CON*ff*IDENCE in Food and Feed: a new European Research Project

Jacob de Jong, Stefan Weigel and Michel Nielen

www.con*ff*idence.eu




RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

SEVENTH FRAMEWORK PROGRAMME

CONFIDENCE

Contents

- Introduction to CON*ff*IDENCE
  - What ?
  - Why ?
  - Who ?
- Methods in CON*ff*IDENCE
  - Detection modes
  - Primary extraction methods
  - Sample preparation
- Method validation
- Expected results

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Contents

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

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CON*ff*IDENCE in a nutshell

Chemical CONtaminants

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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
- 17 partners from 10 countries, representing universities, research institutes, industry and SMEs
- Volume: 7.5 Mio €
- Co-ordinator: RIKILT - Institute of Food Safety, part of Wageningen UR (NL)

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The objectives

- Development and validation of new simplified inexpensive detection methods for chemical contaminants from farm to fork
- Improved exposure assessment through monitoring of selected contaminants
- Contribute to validation of predictive hazard behaviour models
- Dissemination and training of new detection methods to all relevant stakeholders, to advance technology exploitation

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The target contaminants

- POPs: - dioxin-like PCBs + metabolites
  - brominated flame retardants
  - polycyclic aromatic hydrocarbons (PAH)
- Perfluorinated compounds (PFCs)
- Pesticides: paraquat/diquat, dithiocabamates
- Veterinary drugs: - antibiotics, e.g. tetracyclines
  - coccidiostats, e.g. ionophores
- Heavy metals speciation: inorganic arsenic, methyl mercury
- Biotoxins: - alkaloids
  - marine biotoxins
  - mycotoxins

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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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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, e.g. for antibiotics
- To improve inexpensive screening possibilities, e.g. for metal speciation

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Why CONffIDENCE (2) ?

- To speed-up analysis for factory approval of lots

- To contribute to the assessment of risks of emerging contaminants
  - New classes of marine biotoxins: spirolides and palytoxins
  - Plant toxins, e.g. pyrrolizidine alkaloids
  - Perfluorinated compounds

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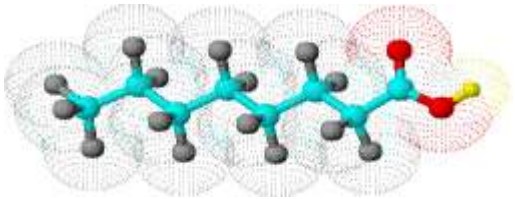
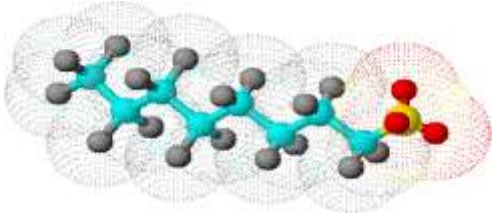
Why CON*ff*IDENCE (3) ?

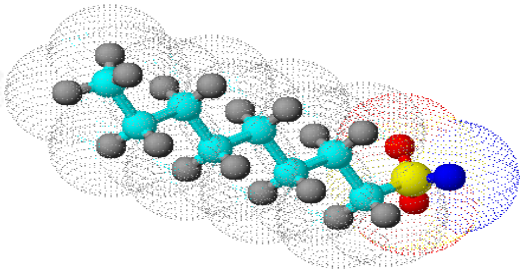
- **Perfluorinated compounds**



PFOA



PFOS



PFOSA

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Why CONffIDENCE (4) ?

- To contribute to the generation of data for exposure assessment, e.g. for PFC's:

efsa
European Food Safety Authority

Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts
Scientific Opinion of the Panel on Contaminants in the Food chain
Adopted on 21 February 2008

"Due to the substantial lack of suitable analytical data, many assumptions have been made in order to derive exposure estimates."

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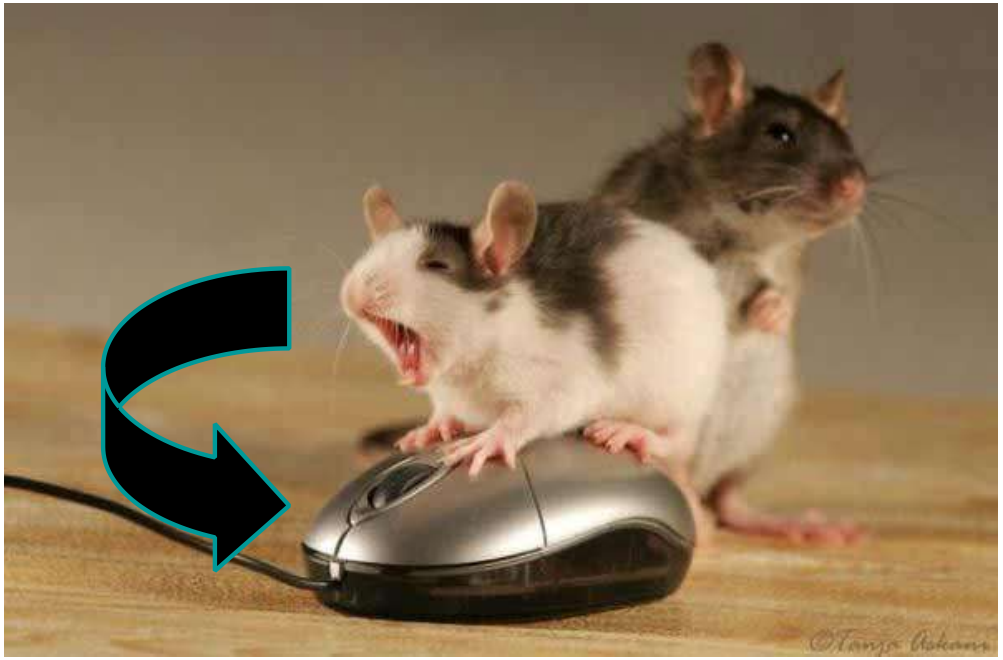
Why CON*ff*IDENCE (5) ?

- To replace animal based bioassays, e.g. the mouse assay for marine biotoxins



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Contents

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  - **Who ?**

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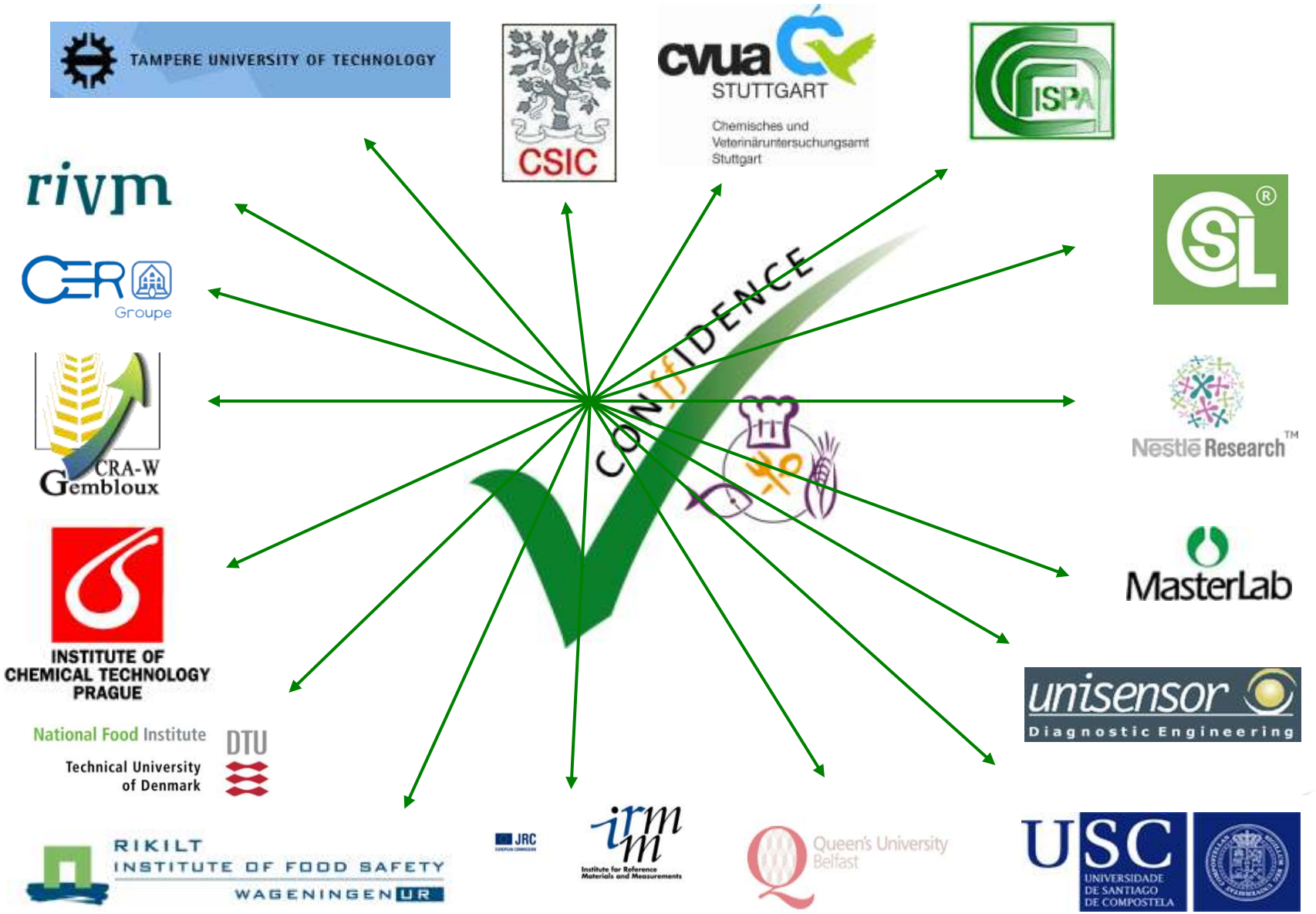
The consortium

