UNIVERSITEIT GENT

FACULTY OF PHARMACEUTICAL SCIENCES

Schnelltests – Stand der Dinge
Rapid Methods – State of the Art

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Outline

1. Introduction
2. Overview of (mycotoxin) rapid screening tests
   1. Typical immunoassays
   2. Biosensors
3. Emerging issues in rapid screening
   1. Alternative receptors
   2. Alternative labels

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Rapid?

- **Different meanings** depending upon the perspective and expectations of the analyst and the context of the analytical environment.
- Assays' speed should **include sample preparation**, extraction, isolation of analyte!

Maragos and Busman. Food Addit Contam 27 (2010) 688-700.

Rapid?

•To deal with an **increasing number** of sample matrices and analytes of interest.

•**Two approaches**:

- Immunochemical rapid screening methods;
- Multi-analyte LC-MS/MS (screening, identification, quantification and confirmation).

•Cost savings

•High throughput

•On-site monitoring

•HACCP approach

•Positive samples need confirmation by a chromatography method

Immunochemical screening tests

Simple to use:

Simple sample extraction; Minimum assay steps; Short assay time; No or minimum toxic solvents; On-site applicability.

Simple to interpret results:

1.Non-instrumental (without any special laboratory equipment) – visual evaluations

- Good contrast between positive and negative results;
- Absence of background coloring.

2.Instrumental (simple, handheld, low cost equipment)

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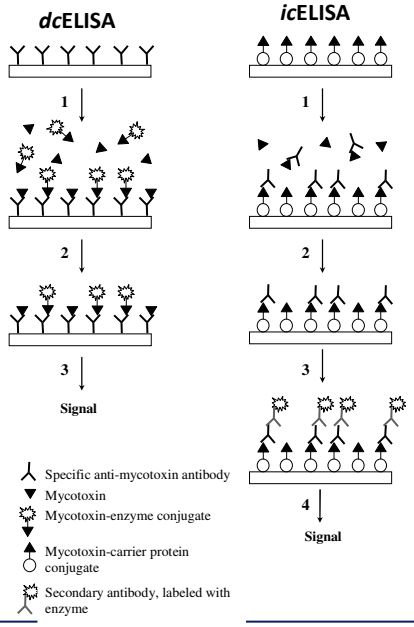
Competitive ELISA principle.

Goryacheva and De Saeger. Determining mycotoxins and mycotoxigenic fungi in food and feed. 2011. Woodhead Publishing.

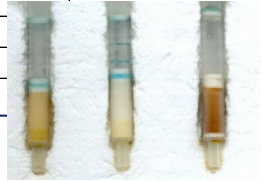


ELISA → microtiterplate
ELISA → tube-based

Microtiterplate ELISA	aflatoxin B1; total aflatoxins	corn, corn meal, corn gluten meal, popcorn, corn/soy blend, soybeans, milled rice, sorghum, wheat, cottonseed, peanuts, paprika, chilli
	aflatoxin M1	milk and milk products
	deoxynivalenol	nuts, cereals and other commodities including animal feeds
	fumonisin B1; total fumonisins	
	zearalenone	
	T-2 toxin	
	ochratoxin A	cereals, cocoa, coffee, wine
Tube-based ELISA	aflatoxin	corn, cereals, feed, peanuts

Membrane tests → lateral flow
Membrane tests → flow-through
Gel-based column tests → flow-through

Lateral flow	aflatoxin B1; total aflatoxins	corn, corn meal, corn gluten meal, popcorn, corn/soy blend, soybeans, milled rice, sorghum, wheat
	aflatoxin M1	milk
	deoxynivalenol	wheat, barley
	zearalenone	corn, cereals, sorghum
Flow-through	aflatoxin B1; total aflatoxins	cereals, soybeans, nuts, derived products
	ochratoxin A	cereals, wine, green coffee
	zearalenone	cereals and derived products



Commercially available diagnostic kits:

www.gipsa.usda.gov
www.aoac.org
www.mycotoxins.org

Lateral Flow Immunoassay (LFD) or immunochromatographic assay

Advantages of LFD:

