfadoxine
SPIRA	macrolides	spiramycin and neospiramycin
STREPTO	aminoglycosides	dihydrostreptomycin and streptomycin
SULFA	sulfonamides	sulfadiazine, sulfamerazine, sulfadimethoxine, sulfamethazine, sulfamethoxazole, sulfaquinoxaline, sulfamonomethoxine, sulfamethoxypyridazine, sulfaethoxypyridazine, sulfapyridine, sulfasalazine, sulfacetamide, sulfachloorpyridazine, sulfaguanidine, sulfathiazole, sulfisoxazole, sulfatroxazole and sulfamethizole
TETRA	tetracyclines	tetracycline, chloortetracycline, oxytetracycline, doxycycline, mynocycline, demeclocycline, sancycline, amicycline, meclocycline and methacycline
TYLO	macrolides	tylosin A, desmycosin and tilmycosin

11

EXTENSO Multiplexing challenges

- No sample prep, no washing or cleaning step

→ Matrice effects with strong signal background

- > 60 bioreagents used in one test
- Long term stability issue
- High workload for testing & validation

12

START FROM MONITORING AND...
DETERMINE THE ANTIBIOTIC RISK PROFILE
THEN ADOPT A SPECIFIC SCREENING PLAN

CHOOSE YOUR CUSTOM CONFIGURATION

CHANNELS							
AFLA		COLI		QUINO	X	TETRA	X
AZINE	X	ERYTHRO	X	SDX		TYLO	
BETA	X	GENTA		SPIRA			
CAP		LINCO		STREPTO			
CEFA		NEO		SULFA	X		

Define your own surveillance plan: check and pay only for the channels corresponding to the contaminants you wish to detect in your sample.

13

Flexibility: >100K combinations in one kit

EXTENSO CHANNELS		TEST CONFIG
1	QUINO	x
2	CAP	x
3	AFLA M1	x
4	QUINO	x
5	AZINE / MELA	x
6	TETRA	x
7	SULFA	x
8	NEO	x
9	GENTA	x
10	STREPTO	x
11	TYLO	x
12	CEFA	x
13	LINCO	x
14	SDX	x
15	SPIRA	x
16	ERYTHRO	x
17	COLI	x

Combinatory analysis = $(2^n)-1$

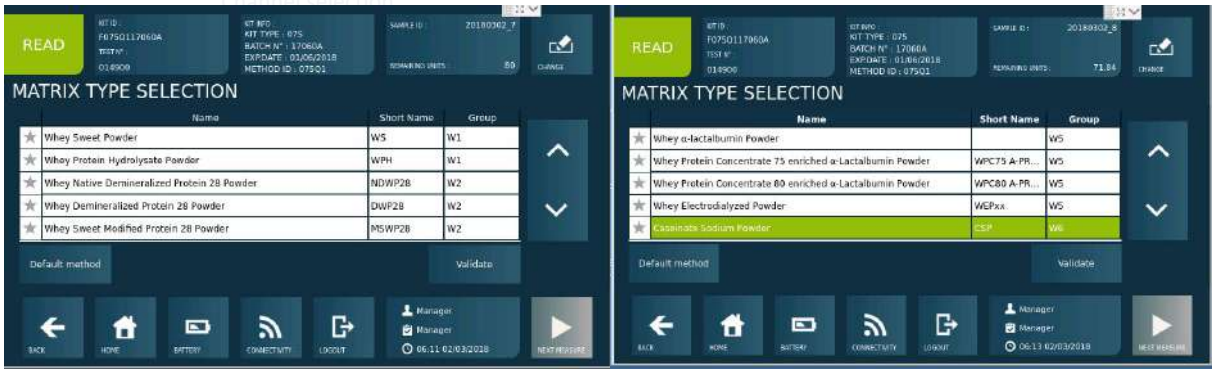
n = 17 types of contaminants

131.071 virtual screening plans in **one Kit**

page 014

14

Flexibility: matrix type selection



>30 optimized dairy matrices (validated by Nestlé)

extenso

page 015

15

RAPID

With EXTENSO, you test the majority of what can be found in a milk sample in only 13 minutes. Actual technologies take several hours.

Time is money!

You deserve the only test that is able to detect so much in so little time.

16

CONNECTED

- Alert instantly your QA Manager via SMS;
- Store locally your data (Internal memory, SD card, or USB stick);
- Import automatically the test method associated to the batch number;
- Export the raw data or as an Excel and Pdf file; or print them;
- Share them through Wi-fi (802.11n/g/b), Mobile data (Sim Card) or Bluetooth 4.0.;
- Send results to a private cloud server, via email or SMS;
- Connect EXTENSO to your quality management system through LIMS;

Full connectivity • Smart data management

- Track any past or present result in your database. EXTENSO ensures traceability for each single test. The biosticks are tagged with a specific barcode for proper batch recognition, sample identification;
- Locate your device thanks to a built in GPS chip.

17

18

PRODUCTS

ONLINE – WEB SERVICES

EXTENSO DEVICE
APP075

INCUBATOR DUO
APP027

EXTENSO ASSAY
KIT075

Extenso Test Keys
KIT175

EXTENSO WEB PORTAL

DEVICE MANAGEMENT

DATA MANAGEMENT

19

EXTENSO ASSAY KIT

Extenso assay

Extenso blister

Extenso biostick

Extenso vial

Extenso pipette 250 µl

20

EXTENSO test principle

extenso

21

Traceability of every sample

extenso

22

EXTENSO WEB PORTAL: Device & Data Management

a2l.io

Extenso DeviceManager

- Softwares
- Device
- Users/Accounts
- ETKs
- Methods

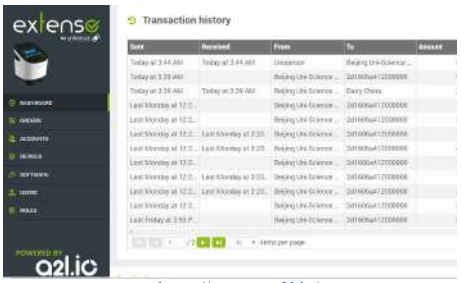
SECURE SERVER
SECURE DATABASE

Extenso DataManager

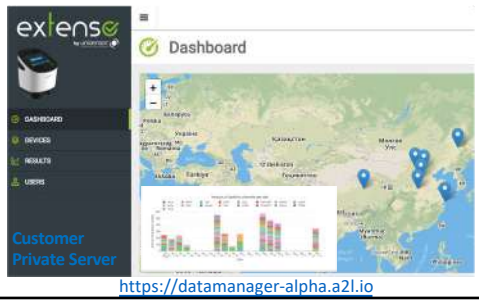
PRIVATE

- Results
- Statistics
- Localization
- Warnings
- ERP/LIMS

PRIVATE SERVER
PRIVATE DATABASE



https://extenso.a2l.io/



https://datamanager-alpha.a2l.io

23

Validation of Extenso platform in Milk by ILVO

Data and tables kindly provided by Dr. Wim Reybroeck, ILVO, Melle, BE

extenso

ILVO
Instituut voor Landbouw-, Visserij- en Voedingsonderzoek

24

AIM: VALIDATION OF EXTENSO™ AT ILVO

According to:

- Commission Decision 2002/657/EC: qualitative screening test
  - detection capability (ccβ)
  - selectivity/specificity
  - applicability/ruggedness/stability

- Guidelines for the validation of screening methods (CRLs, January 20, 2010)

- ISO/IDF Guideline for the validation of qualitative screening tests for the detection of residues of veterinary drugs in milk and milk products. Version 9 (May 3, 2017).

- AFNOR Protocole de validation des méthodes de détection et de quantification des résidus de médicaments vétérinaires dans les produits agroalimentaires. Révision No. 01. Date d'application : 1ier juin 2017.

ILVO

25

DETECTION CAPABILITY (CCβ, in µg/kg)

– Blank raw milk was spiked with the different compounds to test individually.

– The increment between the concentrations tested for each compound is dependent on the level of spiking (ppb → ppm)

– The number of replicates tested at each concentration is based on closeness to the MR(P)L.

Concentration tested	Number of replicates
≤0.5 MRL	20
>0.5 MRL - <0.9 MRL	40
≥0.9 MRL - ≤ MRL	60
> MRL	20

– The **detection capability** is defined as the lowest concentration giving at least **95% of positive** result (19 out of 20 tests, 38 out of 40 tests, or 57 out of 60 tests, respectively).

ILVO

26

OTHER PARAMETERS EVALUATED AT ILVO

⇒ **DAILY CONSISTENCY**
- 4 multi-spiked samples with 17 channel representatives- tested daily

⇒ **FALSE POSITIVE RATE**
- 150 Belgian farm + 150 tanker milks

⇒ **REPEATABILITY**
- Kits, Device

⇒ **Applicability / Test ruggedness**
- Deviations of procedure
  - Temperature
  - Time
  - Sample volume
  - Delay of reading
- Influence of quality parameters of the milk
  - pH
  - Somatic cells
  - Total bacterial count
  - Fat content
  - Protein content
  - Lactation stage
- Influence of different lots

ILVO

27

OTH…LVO

⇒ D…

⇒ F…

⇒ R…

⇒ A…

…tested daily

ILVO

ILVO

Flanders research institute for agriculture, fisheries and food

Technology and Food Science unit
Brusselsestraat 370, 9090 Melle, Belgium
tel. + 32 9 272 30 00 - fax + 32 9 272 30 01
T&VBL@ilvo.vlaanderen.be

PRELIMINARY STUDY RESULTS OF THE EXTENSO (KIT075, UNISENSOR S.A., BE), A MULTIPLEX RAPID SCREENING TEST FOR THE DETECTION OF ANTIMICROBIALS, MELAMINE AND AFLATOXIN M1 IN RAW COWS' MILK.
AFNOR Technical Board of March 22, 2018

Wim Reybroeck & Sigrid Ooghe

ILVO-T&V, Melle, Belgium

Dr. Wim Reybroeck Ir. Sigrid Ooghe

28

GENERAL CONCLUSION

In summary, the results obtained in this preliminary study indicate that EXTENSO™ is a promising, innovative and robust new multi-residue analytical tool for the dairy industry.

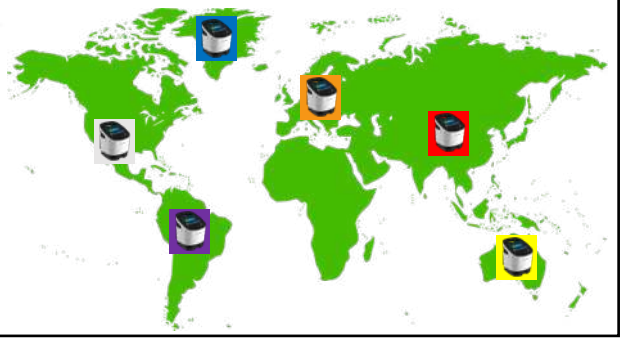
The method has met all the criteria defined in the study project, and has been conclusive. Hence, in March 2018 the AFNOR Technical Board approved the results of this study, so ILVO could proceed with the collaborative study.

ILVO

29

A **global** diagnostic & connected **platform**

Manufacturing **1 single** product for **various** local applications



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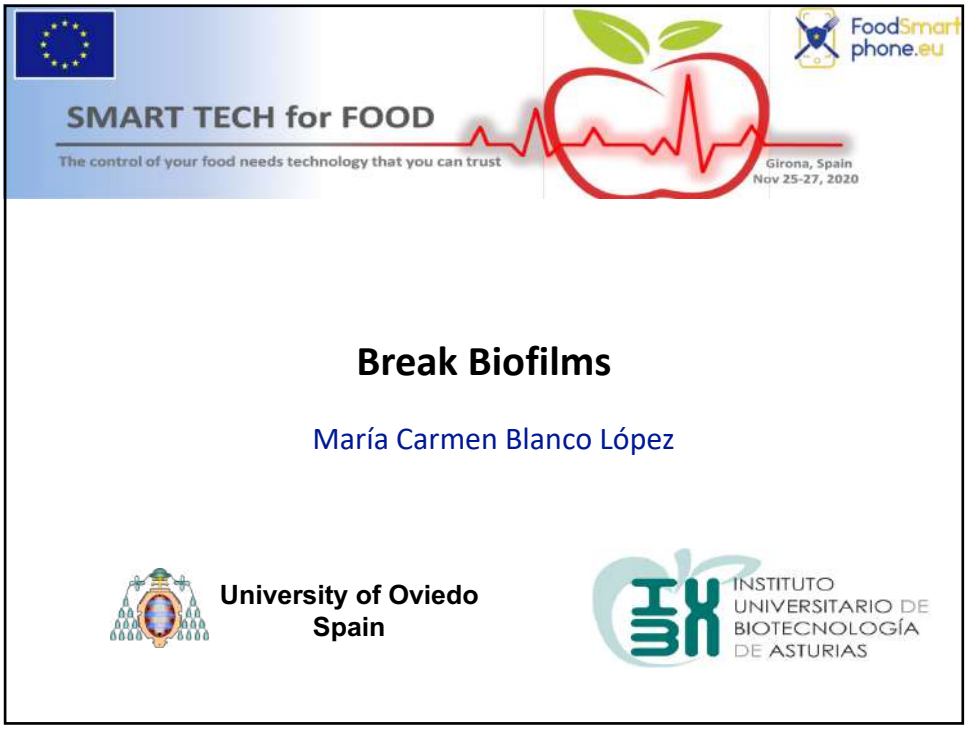
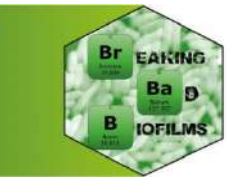

SMART TECH for FOOD
The control of your food needs technology that you can trust
Girona, Spain
Nov 25-27, 2020
FoodSmartphone.eu
Break Biofilms
María Carmen Blanco López
University of Oviedo
Spain
INSTITUTO UNIVERSITARIO DE BIOTECNOLOGÍA DE ASTURIAS

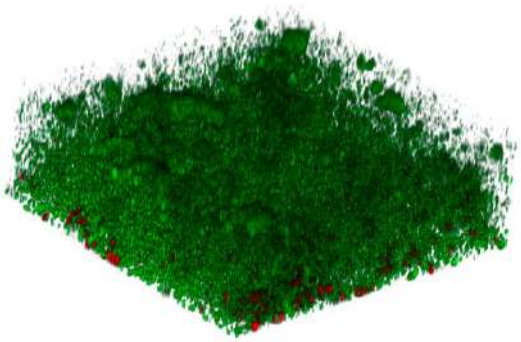
1


MARIE CURIE ACTIONS
BREAKING BIOFILMS
H2020-MSCA-ITN-2018
Grant Agreement number: 813439
Universidad de Oviedo
Universidá d'Uviéu
University of Oviedo
UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO
Abbott Diagnostics
ASIN CAR
BioLogic
Arla
DR.MERK
uulm
DCU
CEEI
pla
Reny Picot
AARHUS UNIVERSITY
WARWICK
Pi
tactica
THERADEP

2

BREAKING BIOFILMS Training Network (H2020-MSCA-ITN)





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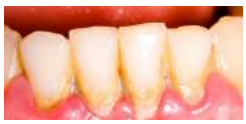


4

Biofilms can grow on any surface:

- Plastic
- Glass
- Wood
- Metal
- Food

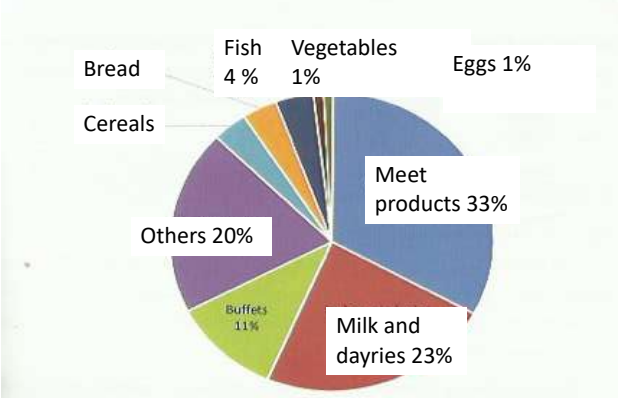
5

Staphylococcus Aureous biofilms in agri-foodstuff

5079 outbreaks in food (20179

43400 people (4541 hospital, 33 died)

European Food Safety Agency 2018



6

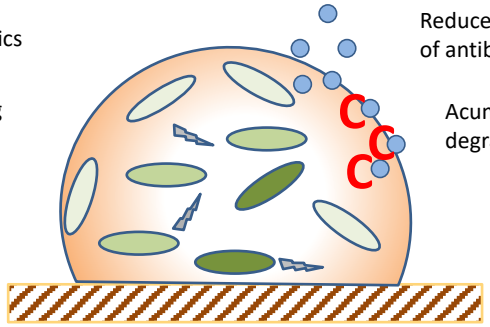
Resistance mechanism of biofilms

Color grade related to the abundance of O and nutrients inside the biofilm

Antibiotics

Antibiotic degrading enzymes

DNA



Reduced penetration of antibiotics

Acumulation of antibiotic degradation enzymes

Bacterial cells with different metabolims

slow

active

7

BREAKING BIOFILMS Training Network (H2020-MSCA-ITN)

Objetives

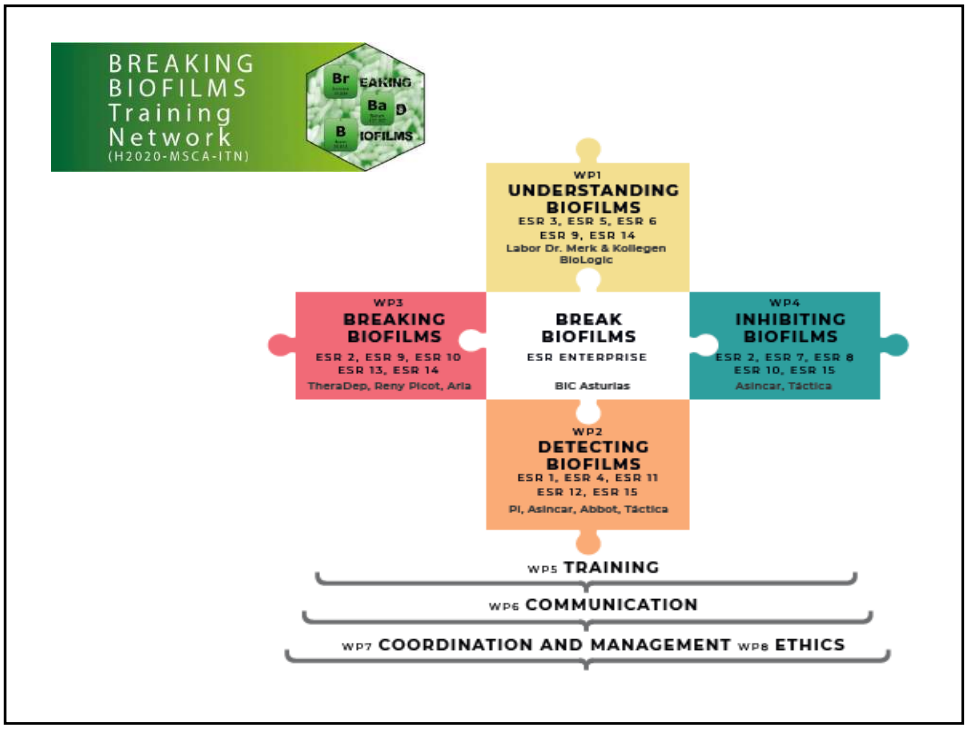
01 ELLUCIDATING ADHESION MECHANISMS
Scanning Eletrochemical. Confocal. Raman. IR. AFM. Fluorescence. Microscopy.

02 BIOFILM DETECTION
Microfluidic DNA array platform, paper devices and diamond electrodes, novel mediators.

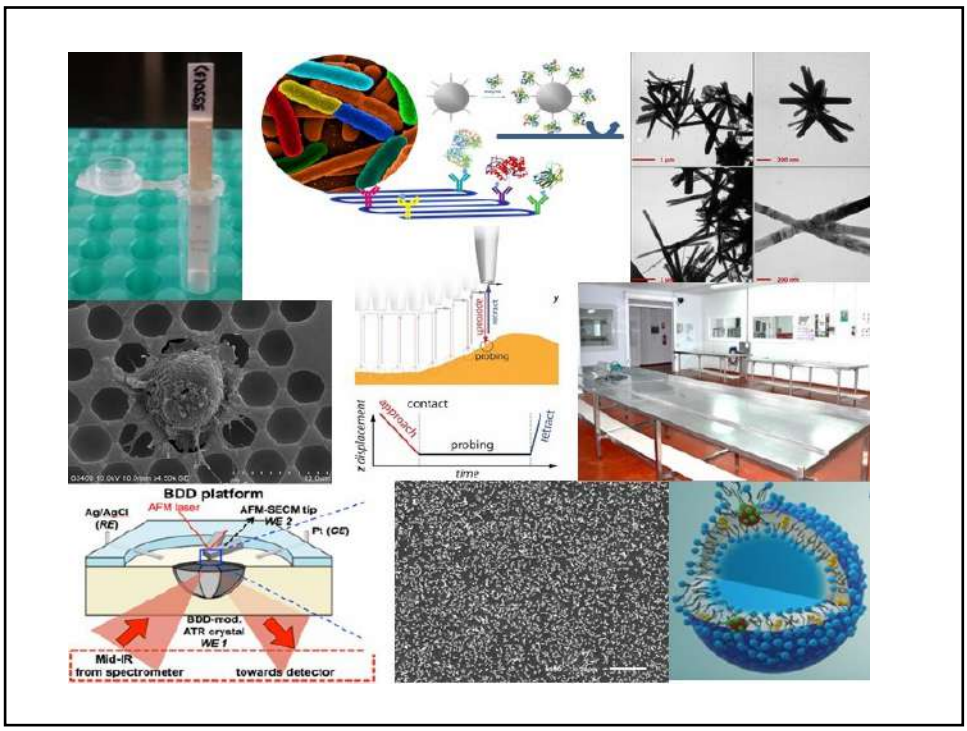
03 BIOFILM DESTRUCTION
Encapsulated antibiotics and enzymes. Bacteriophages. Dendrons.

