Recent progress in rapid methods for food quality and safety control

Jacob de Jong, Stefan Weigel and Michel Nielen

RIKILT–Institute of Food Safety, Wageningen UR (NL)

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SEVENTH FRAMEWORK PROGRAMME

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Recent progress in rapid methods for food quality and safety control

Experiences from CONffIDENCE

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Contents

- ✓ Introduction to CON*ff*IDENCE
  - What ?
  - Why ?
  - Who ?
- ✓ Results and challenges
- ✓ Conclusions

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Contents

- ✓ Introduction to CON*ff*IDENCE
  - **What ?**

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CON*ff*IDENCE in a nutshell

CONtaminants in *f*ood and *f*eed:

Inexpensive DEtectioN

for Control of Exposure

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CON*ff*IDENCE passport

- ✓ FP7 Collaborative Project first call “Food, Agriculture & Fisheries, and Biotechnology”
- ✓ Duration: May 2008 – April 2012
- ✓ 16 partners from 10 countries, representing universities, research institutes, industry and SMEs
- ✓ Coordinator: RIKILT - Institute of Food Safety, part of Wageningen UR (NL)

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The commodities

Food	&	Feed
✓ Fish/shellfish		Fish feed
✓ Cereals		Cereal-based feed
✓ Potatoes/vegetables		
✓ Honey		
✓ Eggs		
✓ Meat		
✓ Dairy products		

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The target contaminants

- ✓ POPs (Persistent Organic Pollutants):
  - dioxin-like PCBs
  - brominated flame retardants
  - polycyclic aromatic hydrocarbons (PAH)
- ✓ Perfluorinated compounds (PFCs)
- ✓ Pesticides
- ✓ Veterinary drugs: - antibiotics
  - coccidiostats
- ✓ Heavy metals speciation: inorganic arsenic, methyl mercury
- ✓ Biotoxins: - alkaloids
  - marine biotoxins
  - mycotoxins

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Contents

- ✓ Introduction to CON*ff*IDENCE
  - What ?
  - **Why ?**

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Why CON*ff*IDENCE (1) ?

- ✓ To assure chemical safety and quality in the European food supply; support of EC policies and competitiveness of food and feed industries
- ✓ To improve multi-detection (“multiplex”) possibilities
- ✓ To improve inexpensive screening possibilities

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Why CON*ff*IDENCE (2) ?

- ✓ To speed-up analysis for factory approval of lots

- ✓ To contribute to the assessment of risks of emerging contaminants
  - e.g. plant toxins such as pyrrolizidine alkaloids

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Why CON*ff*IDENCE (3) ?

What is the challenge ?

- ✓ Fast and cost-effective screening tests for contaminants in food and feed:
  - Product acceptance by companies
  - Official control

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Contents

- ✓Introduction to CON*ff*IDENCE
  - What ?
  - Why ?
  - **Who ?**

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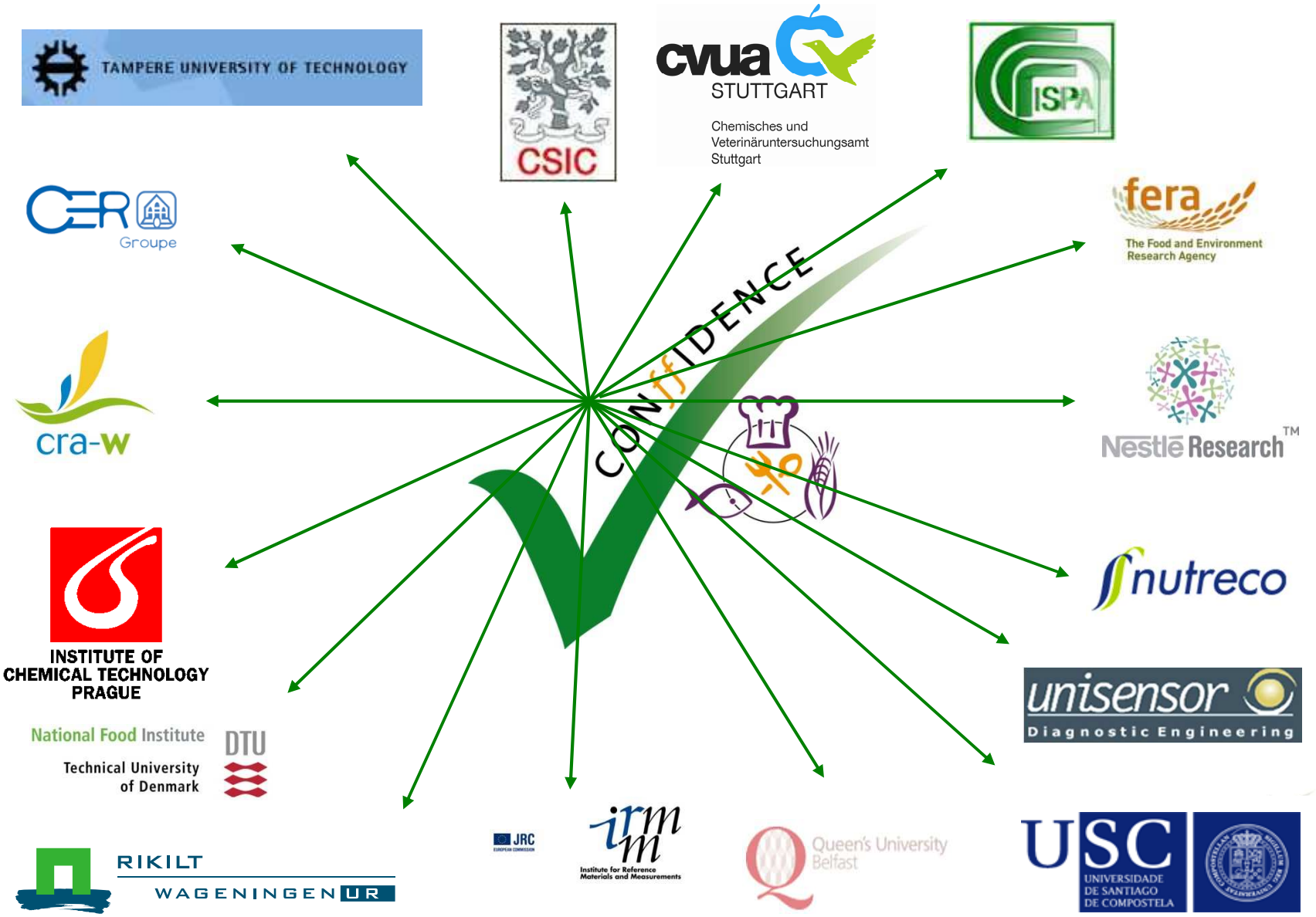
The consortium