1.One-step assay;

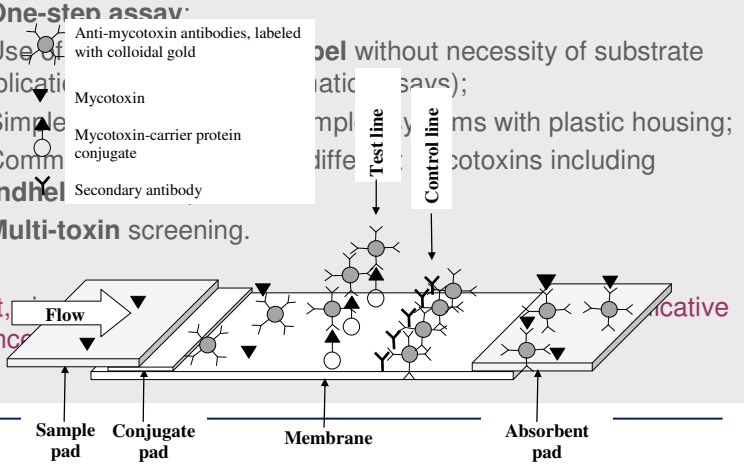
2.Use of **…bel** without necessity of substrate application (…ation …says);

3.Simple … …mpl… …y …ms with plastic housing;

4.Comm… …diffe… …cotoxins including **handhel**…

5.Multi-toxin screening.

But, … …icative conc…



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A biosensor is a bioanalytical device incorporating a molecular recognition element associated or integrated with a physicochemical transducer.

- Optical
    - Colourimetric, fluorescent, chemiluminescent
    - Surface plasmon resonance (SPR)
- Electrochemical
    - Voltametry, amperometry, impedance spectroscopy …
- Piezoelectric sensors (QCM)

Tothill. World Mycotoxin Journal 4 (2011) 361-374.
Maragos and Busman. Food Addit Contam 27 (2010) 688-700.

Advantages of biosensors:

1. **Reusable** for several analyses;
2. **Multiplexing** capabilities;
3. Sample preparation can be incorporated as part of the sensor system (**microfluidic system**);
4. Potential for **miniaturization** (lab-on-a-chip);
5. Towards **label-free** detection systems;
6. Further commercial development of such systems can be expected.

Very active area of research!

Pitfalls for rapid screening tests:

- Very different sample matrices (matrix interference!!);
- Low detection limits are needed;
- False positives/false negatives;
- Limited quality control;
- Cross-reactivity;
- Robustness of on-site test;
- Necessity of matrix-matched calibrations?

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Antibodies:

"Most popular and best established affinity tool, especially in diagnostics. It appears very unlikely that alternative affinity tools will play a significant role in the field of diagnostics soon, simply because of the wealth of antibody-based assays that are readily available".

Ruigrok et al. Biochem. J. 436 (2011) 1-13.

But, researchers always look further than 'soon'!

Some drawbacks of antibodies:

- Development and production costs;
- Development time;
- Small molecules need to be conjugated for immunogen synthesis;
- Degrading bioactivity;
- Thermally and chemically unstable;
- Animal experiments.

Search for alternative « biomimetic receptors » which should bind the target with similar affinity, specificity and reversibility to antibodies.

Molecularly imprinted polymers

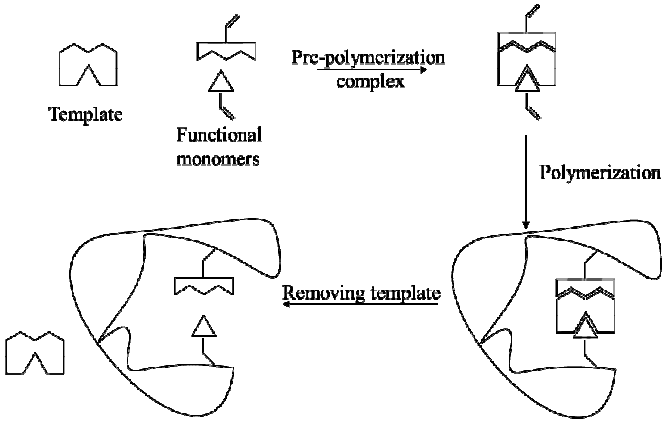
= polymeric matrices capable of preferentially recognizing the template molecules used

