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

MycoRed Training Course

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October 6th 2010




SEVENTH FRAMEWORK PROGRAMME

www.conffidence.eu

CONFFIDENCE

UNISENSOR

Food Diagnostic Engineering

unisensor
www.unisensor.be

Supplying Innovative and Advanced Products for the Rapid Analysis of Food Products.

Organisation

- From R&D to Market
- R&D partner in EU-FP7 (CONffIDENCE)
- International distribution network
- A team of 23 people

Products

- Antibiotics (TetraSensor, TwinSensor, SulfaSensor, QuinoSensor, CapSensor)
- Authenticity : CowSensor, GoatSensor, ChickenSensor, PorkSensor, BeefSensor
- Adulteration : MelamineSensor
- Mycotoxins : MycoSensor, AflaSensor

trisensor
Tetrasensor
Twinsensor BT
mycosensor
aflasensor
melasensor fluo

Plan

- General introduction of the *Conffidence* project
- Our Objectives in the *Conffidence* project
- Introduction on dipstick tests
- Description of the dipstick test for **Maize** (in Lab)
  - Protocol
  - Results interpretation
- Future Plans

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Conffidence

Conffidence

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 *Conffidence*

- Fish/shellfish, fish feed

- Cereals, cereal-based feed and food

- Potatoes/vegetables

- Honey, dairy products

- Eggs, meat

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Objective

Develop Multiplex dipstick tests

for the determination of the *Fusarium* toxins DON, ZEA and T-2/HT-2 in **wheat**, **barley** and **oats**;

for DON, ZEA, T-2/HT-2 and FBs in **Maize** and maize by-products for **feed**;

for DON, ZEA, T-2/HT-2 and FBs in cereal based **food**.

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

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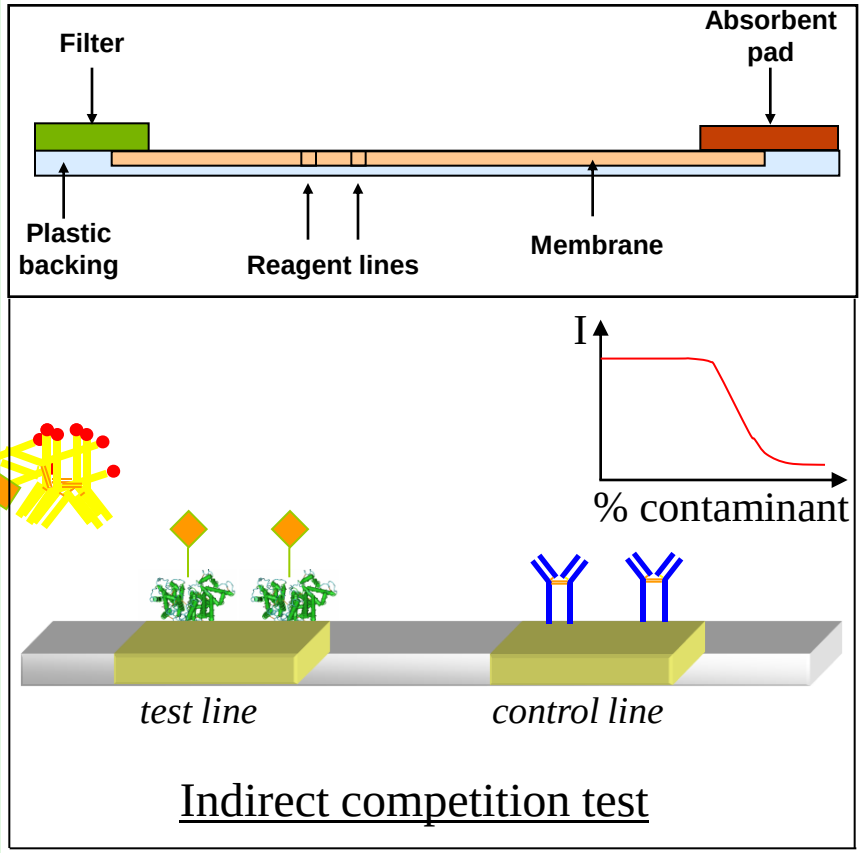
Dipstick Tests

What is a dipstick?