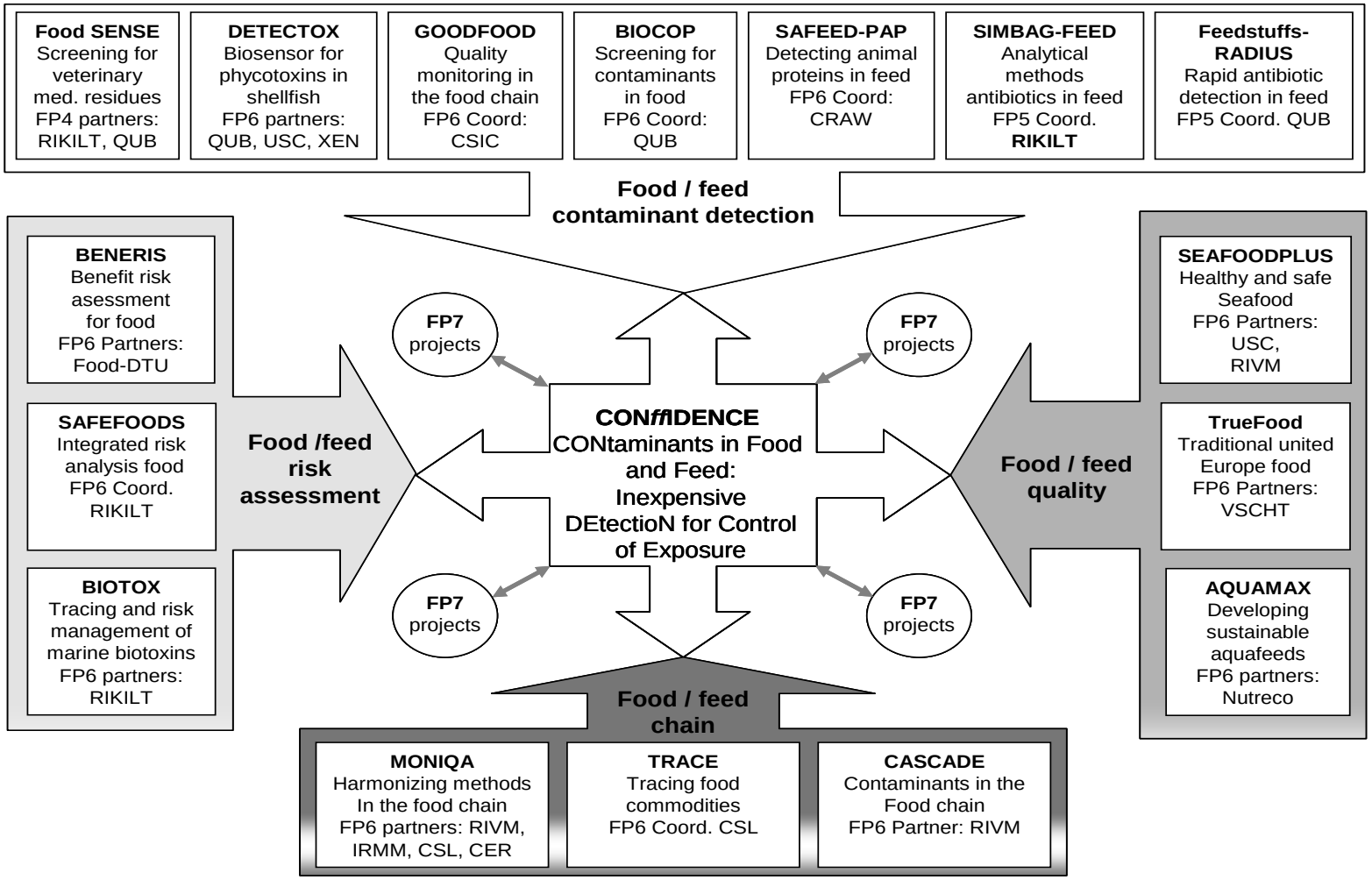


The Advisory Board

Representatives from:

- FAO/IAEA
- DG-SANCO
- EFSA CONTAM panel
- CEN committee Food analysis – Horizontal methods (CEN/ TC 275)

European approach



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- Introduction to CON*ff*IDENCE
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- Methods in CON*ff*IDENCE
  - **Detection modes**

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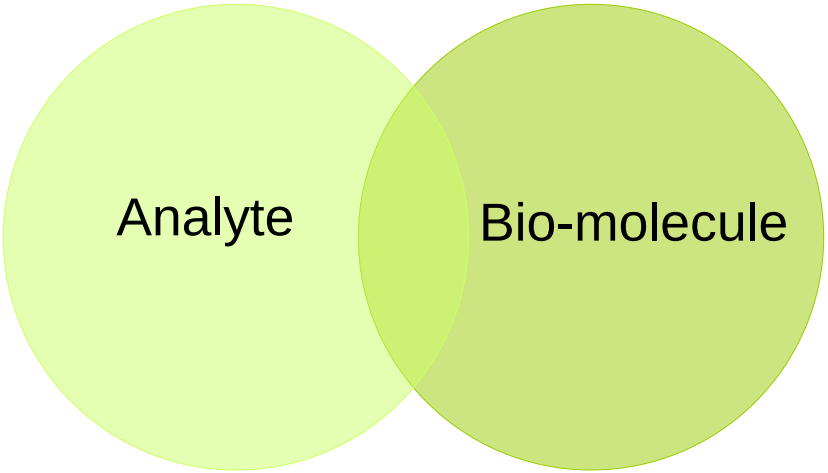
Detection modes

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

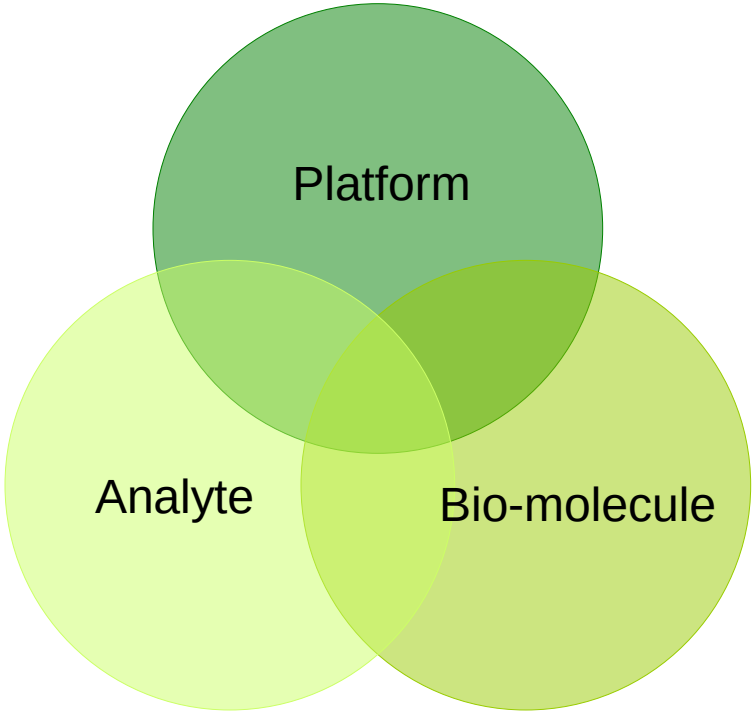
Detection modes

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

Bio-analytical detection



Bio-analytical detection



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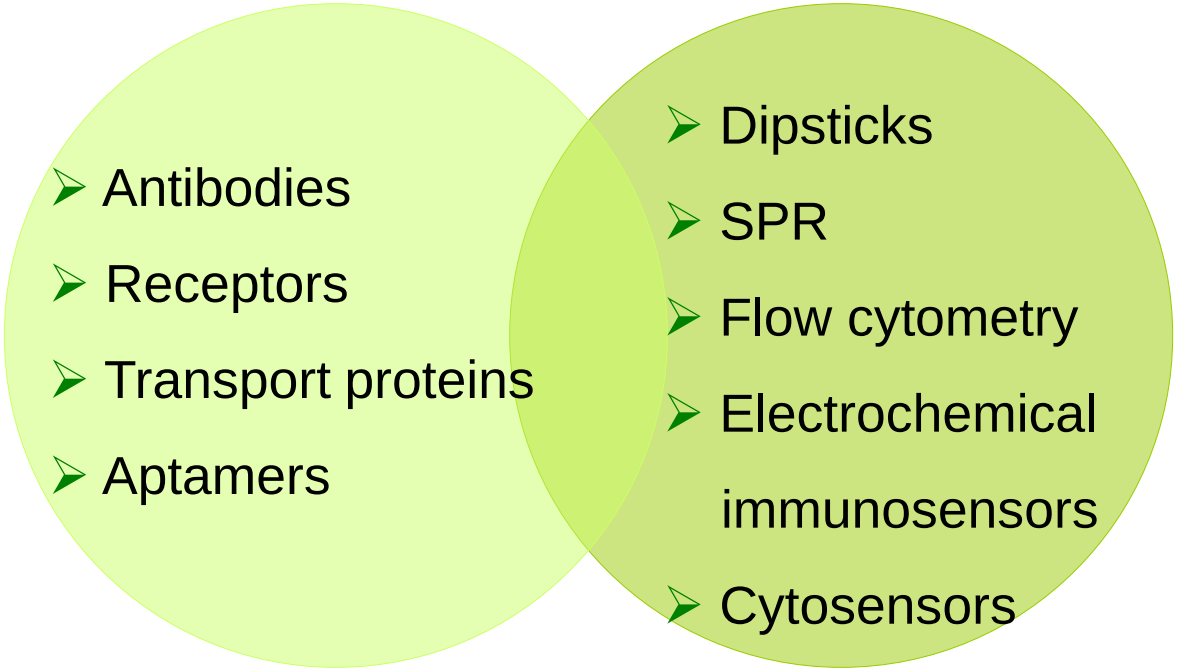
Bio-analytical detection



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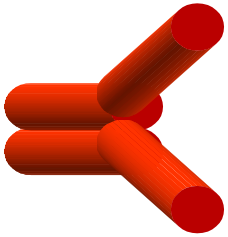
Bio (=binding) molecules

- **Antibodies**
- Receptors
- Transport proteins
- Aptamers

Bio (=binding) molecules

- **Antibodies**
  - Polyclonal *and* Monoclonal
  - Already available *or* produced in CON*ff*IDENCE



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Bio (=binding) molecules

- Antibodies
- **Receptors**
- Transport proteins
- Aptamers

Receptors

- Receptors = proteinic complexes binding analytes with high specificity and affinity

Application in CON*ff*IDENCE:

- Whole-cell, light-emitting microbial sensors for heavy metals
- Reporter gene technology enabling rapid detection of metal bioavailability

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National Food Institute Technical University of Denmark DTU