Aptamers

= < Latin, aptus, i.e. to fit; DNA or RNA oligonucleotides or peptide aptamers; selected from a large random sequence pool to bind to a specific target molecule

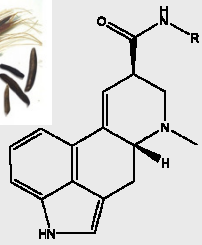
- Advantages over antibodies: stability, simpler and faster production;
- Potential application in rapid tests?

Molecularly imprinted polymers

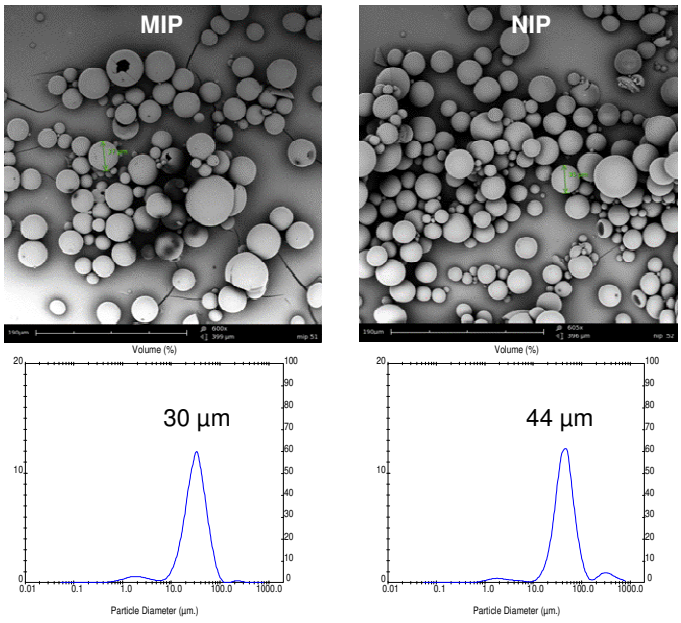


Claviceps purpurea

Example: MIPs towards ergot alkaloids



COMMON STRUCTURE	R-GROUPS		
	ERGOMETRINE	ERGOCORNINE	ERGOCRISTINE
	ERGOSINE	ERGOCRYPTINE	ERGOTAMINE

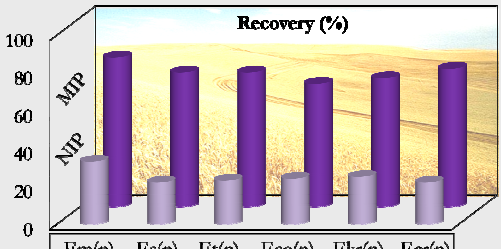


Lenain *et al*, in preparation

Recovery of the MIP and NIP (n = 3)

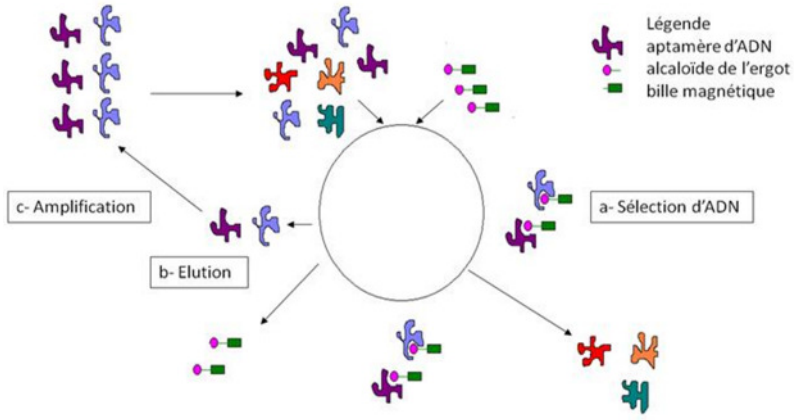
Used abbreviations

Ergometrin(in)e	Em(n)
Ergosin(in)e	Es(n)
Ergotamin(in)e	Et(n)
Ergocornin(in)e	Eco(n)
Ergocryptin(in)e	Ekr(n)
Ergocristin(in)e	Ecr(n)