Need of reagents :

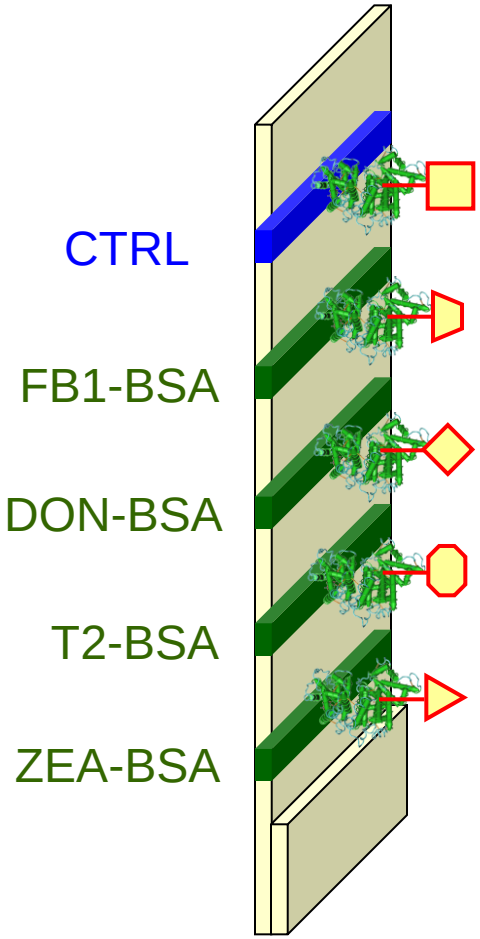
- competitors (ISPA, Unisensor)
- immunogens (Unisensor)
- Antibodies (CER)

For each test line

(ZEA, T2, DON, FB1)

Dipstick Tests

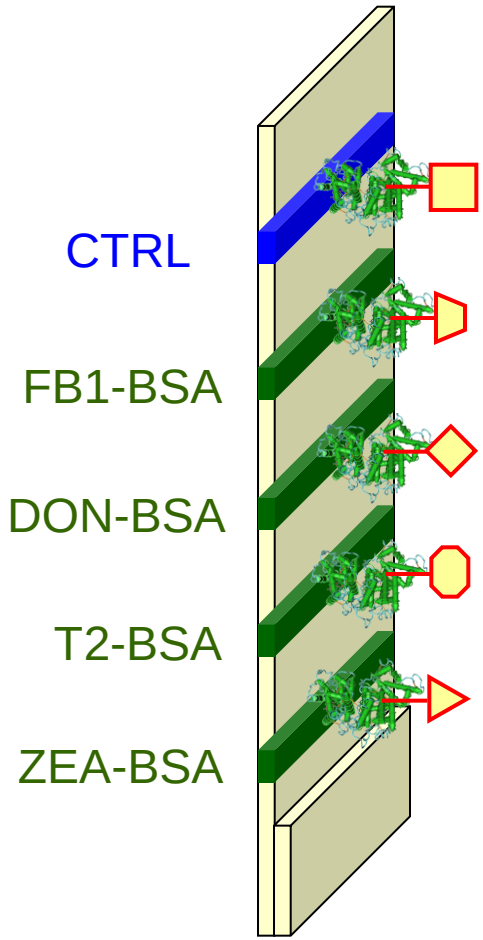
- *Design of Multiplex dipsticks (ZEA, T2/HT2, DON, FBs)*