04 BIOFILM INHIBITION
Development of novel and effective nanoantimicrobials, surface analysis.

8


BREAKING
BIOFILMS
Training
Network
(H2020-MSCA-ITN)
WP1
UNDERSTANDING BIOFILMS
ESR 3, ESR 5, ESR 6
ESR 9, ESR 14
Labor Dr. Merk & Kollegen
BioLogic
WP3
BREAKING BIOFILMS
ESR 2, ESR 9, ESR 10
ESR 13, ESR 14
TheraDep, Reny Picot, Aria
BREAK BIOFILMS
ESR ENTERPRISE
BIC Asturias
WP4
INHIBITING BIOFILMS
ESR 2, ESR 7, ESR 8
ESR 10, ESR 15
Asincar, Táctica
WP2
DETECTING BIOFILMS
ESR 1, ESR 4, ESR 11
ESR 12, ESR 15
PI, Asincar, Abbot, Táctica
WP5 TRAINING
WP6 COMMUNICATION
WP7 COORDINATION AND MANAGEMENT WP8 ETHICS

9


glass
probing
contact
approach
retract
probing
z displacement
time
BDD platform
Ag/AgCl
(RE)
AFM laser
AFM-SECM tip
WE 2
Pt (CE)
BDD-mod.
ATR crystal
WE 1
Mid-IR
from spectrometer
towards detector

10


International Journal of Pharmaceutics

Volume 585, 30 July 2020, 119478

Review

Vesicles as antibiotic carrier: State of art

Verdiana Marchianó [a, b], Maria Matos [b, c], Esther Serrano-Pertierra [a, c], Gemma Gutiérrez [b, c, *], M.C. Blanco-López [a, c, *]

Show more

https://doi.org/10.1016/j.ijpharm.2020.119478

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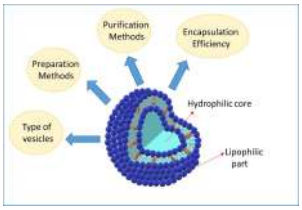
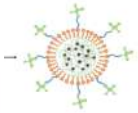
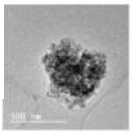


Verdiana Marchianó

Shayesteh Badzefipar





11

Nanoantimicrobials

NPs do not generate antimicrobial resistence

- Cupper, silver, zinc, titanium dioxide

Antimicrobial effects:

1. Plasma membrane permeabilization
2. Membrane lipid peroxidation
3. Alteration of proteins
4. Inhibition of protein assembly and activity
5. Denaturation of nucleic acids

⇨ Broad spectrum antimicrobial, antiviral

12

Universidad de Oviedo
Universidá d'Uviéu
University of Oviedo

University of Bari

nanomaterials

MDPI

Article

Cu Nanoparticle-Loaded Nanovesicles with Antibiofilm Properties. Part I: Synthesis of New Hybrid Nanostructures

Lucia Sarcina [1], Pablo García-Manrique [2,3,4], Gemma Gutiérrez [3,4], Nicoletta Ditaranto [1], Nicola Cioffi [1], Maria Matos [3,4,*] and Maria del Carmen Blanco-López [2,4,*]

1 Department of Chemistry, Università degli Studi di Bari Aldo Moro, via Orabona 4, 70125 Bari, Italy; lucia.sarcina@uniba.it (L.S.); nicoletta.ditaranto@uniba.it (N.D.); nicola.cioffi@uniba.it (N.C.)
2 Department of Physical and Analytical Chemistry, University of Oviedo, Julián Clavería 8, 33006 Oviedo, Spain; garciampablo@uniovi.es (P.G.-M.); gutierrezgemma@uniovi.es (G.G.)
3 Department of Chemical and Environmental Engineering, University of Oviedo, Julián Clavería 8, 33006 Oviedo, Spain
4 Instituto Universitario de Biotecnología de Asturias, University of Oviedo, 33006 Oviedo, Spain
* Correspondence: matosmaria@uniovi.es (M.M.); cblanco@uniovi.es (M.d.C.B.-L.)

Nanomaterials **2020**, *10*, 1542; doi:10.3390/nano10081542

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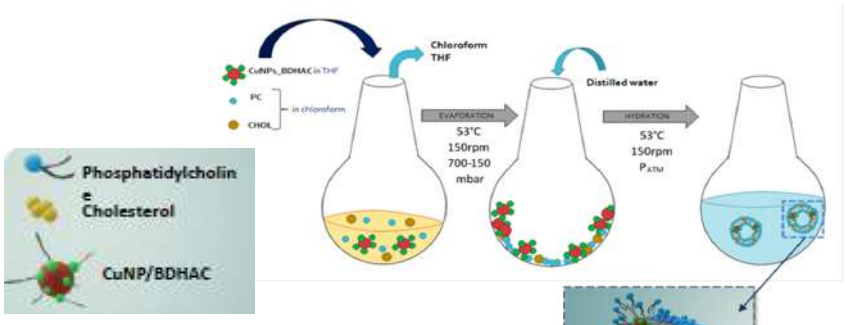
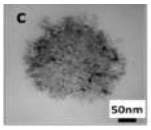
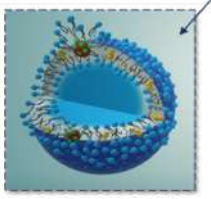
Nanovesicles loaded with CuNPs

Thin film hydration layer



Benzyl-dimethyl-hexadecyl ammonium chloride (BDHAC)





Liposomes

14

- Hybrid nanovesicles could provide a way to control the ionic release of copper through liposomes, which are already used for drug delivery
- Preliminary experiments on antibiofilm efiiciency with Staphylococcus aureus (Gram +), Pseudomonas aeruginosa (Gram -), and Candida parapsilosis (fungus)

→ The stability of liposomes increased with CuNP loading suggesting a combined hindrance and charge-stabilization effect confirmed by dynamic light scattering, -potential measurements and backscattering-monitored precipitation.

Increase CuNP load ↓

SAMPLE	LIP:NPs (*w*/*w*)	ζ-Potential (mV)
NVs	–	−24 ± 5
Hybrid 1	2000:1	−11 ± 4
Hybrid 2	1200:1	5 ± 4
Hybrid 3	800:1	21 ± 6

15

Backscattering-monitored precipitation

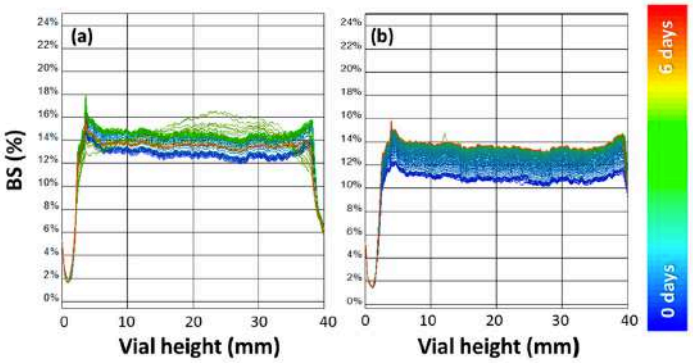


Figure 7. Backscattering profile (%) vs. vials height (mm) acquired over 6 days. Signal of the Cu@BDHAC–NP-loaded NV hybrids at a LIP:NPs ratio of 2000:1 *w*/*w* (**a**) and at a LIP:NPs ratio of 1200:1 *w*/*w* (**b**).

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diagnostics

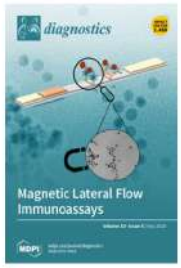
MDPI

Review

Magnetic Lateral Flow Immunoassays

Amanda Moyano [1], Esther Serrano-Pertierra [1], María Salvador [2], José Carlos Martínez-García [2], Montserrat Rivas [2] and M. Carmen Blanco-López [1,*]

[1] Department of Physical and Analytical Chemistry & Institute of Biotechnology of Asturias, University of Oviedo, c/ Julián Clavería 8, 33006 Oviedo, Spain; moyanoamanda@uniovi.es (A.M.); serranoesther@uniovi.es (E.S.-P.)



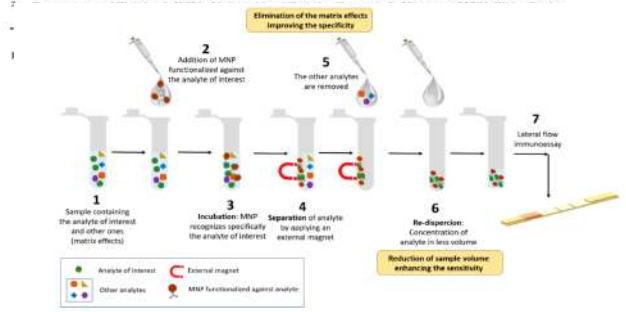


Figure 2. Scheme of the immunomagnetic separation (IMS) procedure.

Diagnostics **2020**, *10*, 288; doi:10.3390/diagnostics10050288

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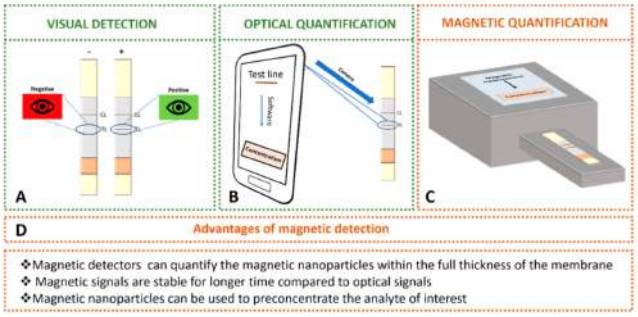

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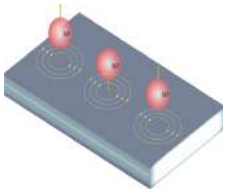
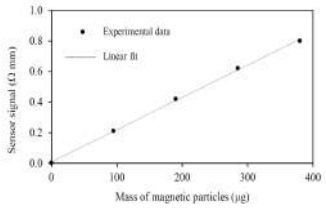
Inductive sensor

Superparamagnetic NP (SPIONs): momentum changes contiously

- Alternate current at conductor circuit
- SPIONs induce" Eddy currents"
- Change at impedance
- Proportional to the number of SPIONs





Montse Rivas et al. *Nanotechnology* **24** (2013) 245501

19

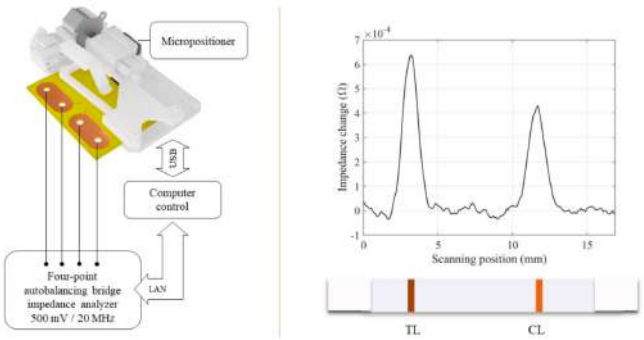


Figure 7. Left: Scheme of the scanning inductive reader for magnetic LFIA. **Right**: Signal recorded for histamine competitive LFIA (blank sample, competitive immunoassay).

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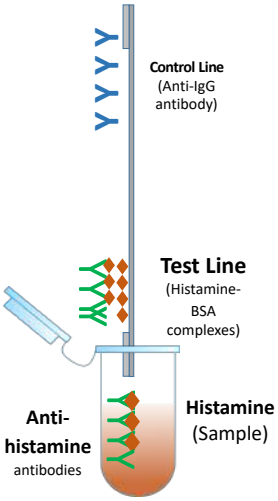
Analytical and Bioanalytical Chemistry (2019) 411:6615–6624
https://doi.org/10.1007/s00216-019-02031-6

RESEARCH PAPER

DENOMINACIÓN DE ORIGEN PROTEGIDA CANGAS

Magnetic immunochromatographic test for histamine detection in wine

Amanda Moyano[1,2] · María Salvador[2,3] · José C. Martínez-García[2] · Vlad Socoliuc[4] · Ladislau Vékás[4] · Miguel A. Alvarez[5] · María Fernández[5] · Montserrat Rivas[2] · M. Carmen Blanco-López[1]



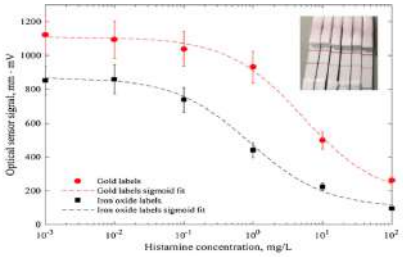


Fig. 3 Quantification of LFIAs by the optical reader using gold (red) and iron oxide (black) NPs. The lines plotted to guide the eye are four-parameter sigmoid curves. Inset: One of the series of standard samples used to obtain the red curve, with the control line on top and the test line below

Validation by HPLC

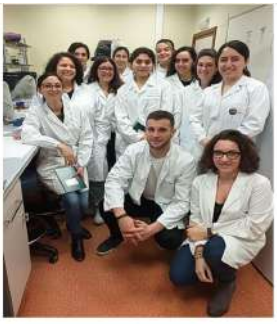
21

BREAKING BIOFILMS Training Network (H2020-MSCA-ITN)

www.breakbiofilms.com

Break Biofilms newsletter

EU vs Virus Hackathon: PADCOV



COVID-19 WON'T STOP US

9-13 March 2020
Lockdown 14th March

https://www.youtube.com/watch?v=AfG4iVTXDYM&feature=youtu.be

22

Analytica Chimica Acta
Special Issue

Detecting emerging biological threats: the power of Analytical Chemistry to fight antimicrobial-resistant microorganisms, biofilms and viruses.

MRS MATERIALS RESEARCH SOCIETY®
Advancing materials. Improving the quality of life.

Editors:

- Nicola Cioffi. University of Bari (Italy).
- Maria Carmen Blanco López. University of Oviedo (Spain)
- Christine Kranz. Ulm University (Germany).
- Elena Ferapontova. Aarhys University (Denmark)
- Julie Macpherson. Warwick University (UK)
- Robert Forster. Dublin City University (Ireland).

23

Thank you

NanoBioAp
CLUSTER OF EXCELLENCE

GOBIERNO DEL PRINCIPADO DE ASTURIAS

DENOMINACION DE ORIGEN PROTEGIDA CANGAS

GOBIERNO DE ESPAÑA MINISTERIO DE ECONOMIA Y COMPETITIVIDAD

FICYT FUNDACION PARA EL FOMENTO EN ASTURIAS DE LA INVESTIGACION CIENTIFICA APLICADA Y LA TECNOLOGIA

PCTI ASTURIAS

24

FoodSmartphone

Plasmonic FoodSmartphone assays for food spoilage

ESR 4. Javier Lou Franco
Institute for Global Food Security (QUB)
jloufranco01@qub.ac.uk

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

Outline

1. TEST – Developing an end-user friendly sensor database
2. Review on Gold nanozymes
3. Synthesis and characterisation of AuNSt
   1. Catalytic properties of AuNSt
   2. Spectroscopic properties of AuNSt
4. Smartphone-based detection of AuNSt
5. Smartphone-based detection of *Mycobacterium bovis*
6. Summary of achievements
7. Research outputs derived from this thesis

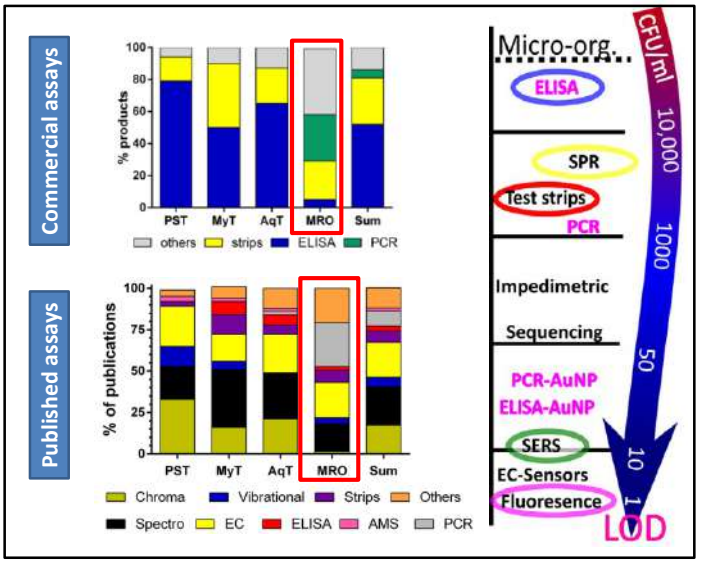
FoodSmartphone is funded by the European Community's Horizon 2020 Framework Programme under Grant Agreement - 720325

1. TEST – Developing an end-user friendly sensor database

An online and interactive database was developed aiming to provide an organised classification of sensors, commercialised or otherwise.

- Aquatic toxins
- Mycotoxins
- Pesticides
- **Microorganisms**

Pathogens: 158 sensors
Spoilage: 39 sensors

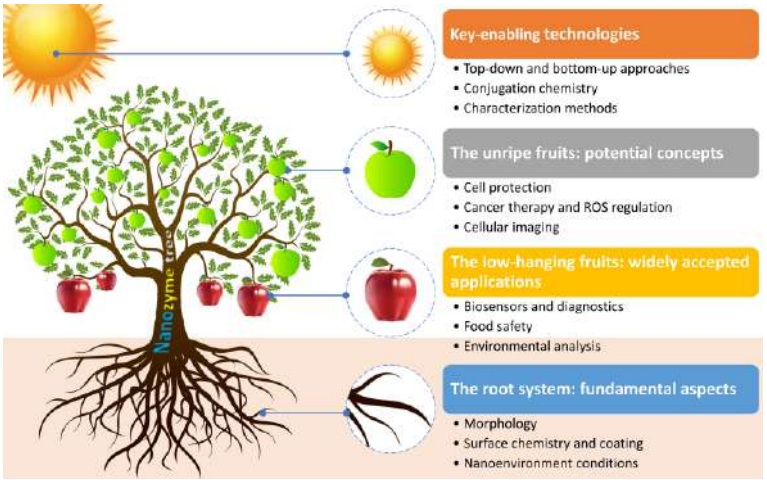


Nelis, J. L. D., Tsagkaris, A. S., Zhao, Y., **Lou-Franco, J.**, Nolan, P., Zhou, H., ... & Campbell, K. (2019). The end user sensor tree: An end-user friendly sensor database. *Biosensors and Bioelectronics*, *130*, 245-253.

2. Review on Gold nanozymes

Over 200 published research papers were reviewed to present the current progress on gold nanozymes and their applications.

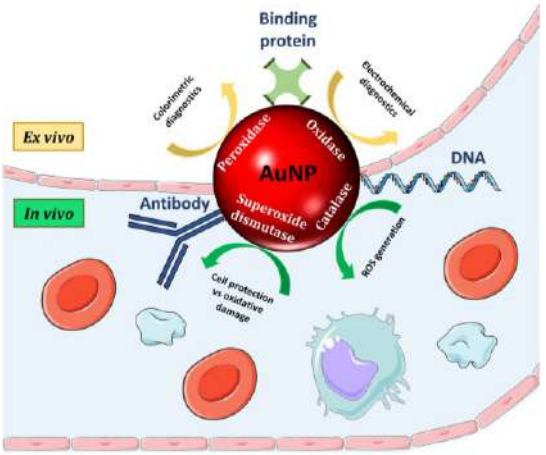
- Catalytic properties of AuNPs
- Biomedical applications
  - Clinical diagnostics
  - ROS generation
  - Cell protection
- Other applications
  - Environmental applications
  - **Food safety applications**
  - Other applications



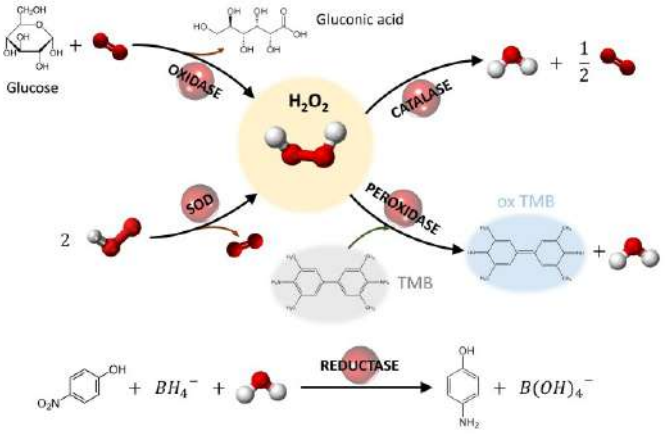
Lou-Franco, J., Das, B., Elliott, C., & Cao, C. (2020). Gold Nanozymes: From Concept to Biomedical Applications. Nano-Micro Letters, 13(1), 1-36.

2. Review on Gold nanozymes

Applications of Au nanozymes



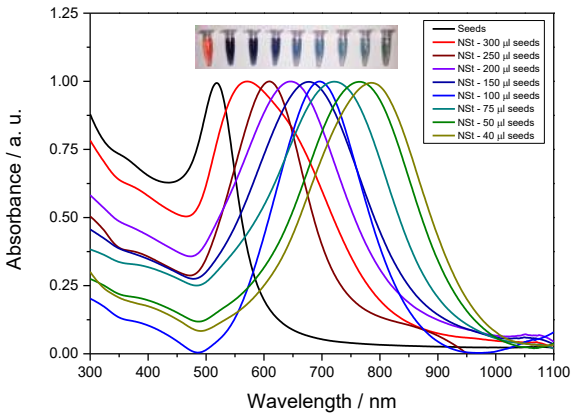
Nanozyme activities of AuNPs



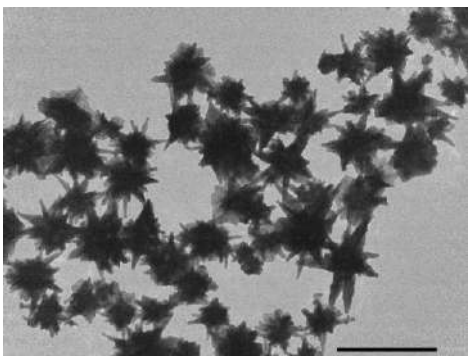
Lou-Franco, J., Das, B., Elliott, C., & Cao, C. (2020). Gold Nanozymes: From Concept to Biomedical Applications. Nano-Micro Letters, 13(1), 1-36.

