



CONFIDENCE: Rapid methods: BSc education modules 4 and 11 October 2011



CONFIDENCE: Rapid methods



BSc education modules

4 and 11 October 2011

Rapid methods for mycotoxins detection and analysis

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Protocol of use of the 4-Mycosensor

Extraction protocol

- 1) Weigh 2 grams of ground maize in a 50 ml falcon
- 2) Add 8 ml of ultra pure water
- 3) Vortex 2 minutes with the maximum speed
- 4) Add 12 ml of methanol
- 5) Vortex 2 minutes with the maximum speed
- 6) Let stand the mixture until no particulate remains (1 minute)
- 7) Add 900 μ l of 4-MycoBuffer in an eppendorf 1.5 ml
- 8) Take 100 μ l of the extract and add them in the eppendorf
- 9) Mix gently 5 times by hands

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Dipstick test procedure

- 1) Place a well containing the freeze-dried reagents in the heatsensor duo
- 2) Take 200 μ l of the diluted extract and add them in the well, homogenize by pipetting up and down 10 times the liquid (avoid foam formation by maintaining the tip well inside the liquid)
- 3) Start the 10 minutes of incubation at 40°C
- 4) Place the strips in the heatsensor Duo, just up to the well
- 5) After the 10 minutes of migration, remove the filter pad and analyze the result with the Readsensor



unisensor

Determination of Deoxynivalenol, Zearalenone, T-2/HT-2 toxins, Fumonisin B1 and B2 in grains by multiplex dipstick immunoassay

CAH Dronten
Dr. N. Nivarlet
October 11th 2011



7 www.confidence.eu

UNISENSOR

Food Diagnostic Engineering **unisensor**
www.unisensor.de



Organization

- From R&D to
- R&D partner
- International
- A team of 25

4-mycosensor alta sensor meta sensor

Plan

- General introduction of the *Confidence* project
- Introduction on dipstick tests
- Development of the mycotoxin dipstick test
- How to use the 4-Mycosensor kit
- Conclusions

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Confidence

Confidence
Contaminants in food and feed : Inexpensive
Detection for Control of Exposure


- FP7 collaborative Project
- Duration : 48 months (2008-2012)
- Participants : 17 partners from 10 countries, representing universities, research institutes, industry and SMEs

The target contaminants

- **Cluster 1 : Organic Pollutants**
 - POPs (WP1a)
 - Perfluorinated compounds (PFCs) (WP1b)
 - Pesticides (WP1c)
- **Cluster 2 : Veterinary pharmaceuticals**
 - Coccidiostats (WP2a)
 - Antibiotics (WP2b)
- **Cluster 3 : Heavy metals speciation (WP3)**
- **Cluster 4 : Biotoxins**
 - Alkaloids (WP4a)
 - Marine Biotoxins (WP4b)
 - Mycotoxins (WP4c)



The commodities in Confidence


- Fish/shellfish, fish feed 
- Cereals, cereal-based feed and food 
- Potatoes/vegetables 
- Honey, dairy products 
- Eggs, meat 

Objective

Development of multiplex dipstick test for ZEA, DON, T-2/HT-2 and FB1/FB2 in grains (wheat, Maize, oats), feed and cereal based-food.

Requirements :

- Easy
- Rapid
- Sensitive
- Selective
- Cost-effective

Dipsticks 

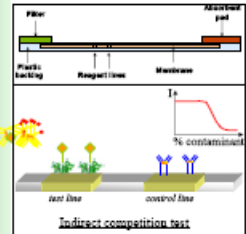
- By developing the multiplex dipstick in buffer
- By adapting the multiplex dipstick in real matrices

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Dipstick Tests

What is a dipstick?



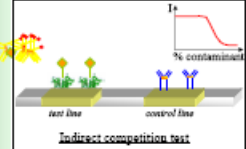
Dipstick manufacturing

Reagents preparation

- Immunogens → antibodies
- Competitors (protein conjugates)
- Gold labelling of antibodies

Dipstick design

- Assembly of all the membranes
- Stripping of the reagents
- Drying and stabilization

Indirect competition test 

Dipstick Tests

Preparation of immunogens and production of antibodies

Large protein carrier (Hemocyanin) → *Limulus Polyphemus* → LPH (1000 active sites)

Targeted molecule → LPH-targeted molecule

Monoclonal antibodies

Polyclonal antibodies

Injection

Dipstick Tests

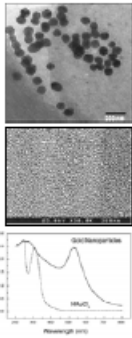
Antibodies gold-labelling

Gold Nps synthesis

HAuCl₄ → Chemical reduction → Stable GNPs

Labeling of Ab with Gold Nps

GNPs + IgG → Electrostatic interaction → GNP-IgG



Dipstick Tests

Preparation of competitors

Test line
control line
competitors

Targeted molecule

BSA
60 active sites

BSA-targeted molecule

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Mycotoxin dipstick test

> Design of Multiplex dipsticks (ZEA, T2/HT2, DON, FBs)

