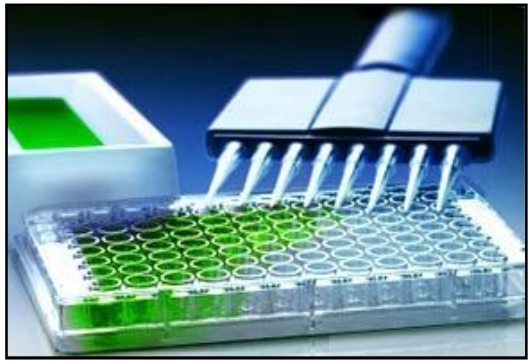
MYCORED AFRICA

2011 CONFERENCE

Innovative and rapid methods for mycotoxin analysis




Angelo Visconti, Veronica Lattanzio, Annalisa De Girolamo,
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Institute of Sciences of Food Production (ISPA)
National Research Council of Italy (CNR)

6th April 2011, CTICC, Cape Town, South Africa

Innovative and rapid methods

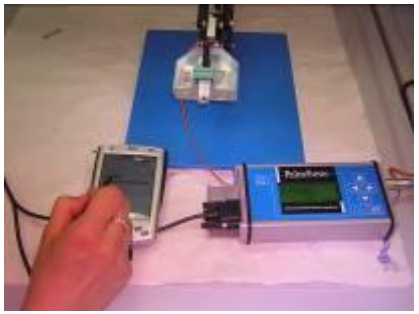
Conventional determination requires:

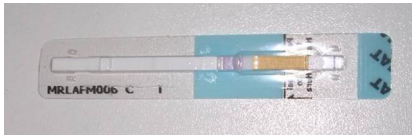
- ✓ Tedious sample preparation
  - Å Grinding of sample
  - Å Extraction
  - Å Clean-up
- ✓ Time consuming separation and detection
  - Å GC-ECD, GC-MS
  - Å HPLC-DAD, HPLC-FD, HPLC-MS
- ✓ High costs
  - Å equipments
  - Å operations
- ✓ Skilled persons

Innovative/Rapid methods for mycotoxin analysis

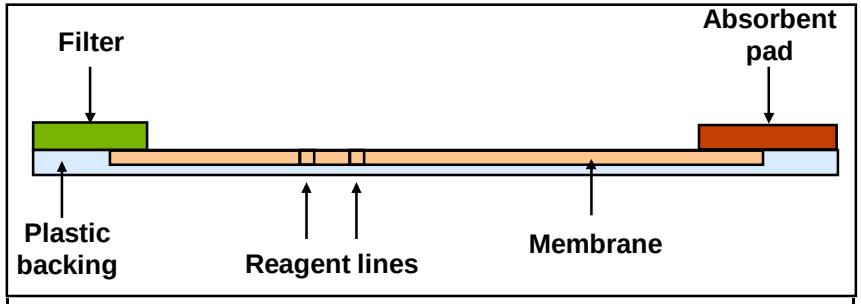
- Immunoassays/immunosensors:
  - Flow through immunoassay (FIA)
  - Lateral flow devices (LFD) or dipsticks
  - Surface plasmon resonance (SPR)
  - Fluorescence polarization immunoassay (FPIA)
  - Electrochemical immunoassay (Screen printed electrodes)
  - Others (Piezoelectric sensors, Fiber optic immunosensor,...)

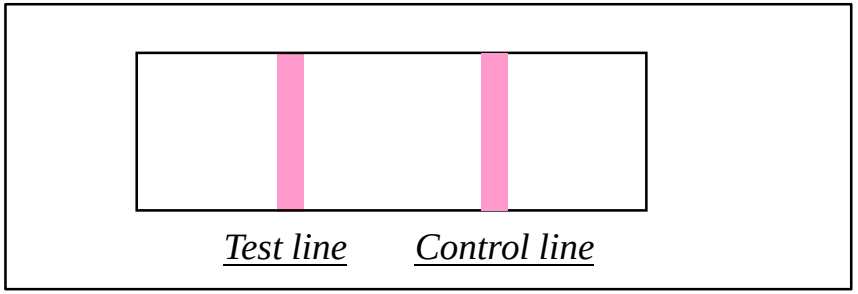
- Methods using alternative receptor (aptamer, MIP, antibody fragment, peptide)
- Infrared spectroscopy (IR)