3. Synthesis and characterisation of AuNSt

UV-Vis analyses of different-sized AuNSt



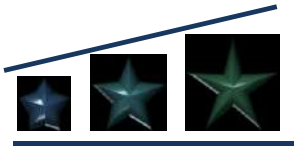
TEM imaging of AuNSt

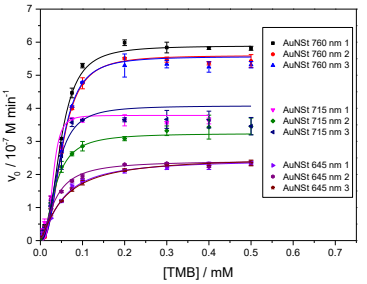


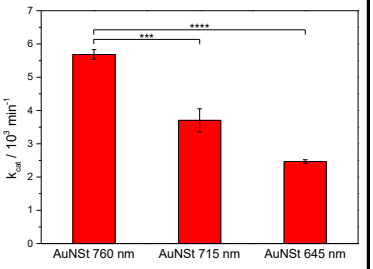
In preparation – Retrieving peroxidase-like activity of gold nanostars for the smartphone-based detection of *Mycobacterium bovis* (**Lou-Franco, J.**, ... & Cao, C.) – *Nano Research*

3.1 Catalytic properties of AuNSt

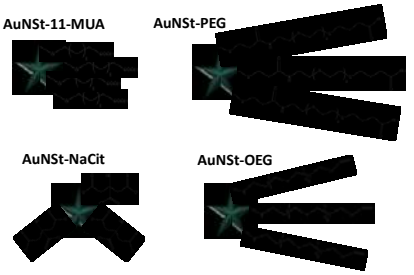
Size-dependence

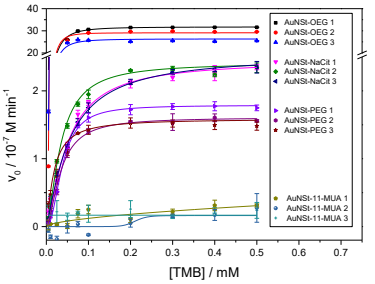


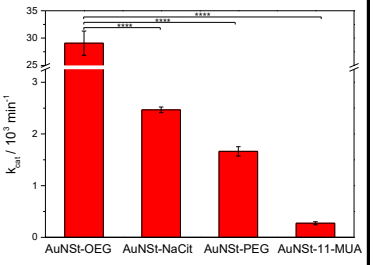




Surface-dependence







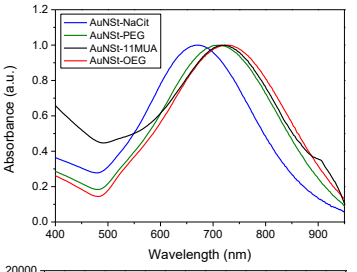
In preparation – Retrieving peroxidase-like activity of gold nanostars for the smartphone-based detection of *Mycobacterium bovis* (**Lou-Franco, J.**, ... & Cao, C.) – *Nano Research*

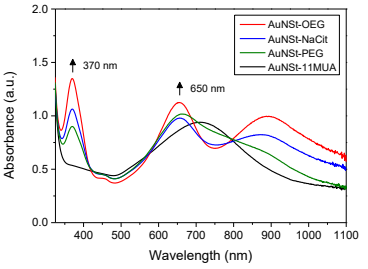
3.2 Spectroscopic properties of AuNSt

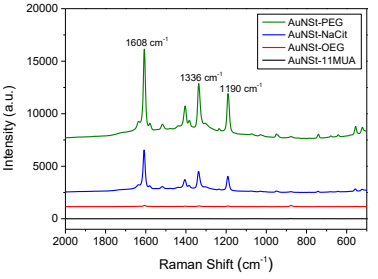
AuNSt stabilised with different coating molecules not only show different efficiency for TMB oxidation, but also to act as Raman enhancers.

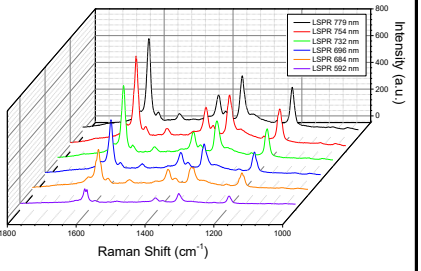
AuNSt-PEG, LSPR ~ 780 nm

Application:
Hg^{2+} detection in seawater samples







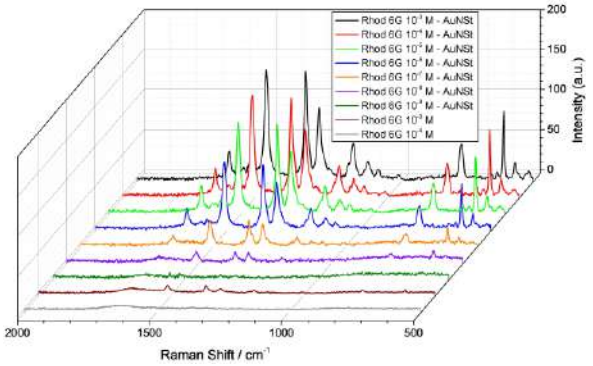


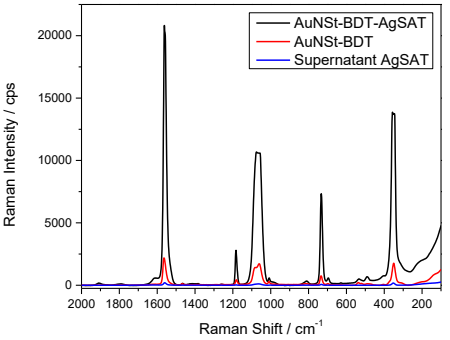
In preparation – SERS-based Detection of Mercury (Hg^{2+}) Ions Using Star-shaped Nanozyme with Inverse Sensitivity (Logan, N.*, **Lou-Franco, J.***, ... & Cao, C.) – *Nano-Micro Letters*

3.2 Spectroscopic properties of AuNSt

AEF (AuNSt) = $2.1 \cdot 10^5$

AEF (AuNSt-AgSAT) = 10 x AEF (AuNSt)

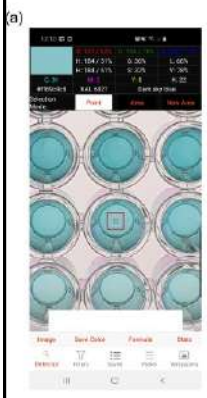


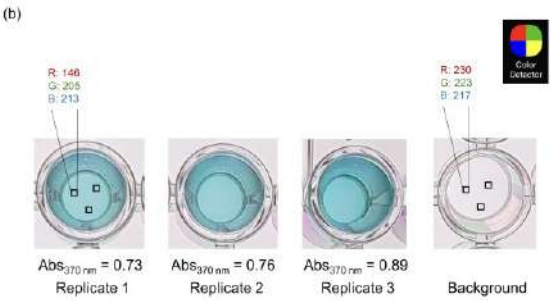


In preparation – 1,4-BDT mediated Gold Nanostar@Silver Satellites: Synthesis, Characterisation and Application in Mercury Sensing (Ellis, M., **Lou-Franco, J.**, ... & Cao, C.)

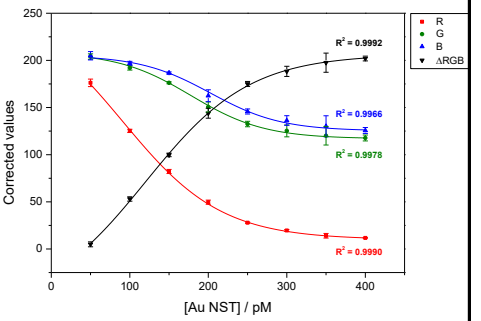
4. Smartphone-based detection of AuNSt

Red (R), Green (G), Blue (B) and ΔRGB values are measured with a smartphone app from a AuNSt sample used to oxidise TMB. Each colour channel is plotted to analyse its suitability to quantify the concentration of oxTMB (parameter directly proportional to the presence of AuNSt and, therefore, to the concentration of target analyte).





$$\Delta RGB = \sqrt{(R_t - R_0)^2 + (G_t - G_0)^2 + (B_t - B_0)^2}$$



4. Smartphone-based detection of AuNSt

Yunfeng Zhao (ESR 5) developed a smartphone app that enables colour quantification after being fed with images for calibration.

- Different diagnostic assays can be saved in the app.
- Provides a final result in the desired units rather than a colour value.

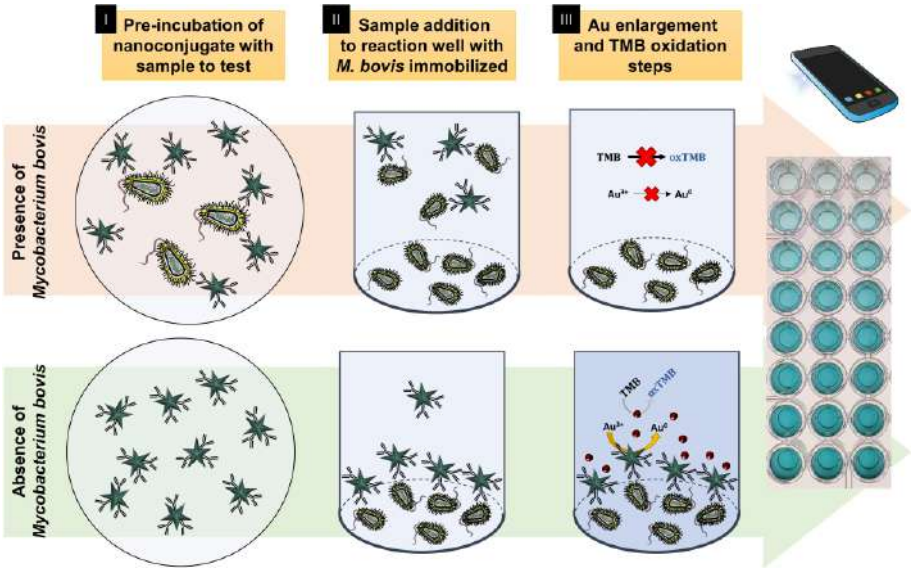
QuantColorimetryApp Calibration for *M. bovis* assay

QuantColorimetryApp Interface with several calibrated assays

QuantColorimetryApp Prediction of new *M. bovis* samples

In preparation – Retrieving peroxidase-like activity of gold nanostars for the smartphone-based detection of *Mycobacterium bovis* (**Lou-Franco, J.**, ... & Cao, C.) – *Nano Research*

5. Smartphone-based detection of *M. bovis*

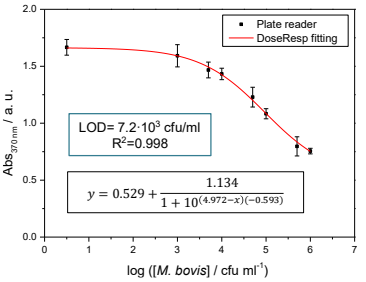


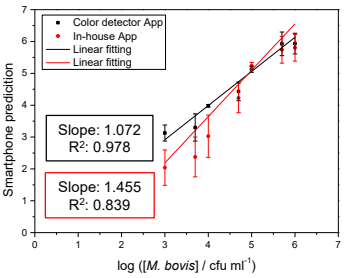
In preparation – Retrieving peroxidase-like activity of gold nanostars for the smartphone-based detection of *Mycobacterium bovis* (**Lou-Franco, J.**, ... & Cao, C.) – *Nano Research*

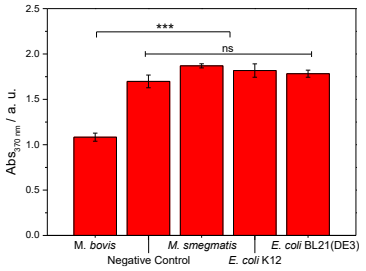
5. Smartphone-based detection of *M. bovis*

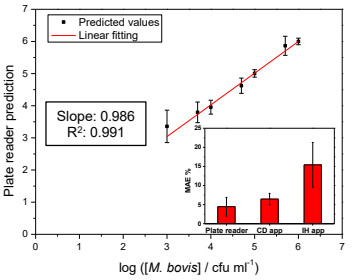
The assay developed uses the peroxidase-like activity of AuNSt to generate a colourimetric signal read with a smartphone camera.

- LOD=7.2 · 10^3 cfu/ml
- Specific for *M. bovis*
- *Color Detector* predictions outperform *QuantColorimtryApp* ones
- Smartphone MAE is not outperformed by plate reader









In preparation – Retrieving peroxidase-like activity of gold nanostars for the smartphone-based detection of *Mycobacterium bovis* (**Lou-Franco, J.**, ... & Cao, C.) – *Nano Research*

6. Summary of achievements

Plasmonic nanomaterials

Review of Gold nanozymes

Study of catalytic properties of AuNSt

Study of SERS properties of AuNSt

Microbiological contamination

Review available sensors & creation of online database

Detection of *M. bovis* using a smartphone

Detection of *L. monocytogenes* and *C. jejuni* through a DNA-based approach

Future implications

Development of ultra-sensitive diagnostic assays exploiting the spectroscopic properties of Au-Ag bimetallic NPs

Applicability of the nanozyme activity of AuNSt for colourimetric sensing approaches

Smartphone-based quantification of colourimetric assays

7. Research outputs derived from this thesis

Publications

1. Nelis, J. L. D., Tsagkaris, A. S., Zhao, Y., **Lou-Franco, J.**, Nolan, P., Zhou, H., ... & Campbell, K. (2019). The end user sensor tree: An end-user friendly sensor database. *Biosensors and Bioelectronics*, *130*, 245-253.

2. Nelis, J. L., Migliorelli, D., Jafari, S., Generelli, S., **Lou-Franco, J.**, Salvador, J. P., ... & Campbell, K. (2020). The benefits of carbon black, gold and magnetic nanomaterials for point-of-harvest electrochemical quantification of domoic acid. *Microchimica Acta*, *187*(3), 164.

3. **Lou-Franco, J.**, Das, B., Elliott, C., & Cao, C. (2020). Gold Nanozymes: From Concept to Biomedical Applications. *Nano-Micro Letters*, *13*(1), 1-36.

4. Zhao, Y., Choi, S. Y., **Lou-Franco, J.**, Nelis, J. L. D., Zhou, H., Cao, C., ... & Rafferty, K. (2020). Smartphone modulated colorimetric reader with color subtraction. *Proceedings of IEEE Sensors*.

Under preparation:

5. SERS-based Detection of Mercury (Hg^{2+}) Ions Using Star-shaped Nanozyme with Inverse Sensitivity (Logan, N.*, **Lou-Franco, J.***, ... & Cao, C.) – *Nano-Micro Letters*
6. Retrieving peroxidase-like activity of gold nanostars for the smartphone-based detection of *Mycobacterium bovis* (**Lou-Franco, J.**, ... & Cao, C.) – *Nano Research*
7. 1,4-BDT mediated Gold Nanostar@Silver Satellites: Synthesis, Characterisation and Application in Mercury Sensing (Ellis, M., **Lou-Franco, J.**, ... & Cao, C.)
8. Nanozymes in Point-of-Care Diagnosis: an Emerging Futuristic Approach for Biosensing (Das, B**., Lou-Franco, J.**, ... & Cao, C.) – *Nano-Micro Letters*
9. Detection of *Mycobacterium bovis* using iELISA by biogenic synthesised gold nanoparticles with peroxidase mimicking activity (Das, B**., Lou-Franco, J.**, ... & Cao, C.)

Posters & Other Research outputs

Lou-Franco, J., ... & Cao, C. (2018). Tuneable Plasmonic and Catalytic Gold Nanostars for Surface-Enhanced Resonance Raman Scattering (SERRS): Synthesis, Characterization and Optimization. *28th World Congress on Biosensors* (Miami).

Lou-Franco, J., ... & Cao, C. (2019). Retrieving Peroxidase-like Activity of Ligand-capped Gold Nanostars for the Detection of *Mycobacterium bovis.* 9th International Symposium RAFA (Prague).

Lou-Franco, J., ... & Elliott, C. (2019). Milk pasteurization: could tuberculosis be slipping into our breakfast bowls? *New Food Magazine, Dec. 2019.*

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Yunfeng Zhao

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

FoodSmartphone

DNA directed immobilization for multiplexed detection platforms integrated with Smartphones

ESR 17 Julian Guercetti

Nanobiotechnology for Diagnostics group,IQAC-CSIC

November 26, 2020

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

Outline

-Introduction

-Fluorescent Microarray based on DNA Directed Immobilization

-Portable SPR Platform with Smartphone integration

antimicrobial resistance

is one of the biggest threats to global health, food security, and development today

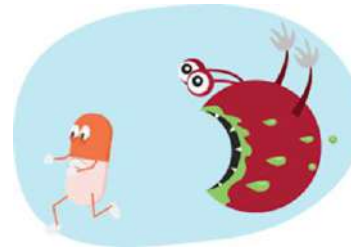
EUROPE

33000 people die each year as a result of hospital infections caused by **5 key resistant bacteria**

GLOBAL A failure to address the problem of antibiotic resistance could result in:

10m deaths by 2050

Costing £66 trillion



www.gov.uk/government/publications/health-matters-antimicrobial-resistance

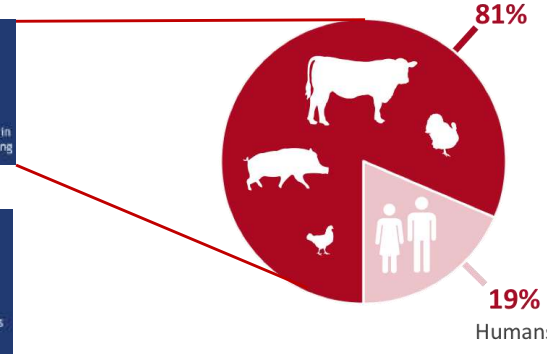
ec.europa.eu/health/sites/health/files/antimicrobial_resistance/docs/amr_2017_factsheet.pdf

CAUSES OF ANTIBIOTIC RESISTANCE

HANDLE ANTIBIOTICS WITH CARE

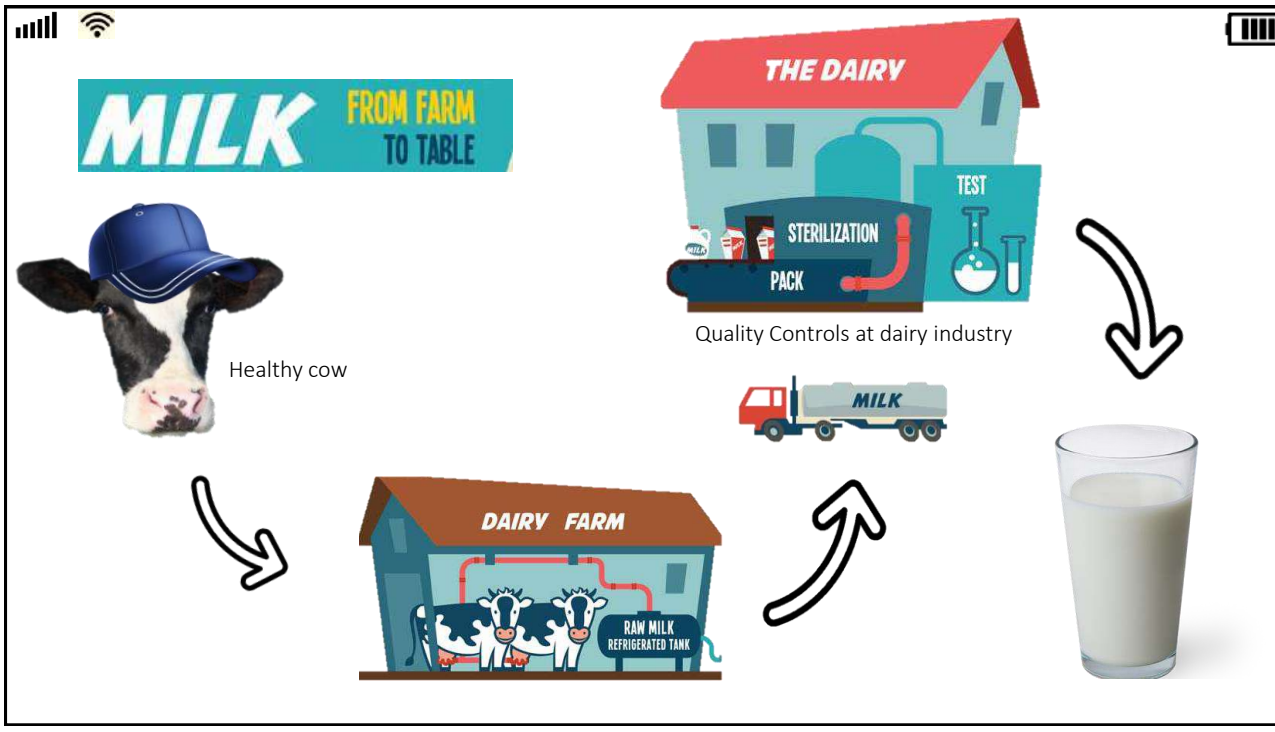
Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause.