Contents

- ✓ Introduction to CON*ff*IDENCE
  - What ?
  - Why ?
  - Who ?
- ✓ Results and challenges

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Current state of progress

- Production and characterization of test materials
- Production of high-quality binders (mainly antibodies)

↓

- Development of the simplified multiplex assays (buffer)
- Development of simplified sample preparation

↓

- Prototype assay protocol
- In-house validation, comparison vs instrumental methods

↓

- Small-scale interlab studies

Year 1

Year 4

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Techniques in CON*ff*IDENCE

- ✓ Bio-analytical techniques
- ✓ Spectroscopic techniques
- ✓ MS-based techniques

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Techniques in CONffIDENCE

- ✓ **Bio-analytical techniques**
- ✓ Spectroscopic techniques
- ✓ MS-based techniques

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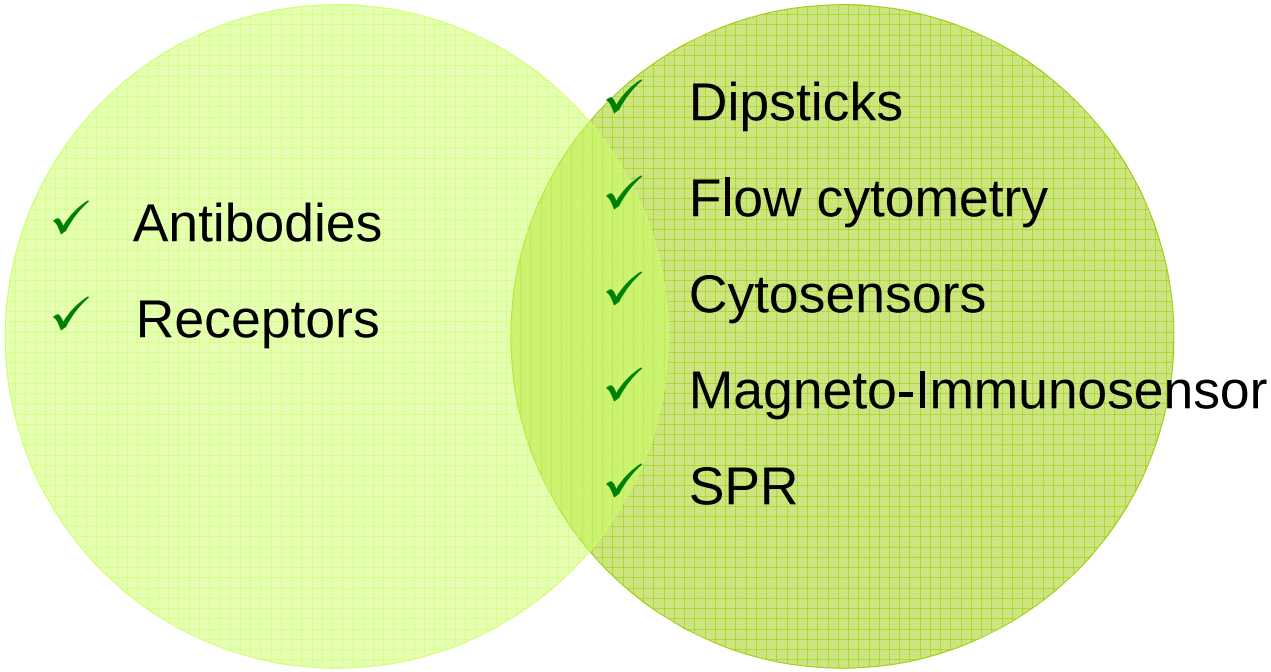
Bio-analytical detection



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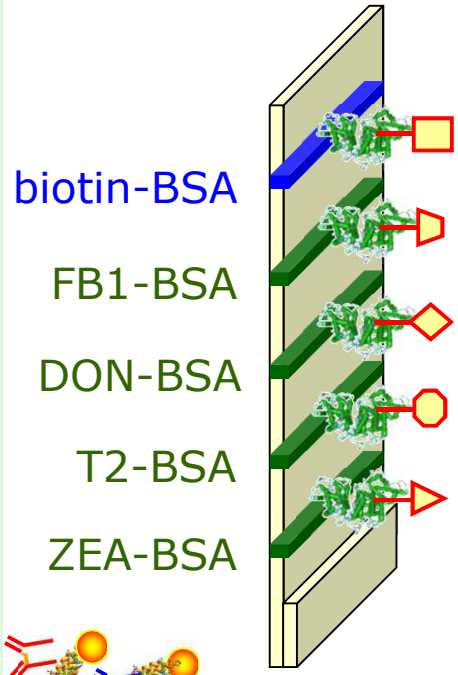
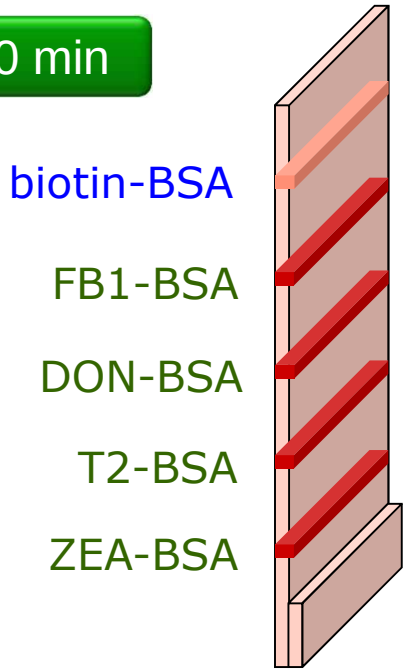
Multiplex dipstick for mycotoxins