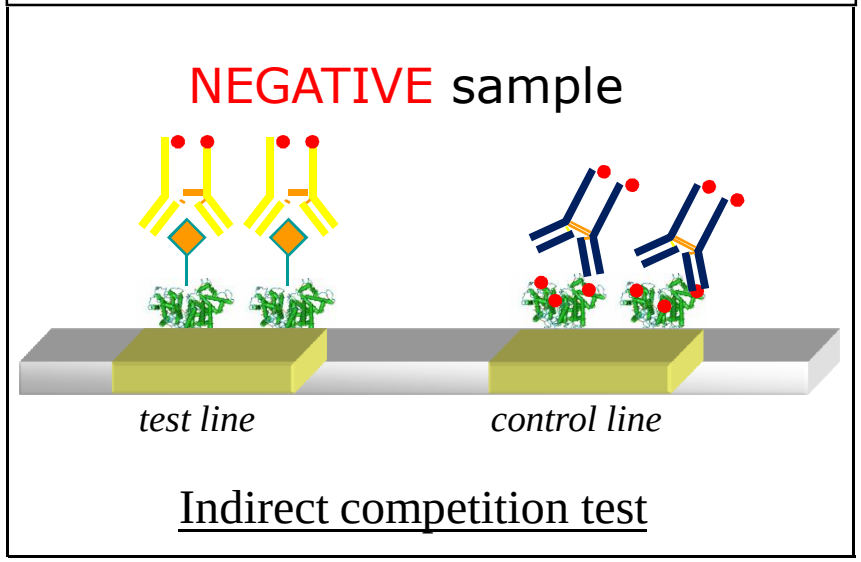




Lateral flow devices (LFD) or dipsticks

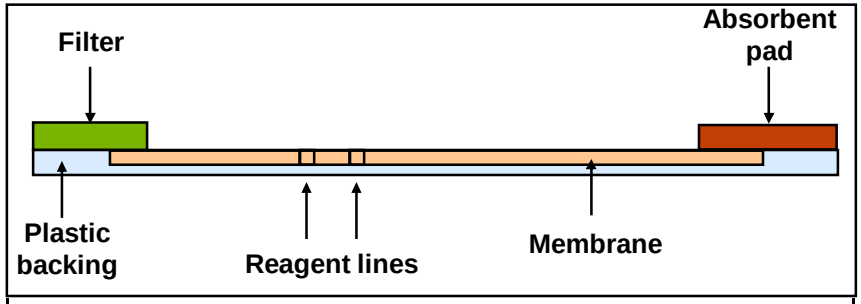


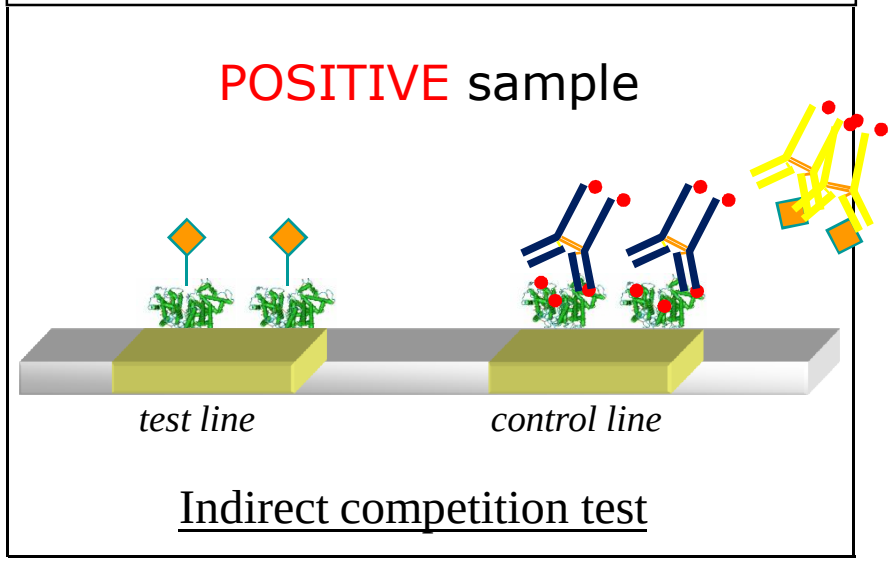




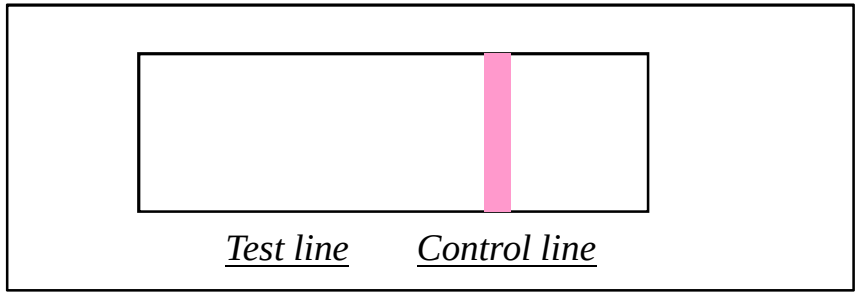
Indirect competition test

Lateral flow devices (LFD) or dipsticks





Indirect competition test



Å LFD/dipsticks commercially available for AFs and FBs in maize, DON in wheat, OTA, ZEA,T-2 and HT-2 in cereal grains

Å Readers allow quantitative analysis

Development of a multiplex dipstick

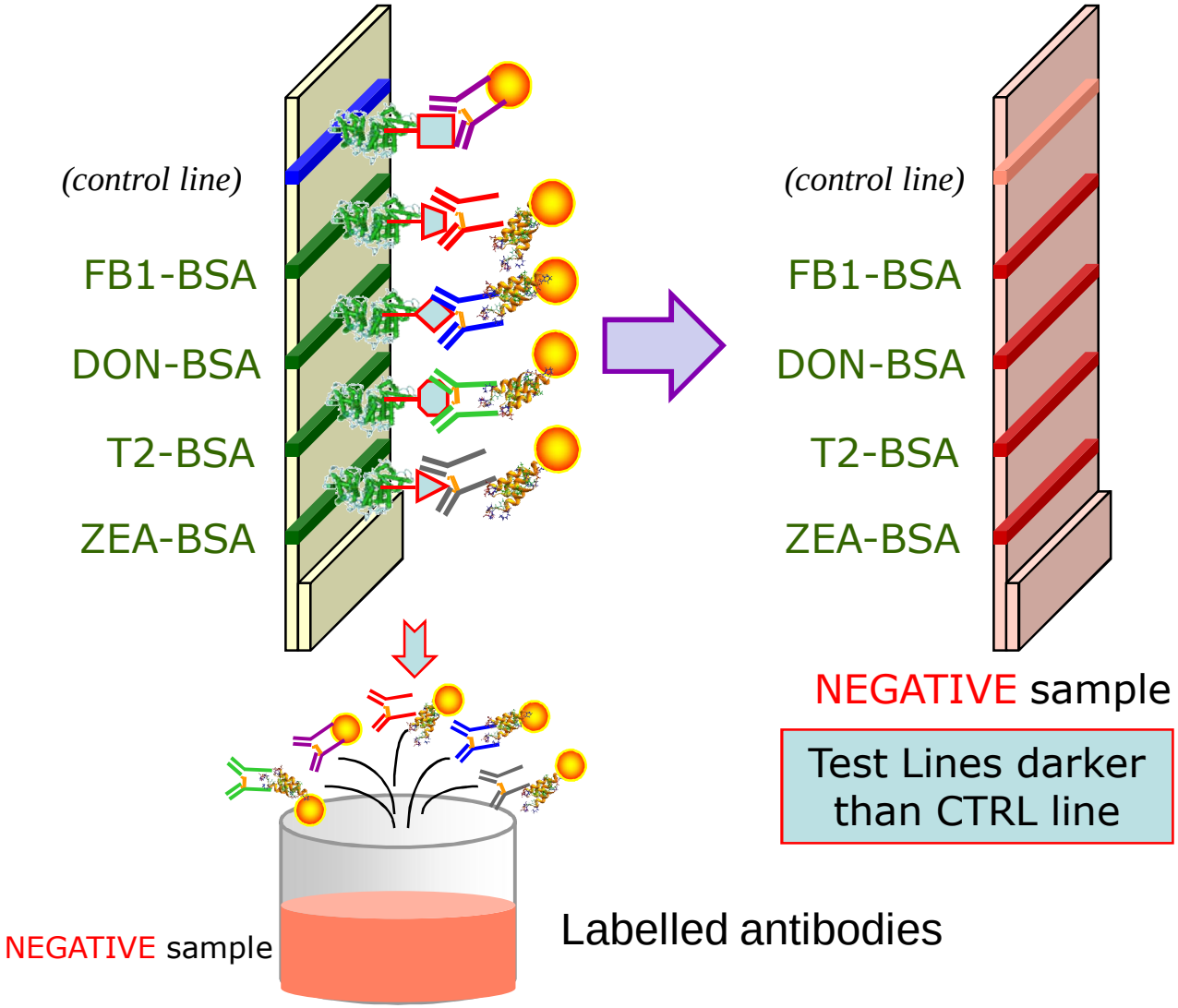
(ZEA, T2/HT2, DON, FB_1/FB_2)

CONffIDENCE project (7 FP): Contaminants in Food and Feed Inexpensive Detection for Control of Exposure

WP4c: Mycotoxins (WP leader Dr. A. Visconti)