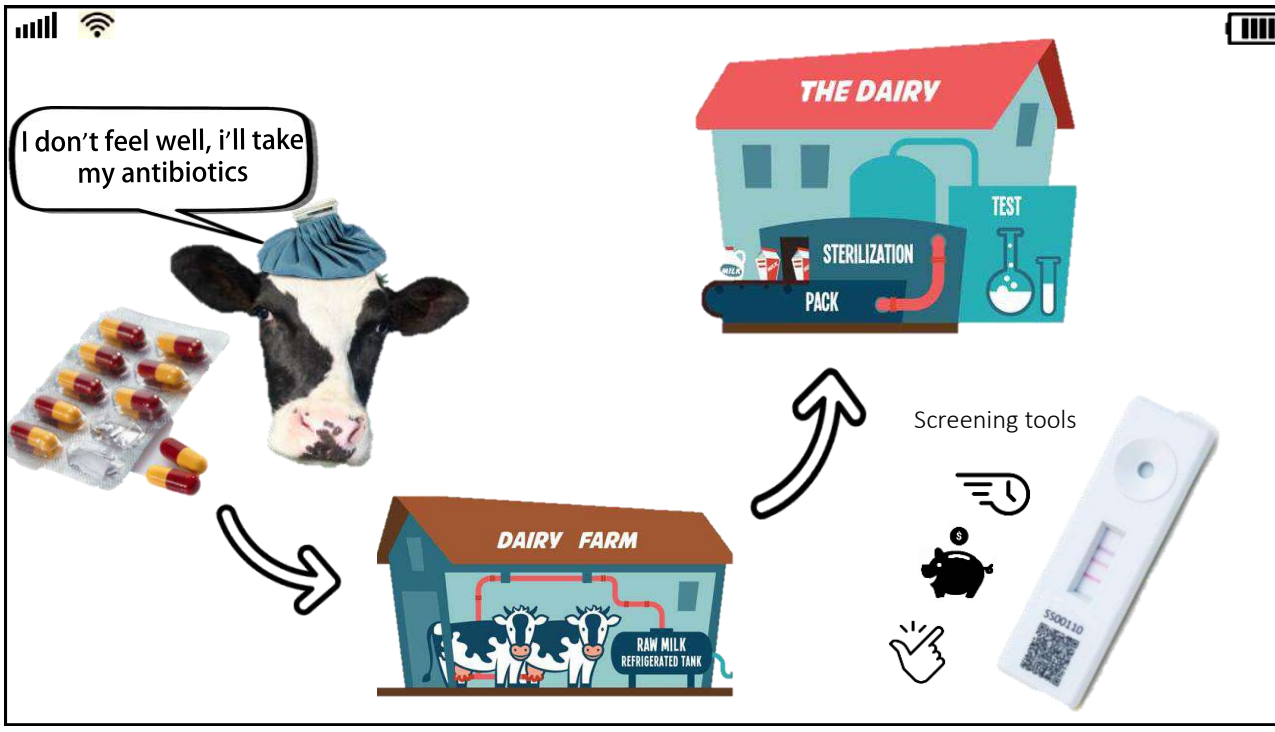
Antibiotic sales in the US



High doses

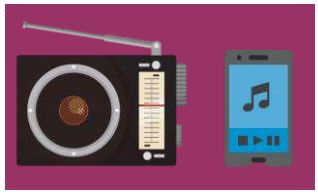
Preventive adminsitration

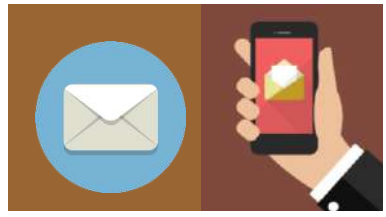

MILK FROM FARM TO TABLE
THE DAIRY
TEST
STERILIZATION
PACK
Quality Controls at dairy industry
Healthy cow
MILK
DAIRY FARM
RAW MILK
REFRIGERATED TANK


I don't feel well, i'll take my antibiotics
THE DAIRY
TEST
STERILIZATION
PACK
Screening tools
DAIRY FARM
RAW MILK
REFRIGERATED TANK

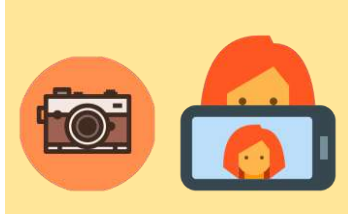
Actions involving animal monitoring

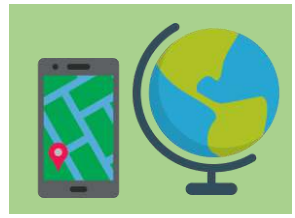
One Health is a collaborative platform with the goal of achieving health outcomes recognizing the interconnection between people, animals, plants, and their shared environment.





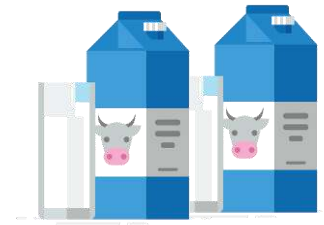
Smartphones in Food Safety Analysis





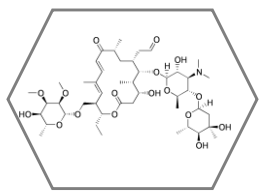
Our aim

Develop multiplexed detection platforms based on DNA-Directed Immobilization for antibiotic residues determination in milk with the use of Smartphones

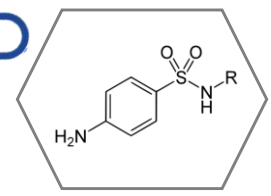


Selected target analytes

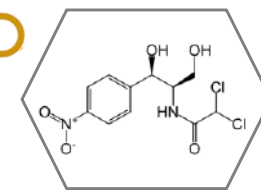
Tylosin



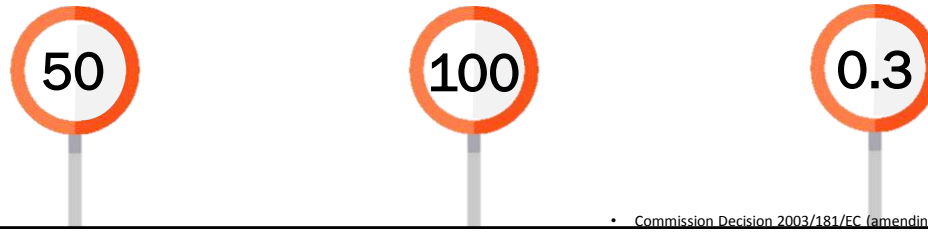
Sulfonamides



Chloramphenicol



Maximum Residue Limits and MRPL (ug/kg) in milk



- Commission Decision 2003/181/EC (amending Decision 2002/657/EC)

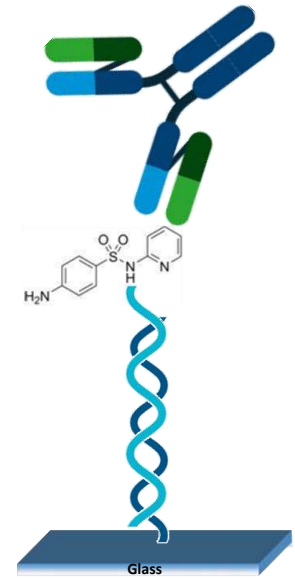
DNA-directed immobilization (DDI)

Uses nucleic acid hybridization for directed immobilization of (bio) molecules on surfaces.

Specific orientation

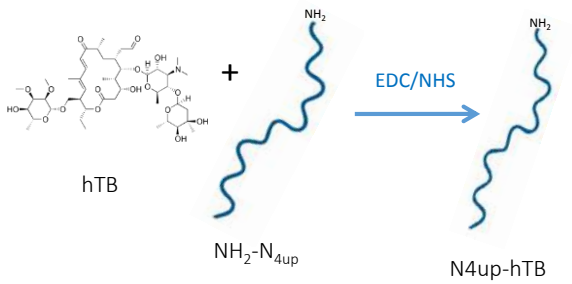
Regenerable surfaces

Allows multiplexation

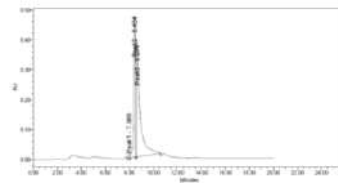


Oligo-hapten conjugation protocol

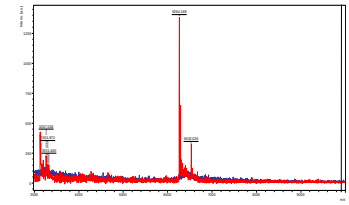
Ester active method



Purification by HPLC



Characterization by MALDI-TOF

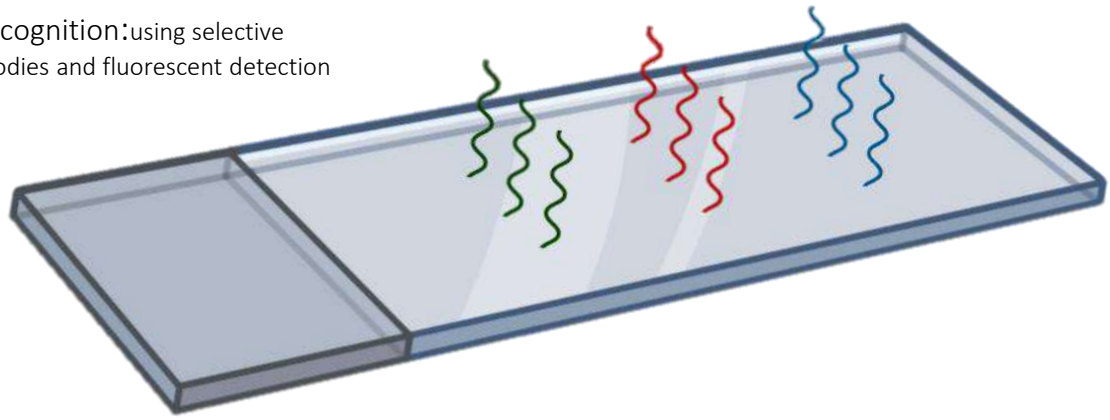


Antibody fluorescent microarray based on DDI

Printing: Nxdown oligos immobilized over a glass slide

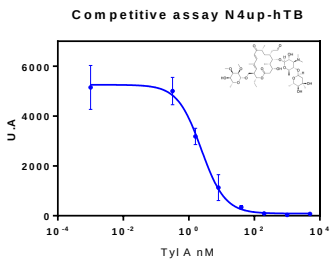
Hibridization: with complementary strands conjugated to haptens

Biorecognition: using selective antibodies and fluorescent detection



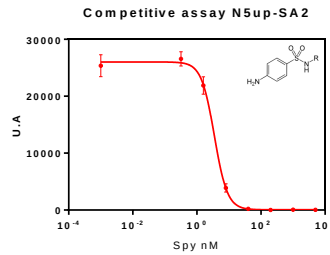
Indirect competitive immunoassays :

Tylosin A detection



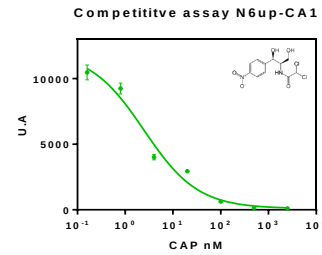
Analytical Parameters	Tyl A
Amax	5254
Amin	88.34
R2	0.998
Slope	-1.197
IC50	2.397
LOD(ug/Kg)	0.545

Sulfapyridine detection



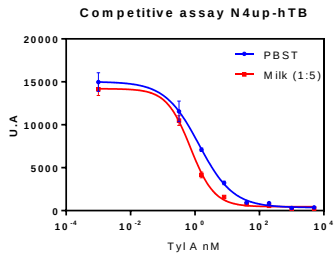
Analytical Parameters	Spy
Amax	28004
Amin	235.6
R2	0.96
Slope	-10.44
IC50	1.468
LOD(ug/Kg)	1.088

Chloramphenicol detection

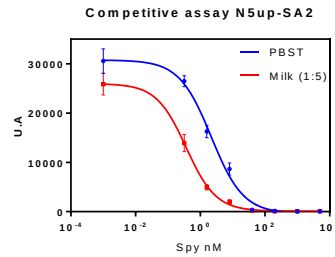


Analytical Parameters	Tyl A
Amax	12051
Amin	94.52
R2	0.9793
Slope	-0.7699
IC50	2.471
LOD(ug/Kg)	0.785

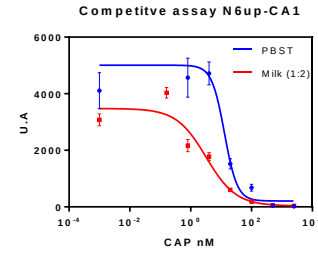
Matrix effect assessment in milk



Analytical Parameters	PBST	Milk 1/5
Amin	355.5	459.1
Amax	15017	14197
R2	0.9992	0.9985
Slope	-0.8208	-1.168
IC50 ug/kg	17.12	4.98
LOD ug/kg	1.26	1.243

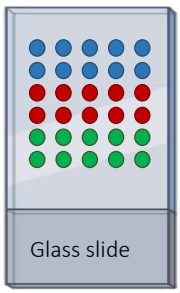


Analytical Parameters	PBST	Milk 1/5
Amin	382.5	98.63
Amax	30755	25970
R2	0.9945	0.9993
Slope	-0.8774	-0.9448
IC50 ug/kg	5.43	1.28
LOD ug/kg	1.083	1.030

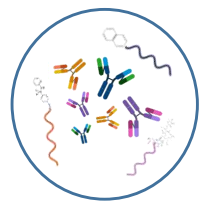


Analytical Parameters	PBST	Milk 1/2
Amin	208.7	35.78
Amax	5008	3477
R2	0.9341	0.9327
Slope	-2.073	-0.8901
IC50 ug/kg	12.88	3.215
LOD ug/kg	1.95	0.89

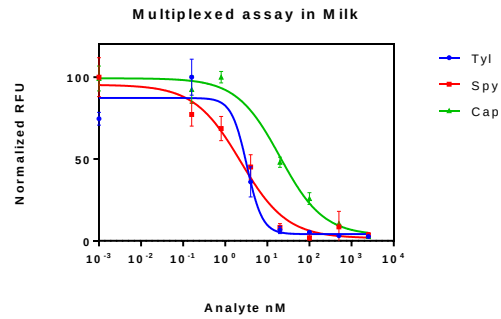
Multiplexed competitive assay

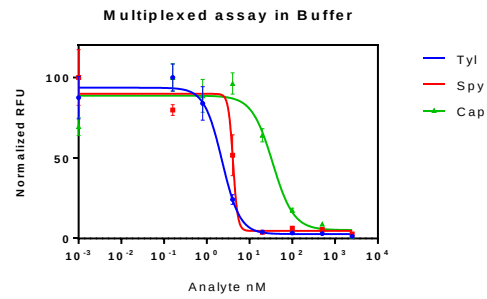


N4down/N4up-hTB
N5down/N5up-SA2 +
N6down/N6up-CA1



Pool of Mab's and oligos

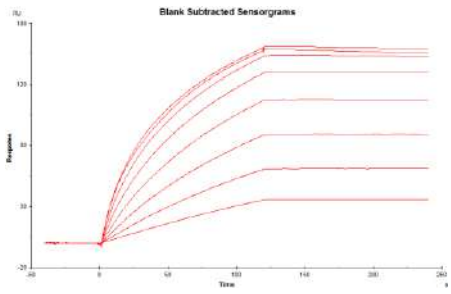




Surface plasmon resonance platform

Kinetic characterization of immunoreagentes with Biacore T200

C6.23.4.2/hTB-BSA



	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
hTB-BSA	3.166E+5	4.328E-5	**1.367E-10**	154.8

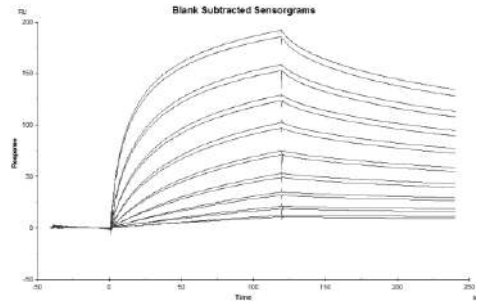
Regeneration: NaOH 10mM Running buffer: PBST

Antibody concentration

\+

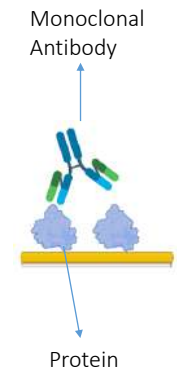
\-

MAb-SA2/SA2-BSA

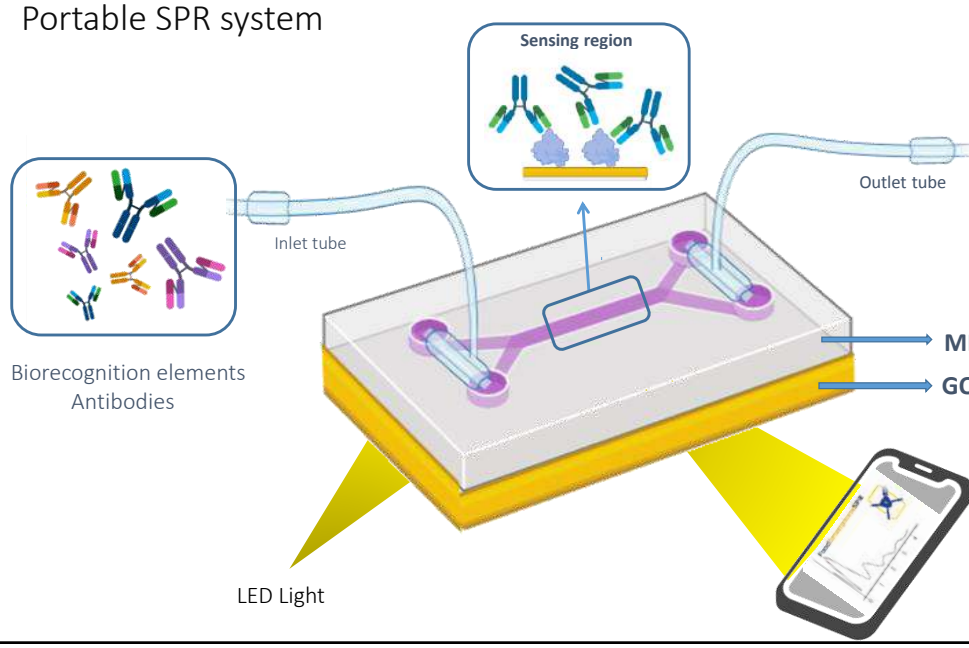


	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
BSA-SA2	2.505E+5	0.002752	**1.098E-8**	166.7

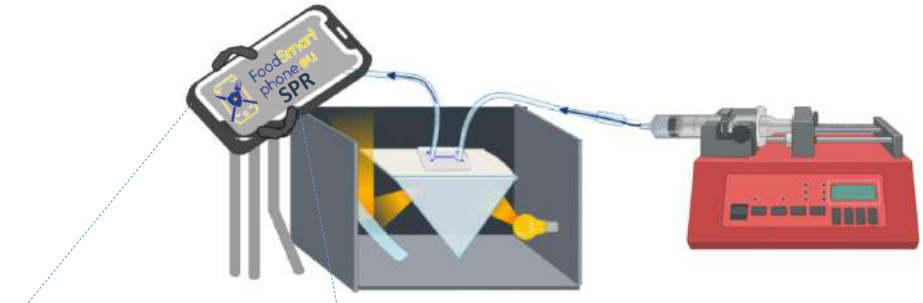
Regeneration: Gyl 10mM, pH 2.5 Running buffer: PBST



Portable SPR system

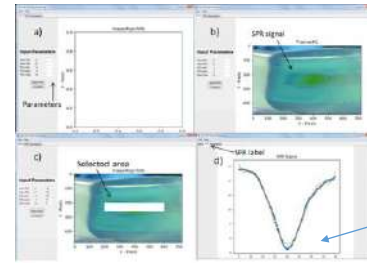


Portable SPR Platfrom with Smartphone detection



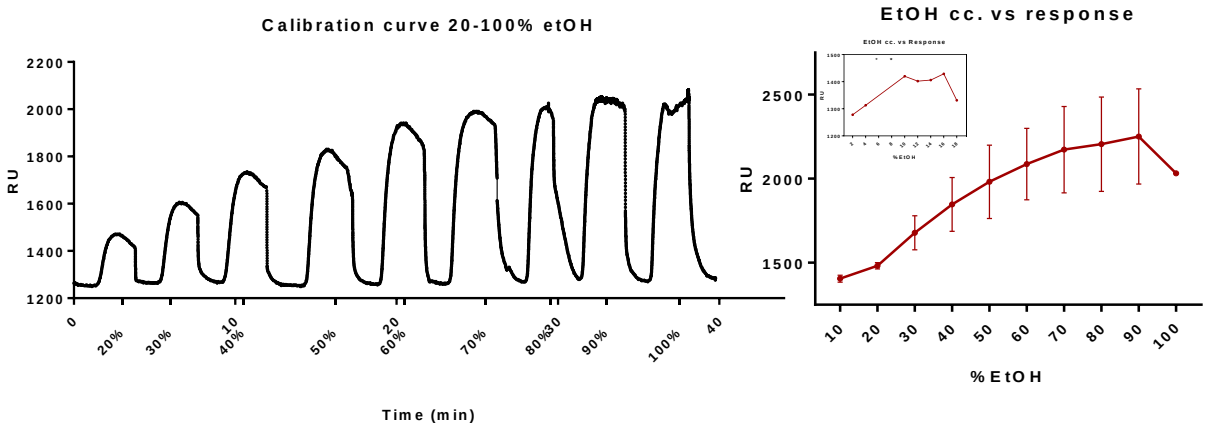
Signal acqusition in real time

Data Analysis

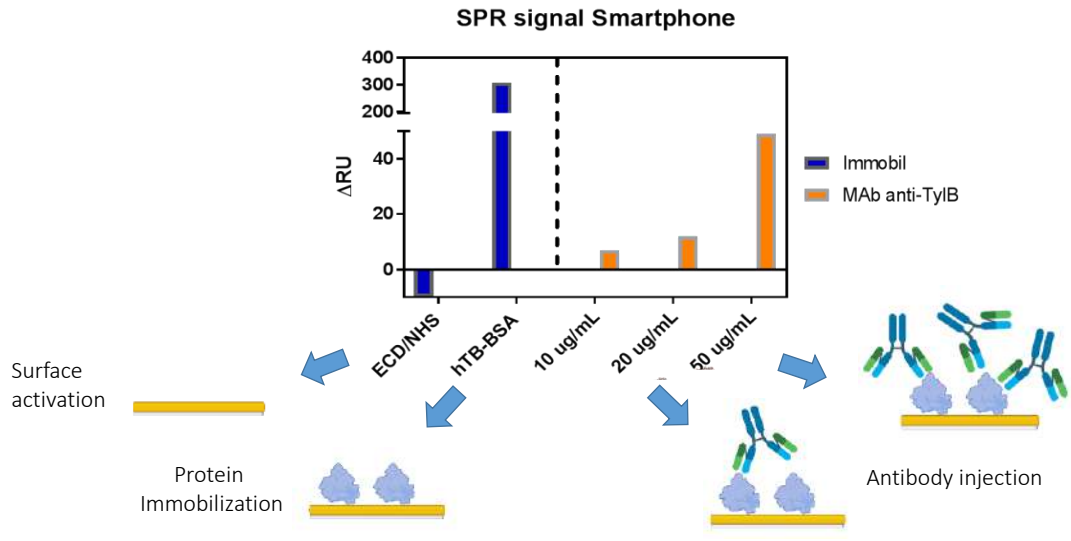


Deep shift

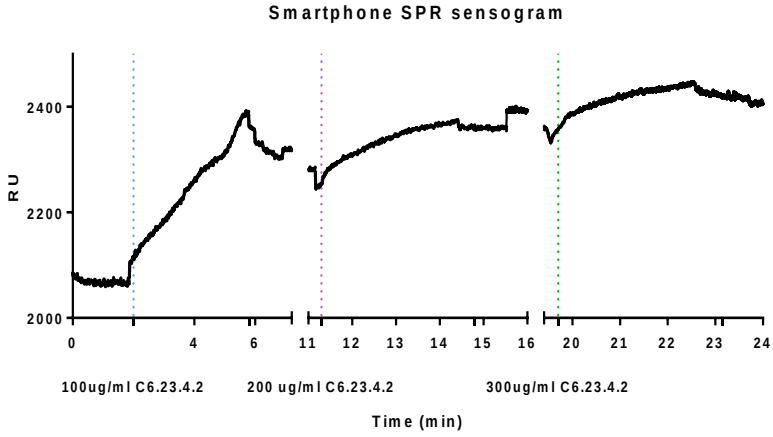
Portable platfrom characterization:
Response to different concentrations of EtOH