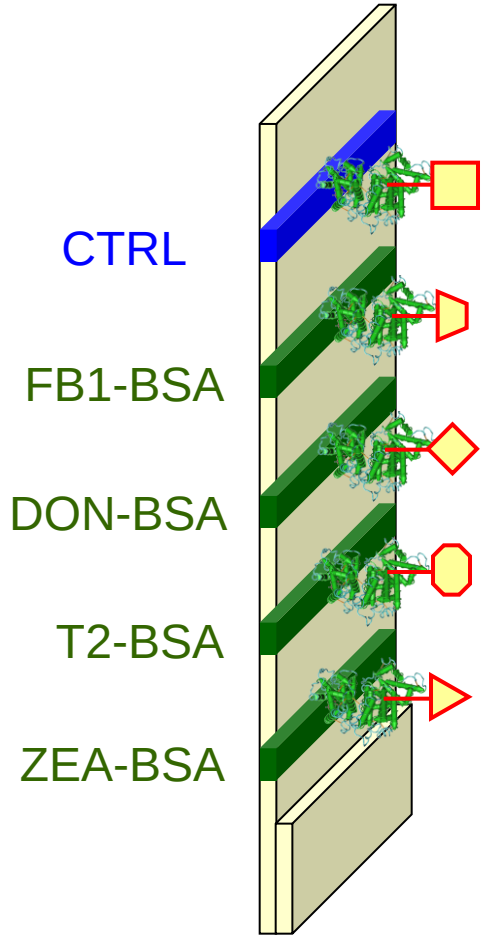
MULTIPLEX DIPSTICK

Dipstick Tests

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



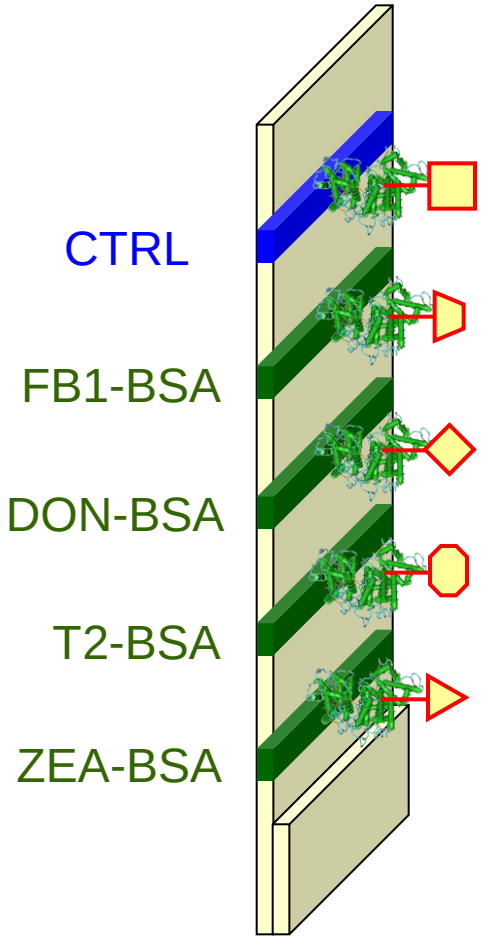
MULTIPLEX DIPSTICK



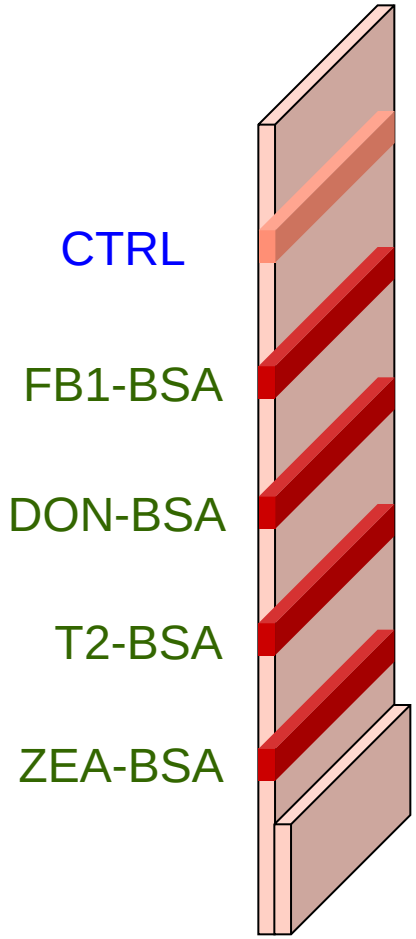
NEGATIVE sample

Dipstick Tests

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



MULTIPLEX DIPSTICK

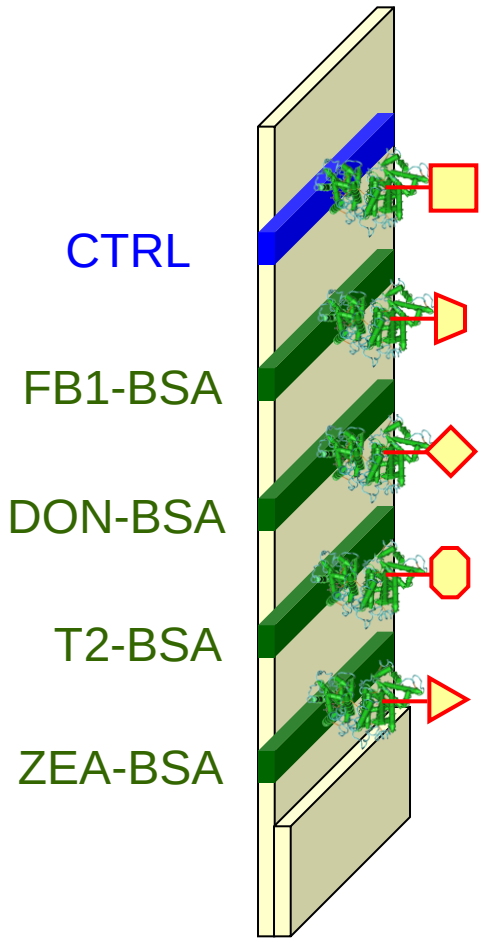