Multiplex dipsticks - Fusarium toxins in cereals, cereal food, maize feed

Prototype multiplex dipstick: *indirect competitive immunoassay*



Multiplex dipsticks - Fusarium toxins in cereals, cereal food, maize feed

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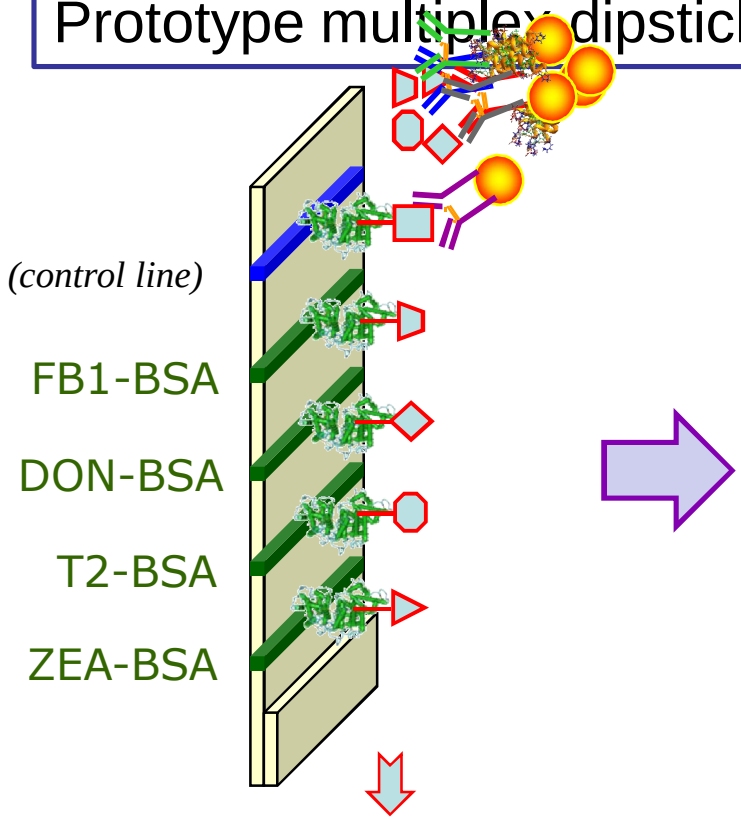
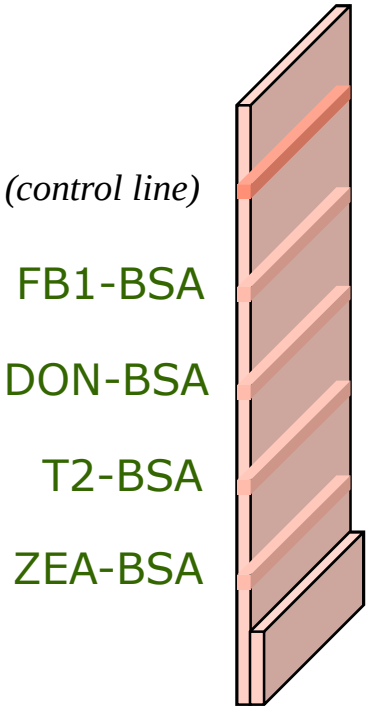
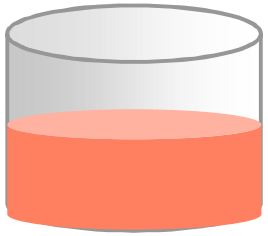




POSITIVE sample

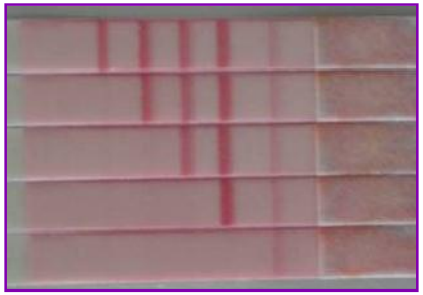
Test Lines lighter than CTRL line



POSITIVE sample

Labelled antibodies

Multi-mycotoxin dipsticks: Protocol of analysis



Negative sample

Positive ZEA

Positive ZEA/T2

Positive ZEA/T2/DON

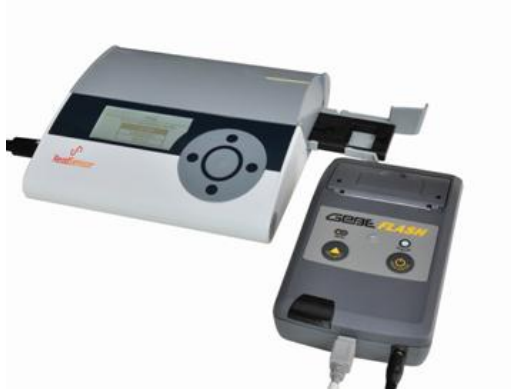
Positive ZEA/T2/DON/FB1

Methanol/water
Blending
(for all mycotoxins)

Dilution
different
dilution factors

Incubation at 40°C
Migration
Optimized conditions

Dipstick reader (*Readsensor*)



Total analysis time:
30 min

Multi-mycotoxin dipsticks: Analysis of Naturally Contaminated Maize Samples

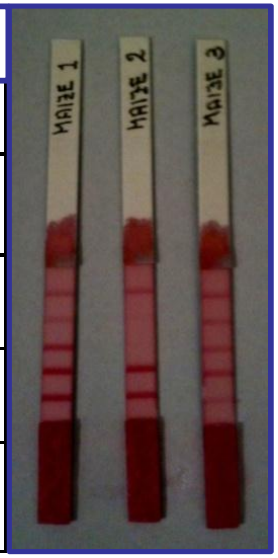
Achieved cut off levels in cereals, cereal foods, maize feed

CUT OFF levels (µg/kg) (fixed at target levels corresponding to 80% of European MRL)

	ZEA	T-2 +HT-2	DON	FB_1+FB_2
WHEAT	80	400	1400	-
OATS	80	400	1400	-
MAIZE	280	400	1400	3200
MAIZE FEED	2400	400	9600	5000
WHEAT based BREAKFAST CEREALS	40	80	400	-
MAIZE based BREAKFAST CEREALS	80	80	400	640

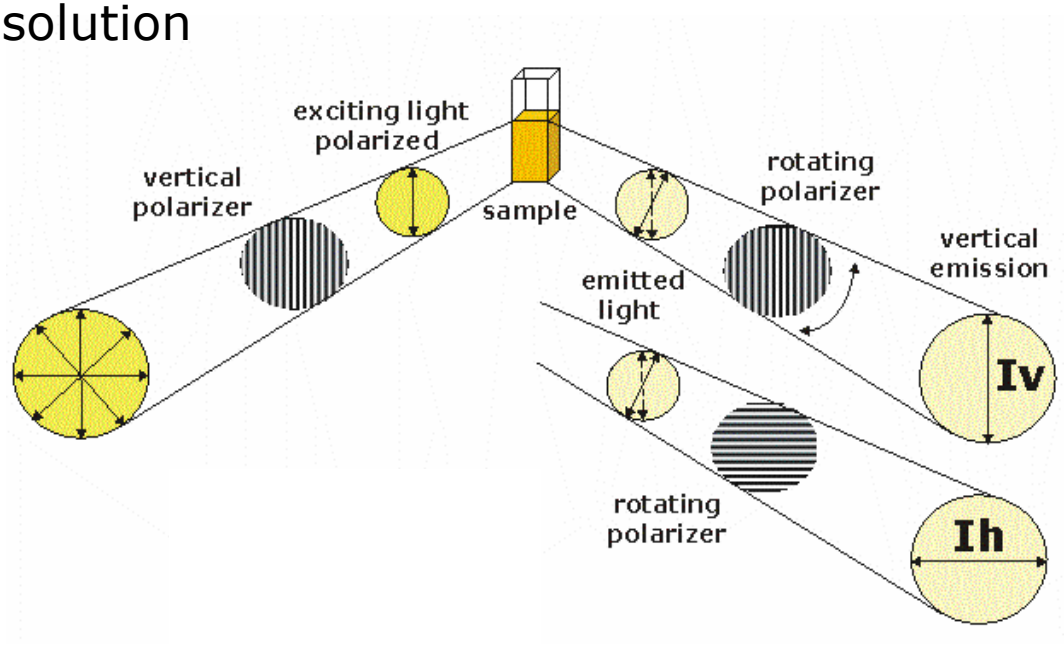
Good agreement between dipstick and LC-MS/MS analysis