- *Indirect competitive immunoassay; 10 min incubation at 40 °C*

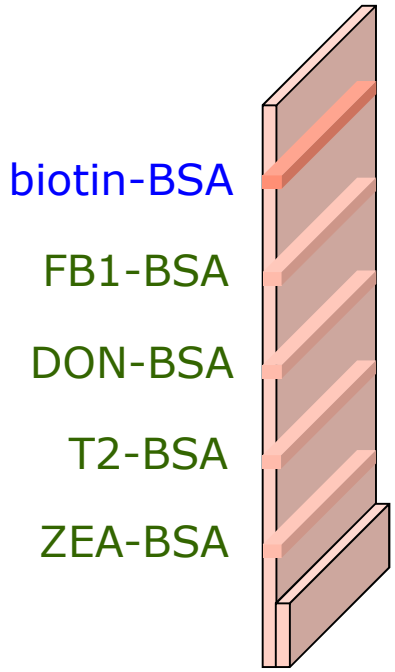


10 min



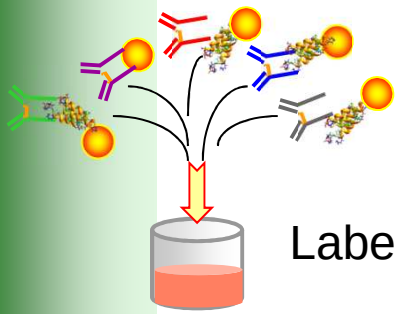
NEGATIVE sample

Test Lines darker than CTRL line



POSITIVE sample

Test Lines lighter than CTRL line



Labelled antibodies

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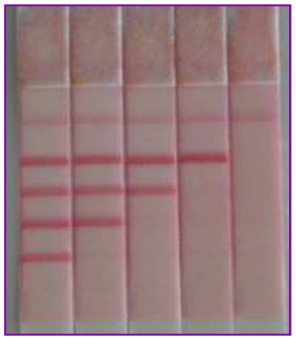
Mycotoxins: procedure for maize feed

Total analysis time: 30 min

- ✓ Add water; 2 min blending
- ✓ Add methanol; 2 min blending

Dilution and analysis

Incubation at 40 C, 10 min
Migration, 10 min



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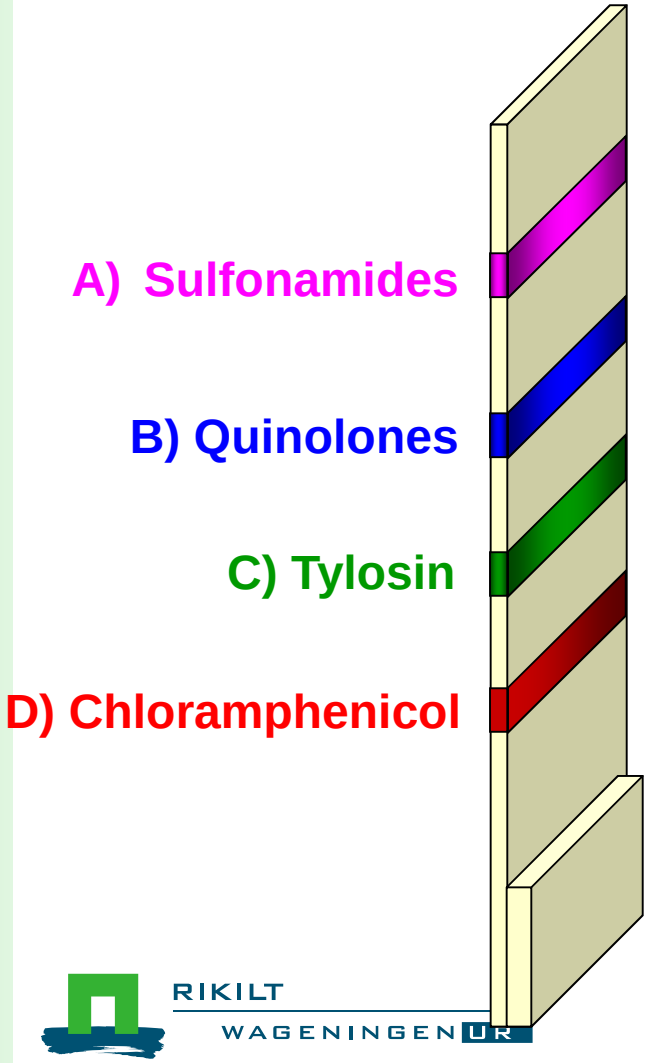
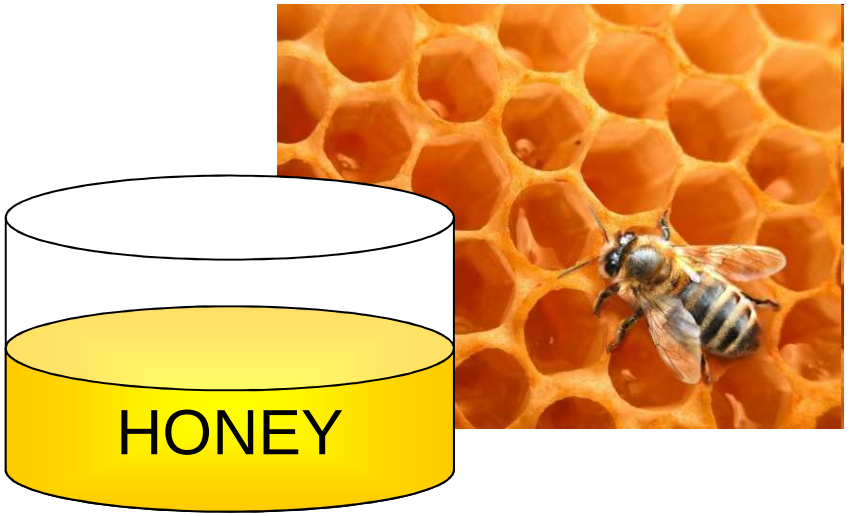
Multiplex dipstick Antibiotics in honey (1)