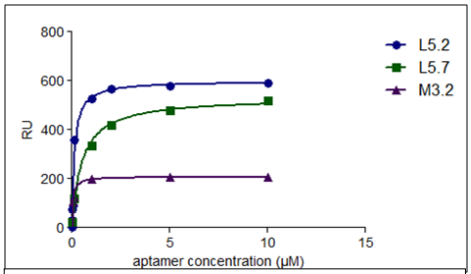
	Em(n)	Es(n)	Et(n)	Eco(n)	Ekr(n)	Ecr(n)
Recovery MIP (%)	79	71	71	65	68	73
Standard Deviation (%)	9	7	6	7	14	15
Relative Standard Deviation (%)	12	10	8	11	21	20
Recovery NIP (%)	33	22	23	24	25	22
Standard Deviation (%)	7	7	3	2	5	4
Relative Standard Deviation (%)	20	30	11	10	19	16

Aptamers



SELEX: Systematic Evolution of Ligands by EXponential enrichment. This iterative process is used to select a recognition element for a target (molecule, cell, bacteria, ...).

Characterization of DNA/ergot alkaloid complexes by surface plasmon resonance (SPR)



SPR responses of the binding of the aptamers to lysergamine

Fitting Model	Two-site specific binding	One-site specific binding	
Best-fit values for	Aptamer M3.2	Aptamer L5.2	Aptamer L5.7
BMax (RU)	205.2	585.8	531.0
K_d	44 nmol²/L²	73 nmol/L	499 nmol/L
R^2	0.997	0.993	0.991

Prof. Ronny BLUST (UA) and Dr. Johan ROBBENS (ILVO), Elsa ROUAH-MARTIN, Jaytry MEHTA, Bieke VAN DORST (UA, ILVO)

Dissociation constants in the nanomolar range were obtained with three selected aptamers.

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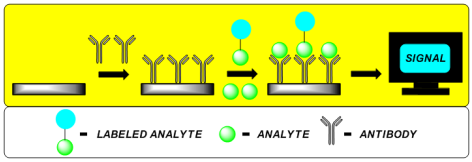
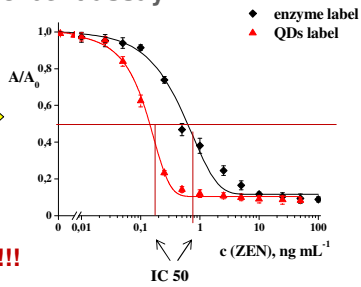
Quantum dots (QDs) as a label in immunoassay

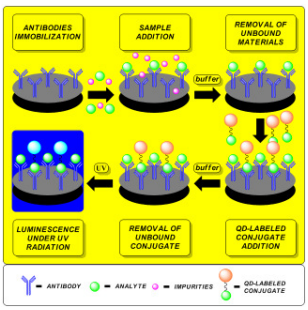
Fluorescent-linkage immunosorbent assay



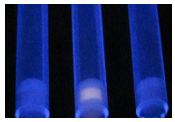


Fourfold decrease in IC 50 with QDs labels!!!

Column-based test-methods



Gel-based immunoassay



frit without antibody 0 5

C(ZEN), ng/mL

Frit-based immunoassay procedure

frit without antibody 0 1 5

C(ZEN), ng/mL

Beloglazova N. et al, ABC, accepted

Acknowledgements

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- Dr Johan Robbens, Elsa Rouah-Martin.

All researchers from the Laboratory of Food Analysis!!!!

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35th MYCOTOXIN WORKSHOP

GHENT, BELGIUM, MAY 22-24, 2013

in cooperation with the Gesellschaft für Mykotoxinforschung (Society for Mycotoxin Research)