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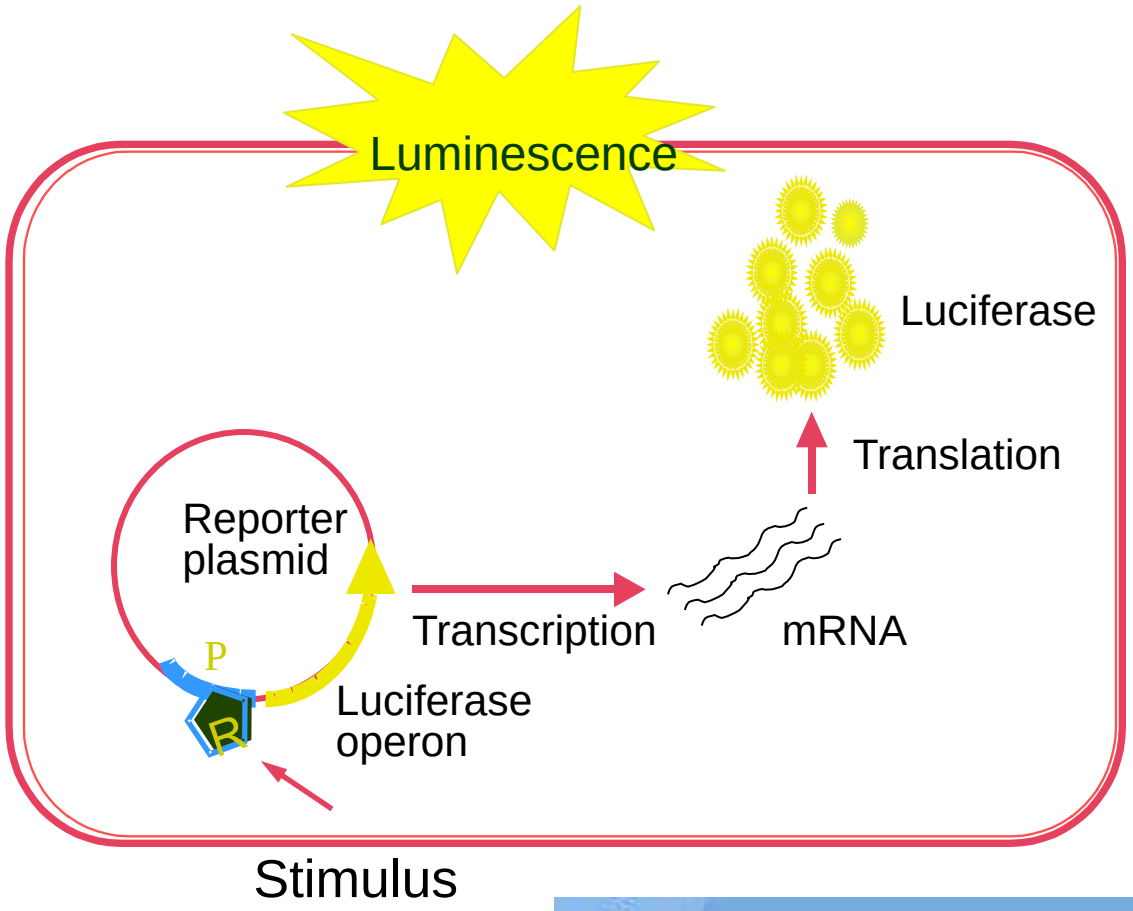
Principle of the luminescence-based sensor strains for specific detection of inorgAs and MeHg



P = promoter

R = regulatory protein

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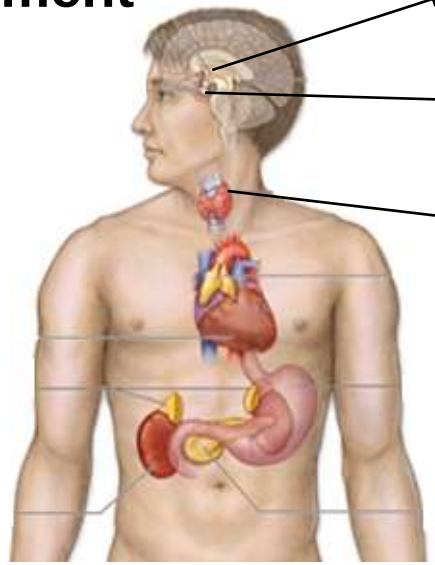
Bio (=binding) molecules

- Antibodies
- Receptors
- **Transport proteins**
- Aptamers

Transport proteins: mechanism

- **Brain Development**
- **Metabolic rate**



Hypothalamus

⇩ TRH

Pituitary

⇩ TSH

Thyroid gland

⇩ **Thyroxine (T4)**

Blood Stream

POPS and Metabolites +

Transport Proteins:

T4 Binding Globulin (TBG) 75%

Transthyretin (TTR) 20%

Human Serum Albumin (HSA) 5%

⇩

T4 Cell Transporter !!

Target Cells

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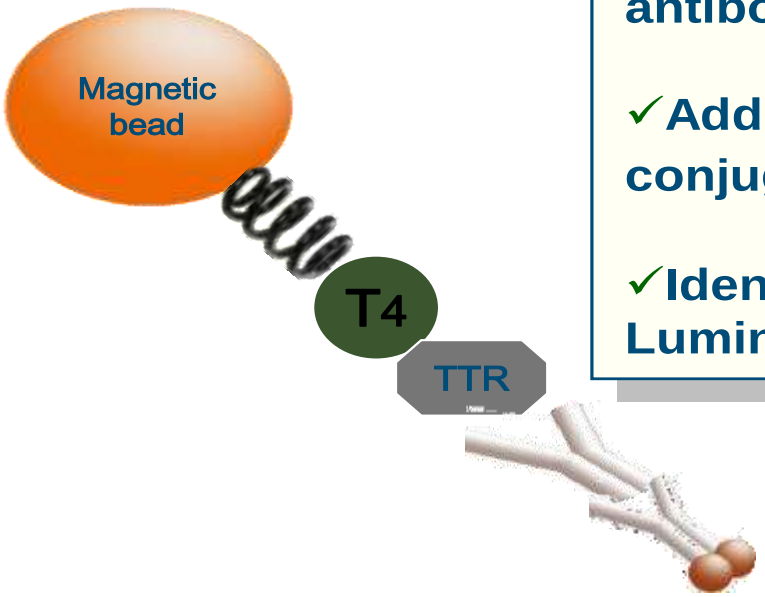
Transport proteins in CON*ff*IDENCE: OH-metabolites of PBDEs

- ✓**Thyroxine (T4) immobilization on the bead surface via spacer.**
- ✓**Addition of transthyretin (TTR).**
- ✓**Bound TTR detection with monoclonal antibody against TTR.**
- ✓**Addition of the secondary antibody conjugated with Phycoerithrin (PE).**
- ✓**Identification and quantification by Luminex.**



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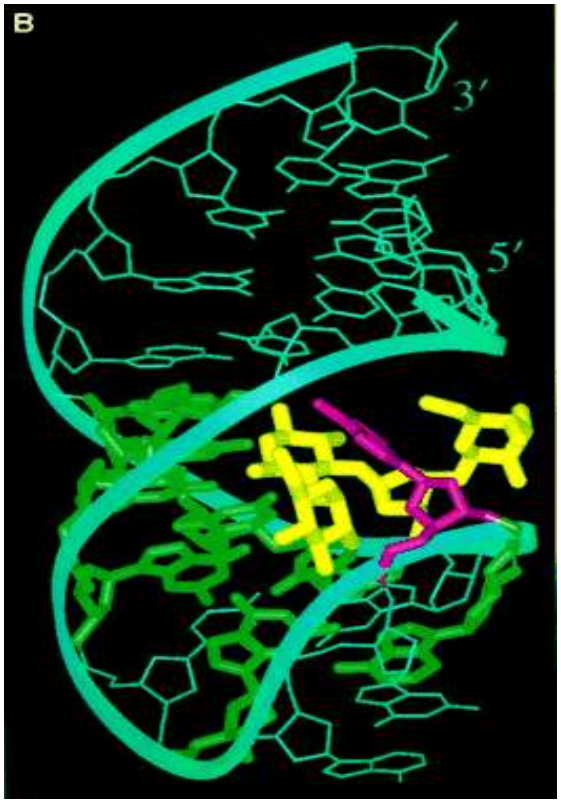
Bio (=binding) molecules

- Antibodies
- Receptors
- Transport proteins
- **Aptamers**

Aptamers

- *Aptus* : to fit, *Meros* : particle

= short single stranded **oligonucleotides** that form 3-D structures that **bind** with **high affinity** and **specificity** to proteine and non-protein targets



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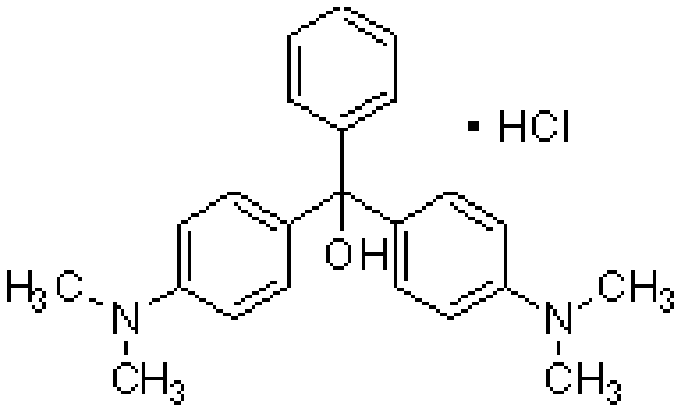
Aptamers in CONffIDENCE:

- Malachite green (MG) in fish tissue and fish feed



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Platforms

- **Multiplex dipstick assays**
- Multiplex SPR biosensor
- Multiplex flow cytometry
- Electrochemical immunosensors
- Cytosensors