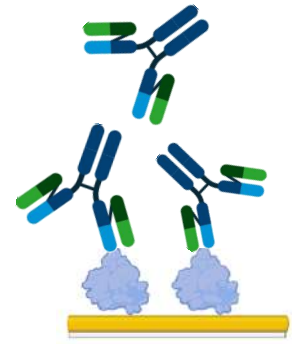


Smarthpone SPR sensor: Protein-conjugates vs antibodies



Immunosensor characterization

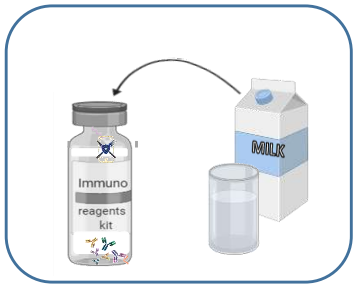


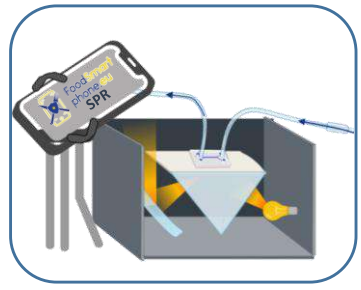


Increasing concentrations of antibody in buffer

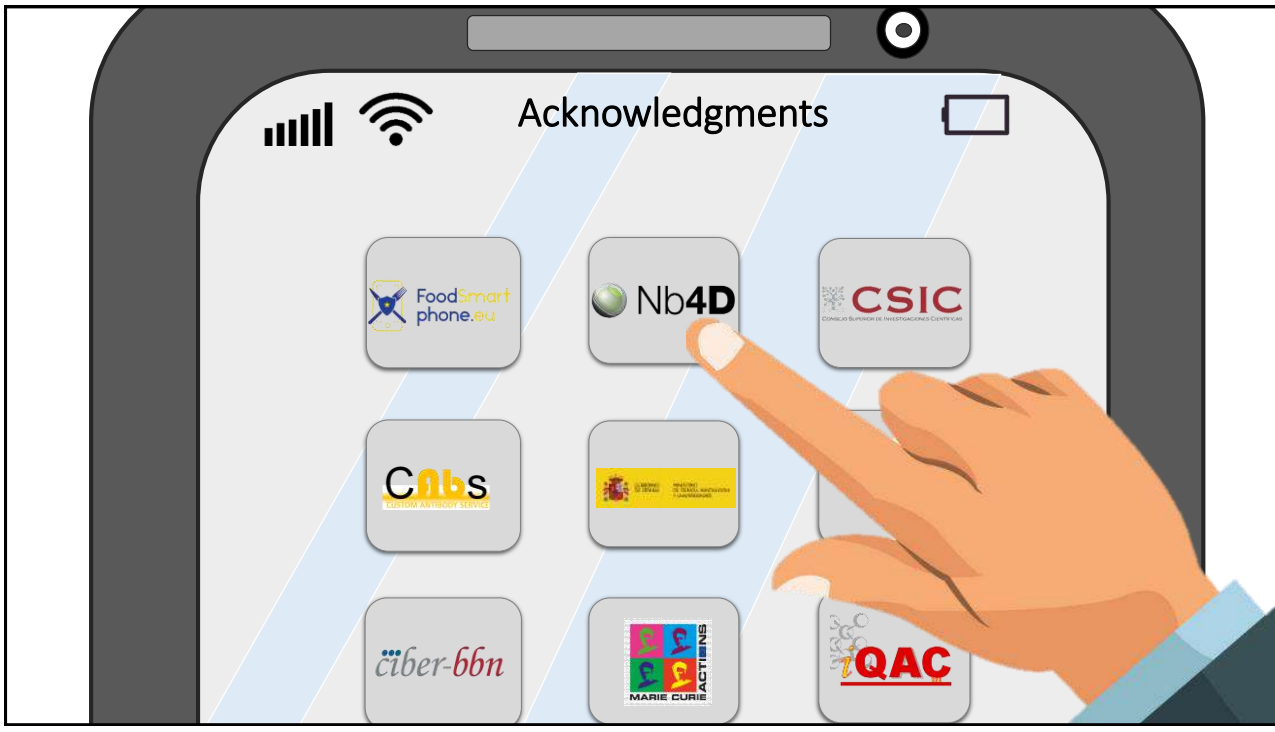
Future perspectives:

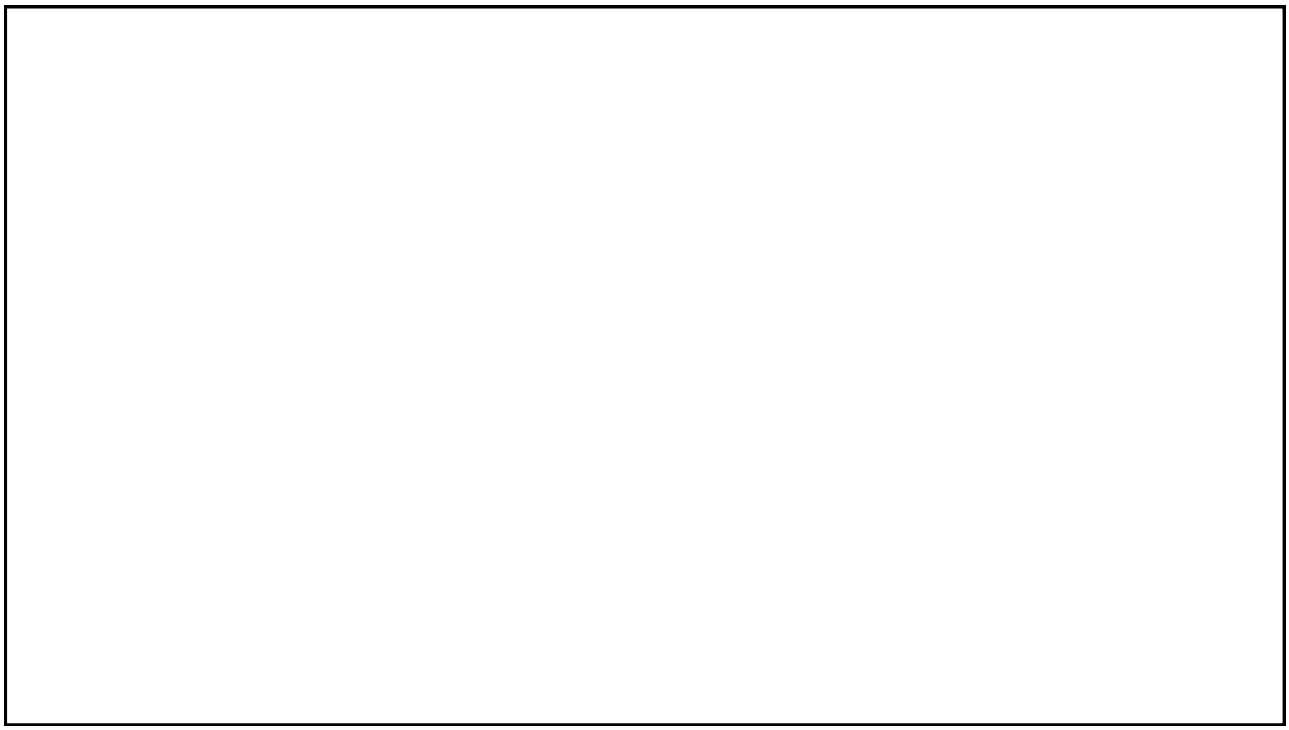
- Integrate DDI approach over the Smartphone SPR system
- Multiplexation of SPR sensor
- Validation in milk samples and App development





Thanks for your attention





BAM
Bundesanstalt für Materialforschung und -prüfung

Sicherheit in Technik und Chemie

26/11/2020

IMMUNOANALYTICAL PLATFORMS FOR ON-SITE ENVIRONMENTAL HEALTH AND FOOD SAFETY TESTING

Rudolf J. Schneider

BAM Federal Institute for Materials Research and Testing
Department of Analytical Chemistry; Reference Materials
Division of Environmental Analysis
Berlin, Germany

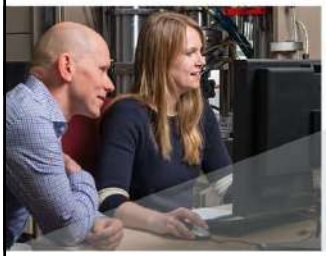
FoodSmartphone.eu

- Online Symposium -
November 25/26, 2020
Application: Pesticides

The Competence Center for Safety in Technology and Chemistry

BAM






BAM is a senior scientific and technical federal authority with responsibility to the Federal Ministry for Economic Affairs and Energy (BMWi)

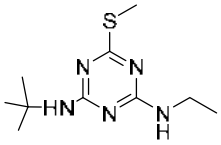
Federal Ministry for Economic Affairs and Energy

Safety creates markets.

Pesticides in drinking water

BAM

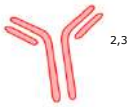
food

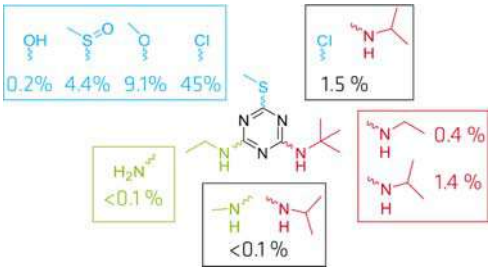


Terbutryn
TBU

… used as a biocide against biofouling in building materials and paints

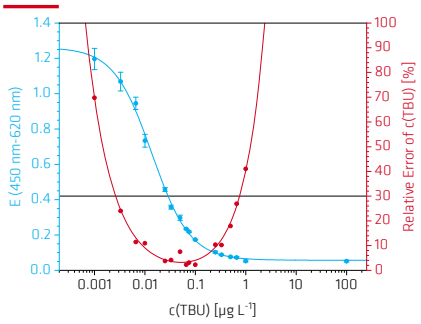
Wash-off and leaching into soil may contaminate surface waters and groundwater resources[1]





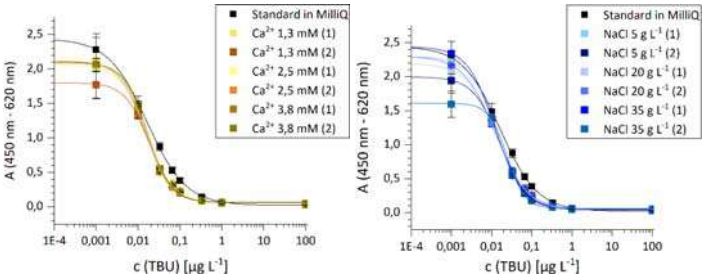
[1] Weil L, **Schneider** RJ, Schäfer O, Ulrich P, Weller M, Ruppert T, **Niessner** R: A Heterogeneous immunoassay for the determination of triazine herbicides in water. Fresenius J Anal Chem 339 (1991) 468-469
[2] Giersch T, **Hock** B: Production of monoclonal antibodies for the determination of s-triazines with enzyme immunoassays. Food Agric Immunol 2 (1990) 85-97
[3] Giersch T, Kramer K, Weller MG, Hock B: Improvement of a monoclonal antibody-based immunoassay for the determination of terbutryn. Acta hydrochim hydrobiol 21 (1993) 312-315

A terbutryn ELISA



Improved sensitivity due to new tracer, optimized conditions and sample buffer

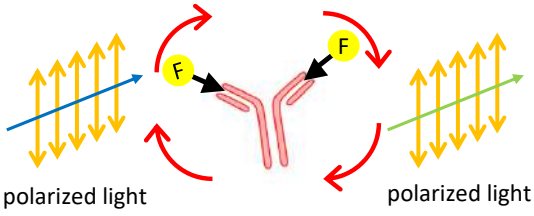
LOD assessment via precision profile, measurement range: 2.6 - 75 ng L^{-1}
Lower cross reactivity than reported before



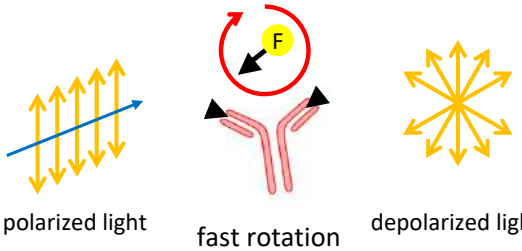
P Lehmann, Master Thesis, HTW Berlin, 2020
unpublished

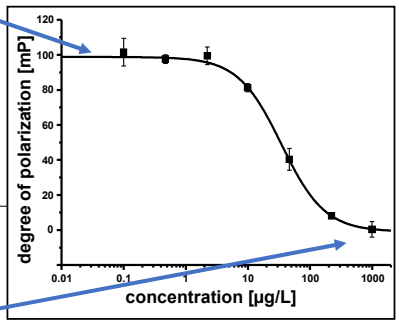
Fluorescence Polarization Immunoassay (FPIA) (a homogeneous assay)

a)
no analyte
(resp. low
concentration)



b)
excess analyte
(resp. high
concentration)





analyte

F fluorophore conjugate

FP platforms

Microtiter plate

Well strips

Cuvette

Synergy H1

M5

Sentry 2000Si

Sentry 201

FP470

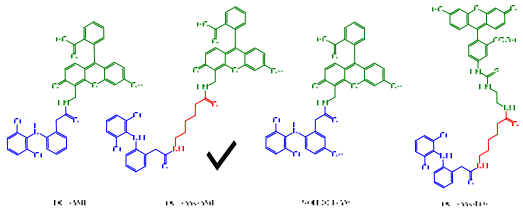
microplate readers
filter-based monochromator-based

strip reader

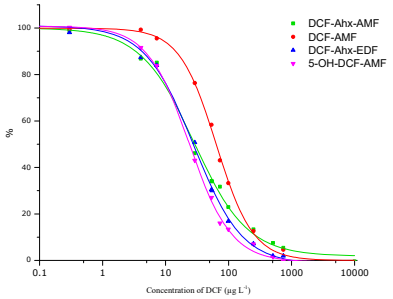
portable
cuvette reader

semiautomatic (w. LHW)
kinetic measurements

Application: Diclofenac in wastewater



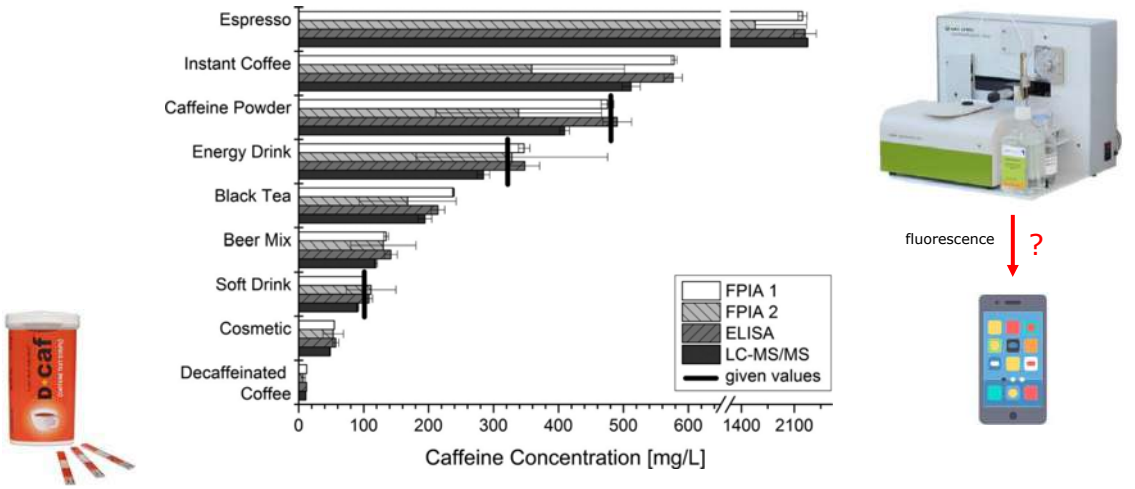
Sentry 2000Si



No.	Wastewater treatment plant, influent/effluent	*c* (DCF) ± SD (µg L^{-1})		
		FPIA	ELISA	LC-MS/MS
1	Ruhleben, influent	2.6 ± 0.5	3.2 ± 1	2.1 ± 0.1
2	Schönerlinde, influent	3.3 ± 0.9	5.4 ± 2	4.5 ± 0.1
3	Waßmannsdorf, influent	3.7 ± 0.1	6.5 ± 3	4.5 ± 0.1
4	Ruhleben, effluent	n.d.	2.8 ± 0.6	2.4 ± 0.1
5	Schönerlinde, effluent	2.7 ± 0.3	4.3 ± 1	3.3 ± 0.1
6	Waßmannsdorf, effluent	1.8 ± 0.8	5.5 ± 2	4.3 ± 0.1

Raysyan A, Moerer R, Coesfeld B, Eremin SA: Fluorescence polarization immunoassay for the determination of diclofenac in water, Anal Bioanal Chem (2020); 10.1007/s00216-020-03058-w

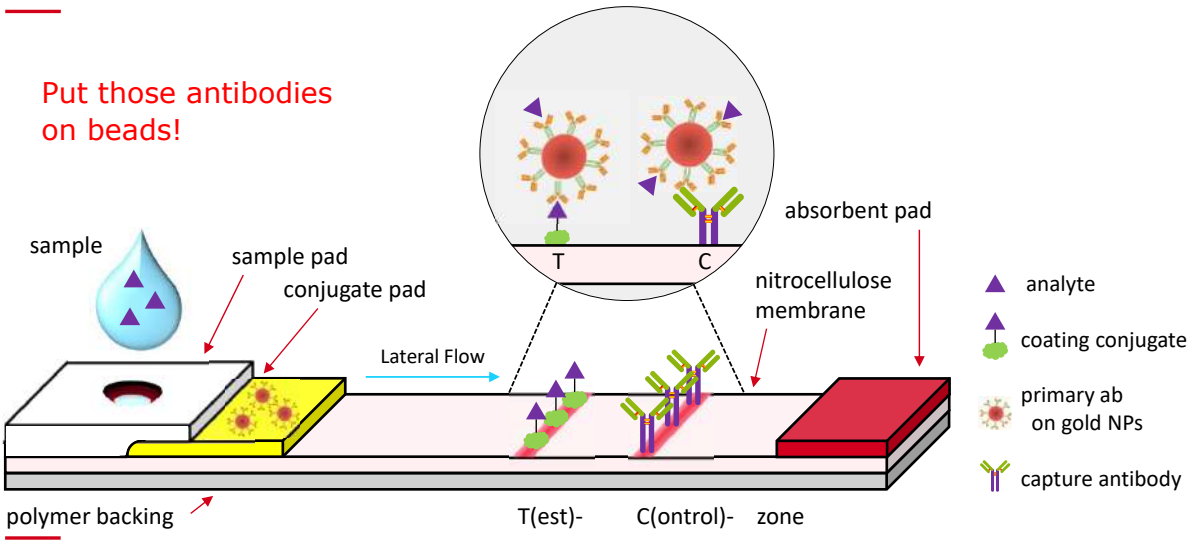
Application:
Caffeine in beverages and consumer products



L. Oberleitner, J. Grandke, F. Mallwitz, U. Resch-Genger, L.-A. Garbe, and R. J. Schneider, J. Agric. Food Chem., 2014, 62, 2337

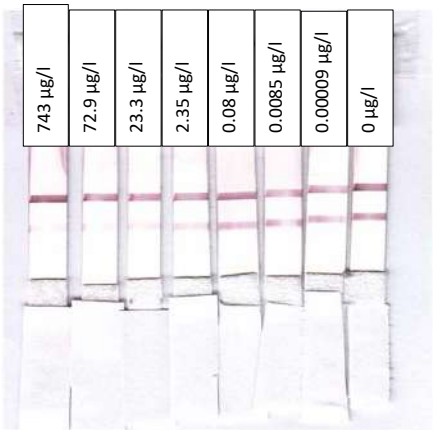
Lateral Flow Immunoassays
aka dipstick assays, "pregancy test"-like assays

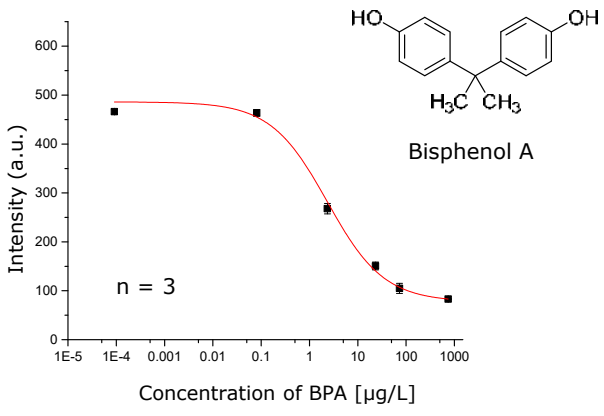
Put those antibodies on beads!