Sample	ZEA		T-2 +HT-2		DON		FB_1+FB_2	
	dipstick	*LCMSMS µg/kg*	dipstick	*LCMSMS µg/kg*	dipstick	*LCMSMS µg/kg*	dipstick	*LCMSMS µg/kg*
1	**NEG**	*n.d.*	**NEG**	*n.d.*	**NEG**	*n.d.*	**NEG**	*725*
2	**NEG**	*n.d.*	**NEG**	*n.d.*	**POS**	*24200*	**POS**	*8150*
3	**POS**	*420*	**POS**	*392*	**NEG**	*298*	**NEG**	*725*



Fluorescence Polarization Immunoassay (FPIA)

Fluorescence Polarization measures the size of fluorescent molecules in solution



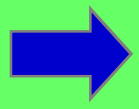
$$P = \frac{I_v - I_h}{I_v + I_h}$$

I_v = Intensity, vertical

I_h = Intensity, horizontal

$$P \propto \tau = \frac{3\eta V}{RT}$$

Rapid rotation

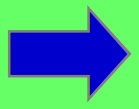


low

P value

Fluorescent tracer

Slow rotation



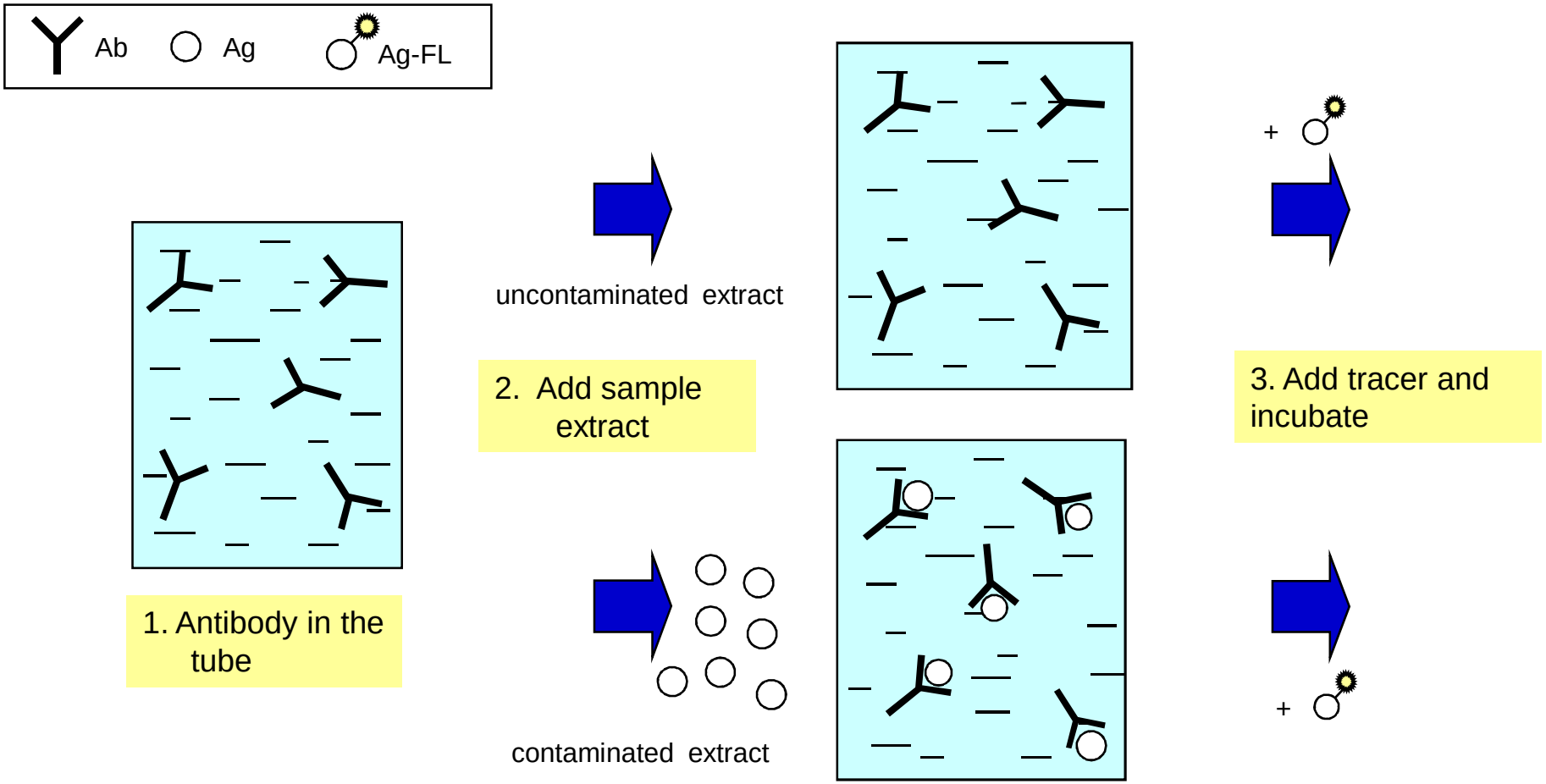
high

P value

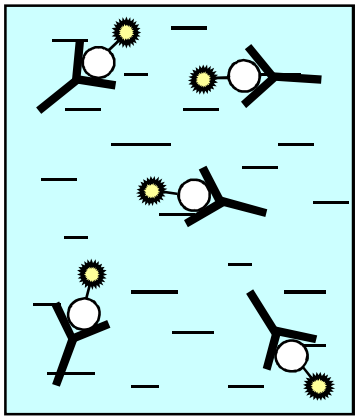
Large complex

FPIA ï basic principles

FPIA is a homogeneous competitive fluorescence immunoassay



FPIA ï basic principles

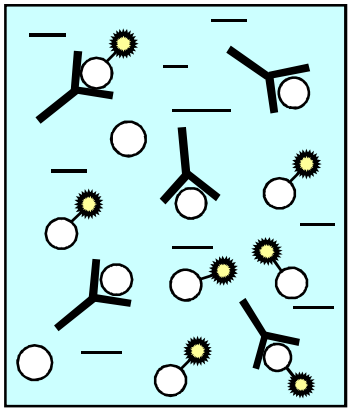


uncontaminated extract

Slow rotation

High P value

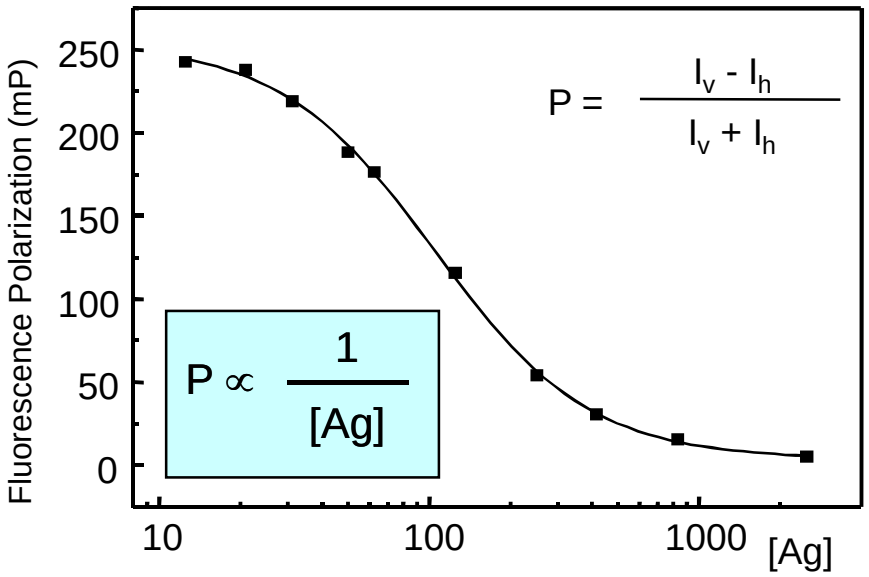
4. Mesure fluoresecence polarization



contaminated extract

Fast rotation

Low P value



✓ P is inversely related to free antigen content in solution that competes with the tracer

FPIA ï DON in wheat and derivative products