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Nestlé Research™

fera The Food and Environment Research Agency

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Multiplex dipstick Antibiotics in honey (2)

Two approaches under development:

1. A rapid laboratory based procedure incorporating acid hydrolysis (for sulfonamides), a generic ethyl acetate extraction and concentration step; > 80 % of the target analytes can be detected below recommended ML's; **< 90 min**
2. A simplified field based procedure incorporating an acid hydrolysis / buffer dilution step for detection of gross contamination in raw honey under field test conditions; **< 30 min**

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Requirements for dipstick assays

- ✓ Good quality antibodies or receptors
- ✓ Conjugates for:
  - ➢ Production of antibodies
  - ➢ Assay competitors
  - ➢ ELISA characterization

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Queen's University Belfast

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Requirements for dipstick assays

- ✓ Good quality antibodies or receptors
- ✓ Conjugates for:
  - ➢ Production of antibodies
  - ➢ Assay competitors
  - ➢ ELISA characterization
- ✓ Expertise in dipstick production

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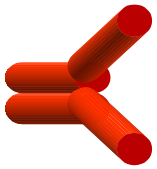
Dipstick experiences in CONffIDENCE

- ✓ Good quality antibodies (and prototype dipsticks !) have been produced for e.g.
  - Mycotoxins
  - Ergot alkaloids



- ✓ Sensitivity may be a problem, viz. for chloramphenicol: MRPL in honey: 0.3 µg/kg !

- ✓ Sensitivity of antibodies may differ between ELISA characterization and dipstick prototype, e.g. for pyrrolizidine alkaloids ELISA results are better

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Dipstick demonstrations (+ posters)

CON*ff*IDENCE Open Day

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Dipstick demonstrations (+ posters)

CON*ff*IDENCE Open Day

- ✓ Mycotoxins in wheat, N. Nivarlet
- ✓ Antibiotics in honey, V. Chabottaux

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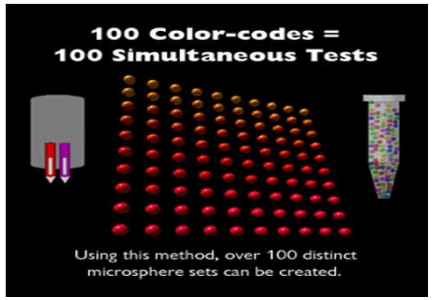
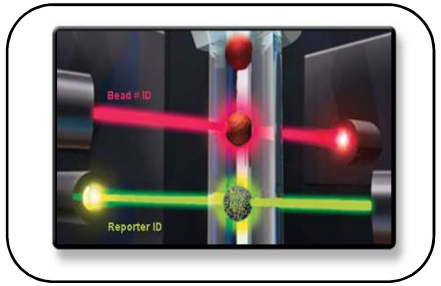
Multiplex flow cytometry

- ✓ Flow cytometry in combination with the xMAP technology (Luminex)
- ✓ Principle: see presentation of A. Meimaridou (RIKILT)
- ✓ Applications in CON*ff*IDENCE:
  - POP's in fish
  - Coccidiostats: cross-contamination of feed and transfer to eggs



http://www.luminexcorp.com/



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Multiplex flow cytometry: results

- ✓ Good results both for
  - ➢ POP's in fish: see presentation A. Meimaridou
  - ➢ Coccidiostats:
    - ✓ Cross-contamination in feed and residues in eggs
    - ✓ Target analytes: lasalocid A, monensin, salinomycin, narasin, nicarbazin and diclazuril
    - ✓ Most probably it will be possible to reach the MRL's in egg and feed (exception: diclazuril in feed ?)
    - ✓ Multi-screening of 40 samples per day per analyst
    - ✓ See poster + video + PPT M. Bienenmann-Ploum, Open Day