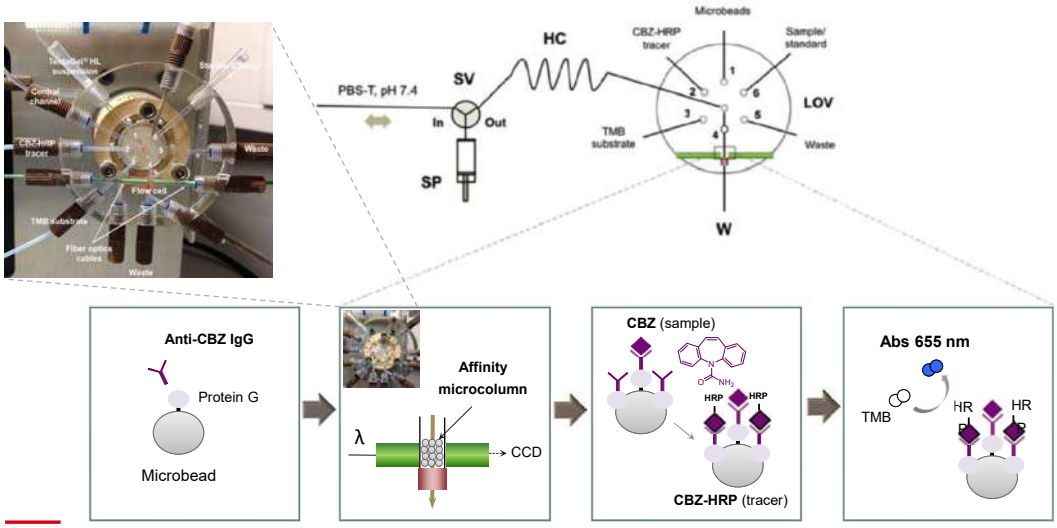
Evaluation via line scanners or via smartphone picture analysis

Satisfactory sensitivity





Lab-on-valve Immunoassay (LOVIA)*



Lab-on-valve Immunoassay (LOVIA)*

CBZ concentration (μg L^{-1}) in wastewater samples obtained by μ-BIS-LOV and reference methods.

Sample[a]	$C_{\mu\text{-BIS-LOV}}$ (reaction rate)[b]	$C_{\mu\text{-BIS-LOV}}$ (fixed time)[c]	C_{ELISA}	$C_{LC\text{-}MS/MS}$
Influent	2.2 ± 0.3	2.4 ± 0.6	2.9 ± 0.3	2.22 ± 0.03
Effluent	1.7 ± 0.5	1.4 ± 0.4	2.3 ± 0.2	2.14 ± 0.03

* … or: μ-BIS-LOV
(Micro-bead injection spectroscopy)

Ramos II, Carl P, Schneider RJ, Segundo MA: Automated lab-on-valve sequential injection ELISA for determination of carbamazepine. Anal Chim Acta 1076 (2019) 91-99

Carl P, Ramos II, Segundo MA, Schneider RJ: Antibody conjugation to carboxyl-modified microspheres through N-hydroxysuccinimide chemistry for automated immunoassay. PLoS ONE 14(6) (2019): e0218686

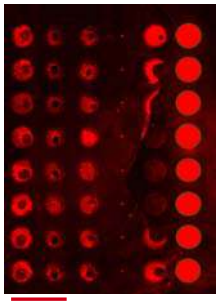
Multiplexing ⟶ „Arrays“

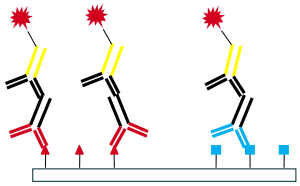
BAM

Problem: Standard immunoassays are only capable of single analyte analysis

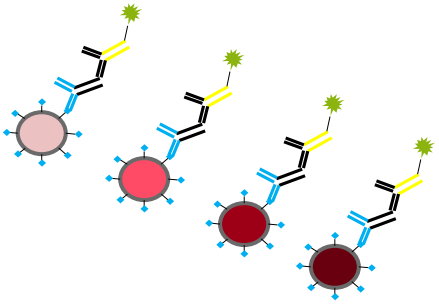
Solution

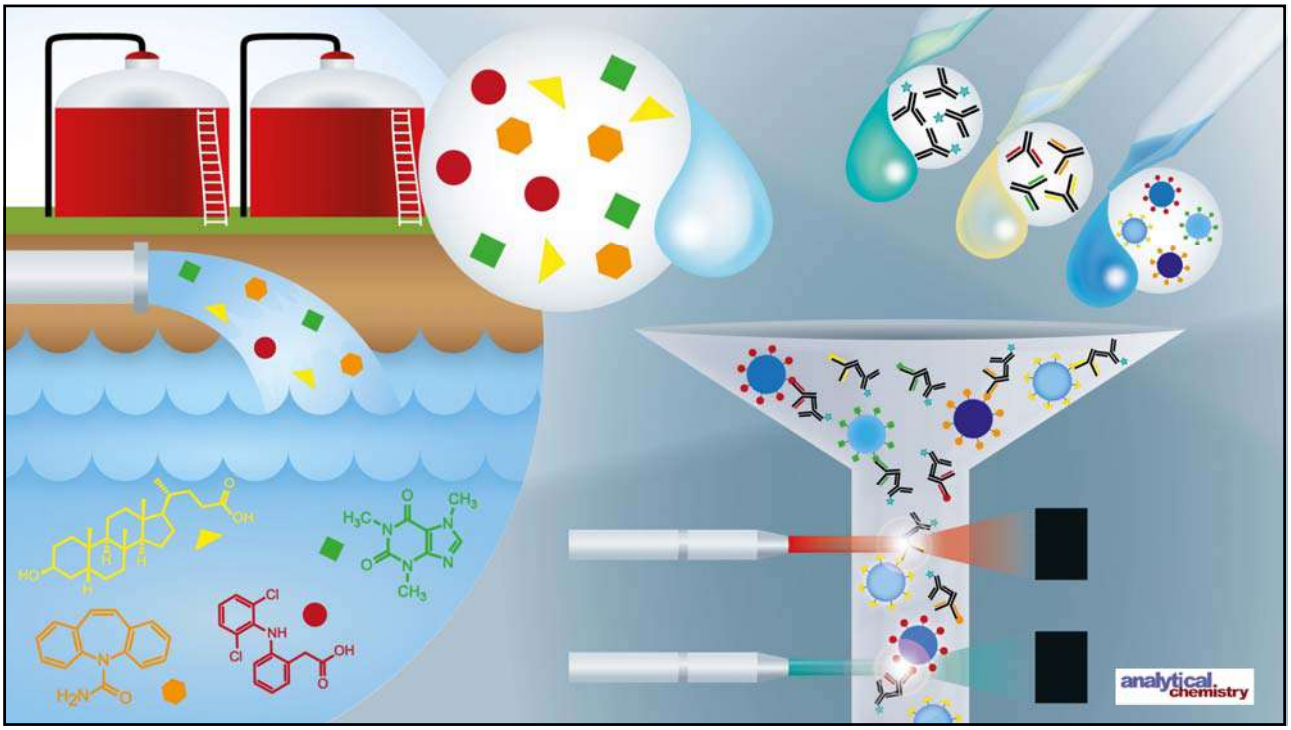
Flat (chip-based) arrays:
encoding by spatial grid



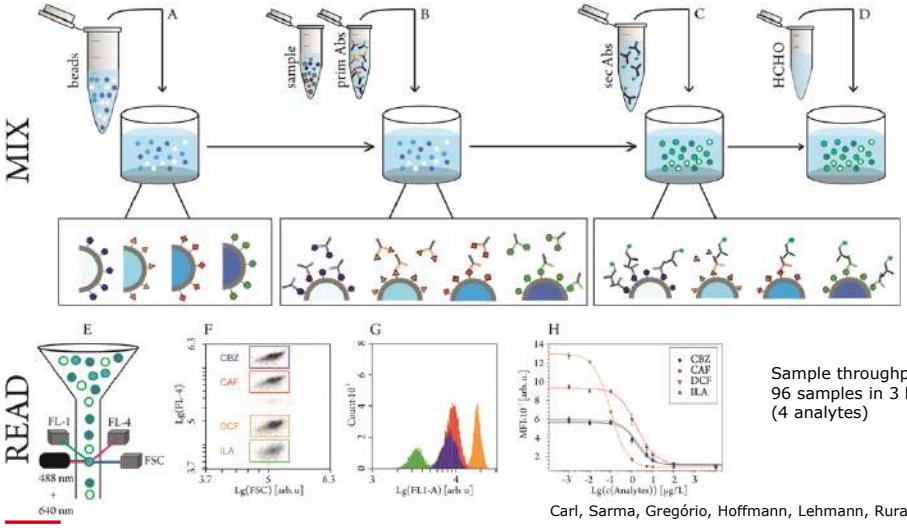


Suspension (bead-based) arrays:
encoding by diff. fluorescent particles





Suspension Array Fluorescence Immunoassay SAFIA

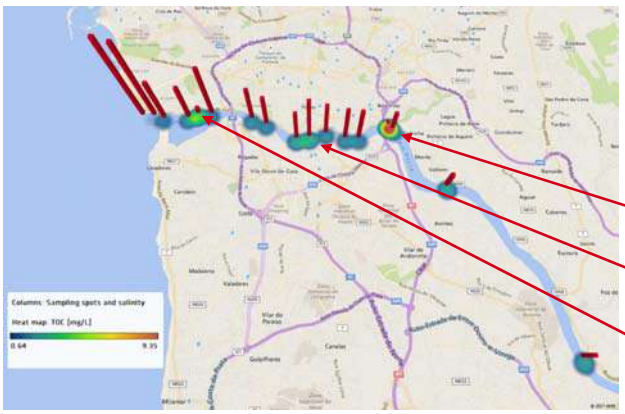


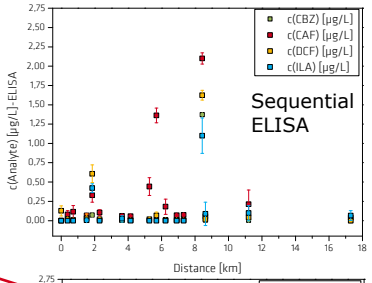
Anthropogenic markers
CBZ … carbamazepine
CAF … caffeine
DCF … diclofenac
ILA … isolithocholic acid

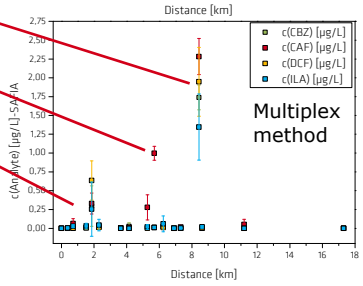
Sample throughput:
96 samples in 3 h
(4 analytes)

Carl, Sarma, Gregório, Hoffmann, Lehmann, Rurack, Schneider, Anal Chem 2019

Real-world surface water samples (Douro, Porto) … hot spot screening







Analytical practice in mycotoxin analysis

BAM

Immunoassay

simple

inexpensive

1 mycotoxin

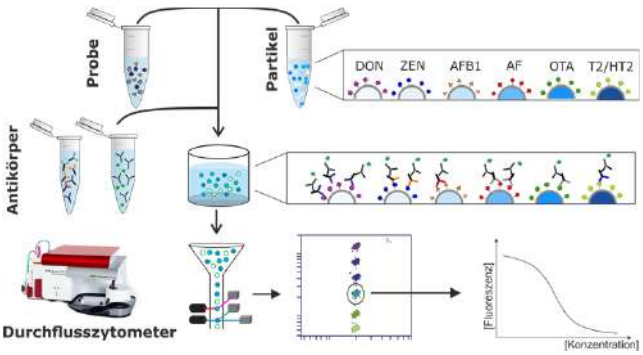
LC-MS / MS

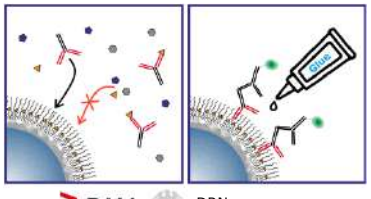
Lab method

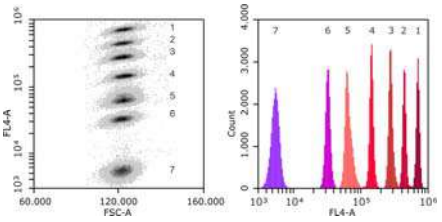
expensive

Taking the SAFIA principle forward …

BAM



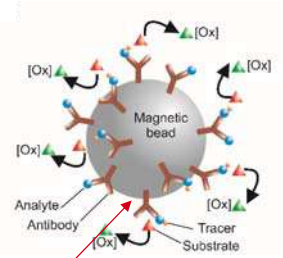




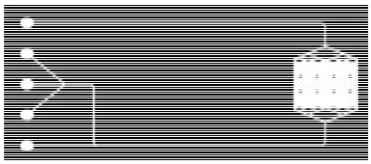
SAFIA

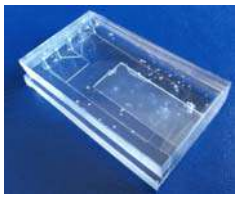
Bead-based microfluidic electrochemical immuno-”Sensor” (Lab-on-chip Immunoassay: LoCIA)

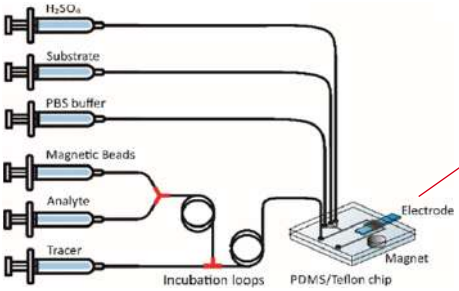
BAM

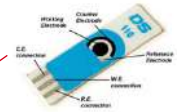


Superparamagnetic particles, iron oxide core, silane coating, amino-functionalized, Ø 1 µm glutaraldehyde cross-linking





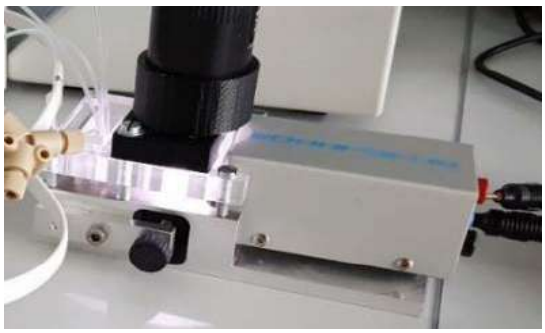


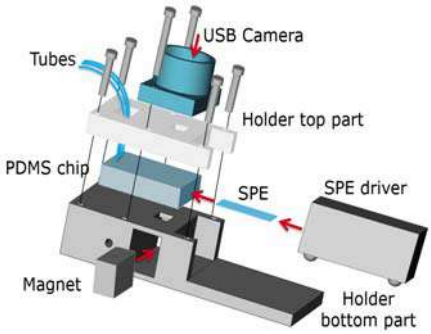


courtesy: Dr. J. Bell, BAM

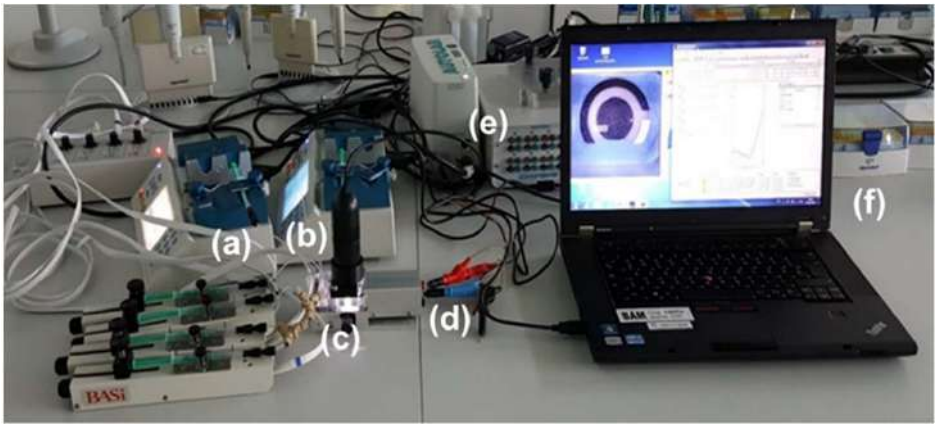
Bead-based microfluidic electrochemical immuno-”Sensor” (Lab-on-chip Immunoassay: LoCIA)

BAM



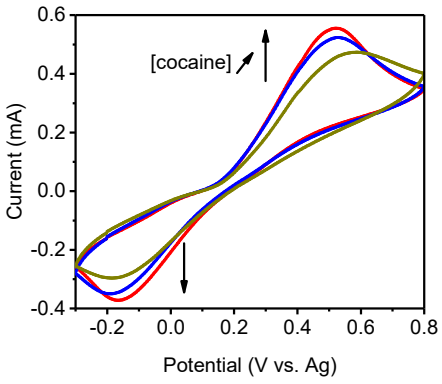


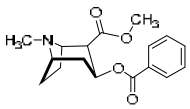
Bead-based microfluidic electrochemical immuno-”Sensor” (Lab-on-chip Immunoassay: LoCIA)



(a) syringe pumps, (b) microfluidic chip, (c) chip holder,
(d) SPE connector, (e) potentiostat, (f) display/data analysis

CV read-out is concentration-dependent, excellent sensitivity





Cocaine,
a marker for
wastewater epidemiology

Sample	Spiked Cocaine ng/L	Found Cocaine ng/L	Recovery %
Saliva	1.00 ± 0.01	1.07 ± 0.04	106
	100.0 ± 0.6	88.1 ± 6.0	88
Urine	1.00 ± 0.01	0.94 ± 0.03	94
	100.0 ± 0.6	89.2 ± 8.6	89

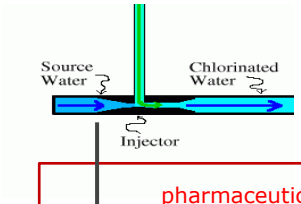
Abdelshafi et al., Drug Test Anal 2019

Integrated Diagnostics of Contaminants in Water Supply and Management System

BAM

IGSTC
INDO-GERMAN SCIENCE AND TECHNOLOGY CENTRE
IGSTC 2+2 Projects

IDC-Water



Water pipeline

Integrated System

pharmaceuticals

In-line Collection

Affinity enrichment

Microfluidic immunosensor

Cell Culture

Cell/DNA detection

micro-PCR

pathogens

Early Warning Data Mapping

Laboratory

sifin

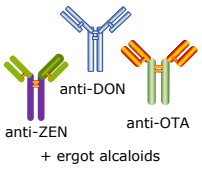
BAM

IISc

bigtec

Modular, multiplexed, antibody-based Lab-on-Chip Analyser for food control

Putting the ELISA on beads



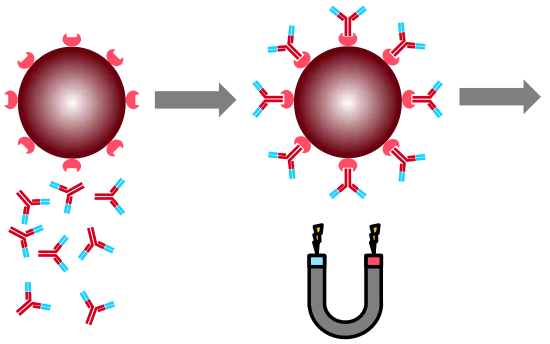
BAM

Project MamaLoCA

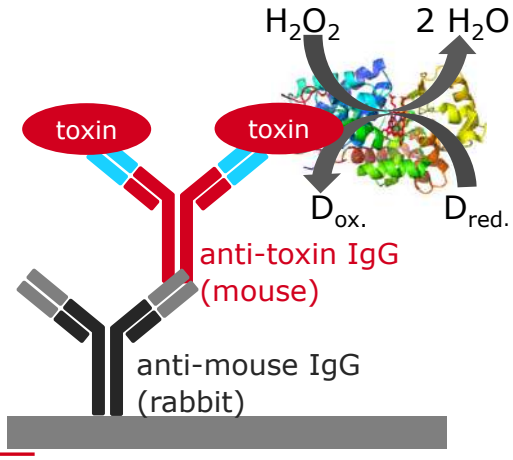
anti-OTA + Protein G modified magnetic beads

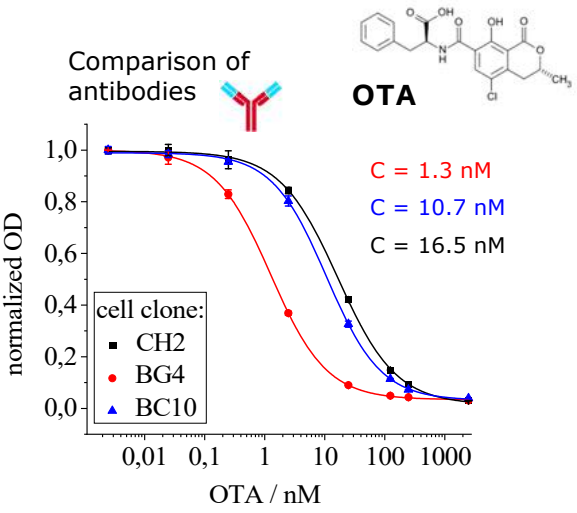
washing

ELISA on beads

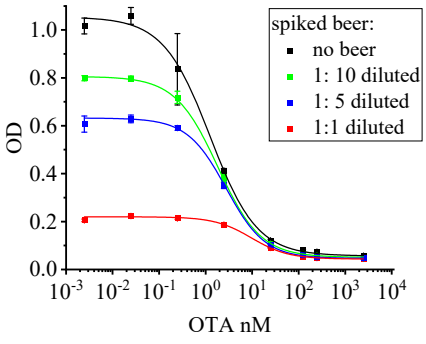


Moving from colorimetric to amperometric detection





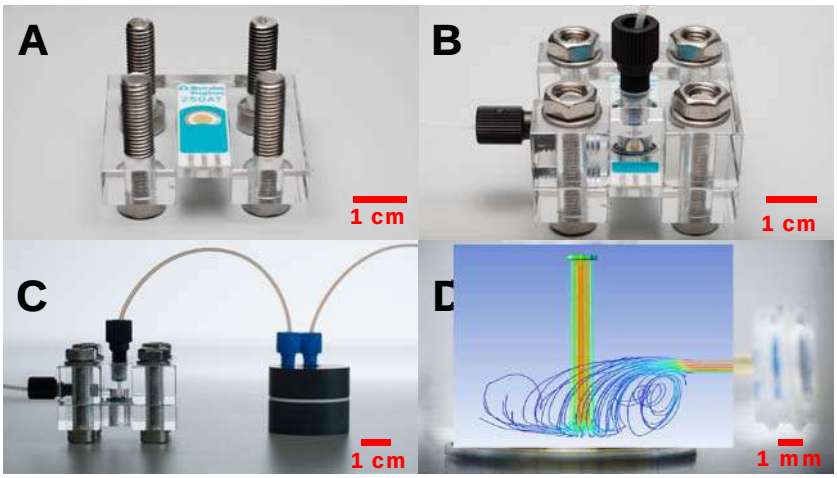
Immunomagentic OTA assay in spiked beer



C = 1.2 nM
C = 1.8 nM
C = 2.8 nM
C = 9.7 nM

With 1:5 dilution OTA concentrations down to 2 µg L^{-1} can be quantified!