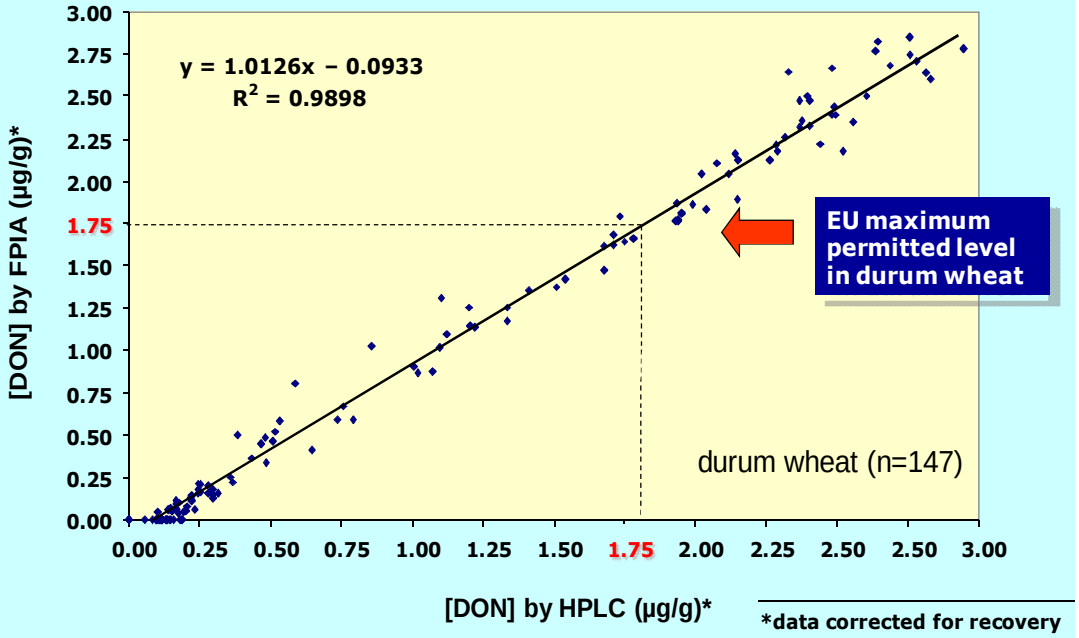
✓ **applicability:** **durum wheat, common wheat, semolina, pasta**

✓ **detection limit:** **0.08 µg/g**

✓ **accuracy:** **98-102%**

✓ **precision:** **≤ 4%**

✓ **time of analysis:** **≤ 10 min**



✓ **linearity range:** **0.1 ï 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

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 (<5%)
- Useful and practical alternative to HPLC
- More convenient than HPLC for routine analyses due to higher throughput (15 samples / 3 h *vs.* 1 sample / 3 h)

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* European Patent Application No. 1882938A2. Visconti A., Pascale M., Lippolis V., Ranieri R., Silvestri M. e DÔAlessandro A.

FPIA ï OTA in wine

A rapid FP immunoassay with SPE clean-up has been developed for semi-quantitative screening of OTA in red wine

Dilution
(methanol)

↓

SPE purification
(aminopropyl phase)

↓

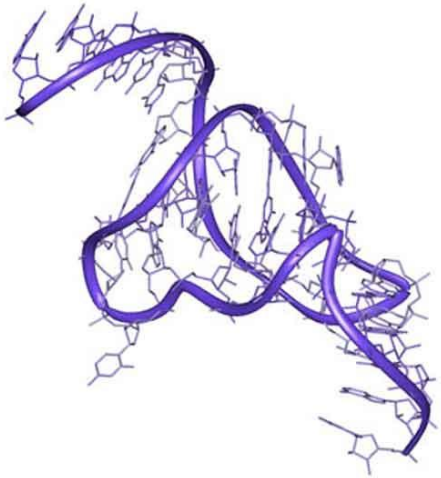
FPIA determination

- ✓ **detection limit:** **0.7 ng/mL**
- ✓ **accuracy:** **79%**
- ✓ **precision:** ≤ **11%**
- ✓ **time of analysis:** ≤ **10 min**
- ✓ **linearity range:** **1 - 5 ng/mL** (2 ng/mL is EU MRL)
- ✓ **validation:** on 154 naturally contaminated or spiked red wine by comparison with HPLC/IAC analysis showing a good correlation (r = 0.9222).