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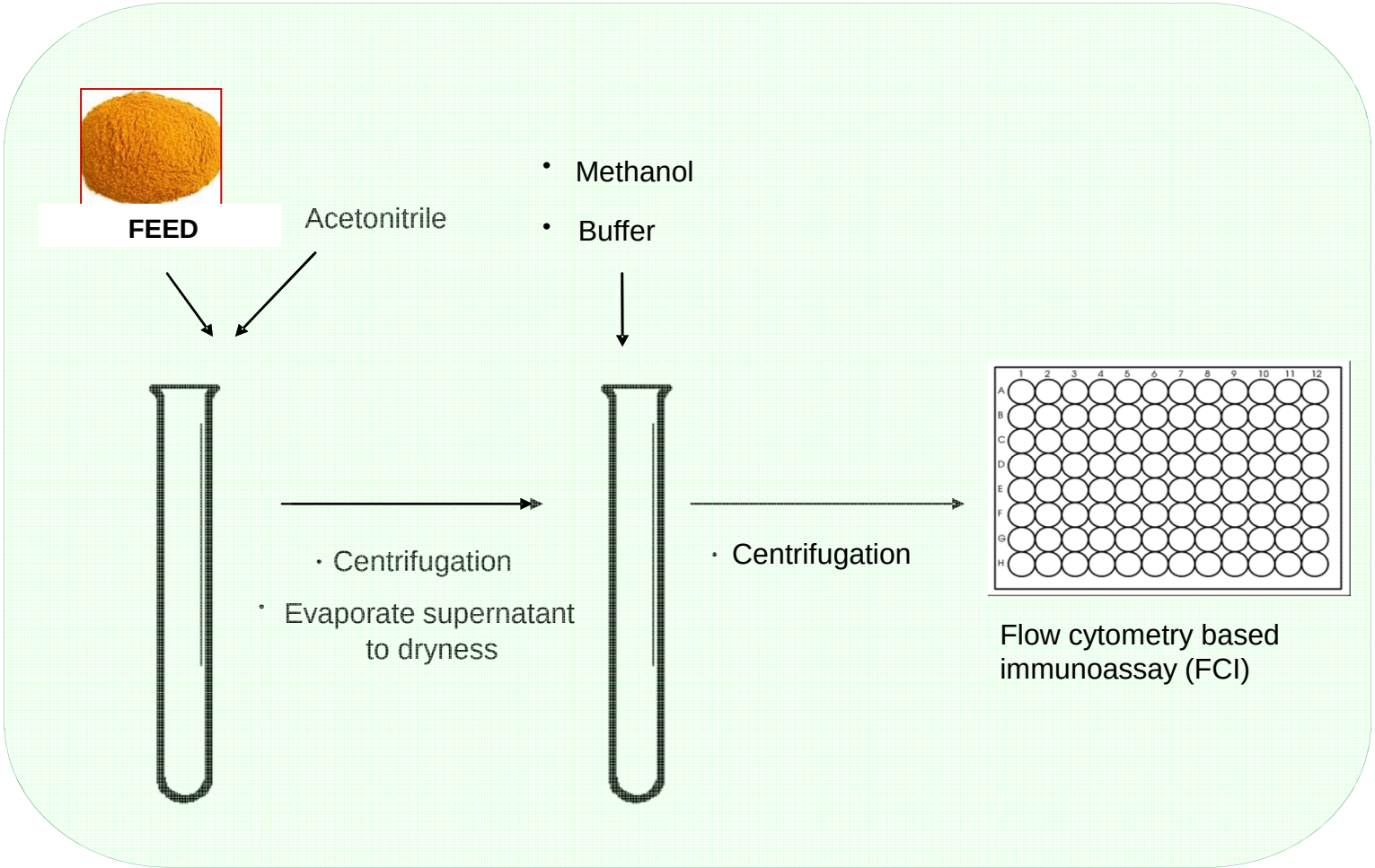
5-Plex assay for coccidiostats



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JRC

irmm Institute for Reference Materials and Measurements

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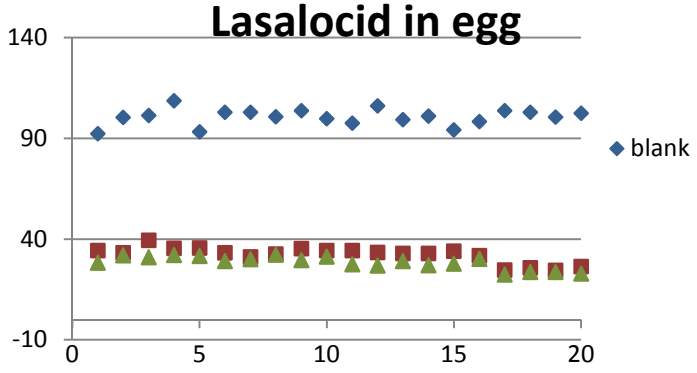
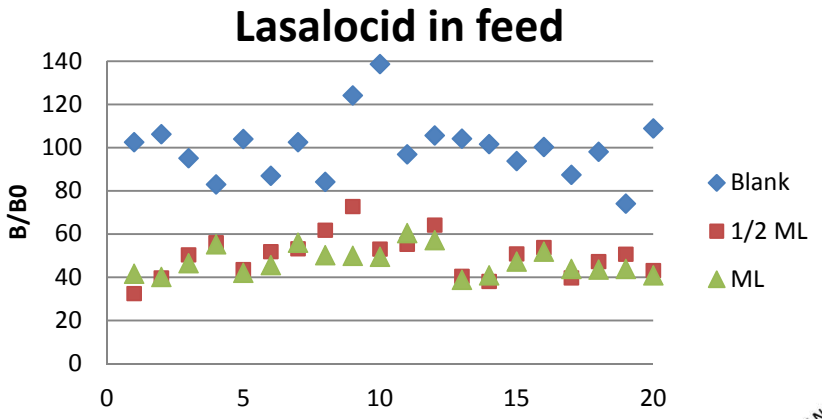
Flow cytometry experiences in CONffIDENCE - Coccidiostats

- ✓ Performance of antibodies may differ between ELISA characterization and flow cytometry: in most cases better results were obtained with flow cytometry
- ✓ Difference between matrix EGGS and FEED