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Principle of Dipstick Assay

Lateral Flow

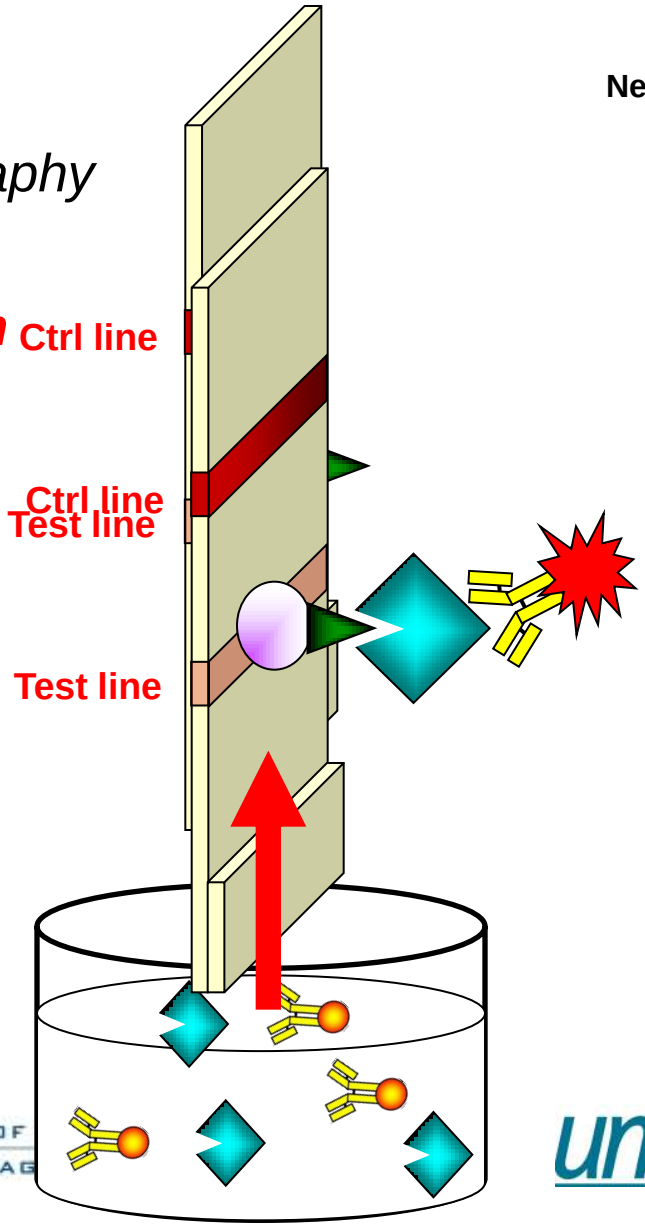
Chromatography

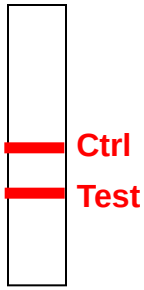
Indirect

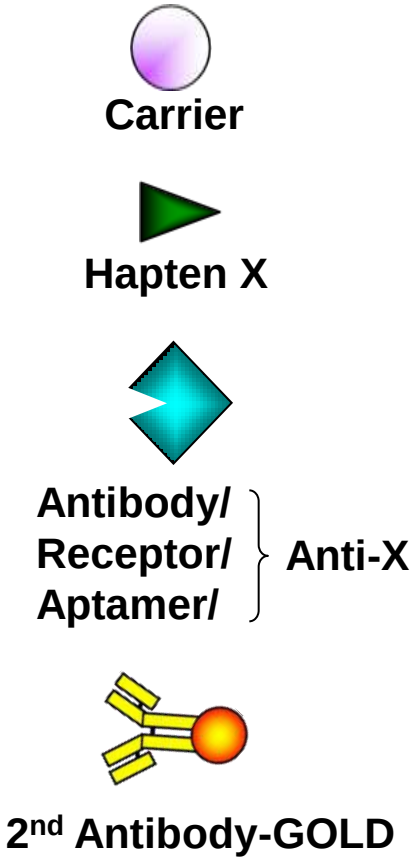
Competition

Dipstick

Assay







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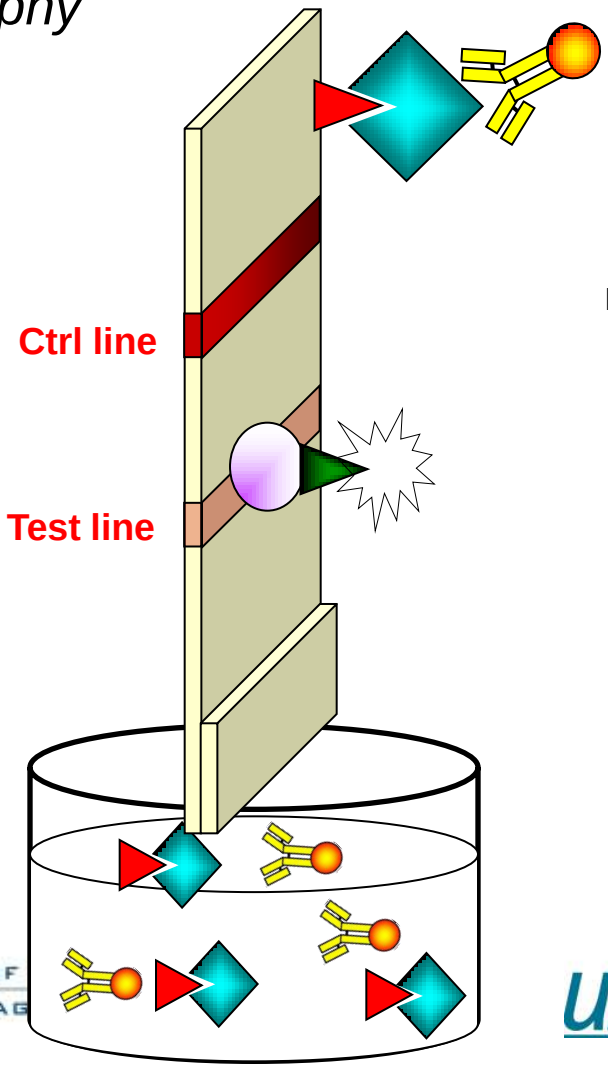
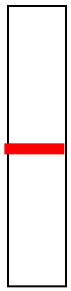
Principle of Dipstick Assay

Lateral Flow
Chromatography
Indirect
Competition
Dipstick
Assay

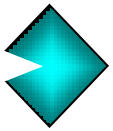


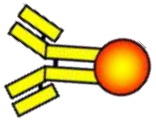
Positive test




Carrier


Hapten X


Antibody/ Receptor/ Aptamer/ Anti-X


2nd Antibody-GOLD


Contaminant X

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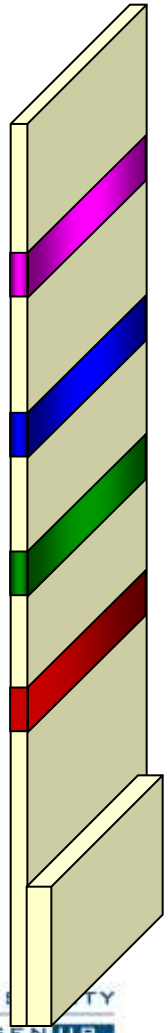
Multi-dipsticks in CONffIDENCE: antibiotics in honey

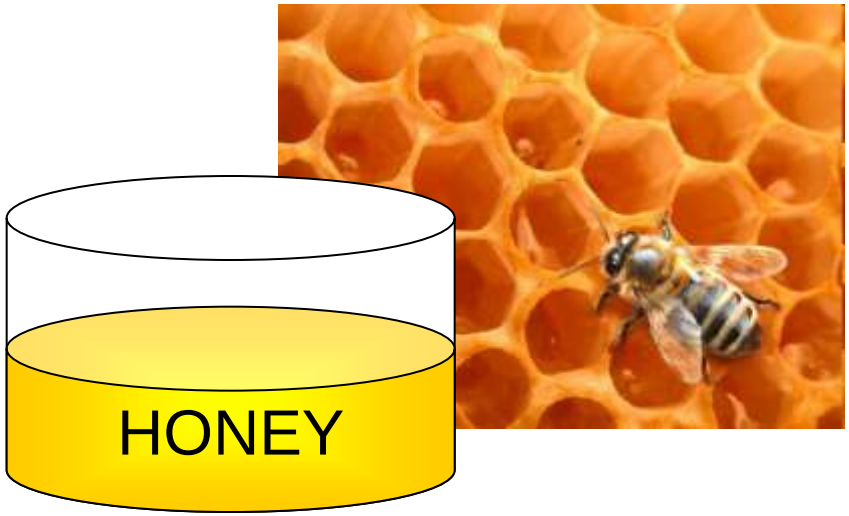
A) Sulfonamides

B) Quinolones

C) Tylosin

D) Chloramphenicol





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Nestlé Research™

Platforms

- Multiplex dipstick assays
- **Multiplex SPR biosensor**
- Multiplex flow cytometry
- Electrochemical immunosensors
- Cytosensors

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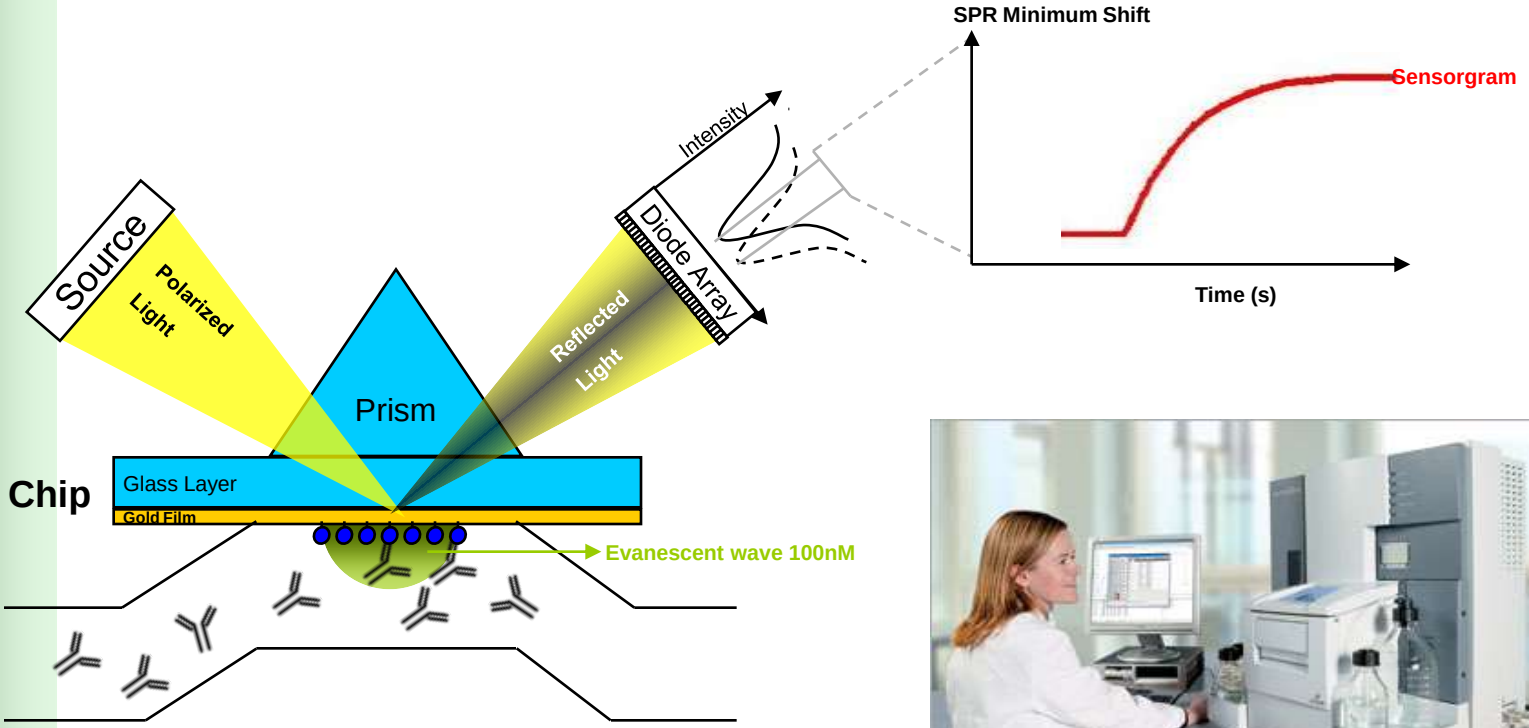
Surface Plasmon Resonance (SPR)