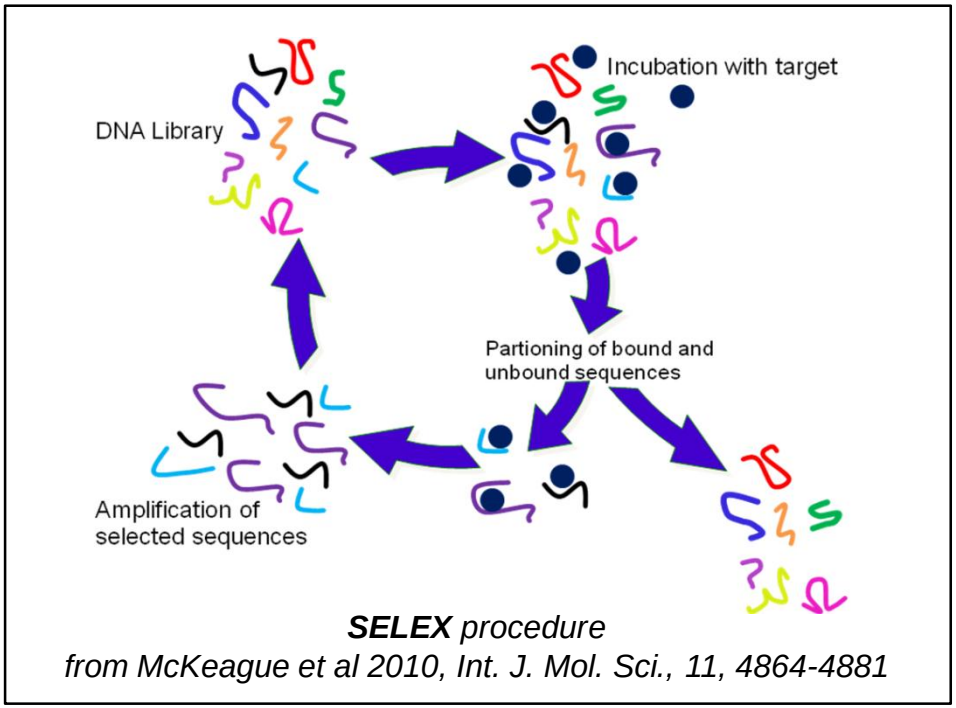
Confirmatory analysis is required for OTA levels of 1-3 ng/mL

Zezza F., Longobardi F., Pascale M., Eremin S.A., Visconti A., Anal. Bioanal. Chem., 2009, 395, 1317-1323

Novel materials for mycotoxin analysis: Aptamers



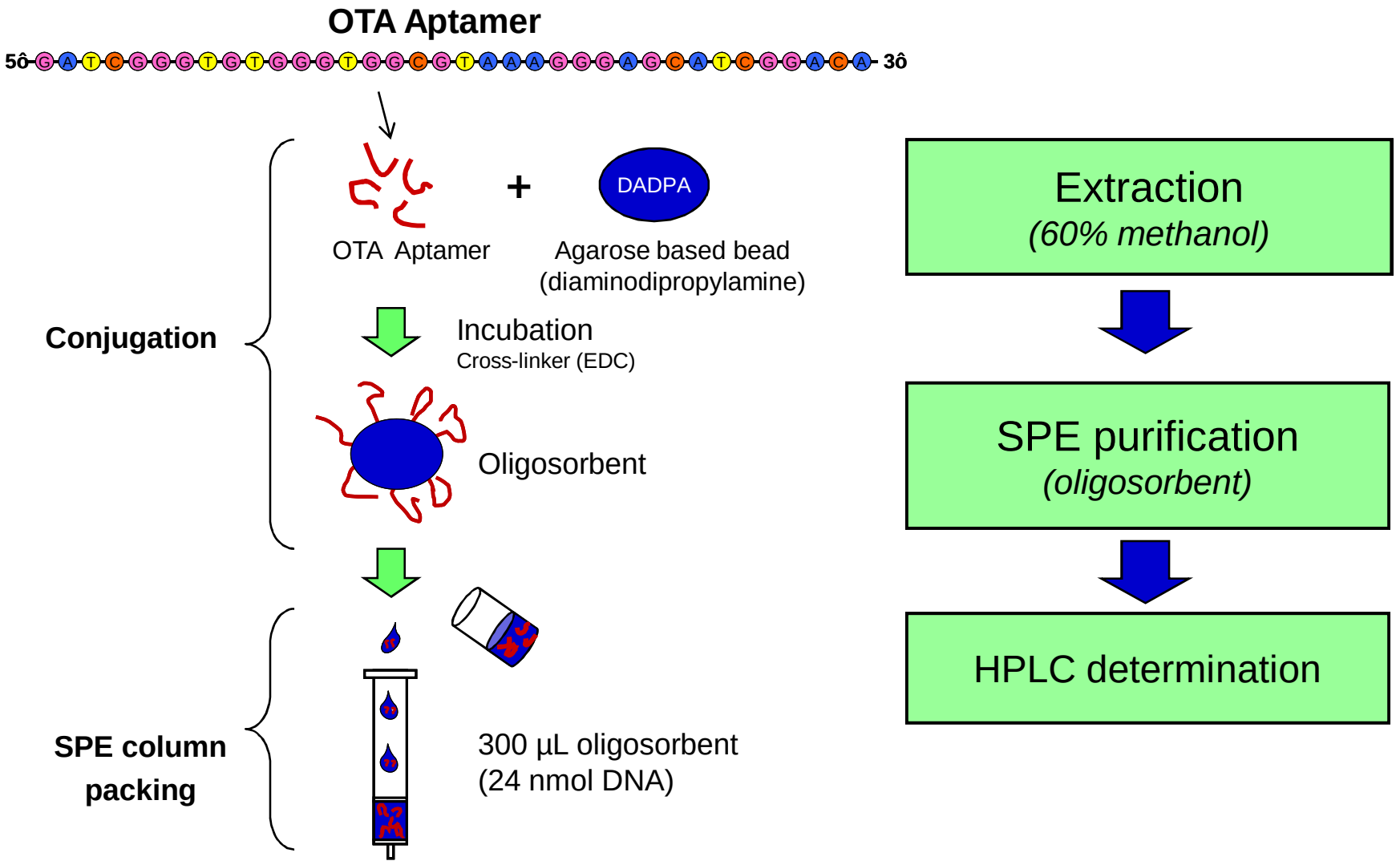
Aptamer



***SELEX** procedure*
from McKeague et al 2010, Int. J. Mol. Sci., 11, 4864-4881

- **Aptamers** are single-stranded oligonucleotides (DNA or RNA) that bind with **high affinity** and **specificity** to specific targets.
- Aptamers are produced by an ***in vitro* selection** process called **SELEX** (*Systematic Evolution of Ligands by Exponential)*.
- Aptamers, like antibodies, have potential in a broad range of **applications** including **biosensors**, **affinity chromatography**, **lateral flow devices**.
- Aptamers for OTA and FB_1 have been produced.

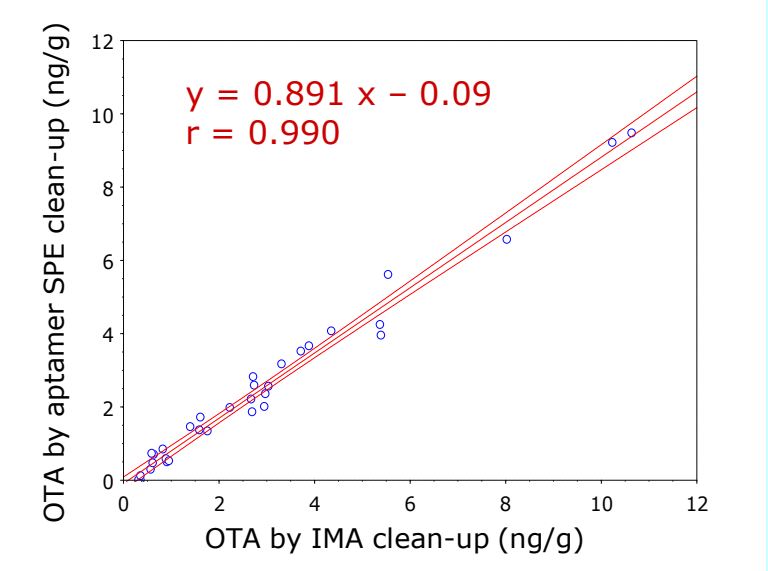
DNA aptamer-SPE column clean-up ï OTA in wheat



De Girolamo A, McKeague M, Miller DJ, DeRosa MC, and Visconti A, 2011. *Food Chem.*, 127, 1378-1384.