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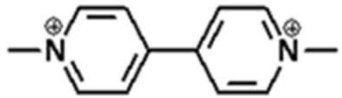
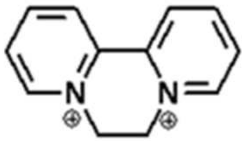
Electrochemical immunosensor (1)

- ✓ Objective: multiplex sensor for paraquat (PQ) and diquat (DQ)
- ✓ Specific antibody against paraquat could be produced but for diquat despite many attempts it proved to be impossible to synthesize a suitable hapten



Paraquat



Diquat

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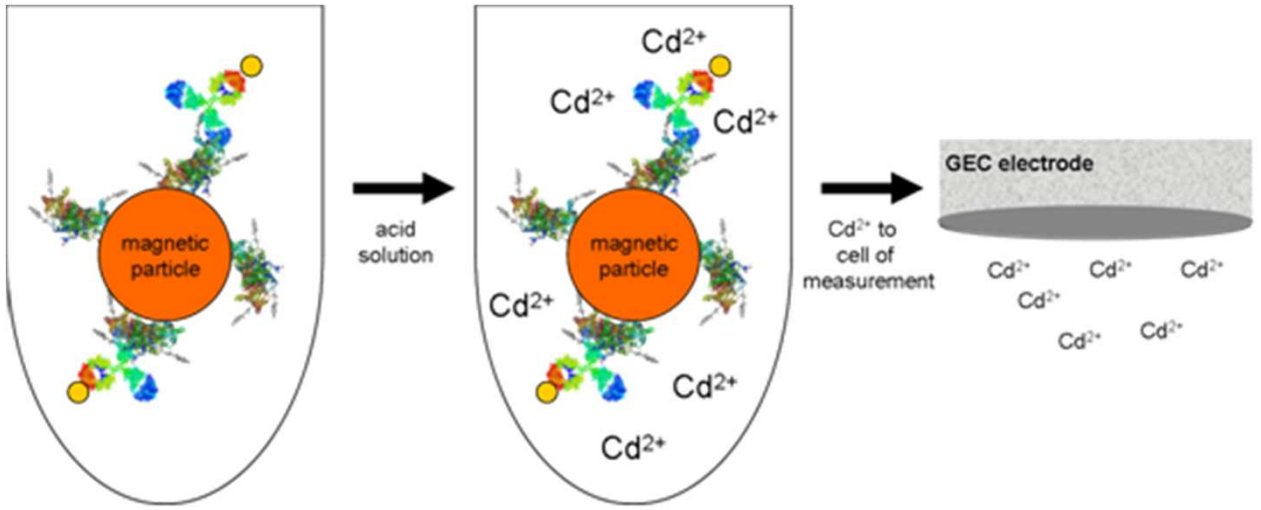
Electrochemical immunosensor (2)

- ✓ LOD PQ in potato samples: < 0.001 mg/kg
- ✓ See poster E. Valera, CON*ff*IDENCE Open Day



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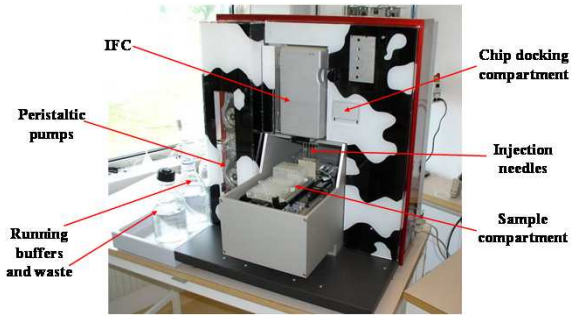
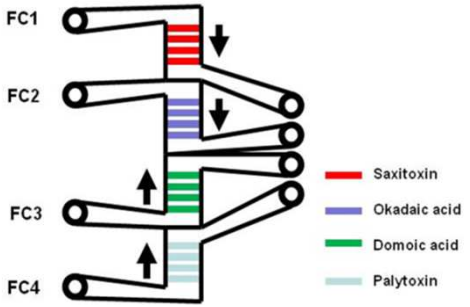
Multiplex Surface Plasmon Resonance (SPR)

- ✓ Multiplex Immunoassay based on optical SPR biosensors
- ✓ Marine Biotoxins: high-throughput multiplex method established for representatives from PSP / DSP / ASP shellfish toxin classes + Palytoxin (emerging)
- ✓ See presentation K. Campbell, Natural Toxins II session
- ✓ See poster + video K. Campbell, Open Day





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Heavy metal speciation

Objective:

- ✓ Speciation of inorganic arsenic and methyl mercury in food and feed

Two different approaches:

1. Cytosensor platform: Whole-cell, light-emitting microbial sensors; to be incorporated into a portable device
2. Selective separation by SPE, followed by AAS

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National Food Institute
Technical University of Denmark
DTU