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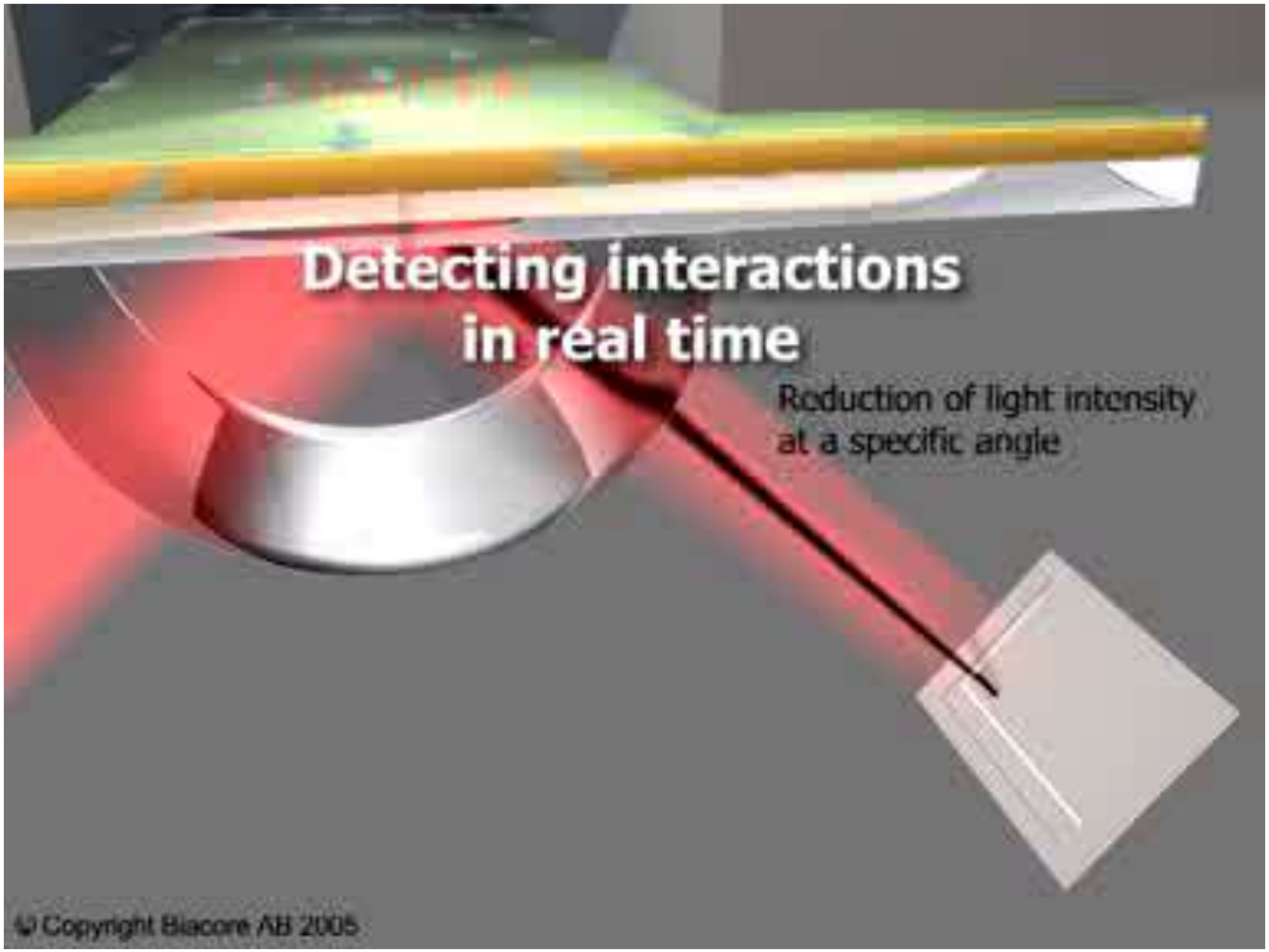
Demo of SPR



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Belfast

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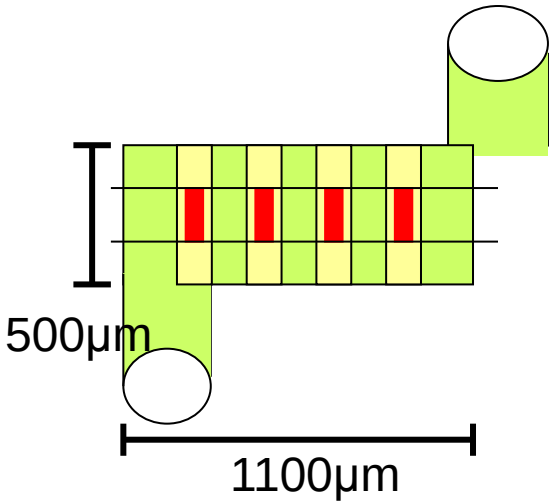
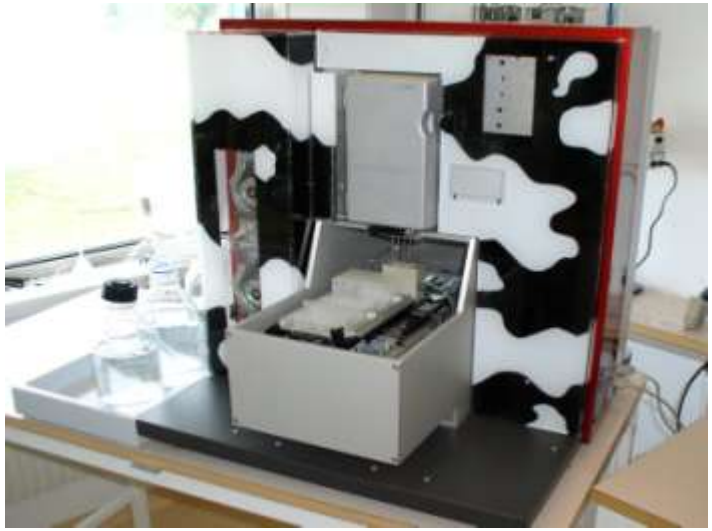
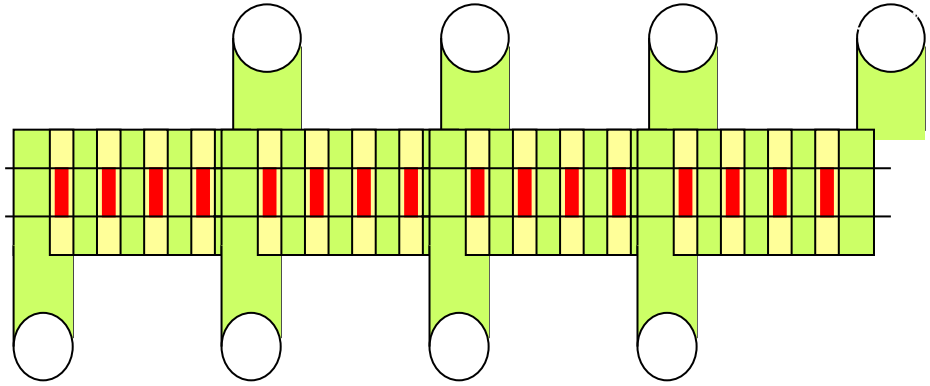
SPR Multiplexing







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SPR application in CON*ff*IDENCE

- Development of
  - a rapid multiplex assay capable of detecting a combination of PSP and DSP toxins in shellfish based on interaction with specific antibodies
  - assays for emerging toxins palytoxin and spirolides, to add to the multiplex assay (based on risk assessment evaluations)

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CER Groupe

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

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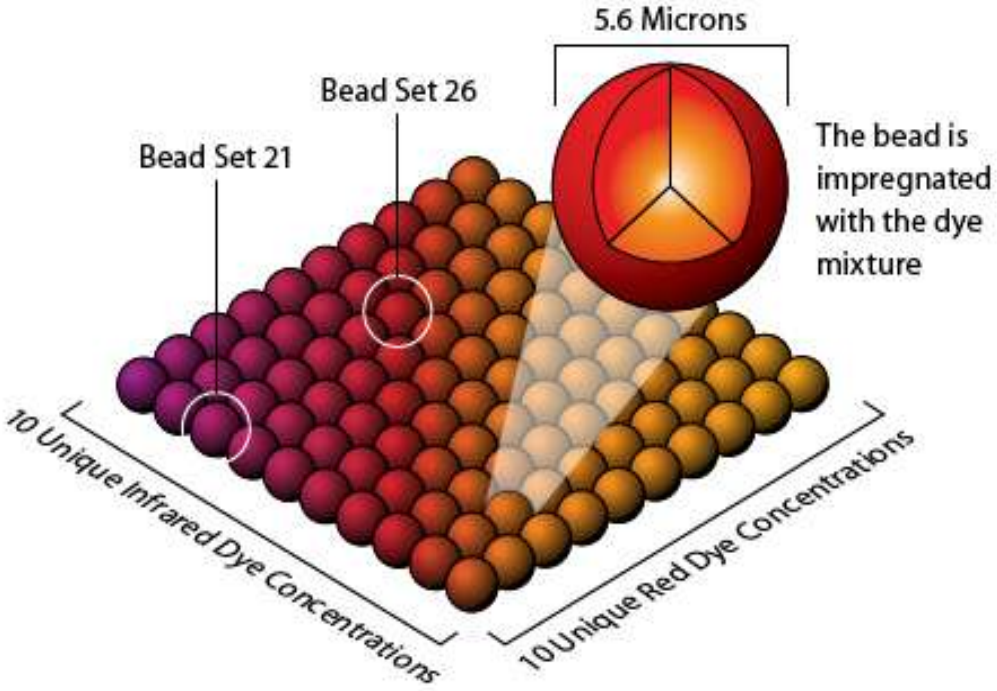
Platforms

- Multiplex dipstick assays
- Multiplex SPR biosensor
- **Multiplex flow cytometry**
- Electrochemical immunosensors
- Cytosensors

Principles of multiplex flow cytometry



Luminex® (xMAP) Technology

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Flow cytometry application in CON*ff*IDENCE : coccidiostats

- Development of a multiplex **immunoassay** for residues of the following **coccidiostats** in eggs and their cross-contamination in non-targeted feed (laying hens feed):
  - Lasalocid
  - Monensin
  - Salinomycin
  - Narasin
  - Nicarbazin
- **Transfer study** for lasalocid from

  feed ⇨ eggs

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JRC

irmm Institute for Reference Materials and Measurements

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Flow cytometry application in CON*ff*IDENCE : coccidiostats

- A multiplex **immunoassay** for residues of the following **coccidiostats** in eggs and their cross-contamination in non-targeted feed (laying hens feed):
  - Lasalocid: **bead set 1**
  - Monensin: **bead set 2**
  - Salinomycin: **bead set 3**
  - Narasin: **bead set 4**
  - Nicarbazin: **bead set 5**