DNA aptamer-SPE column clean-up ï OTA in wheat

- ✓ **detection limit:** 0.02 µg/kg
- ✓ **accuracy:** 84%
- ✓ **precision:** ≤ 8%
- ✓ **linearity range:** 0.08 - 50 µg/kg
- ✓ **validation:** comparison with HPLC/IMA analysis (33 naturally contaminated wheat samples)



Extraction
(80% methanol)

↓

SPE purification
(oligosorbent)

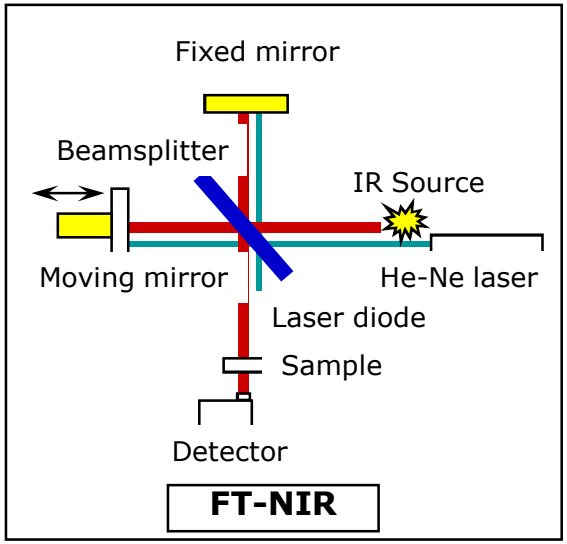
↓

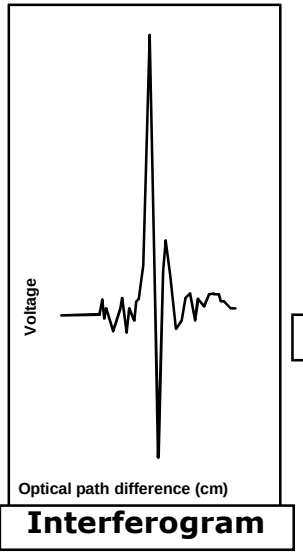
HPLC determination

De Girolamo A, McKeague M, Miller DJ, DeRosa MC, and Visconti A, 2011. *Food Chem.*, 127, 1378-1384.

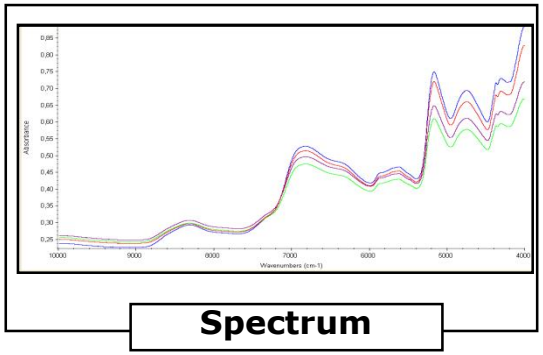
Fourier Transform Near Infrared Spectroscopy (FT - NIR)

- FT-NIR are faster, more sensitive and measure simultaneously the entire spectrum.
- FT-NIR spectra show higher resolution.

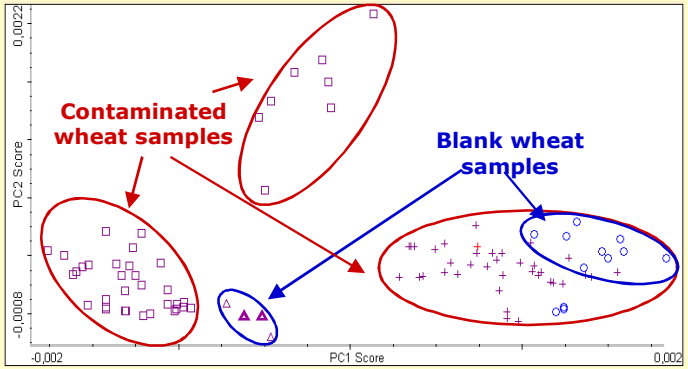




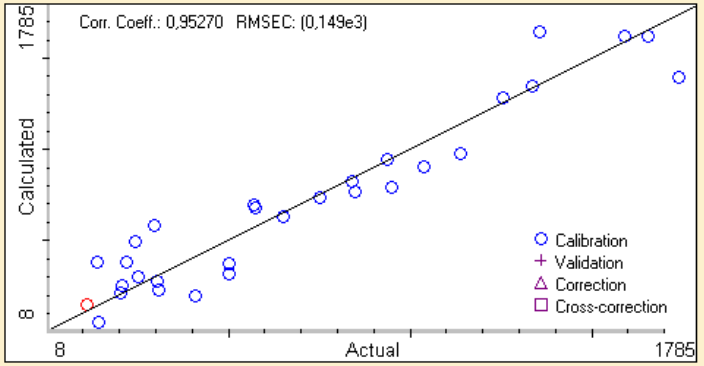
FT Transform



Qualitative analysis



Semi-quantitative analysis

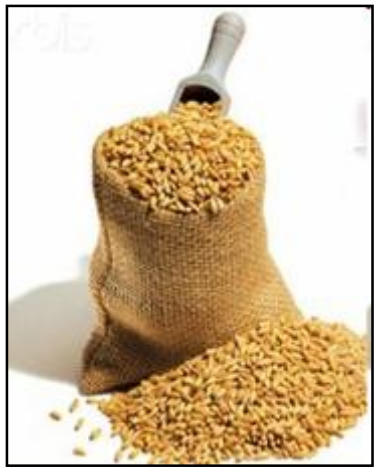


FT NIR ï DON in wheat

Sample preparation

Time < 3 min

Sample collection



wheat infected by
Fusarium species
(DON contamination)