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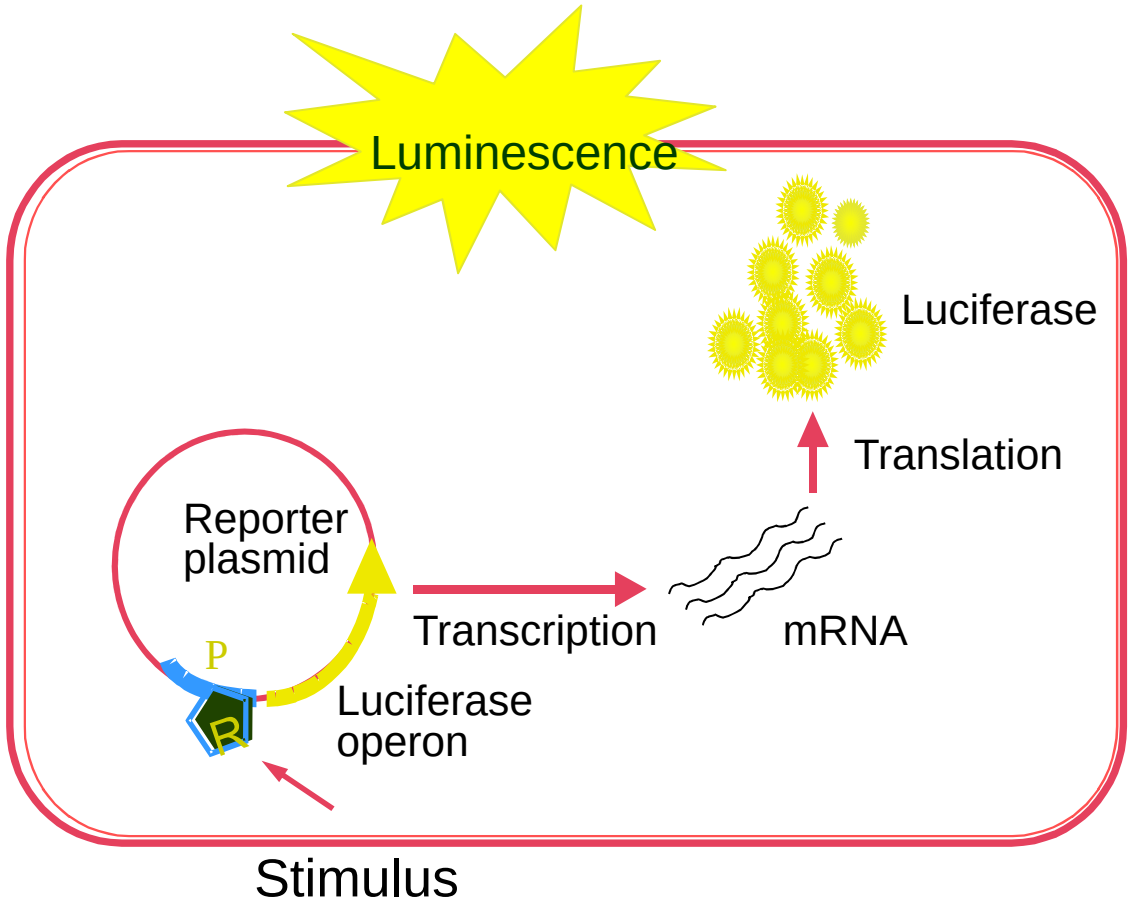
Principle of the luminescence-based sensor strains for specific detection of inorgAs and MeHg



P = promoter

R = regulatory protein

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Results Cytosensor platform – iAs (1)

- ✓ Specific cytosensor for inorganic arsenic (iAs) was available prior to the start of CON*ff*IDENCE – was already successfully applied in environmental (sediment) analysis
- ✓ Dilute acid (HCl, HNO3) not compatible with sensor cells → boiling with water applied; sufficient recovery

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Results Cytosensor platform – iAs (2)

- ✓ At target LOD's (0.5 ppm for food and 1 ppm for feed) signal is below quasi-linear range of the calibration curve
- ✓ Inhibition of luminescence signal: strongly dependent on matrix type, e.g.
  - ➢ fish meal ←→ fish fillet
  - ➢ mussel ←→ cockney
- ✓ Conclusion: not possible to develop a rapid and robust test

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Techniques in CONffIDENCE

- ✓ Bio-analytical techniques
- ✓ Spectroscopic techniques
- ✓ MS-based techniques

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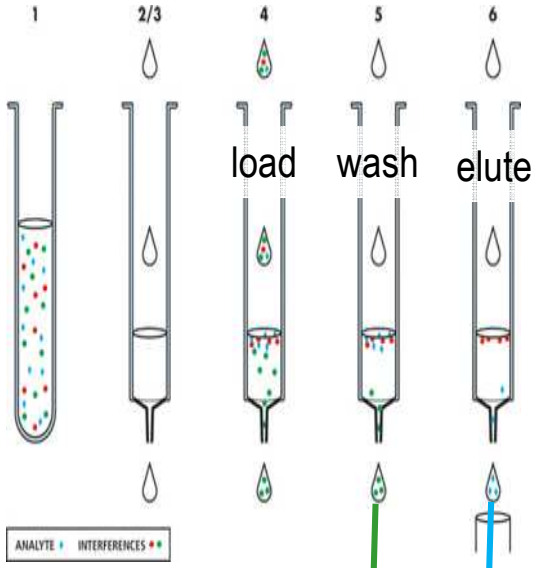
SPE-HG-AAS – a novel speciation alternative…

μ-wave extraction → Separation by SPE → Detection by HG-AAS

Inexpensive detection system



Sequential elution for selective separation of inorg As from organo As species by SPE

See presentation R. Rasmussen

See poster + demo R. Rasmussen

Open Day

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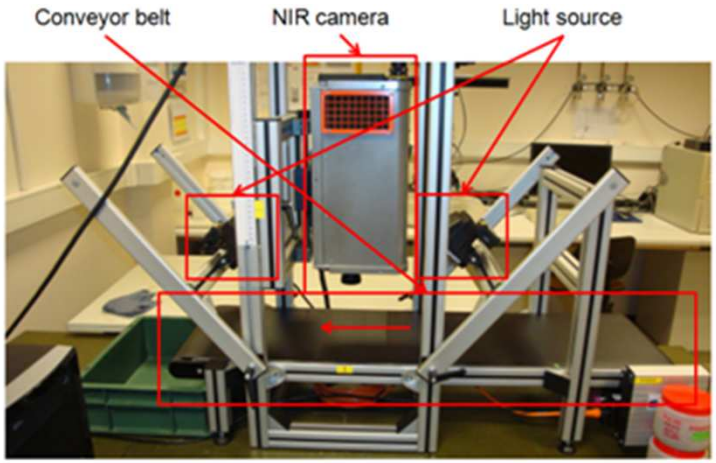
NIR Hyperspectral imaging (1)