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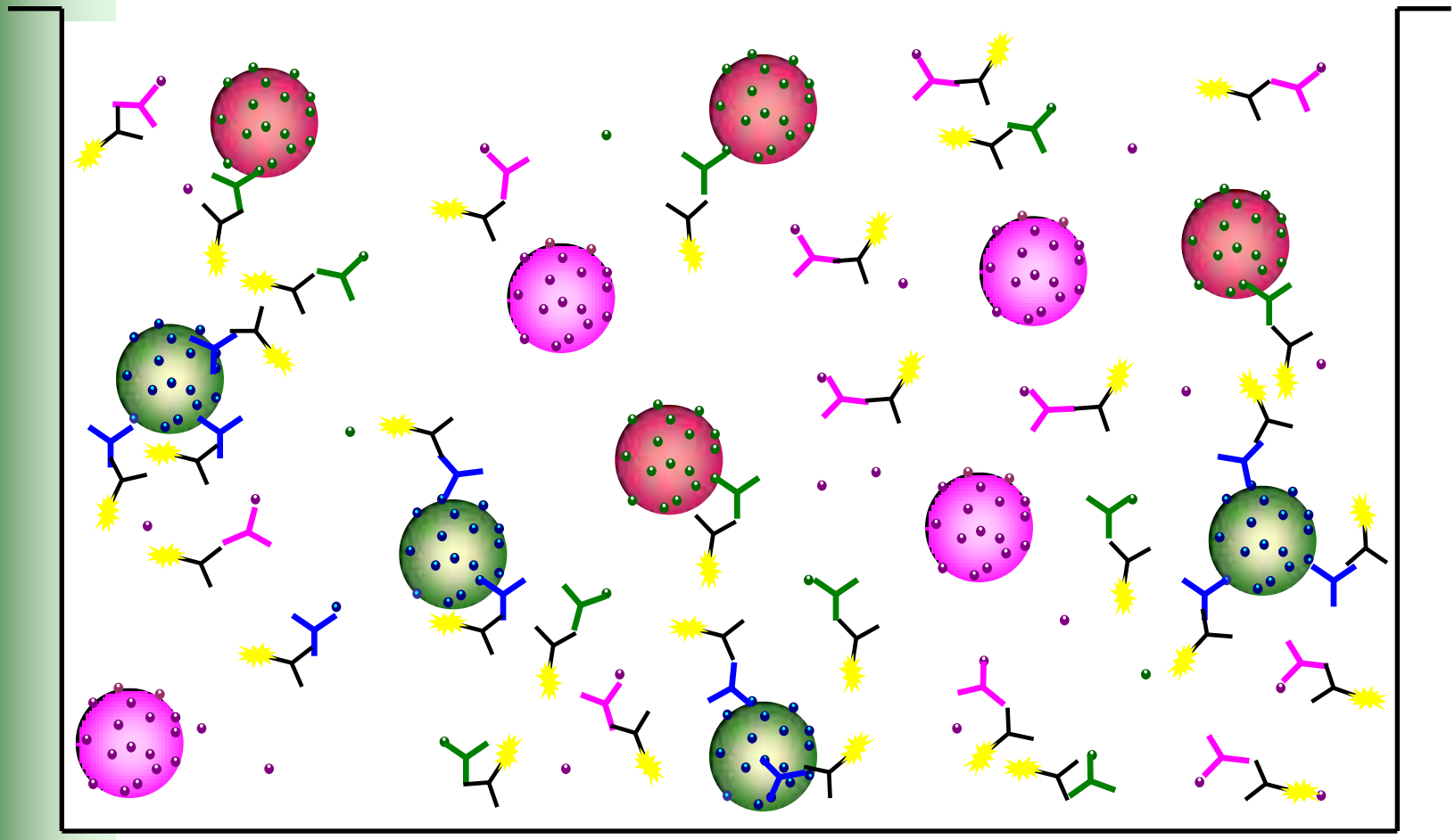
Example for 3 coccidiostats



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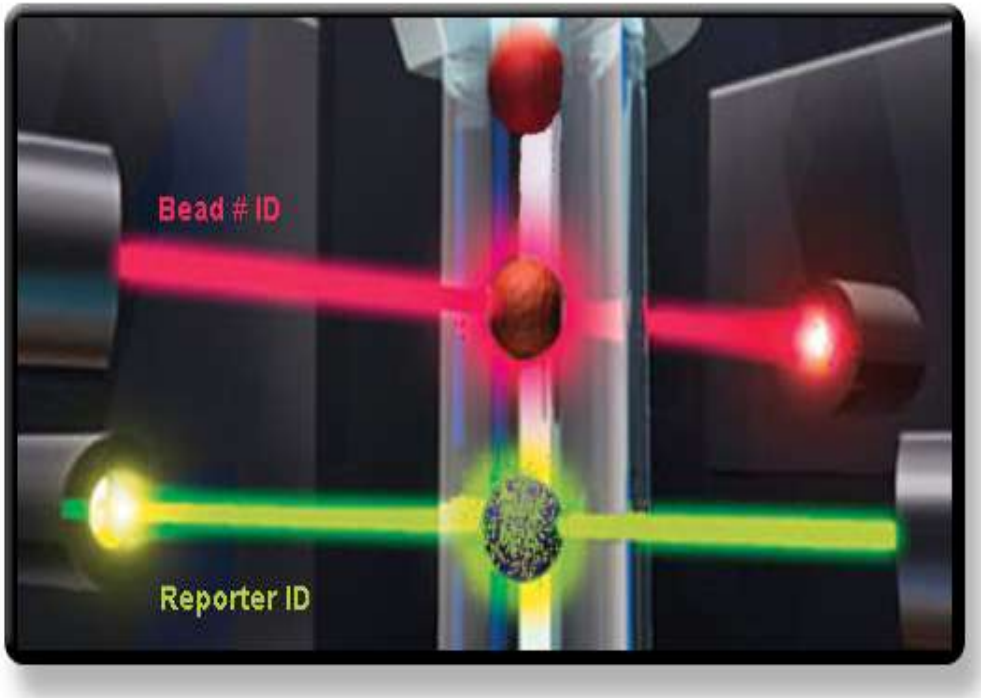
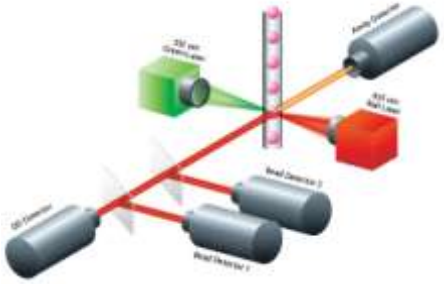
The flow cell



Red laser reads the bead, i.e. the target

Green laser detects the amount of the target

Luminex® (xMAP) Technology



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Platforms

- Multiplex dipstick assays
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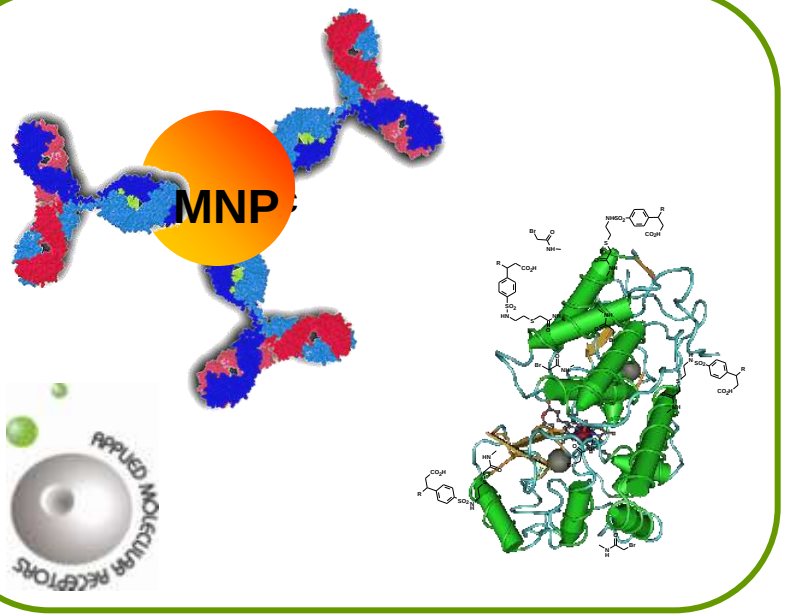
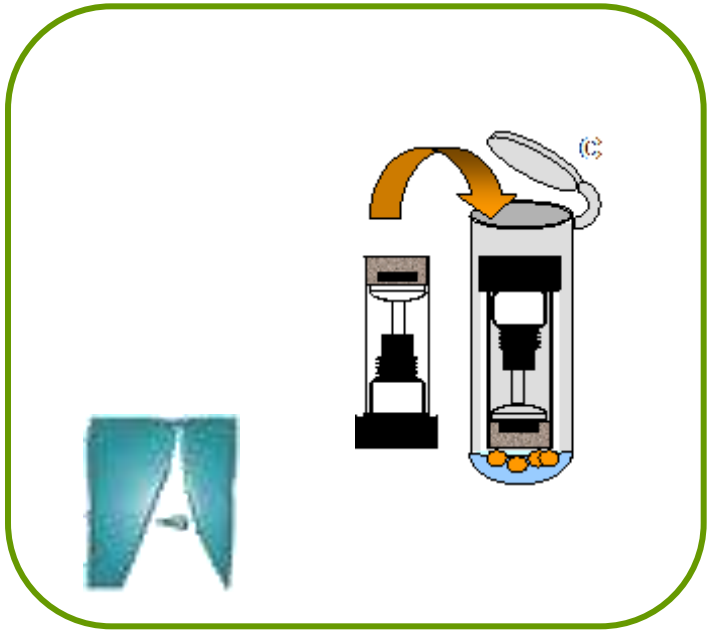
- **Electrochemical immunosensors**
- Cytosensors