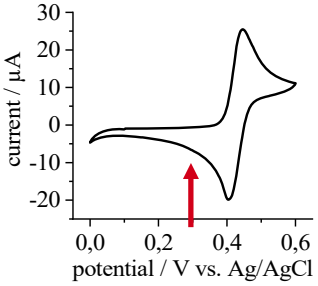
Mesofluidic test set-up

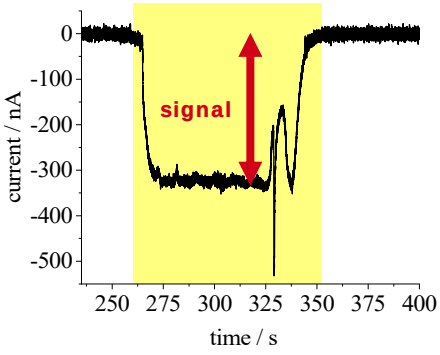


Amperometry (fixed potential, forced convection)

gold

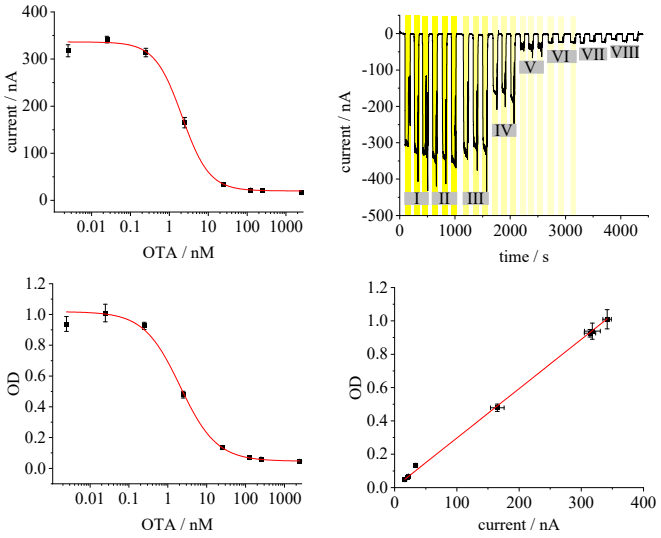
Amperometry:
300 mV vs. Ag/AgCl
flow rate 600 µl min $^{-1}$

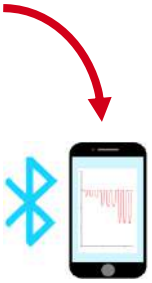


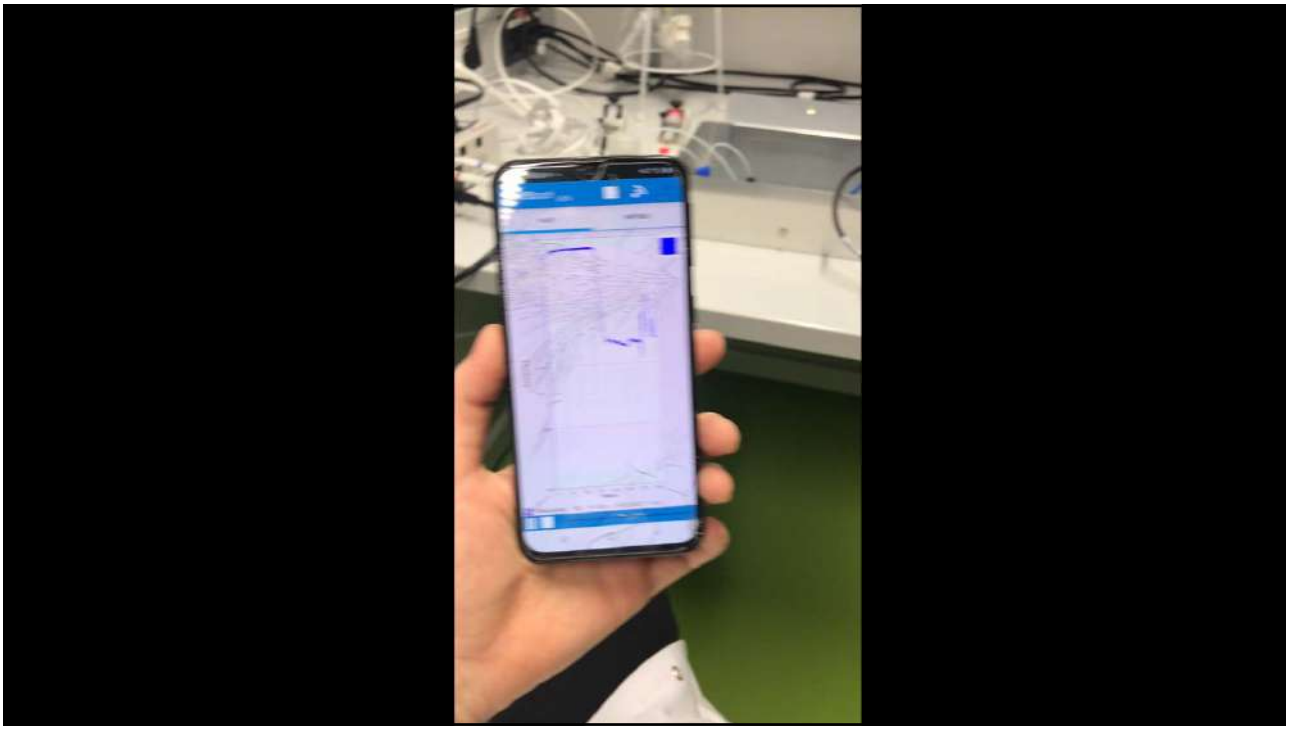


- reversible/ quasi reversible electrochemistry of TMB at pH 1 on gold electrodes was observed
- Surface characterization of electrodes after electrochemical reaction with TMB (SEM, EDX, Tof SIMS)

Electrochemical vs colorimetric detection







Take-Home Messages

- Immunoanalytical platforms, in a variety of formats, are well suited for environmental health and food safety testing
- Selectivity and sensitivity can only be obtained by the availability high-affinity, selective antibodies (which for small analytes are still not easy to obtain)
- Many formats benefit from the trends of miniaturisation, automation, microfluidics, so that they can be turned into online sensors.
- Progress in „materials sciences", e.g. nanotechnology, surface chemistry etc. allows for further improvements.
- **Smartphone analyzers for on-site testing of food quality and safety will become a daily reality.**

Acknowledgements

Anna Raysyan

SAFIA eXIST

Dr. Peter Carl & Team SAFIA

Dr. Margarida Carvalho

Dr. Nahla Abdelshafi

Robin Moerer

Petra Lehmann

Soraya Höfs

MARIE CURIE ACTIONS 7 PEOPLE SOS

BAM Division 1.9

Dr. Jérémy Bell

Dr. Dominik Sarma

Dr. Knut Rurack

Dr. Cinthya Véliz Montes

Dr. Lidia Oberleitner

Federal Ministry for Economic Affairs and Energy

DAAD

DFG Deutsche Forschungsgemeinschaft

Quality System and Food Safety

Esmeralda Payan

26/11/2020

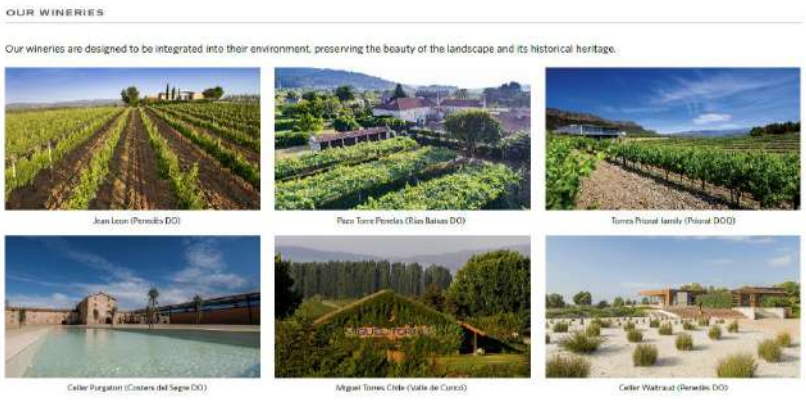
1

1. Familia Torres
2. Quality system approach
3. QS in vineyard
4. QS in winemaking process
5. QS in filling process and final product
6. Conclusions

2

FAMILIA TORRES

- Family-owned winery founded 1870
- 150th Anniversary - 5 generations – Member of PFV
- Historical connection Penedès, Conca de Barberà, Priorat, Costers del Segre
- Later also Rioja, Ribera del Duero, Rueda, Rías Baixas + Chile/California
- 2.432 hectares (2.000 Spain, 400 Chile, 32 California); Catalonia 1.800 ha, of which 850ha certified organic by CCPAE
- Focus on singular wines that express the landscape of each wine region we are passionate about, recovery of ancestral varieties and climate change.
- Balance between tradition and innovation with special focus on sustainability (Torres & Earth - IWCA)



3

QUALITY SYSTEM APPROACH

OBJECTIVE

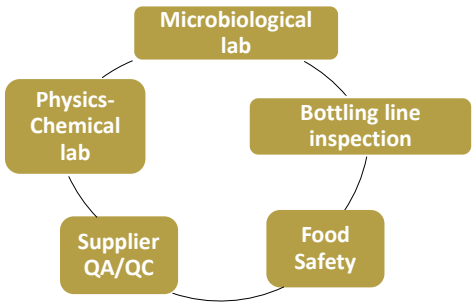
- Know the real risks of the products we handle and the processes in which we intervene.
- Ensuring that hygiene measures and established controls are adequate for the risks detected.

Vineyard → Winemaking process → Wine filling

LEGAL AND CUSTOMER REQUIREMENTS

4

QA/QC AND FS DEPARTMENT



- 15 bottling lines (Spain)
- Export to more than 150 countries
- 25 technicians in QAFS department

5

QUALITY SYSTEM IN VINEYARD

Grape suppliers' advice:

- Phytosanitary treatments
- Grape Maturity
- Grape transportation
- Documentation

Grape quality controls:

- Maturity control (ºbrix: probable alcohol degree, acidity and pH)
- Sanitary status (Gluconic acid)
- Phytosanitary residues. Random test before harvesting
- Documentation (ORGANIC, DO, VARIETY..)

6

QUALITY SYSTEM IN VINEYARD

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- Sanitary status (Gluconic acid)
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- Documentation (ORGANIC, DO, VARIETY..)

MAIN SAFETY CONCERNS IN FINAL PRODUCT

- Phytosanitary over the legal limit. ORGANIC PRODUCTS
- Production of Ochratoxin A.
- Labelling mistakes. (Origin, organic, variety)

7

QUALITY SYSTEM IN WINEMAKING PROCESS

Quality controls:

- Twice a day fermentation control (alcohol degree, volatile acidity, glucose-fructose, etc.
- Legal parameters. (Ochratoxin A, Ethyl carbamate, phytosanitary, Biogenic amines, metals, methanol, SO_2, etc.
- Microbiological control. Inoculum maintenance during the alcoholic and malolactic fermentation
- Traceability of oenological products (vegan, allergenics)

8

QUALITY SYSTEM IN WINEMAKING PROCESS

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- Twice a day fermentation control (alcohol degree, volatile acidity, glucose-fructose, etc.
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- Microbiological control. Inoculum maintenance during the alcoholic and malolactic fermentation
- Traceability of oenological products (vegan, allergenics)

MAIN SAFETY CONCERNS IN FINAL PRODUCT

- Parameters over the legal limit. ORGANIC PRODUCTS.
- Labelling mistakes. (Origin, organic, variety, etc.)
- Healthy concerns: allergenics (milk, egg, SO_2..)
- Organoleptic profile
- Product recall

TAKE YOUR TIME BEFORE THE FILLING PROCESS

BE AWARE OF THE LEGAL CHANGES (Eurolex, wine association, legal platforms)

QUALITY SYSTEM IN WINEMAKING PROCESS

1. **BARRELS**
   - Direct and continuous communication between coopers and our wine makers.
2. **CORK**
   - TCA (2,4,6-Trichloranisole)
   - Microbiological contamination
   - Mechanical properties (ex. Density, weight, height, extraction force, hermetic sealing..)
3. **YEAST AND BACTERIA**
   - Viability and purity yeast bacteria batches
   - Yeast bacteria growth

QUALITY SYSTEM IN FINAL PRODUCT

FILLING PROCESS:

- Routine checklist
- Foreign body
- Microbiological stability. Quarantine.

BE READY TO ACT

TRACEABILITY, BATCH NUMBER MANAGEMENT

11

QUALITY SYSTEM IN FINAL PRODUCT

FILLING PROCESS:

- Routine checklist
- Foreign body
- Microbiological stability. Quarantine.

BE READY TO ACT

TRACEABILITY, BATCH NUMBER MANAGEMENT

LABELLING AND CUSTOMER COMPLIANCE:

- Different country legislation (varieties, allergenic, sugar…)
- Product category
- Organoleptic profile
- Documentation (ORGANIC, DO, VARIETY..)

MANDATORY LABEL INFORMATION (EU):

- Product category*/DOP/IGP
- % vol.
- Origin*
- Volume
- Operator responsible (importer)
- Sugar content (sparkling)
- Batch number
- Allergenics

12

CONCLUSIONS

MOST COMMON ISSUES:

- Foreign bodies in bottles
- Analytics parameters out of range (SO_2, % vol..)
- Microbiological stability. Quarantine.

QUALITY CHALLENGES:

- Parameters detection online in the cellar (alcoholic fermentation monitoring)
- Glass detection in glass bottles
- Fast detection of microorganisms
- Phytosanitary detection

CONCLUSION

- System based on risk assessment
- Monitoring of regulatory changes
- Keep in contact with costumers

FAMILIA TORRES

13

14

Smartphone-based enzyme assays for cholinesterase inhibitors screening

Name: Aristeidis Tsagkaris, Ph.D.
Institute: UCT Prague
tsagkara@vscht.cz

Daily supervisor: Prof. Jana Pulkrabova
Supervisor: Prof. Jana Hajslova

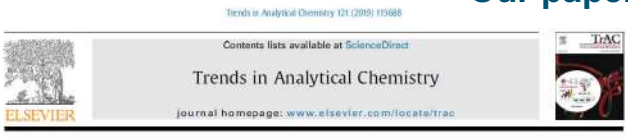
SMART TECH for FOOD workshop, online event, 25 & 26 November 2020

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325

Background

Screening methods, 2002/657/EC
methods that are used to detect the presence of a substance or class of substances at the level of interest.

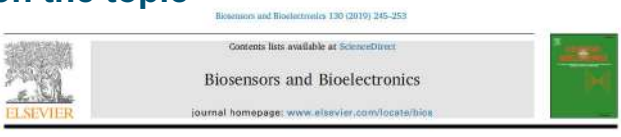
Our papers on the topic


Trends in Analytical Chemistry

Critical assessment of recent trends related to screening and confirmatory analytical methods for selected food contaminants and allergens

A.S. Tsagkaris, Y. Zhao, K. R, M.P. Marco

Under review


Biosensors and Bioelectronics

The end user sensor tree: An end-user friendly sensor database

J.L.D. Nelis, A.S. Tsagkaris, Y. Zhao, J. Lou-Franco, P. Nolan, H. Zhou, C. Cao, K. Rafferty, J. Hajslova, C.T. Elliott, K. Campbell

Under review


foods

Review

Optical screening methods for pesticide residue detection in food matrices: Advances and emerging analytical trends

Aristeidis S. Tsagkaris, Jana Pulkrabova and Jana Hajslova

Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6 – Dejvice, Prague, Czech Republic

Correspondence: tsagkara@vscht.cz

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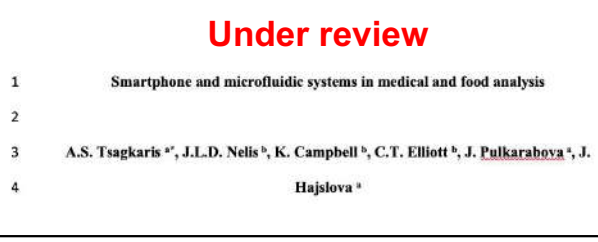
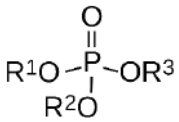

Smartphone and microfluidic systems in medical and food analysis

A.S. Tsagkaris, J.L.D. Nelis, K. Campbell, C.T. Elliott, J. Pulkarabova, J. Hajslova

Motivation

Organophosphates **Car**



NEUROTOXIC

LC-MS/MS **GC-MS/MS**

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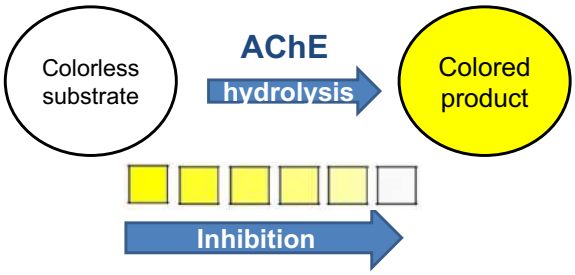
journal homepage: www.elsevier.com/locate/trac

Smartphone-based optical assays in the food safety field

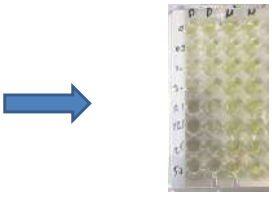
J.L.D. Nelis [a, *, 1], A.S. Tsagkaris [b, 1], M.J. Dillon [a], J. Hajslova [b], C.T. Elliott [a]

[a] Institute for Global Food Security, School of Biological Sciences, Queen's University, 19 Chlorine Gardens, Belfast, BT9 5DL, United Kingdom
[b] Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6 – Dejvice, Prague, Czech Republic



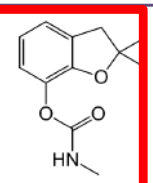
Optimization stage

A. Microplate assay

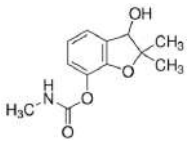


B. Specificity testing

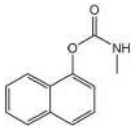
Carbamates



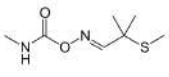
carbofuran



carbofuran-3-OH

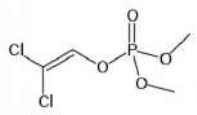


carbaryl

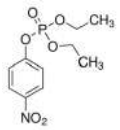


aldicarb

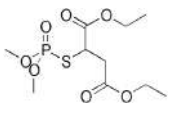
Oxon forms (P=O)



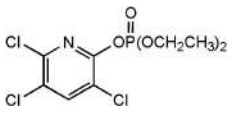
dichlorvos



paraoxon

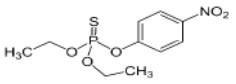


malathion

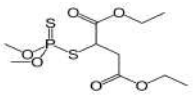


chlorpyrifos-oxon

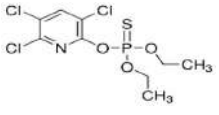
Thiono forms (P=S)



parathion

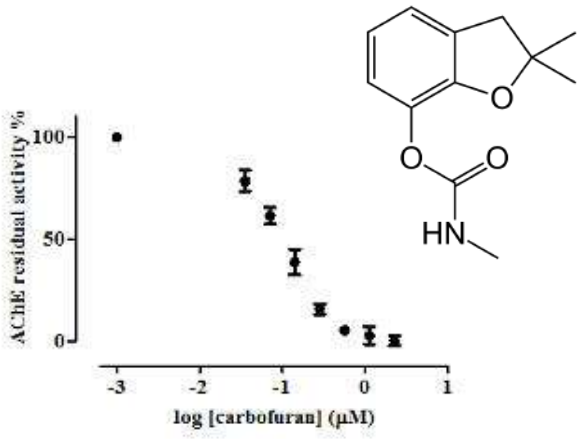


malathion



chlorpyrifos

Why carbofuran??



IC_{50}= 22 ng mL –1 (20 – 24 ng mL –1), n=9

applied sciences

MDPI

Article

Screening of Carbamate and Organophosphate Pesticides in Food Matrices Using an Affordable and Simple Spectrophotometric Acetylcholinesterase Assay

Aristeidis S. Tsagkaris, Leos Uttl, Jana Pulkrabova and Jana Hajslova *

Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6- Dejvice, Prague, Czech Republic; tsagkara@vscht.cz (A.S.T.); uttll@vscht.cz (L.U.); pulkrabj@vscht.cz (J.P.)
* Correspondence: jana.hajslova@vscht.cz; Tel.: +420-220-443-185

C. Benchmarking towards LC-MS/MS

Method	AChE Assay				LC-MS/MS			
Matrix	Lettuce		Strawberry		Lettuce		Strawberry	
Spiking Level (mg Kg^{-1})	0.020	0.100	0.020	0.100	0.020	0.100	0.020	0.100
R% [1]	91	94	71	78	79	91	81	88
RSD_r% [2]	5	5.2	19	10	1.9	1.2	1.5	2.4
LOD (mg Kg^{-1})	0.013		0.012		0.0014		0.0012	

[1] R%: recovery %. [2] RSDr%: relative standard deviation under repeatability conditions %.