Objective:

- ✓ Detection and quantification of ergot sclerotia in wheat
- ✓ Moving belt system – transfer to a feed mill lab



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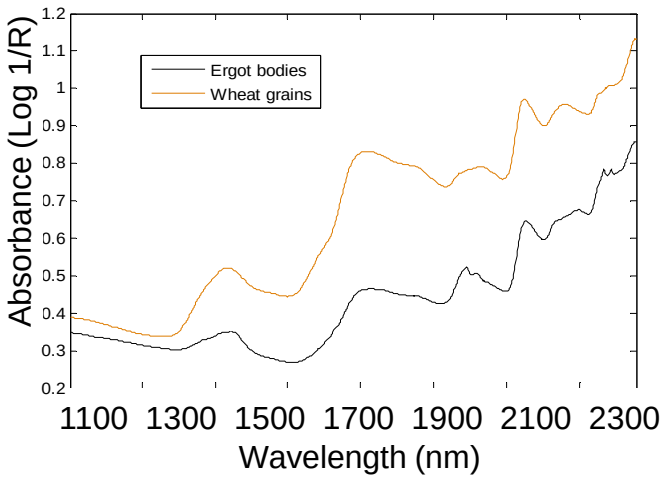
NIR Hyperspectral imaging (2)

Results:

- ✓ Proof of principle has been shown
- ✓ Satisfactory LOQ (0.5 mg/kg)
- ✓ Rapid: 250 g sample in 1 min (microscopy: 30 to 60 min)
- ✓ See poster + movie P. Vermeulen Open Day



Ergot bodies

Wheat grains

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Techniques in CONffIDENCE

- ✓ Bio-analytical techniques
- ✓ Spectroscopic techniques
- ✓ MS-based techniques

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Results for GC-MS of POPs

POPs: - PCBs (dioxin-like and non-dioxin-like)
- brominated flame retardants
- polycyclic aromatic hydrocarbons (PAHs)

✓ Simplified and rapid determination of PCBs, PBDEs and PAHs in seafood and fish feed integrated into a single method

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Jana Hajslova, Jana Pulkrabova and Lucie Drabova

✓ See poster Lucie Drabova, Open Day

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Integrated sample preparation

BFR | PCB | PAH | Non-*ortho* PCB

Extraction
Shaking (H_2O + ethylacetate)

↓

Partition (transfer into organic phase) induced by $MgSO_4$ + NaCl

↓

Clean up
Silica minicolumn

↓

Identification & quantification
GC-TOFMS (EI)
GC GC-TOFMS (EI)

Isolation
10 min

Clean up
30 min

Identification & quantification
1 h

6 SAMPLES / < 1 HOUR

SINGLE GC INJECTION

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Oil Spill - Gulf Of Mexico 2010

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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 hydrocarbon (PAH) "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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AOAC INTERNATIONAL

NEWS FLASH

PAH Update: Candidate Method to Enter Collaborative Study

Due to the urgent need for rugged, reliable methods to determine polycyclic aromatic hydrocarbon (PAH) compounds in seafood from the Gulf, AOAC expedited a process that, ultimately, led to a candidate method ready for AOAC validation. AOAC facilitated a stakeholder panel and working group meetings; established a fitness-for-purpose statement; issued calls for methods and collaborators; evaluated available methodology purported to meet fitness for purpose; and selected the best candidate method for further evaluation and validation—all within 3 months. Further, AOAC has developed, and is currently finalizing, a validation study protocol, and the method is about to enter into collaborative study. AOAC validation of a method to detect PAHs in seafood is expected to take less than 6 months from start to finish.

In choosing a candidate method, AOAC reviewed approximately 30 methods for the detection of PAHs. Consequently, the PAH Working Group on Quantitative Methods, chaired by **Gina Ylitalo**, NOAA NWSFC, recommended a method by **Lucie Drabova et al.** at the Institute of Chemical Technology in Prague, Czech Republic as the most promising candidate method for further evaluation and, ultimately, validation as an AOAC-approved method.

In general, the method (Rapid Method for Simultaneous Determination of PAHs, Polychlorinated Biphenyls, and Polybrominated Diphenyl Ethers in Fish and Seafood Using GC-TOF/MS) is easy to perform, uses common laboratory equipment, and meets fitness-for-purpose and AOAC single-laboratory validation (SLV) requirements. The method uses a gas chromatography system coupled to a mass spectrometer detector that allows identification and quantification of all target PAHs.

Contents

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- ✓ Conclusions

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Conclusions

- ✓ The CON*ff*IDENCE project contributes to improved safety of food and feed through the production of rapid, simplified, cost-effective and high-throughput methods for a wide variety of chemical contaminants
- ✓ Progress has been made for a variety of bio-analytical, spectroscopic and MS-methods
- ✓ Part of these methods (the dipsticks) can be used under "field" conditions; other methods require well equipped laboratories
- ✓ Some challenges ahead, e.g. heavy metal speciation

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More information

Website: **www.conffidence.eu**

Contact:

coordination@conffidence.eu

e-newsletter

(registration on website)

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Open Day CONffIDENCE

CONffIDENCE: Safer food through rapid and cost-efficient tests for chemical contaminants in the food chain

Open Day at RAFA 2011

3 November 2011

Stella Hall: 13:00 – 16:00

Posters (23)

Demonstrations (8)

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Acknowledgements

- ✓ Many colleagues from CON*ff*IDENCE partners
- ✓ The CON*ff*IDENCE project has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n°KBBE-211326

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Thank you for your attention !

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