CTRL
FB1-BSA
DON-BSA
T2-BSA
ZEA-BSA

MULTIPLEX DIPSTICK

Mycotoxin dipstick test

> Design of Multiplex dipsticks (ZEA, T2/HT2, DON, FBs)

CTRL
FB1-BSA
DON-BSA
T2-BSA
ZEA-BSA

MULTIPLEX DIPSTICK

NEGATIVE sample

Mycotoxin dipstick test

> Design of Multiplex dipsticks (ZEA, T2/HT2, DON, FBs)

CTRL
FB1-BSA
DON-BSA
T2-BSA
ZEA-BSA

MULTIPLEX DIPSTICK

NEGATIVE sample

Test Lines darker than CTRL line

Mycotoxin dipstick test

> Design of Multiplex dipsticks (ZEA, T2/HT2, DON, FBs)

CTRL
FB1-BSA
DON-BSA
T2-BSA
ZEA-BSA

MULTIPLEX DIPSTICK

POSITIVE sample

Mycotoxin dipstick test

> Design of Multiplex dipsticks (ZEA, T2/HT2, DON, FBs)

CTRL
FB1-BSA
DON-BSA
T2-BSA
ZEA-BSA

MULTIPLEX DIPSTICK

POSITIVE sample

Test Lines lighter than CTRL line

Mycotoxin dipstick test

> Design of Multiplex dipsticks (ZEA, T2/HT2, DON, FBs)

CTRL
FB1-BSA
DON-BSA
T2-BSA
ZEA-BSA

MULTIPLEX DIPSTICK

LOW POSITIVE sample

Test Lines equal to CTRL line

Mycotoxin dipstick test

The CONTROL line will be used to validate the dipstick test and also to compare all the test lines to an internal reference.

No CTRL line = Invalid
CTRL line = Valid

Ratio (test line/control line)
Ratio < x Positive
Ratio > x Negative
x depends on the toxin and the matrix

ZEA T-2 DON FB CTRL

Mycotoxin dipstick test

Cut off ratios for maize and wheat

Maize			Wheat		
Toxin	Ratio (x)	Result	Toxin	Ratio (x)	Result
ZEA	1.0 x ± 0.5	LPOB	ZEA	1.0 x ± 0.5	LPOB
	< 1.0	POB		< 1.0	POB
	> 1.0	NBO		> 1.0	NBO
T2/HT2	1.0 x ± 0.18	LPOB	T2/HT2	1.0 x ± 0.18	LPOB
	< 1.0	POB		< 1.0	POB
	> 1.0	NBO		> 1.0	NBO
DON	1.0 x ± 0.14	LPOB	DON	0.8 x ± 0.12	LPOB
	< 1.0	POB		< 0.8	POB
	> 1.0	NBO		> 1.0	NBO
FB1/FB2	0.5 x ± 0.08	LPOB			
	< 0.5	POB			
	> 0.5	NBO			

Sensitivity of the multiplex dipstick test
80% of the EU MRLs

Detection Limit (µg/kg)	ZEA	T-2/HT-2	DON	FB1/FB2
Maize	280	400	1400	3200
Wheat or Rye	80	400	1400	-

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Protocol

2g of ground sample + 8ml of Water

EXTRACTION

vortex 2 minutes

+ 12 ml of Methanol

vortex 2 minutes

Dilution 10 times in 4-Mycobuffer

Run the dipstick test

Protocol

2g of ground sample

10ml of MeOH/Water (80:20)

EXTRACTION

vortex 2 minutes

Dilution 20 times in 4-MycoBuffer

Run the dipstick test

Protocol

- 1) Dilute the extract 10/20 times in 4-MycoBuffer
- 2) Add 200 µl of the diluted extract in the well, mix 15 times to homogenize and let incubate 10 minutes @ 40°C
- 3) Add the strips in the wells and wait for another 10 minutes
- 4) Remove the filter and read the strips with the Readsensor

Protocol

- 1) Dilute the extract 10/20 times in 4-MycoBuffer

Malze

CTRL					
FBs					
DON					
T-2HT-2					
ZEA					
Blank	ZEA 200ppb	T2 400ppb	DON 1400ppb	FB1 2000ppb	

Conclusions

4myco'sensor

- Detects 4 mycotoxins in grains (ZEA, DON, T-2HT-2, FB1/FB2) at 80% of their MRL in only 20 minutes and in one single step
- Rapid, sensitive and selective test
- Low price
- One single extraction method
- Easy to run
- Very Simple test, no particular skill

> **General Informations:** info@unisensor.be; noan.nivariet@unisensor.be

> **Commercial Purposes:** contact olivier.heymen@unisensor.be

Thanks to

Thank you !