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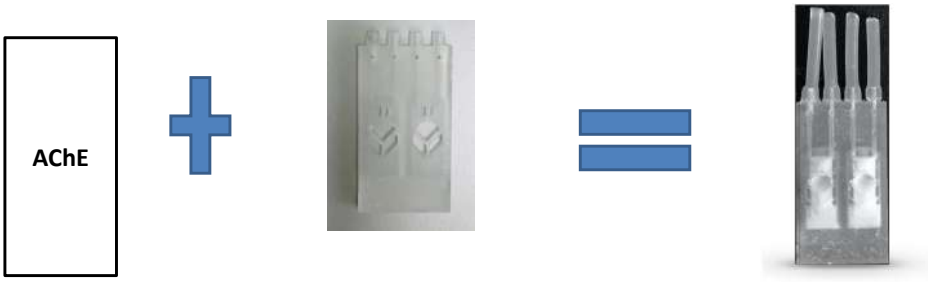
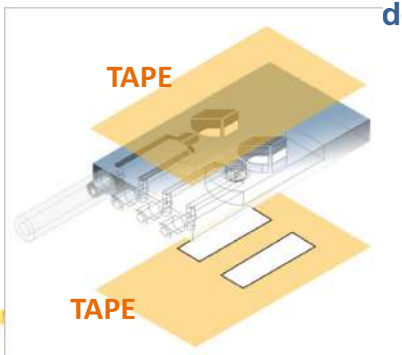
1 European Food Safety Authority. "The 2018 European Union report on pesticide residues in food." EFSA Journal (2020), https://doi.org/10.2903/j.efsa.2020.6057
2 https://webgate.ec.europa.eu/rasff-window/portal/?event=SearchByKeyword&NewSearch=1&Keywords=carbofuran, last accessed 20/11/2019
3 Radhakrishnan, Sreejith. "A note on wildlife poisoning cases from Kerala, South India." European journal of wildlife research 64.5 (2018): 58.

Hybrid paper-lab-on-a-chip injector



3D-printed lab-on-a-chip (LOC) device

Hybrid injector



- Apparatus: SLA 3D-printer
- Fast manufacturing: < 1h per run (2-4 devices)
- Cost per chip: 0.3 EUR
- 4 active channels
- 1 test strip & 1 reference strip
- Silicone tubing injectors

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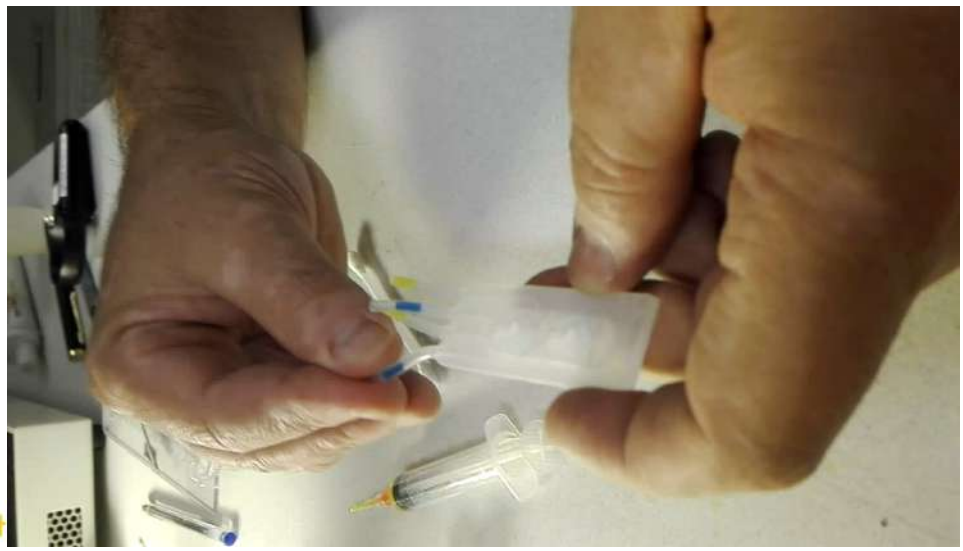
Silicone tubing= Finger pumping

samples and substrates were put in the silicone tubes, which can be used as a finger-pump



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In-house smartphone color reader

- **In-house prototype**
- **Apparatus: FDM 3D-printer (150 EUR) & smartphone (100 EUR)**
- **Fast manufacturing: < 2h per run**
- **Cost per "box": < 1 EUR**
- **Ambient light effect elimination**
- **Standardized light conditions, flash on**

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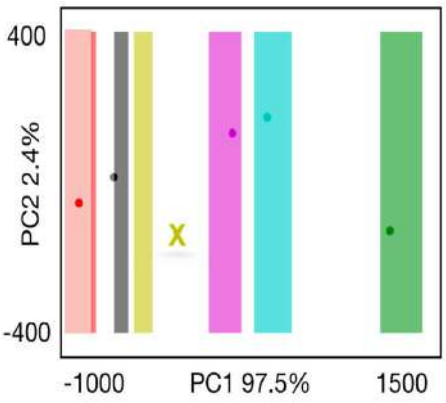
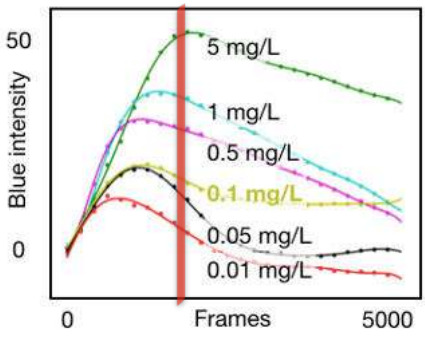
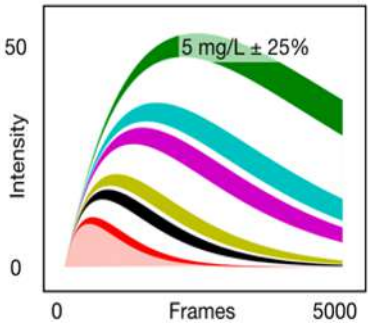
Calibration curve, 0.010 – 5.0 mg L^{-1} in PBS

PCA system - quality control



Experimental data





Predictive theoretical model

$$s(t) = A * \left(1 - e^{\frac{-t}{\tau_1}}\right) * e^{\frac{-t*c^{\gamma}}{\tau_2}}$$

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Carbofuran screening in apple extracts

sensors

MDPI

Article

A Hybrid Lab-on-a-Chip Injector System for Autonomous Carbofuran Screening

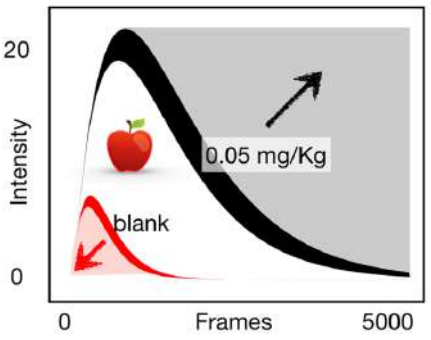
Aristeidis S. Tsagkaris [1,*], Jana Pulkrabova [1], Jana Hajslova [1] and Daniel Filippini [2,*]

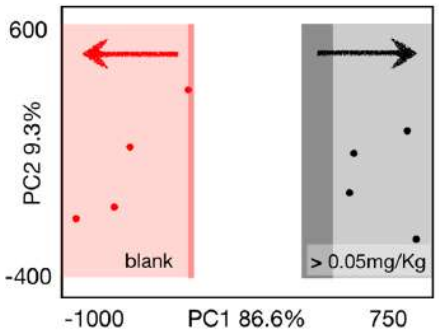
1 Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Technická 5, 6–Dejvice, 166 28 Prague, Czech Republic; pulkrabj@vscht.cz (J.P.); hajslovj@vscht.cz (J.H.)

2 Optical Devices Laboratory, Department of Physics, Chemistry and Biology—IFM, Linköping University, S-58183 Linköping, Sweden

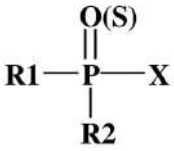
* Correspondence: tsagkara@vscht.cz (A.S.T.); daniel.filippini@liu.se (D.F.)

carbofuran concentration	sample	spiked (mg Kg^{-1})	found (mg Kg^{-1})
blank	a	0	not detected
	b	0	not detected
	c	0	not detected
	d	0	not detected
0.050 mg/kg	a	0.050	0.049
	b	0.050	0.048
	c	0.050	0.049
	d	0.050	0.051





Organophosphate intoxication



- Acetylcholinesterase inhibition
- Terrorist attacks (Tokyo, 1995)
- Recent poisoning of a Russian politician
- 3 million life-threatening human poisonings each year
- Workers exposure due to protective equipment misuse

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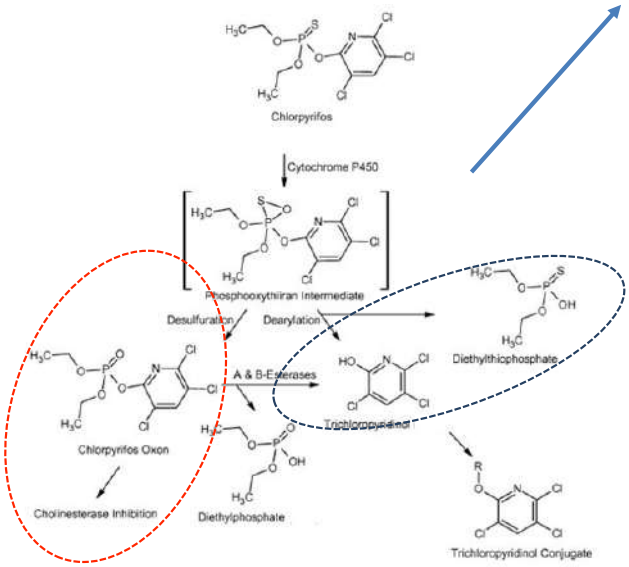
Chlorpyrifos metabolism



- Common matrix: urine
- Up to 48 h to be released in urine
- Common detection method: chromatographic methods

Problems

1. Intoxication cannot be monitored during the early stage
2. Chrom. methods: rather costly, time-consuming and need highly qualified laboratory staff

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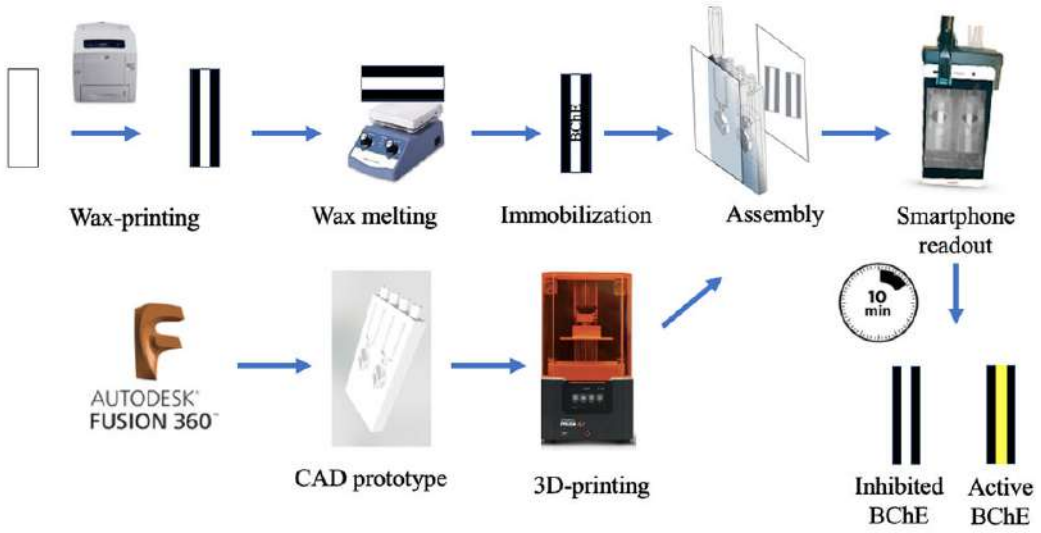
Our approach



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Microfluidic paper-based analytical device (μPAD)

μPAD validation, 2002/657/EC & 98/79/EC

A. Specificity – Cross reactivity %

B. CCβ & ruggedness

Talanta 222 (2021) 121535

Contents lists available at ScienceDirect

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Check for updates

Result

- Negli
  chlorp
  reacti
- strong
  oxon
  compa

A microfluidic paper-based analytical device (μPAD) with smartphone readout for chlorpyrifos-oxon screening in human serum

A.S. Tsagkaris[a,**], D. Migliorelli[b], L. Uttl[a], D. Filippini[c], J. Pulkrabova[a], J. Hajslova[a,*]

[a] Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Technická 5, 166 28, Prague 6 – Dejvice, Prague, Czech Republic
[b] CSEM SA, Center Landquart, Bahnhofstrasse 1, Landquart, Switzerland
[c] Optical Devices Laboratory, Department of Physics, Chemistry and Biology—IFM, Linköping University, S-581 83, Linköping, Sweden

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To sum up

- 3D-printed devices were prototypes and provided integrated sample handling using a smartphone as the analytical detector
- The µPAD can be used as a complementary early warning tool in OP intoxication incidents.
- The achieved work contributes to the establishment of portable chemical analysis

---

- 9 publications (6 published, 2 under review, 1 in preparation) in scientific journals with significant impact in the field
- Several oral and poster presentations in international conferences
- 2 awards (AOAC/Eurofins "Testing for Life" & NACRW student award)
- Co-chairman of the 1st European workshop on portable food analysis

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Acknowledgements

Prof. D. Filippini

Dr J.Nelis and Prof. C. Elliott

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Prof. J. Hajslova
Prof. J. Pulkrabova

Dr. D. Migliorelli

Dr P. Salvador
Prof. M.P. Pillar

Dr M. Suman

Thank You!

PHOTONFOOD – Flexible mid-infrared photonics solutions for rapid farm-to-fork sensing of food contaminants

Prof. Dr. Achim Kohler, PHOTONFOOD Coordinator

SMART TECH for FOOD | Online

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 101016444.

Photonics Public Private Partnership
www.photonics21.org

Contents

Preceding EU projects led by consortium members

Preceding EU projects led by consortium members

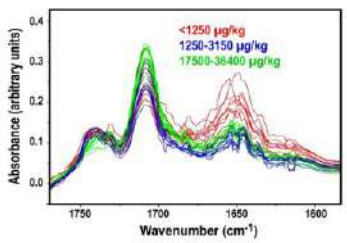
Project	Relevance for PHOTONFOOD
FUST - Source tracking and monitoring of mould contamination in food production (FP7-SME-2012-315271-FUST) (2012-2014).	Development of automated lab system for source tracking of microorganisms along the food production chain. Benchmarking.
MYCOSPEC - Novel infrared Spectroscopic tools for Mycotoxin Determination (FP7-SME-2012-314018 - MYCOSPEC) (2013-2016).	QLCs and thin-film GaAs/AlGaAs waveguide technology. Benchmarking for mycotoxin contamination in PHOTONFOOD.

FUST: Sample preparation for high-throughput FTIR

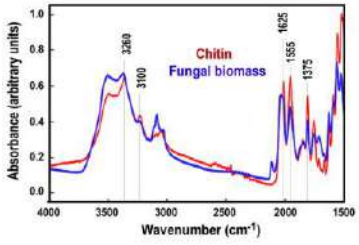
Detection of mycotoxins and fungi by MIR spectroscopy has been demonstrated by the consortium

PHOTON FOOD

Selecting spectral regions for building light sources



QCL IR spectra of 24 maize extracts with different deoxynivalenol contamination levels (Reprinted from Sieger et al. J Scientific reports 2017, 7:44028).

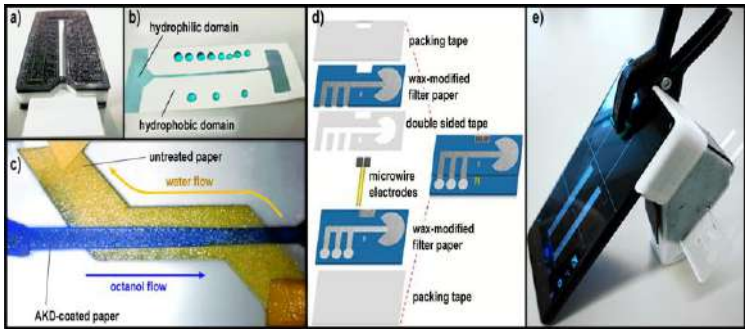


FTIR spectra of chitin and fungal biomass with the characteristic spectral biomarkers for detection of fungal contaminants.

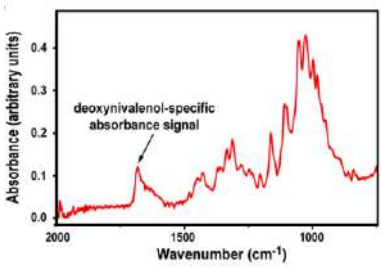
PHOTONFOOD goal

- To provide a **handheld analyser (MI-FI) for monitoring** and a **portable device for reference analysis (HI-FI)**.

Separation and pre-concentration by paper-based microfluidics



(a) 3D-printed polymer scaffolding used for the fabrication of alkyl ketene dimer (AKD) patterned paper microfluidic device[42]. (b) Paper device with untreated (hydrophilic) and AKD-coated (hydrophobic) domains. An aqueous blue dye solution was applied to both domains to demonstrate wettability by water, creating wetted hydrophilic domain and droplets on top of the hydrophobic domain[42]. (c) Demonstration of 3D paper microfluidic device with two-phase countercurrent flow of water (with hydrophilic yellow dye) and octanol (with hydrophobic blue dye)[43]. (d) Illustration of 3D paper network with integrated electrode sensor[44]. (e) Fully integrated 3D-printed system, containing 3D printed microfluidics and paper-based testing (to be published soon



Deoxynivalenol on a paper-based sampling strip

New light sources

New light sources and photonics devices

- MIR light emitting diode (IC-LED) light sources and ICL/QCL lasers combined with novel waveguides, for the detection of microbial and chemical contamination in food and commodities along the food value chain.

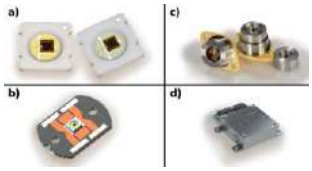
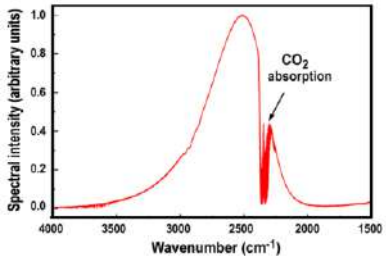


Figure 9: **Light sources for the MI-FI and HI-FI instruments.** (a) Photos of IC-LED chips mounted into SMD (surface mountable device) packages based on AlN for excellent heat removal. (b) CAD model of an AlN package with an IC-LED chip soldered onto a metal PCB, e.g. for integration into a measurement set-up or high-power optical characterization (c) TO3 housings for QCL chips with and without collimation lens. (d) The likely format of tunable laser package; 5-6 electrical drive currents might be used to achieve the required wavelength.



Emission spectrum of an IC-LED designed for the 2500 wavenumber (the strong absorption features around 2300 cm-1 are caused by CO2 present in the measurement path).

New photonics devices

New photonics devices

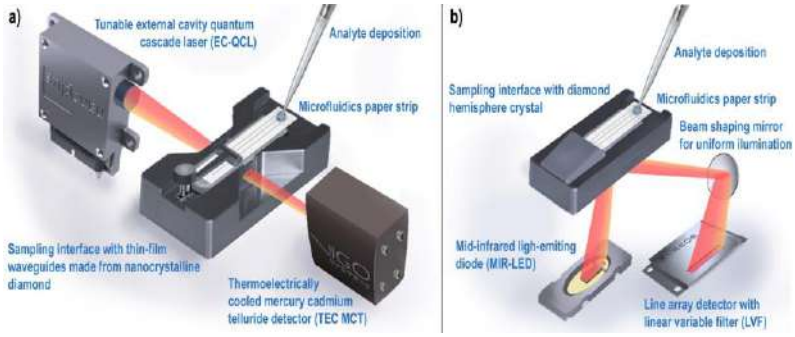


Figure 10: Simplified scheme of the working principles of the HI-FI (a) and MI-FI (b) devices are illustrated focusing on the key innovative components developed within PHOTONFOOD and omitting additional optics/electronics. The HI-FI device is based on an ICL/QCL laser, while the MI-FI device is based on low-cost MIR-LEDs.

Hand-held and portable solution

New solution

a) The use of the MI-FI device for daily monitoring, and

b) the HI-FI device for reference analysis along the value chain. Compared to existing testing off-site, considerably more data will be collected.

c) Data transfer to platforms and data integration will define a new paradigm for food safety control and reduction of food waste, and thereby represent a disruptive technology in agronomy and food industries.

d) PHOTONFOOD devices will feature two different types of photonic technologies (IC-LED and ICL/QCL), and waveguides.

e) a paper-based microfluidic sample handling unit, for detection of minute concentrations of contaminants

Validation

Validation

Photonic based monitoring solution for highly important contamination problems in the food production chain

- Fungal and water mould (oomycetes) contamination and mycotoxins in wheat, nuts, dried fruits and aquaponic-based herbs
- Pesticides and antibiotics in aquaponic-based herbs


Wheat


Nuts


Dried fruits


Aquaponic-based herbs

Key numbers

Key numbers

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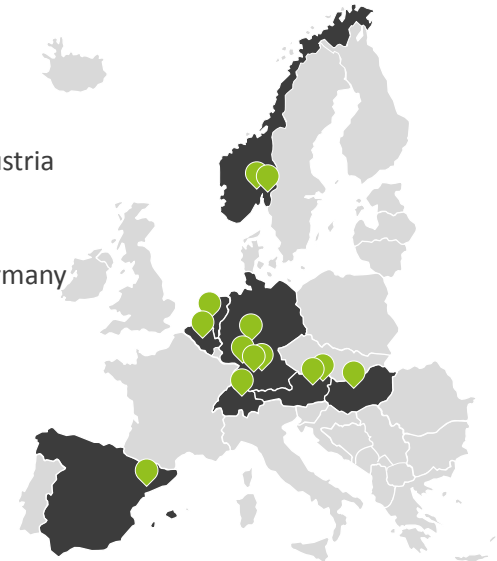
www.photonfood.eu

Partners

- Norwegian University of Life Sciences, Norway
- Ulm University, Germany
- University of Natural Resources and Life Sciences, Austria
- Wageningen University, Netherlands
- National Food Chain Safety Office, Hungary
- nanoplus Nanosystems and Technologies GmbH, Germany
- Romer Labs, Austria
- IRIS Technology Solutions S.L., Spain
- accelopment Schweiz AG, Switzerland
- BIGH Anderlecht SPRL, Belgium
- Seeberger GmbH, Germany
- BAMA Gruppen AS, Norway
- Hahn-Schickard, Germany



Contact

Prof. Dr. Achim Kohler, PHOTONFOOD Coordinator

PHOTONFOOD Coordinator, Norwegian University of Life Sciences

achim.kohler@nmbu.no

Flexible mid-infrared photonic solutions for rapid farm-to-fork sensing of food contaminants

Under construction: www.photonfood.eu

Photonics Public Private Partnership

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