Electrochemical immunosensor





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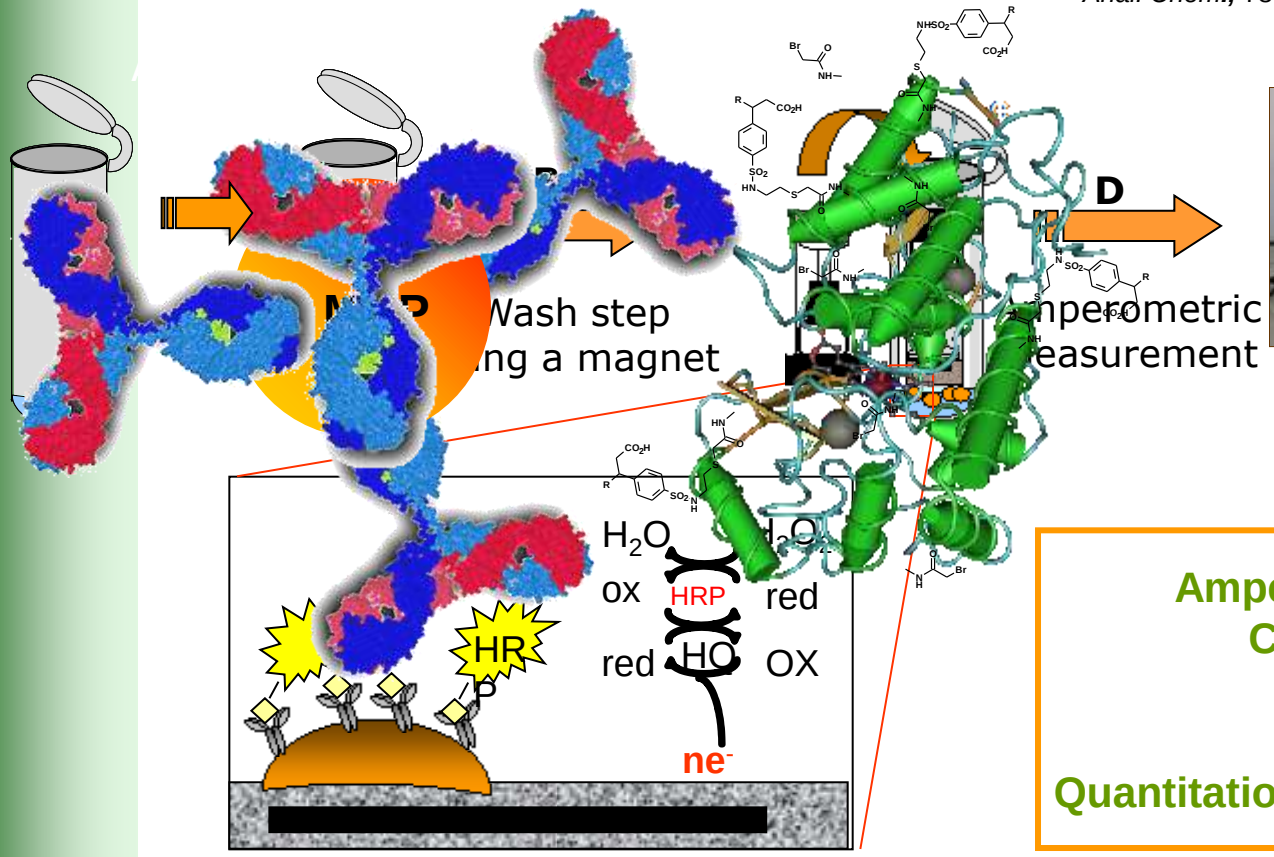
Electrochemical immunosensor

E. Zacco, M. I. Pividori, S. Alegret, R. Galve and M.-Pilar Marco
Anal. Chem., 78, 1780-1788, 2006



Amperometric Current

Quantitation of the Analyte

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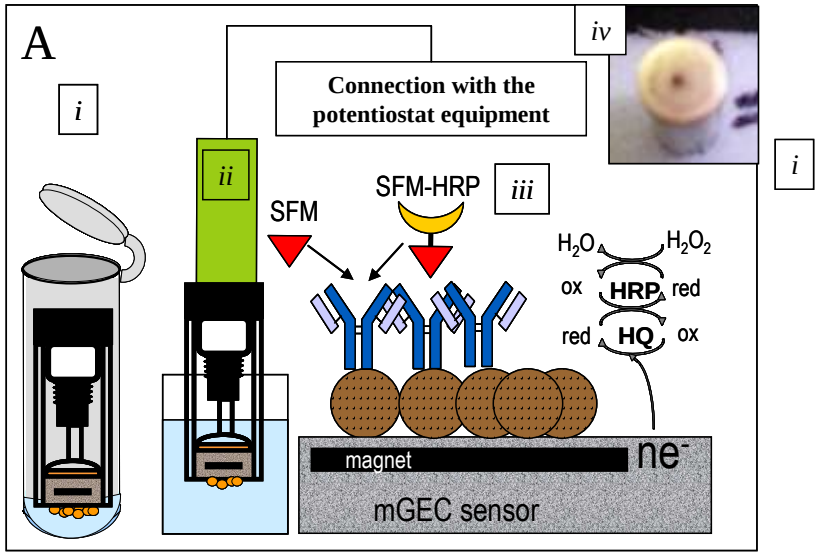
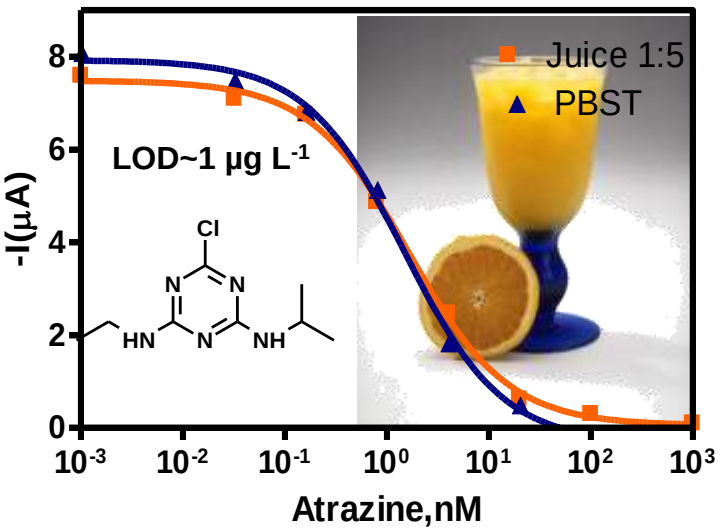
Electrochemical immunosensor





(1) Zacco, E.; Pividori, M. I.; Alegret, S, R. Galve and M.-Pilar Marco; *Analytical Chemistry* 2006, 78, 1780-1788.

(2) Zacco, E.; Galve, R.; Marco, M. P.; Alegret, S.; Pividori, M. I. *Biosensors & Bioelectronics* 2007, 22, 1707-1715.

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Electrochemical immunosensors in CON*ff*IDENCE:

- Development of a method for paraquat and diquat in potato and cereals

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Platforms

- Multiplex dipstick assays
- Multiplex SPR biosensor
- Multiplex flow cytometry
- Electrochemical immunosensors
- **Cytosensors**

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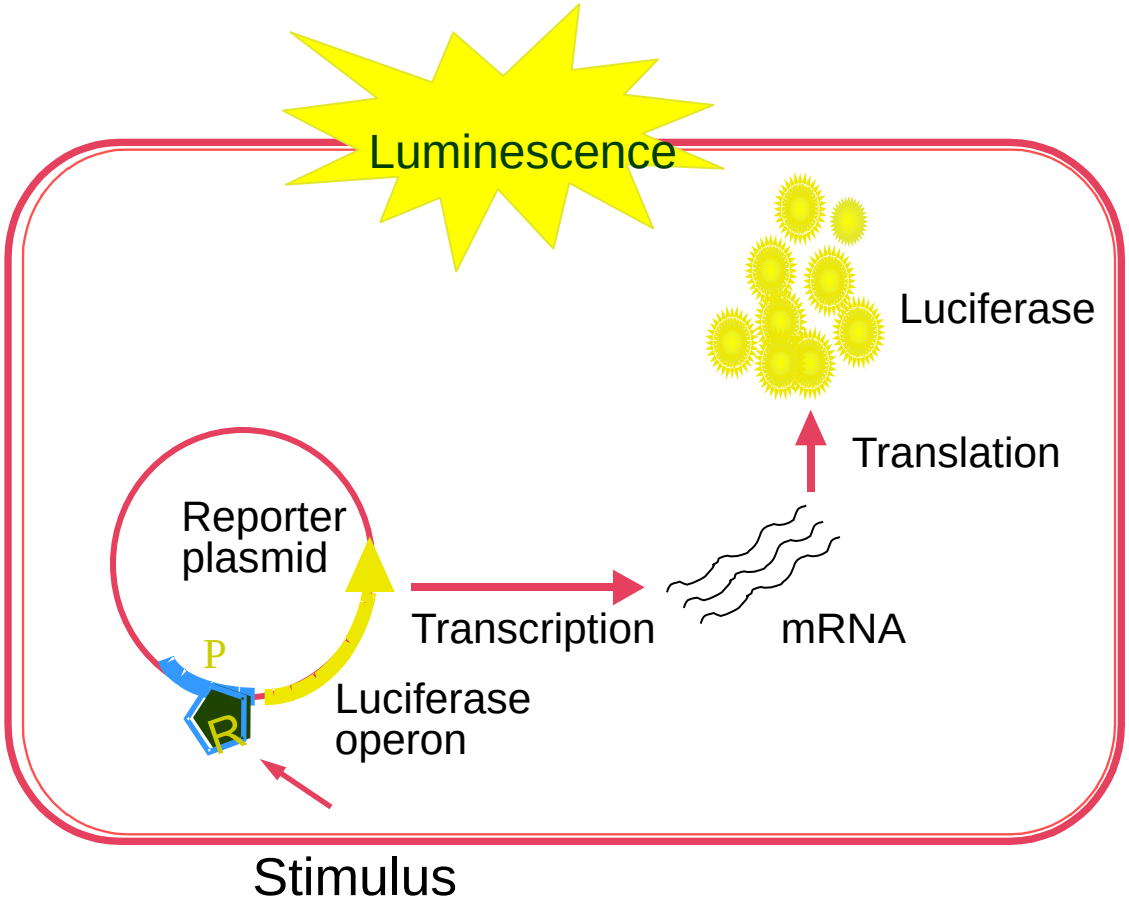
Cytosensor for heavy metal speciation: portable device for field usage



P = promoter

R = regulatory protein

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Detection modes

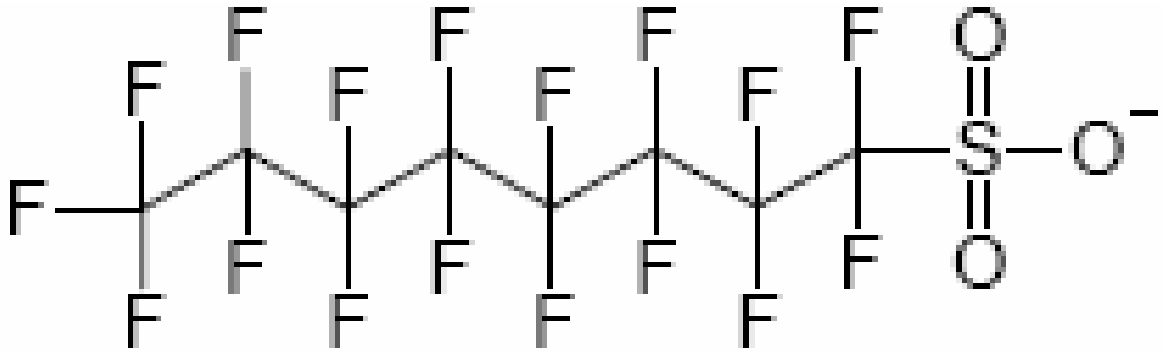
- Bio-analytical
- MS-based
- Spectroscopic

MS-based detection

- LC-MS or LC-MS/MS

  Example: Perfluorinated compounds

  NOTE: bio-analytical methods are not feasible due to impossibilities to find appropriate binding molecules !