Milling

Particle size < 500 µm

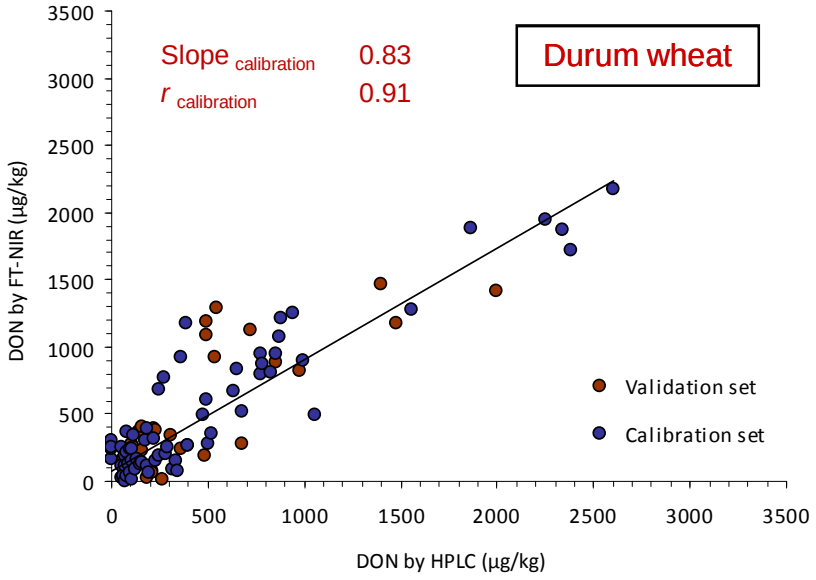
FT-NIR Analysis

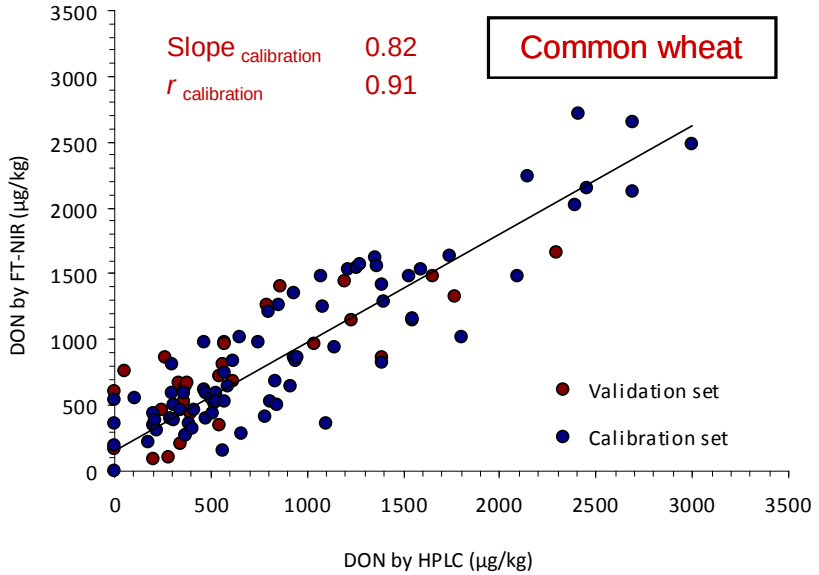
Time < 2 min

Nicolet Antaris II, Thermo Scientific Corp.
NIR region, 10000-4000 cm^{-1}
Resolution, 8 cm^{-1}
Scans, 128/sample
Detector, InGaAs

RAPID/NOT DESTRUCTIVE

FT NIR ï DON in wheat



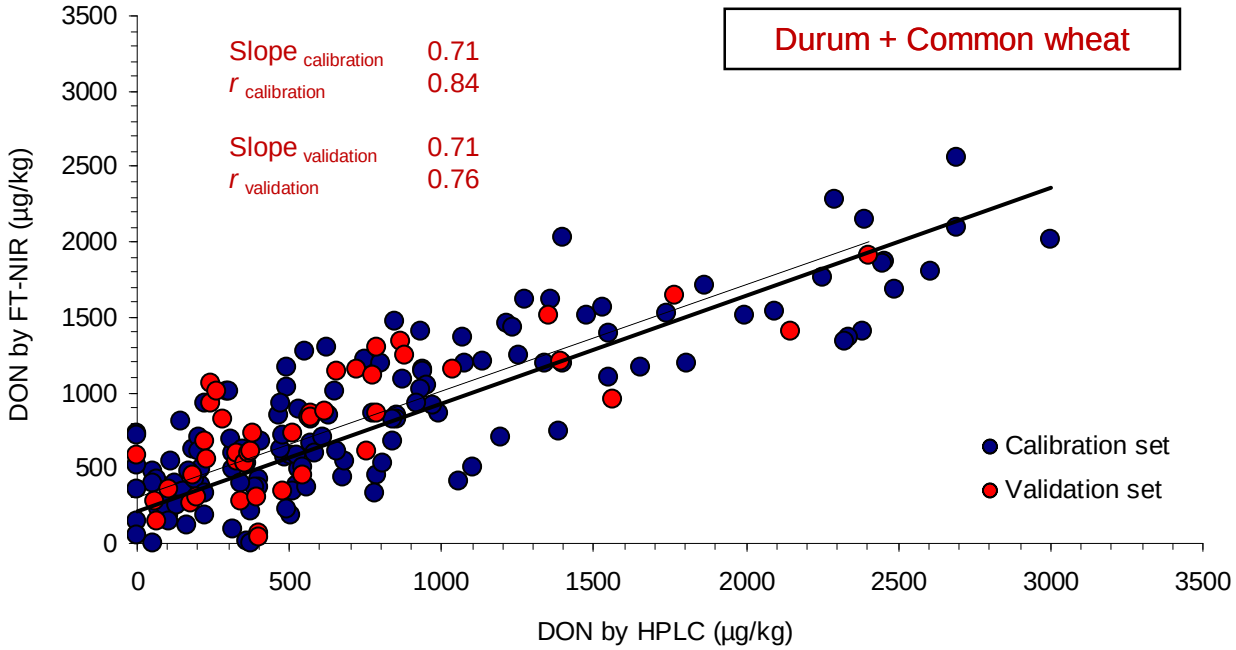


Wheat	Calibration/ validation set	Range calibration set (µg/kg)	RMSEC* (µg/kg)	RMSEP* (µg/kg)	RMSECV* (µg/kg)
Durum	**76 / 30**	**0 - 2600**	**240**	**306**	**470**
Common	**77 / 30**	**0 - 3000**	**303**	**348**	**516**
Durum + Common	149 / 48	0 - 3000	386	379	555

*Root Mean Square Error of Calibration (RMSEC), of Prediction (RMSEP), of Cross-Validation (RMSECV)

De Girolamo A., Lippolis V., Nordkvist E., and Visconti A., 2009. *Food Add Contam.*, 26, 6, 907-917.

FT NIR ï DON in wheat



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De Girolamo A., Lippolis V., Nordkvist E., and Visconti A., 2009. *Food Add Contam.*, 26, 6, 907-917.

CONCLUSIONS

Method	Advantages	Disadvantages
Multi-mycotoxin dipsticks	- Rapid - Practical - No clean-up	- Semi-quantitative - Antibody cross-reactivity - Matrix-interference problems
FPIA	- Rapid - Automated - No clean-up	- Antibody cross-reactivity - Calibration models needed - Matrix effects - No application to multi-mycotoxin analysis
FT-NIR	- Rapid - Not destructive - Practical	- Qualitative/semi-quantitative - Expensive equipment - Specific Calibration models needed - Statistics basis required
Aptamer-SPE	- Good selectivity - Good stability - Re-usability - Good batch-to-batch reproducibility	- Sensitivity - No commercial availability

Acknowledgements

United States Department of Agricultural
Agricultural Research Service

- Chris M. Maragos

Carleton UNIVERSITY Chemistry Department

- David J. Miller
- Maria C. DeRosa
- Maureen McKeague

Basilica of St. Nicholas, Bari, Italy

IFA TULLN Department for Agrobiotechnology, IFA-Tulln
University of Natural Resources and Applied Life Sciences BOKU

- Rudolf Krska