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MS-based detection

- Simplified ambient mass spectrometric (MS) methods
  Example: DESI-MS and DART-MS for dithiocarbamates in crops

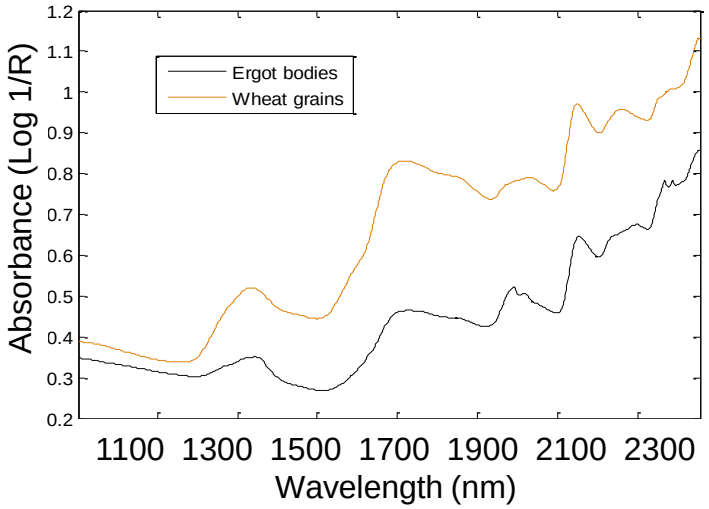
Detection modes

- Bio-analytical
- MS-based
- Spectroscopic

Detection of ergot bodies in wheat grains by NIR spectroscopy

Ergot bodies

Wheat grains



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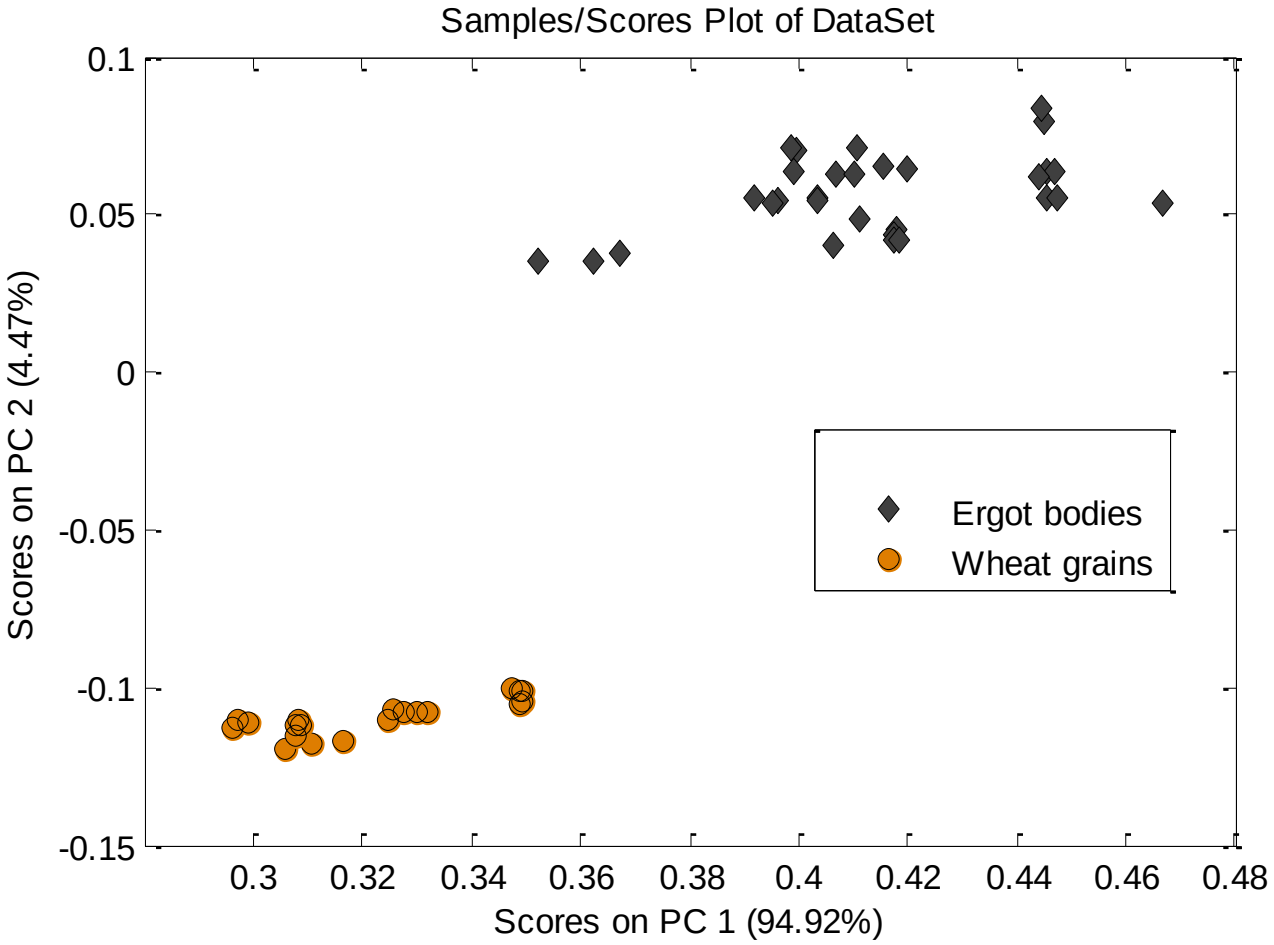
Discrimination between ergot bodies and wheat grains by NIR spectroscopy



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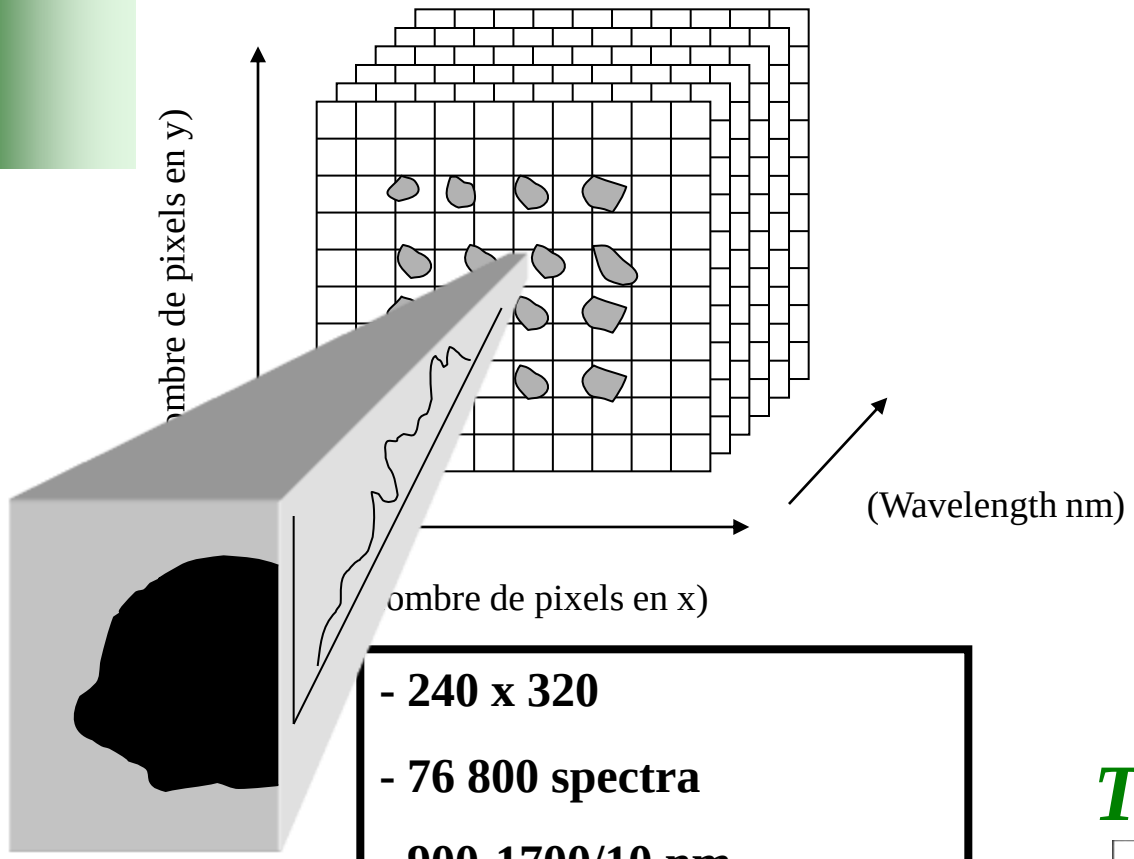
Infrared Hyperspectral Imaging System



- **240 x 320**
- **76 800 spectra**
- **900-1700/10 nm**
- **3 to 5 minutes**

The NIR camera

CRA-W Gembloux

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Contents

- Introduction to CON*ff*IDENCE
  - What ?
  - Why ?
  - Who ?
- Methods in CON*ff*IDENCE
  - Detection modes
  - **Primary extraction methods**

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Primary extraction methods

- Multi-compound & Multi-class: generic extraction methods, a challenge for e.g.
  - POP's and their metabolites
  - Mycotoxins
  - Plant alkaloids

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- Rapid methods, e.g. Pressurized solvent extraction

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  - Primary extraction methods
  - **Sample preparation**

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Sample preparation

- **No** clean-up as far as possible
- Fast and simple clean-up, e.g.
  - Magnetic beads: integrated sample preparation for
    - Flow cytometry
    - Electrochemical immunosensor
  - SPE with selective sorbents: separation of organic vs. inorganic arsenic

National Food Institute
Technical University of Denmark
DTU

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  - Sample preparation
- **Method validation**

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Method validation

Method development

In-house method validation

Validation by means of small-scale interlab studies

← Need for well characterised test materials

MasterLab

Nestlé Research™

JRC

irmm Institute for Reference Materials and Measurements

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Contents

- Introduction to CON*ff*IDENCE
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  - Sample preparation
- Method validation
- **Expected results**

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Expected results (Summary)

- Increased food and feed safety through more effective chemical contaminant monitoring
- Excellent screening tools for statutory control and industry
- Simple, fast, inexpensive multiplex assays (multi-analyte & multi-class detection), validated at 50 % of the regulatory limits
- Surveys that will enable exposure assessments
- Contributions to the validation of predictive models for transfer from feed to food

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Acknowledgements

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More information

Website: **www.conffidence.eu**

Contact:

coordination@conffidence.eu

e-newsletter

(registration on website)

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SEVENTH FRAMEWORK
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