NEGATIVE sample

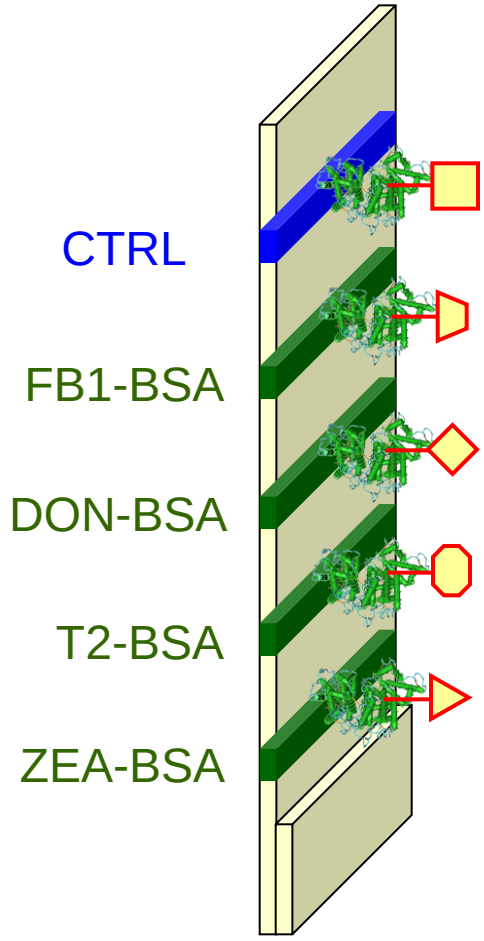
Test Lines darker than CTRL line

Dipstick Tests

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



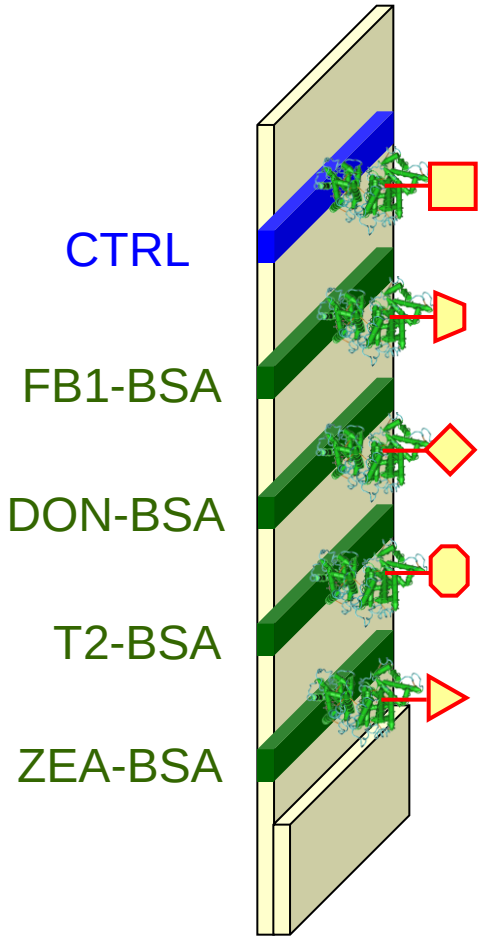
MULTIPLEX DIPSTICK



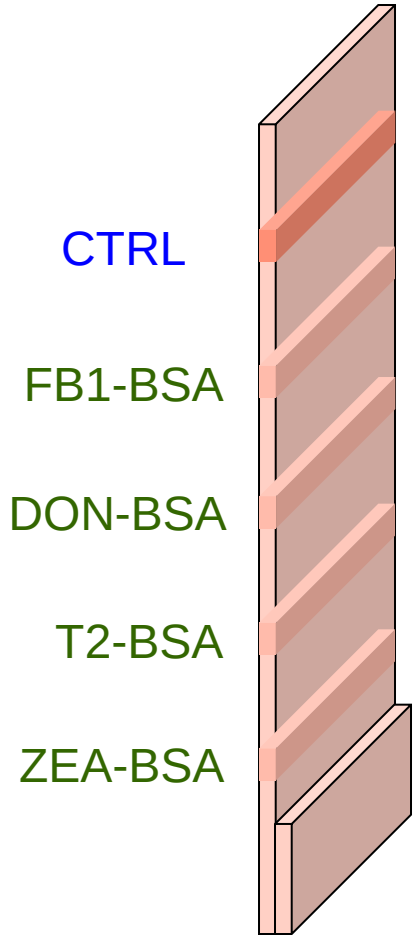
POSITIVE sample

Dipstick Tests

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



MULTIPLEX DIPSTICK

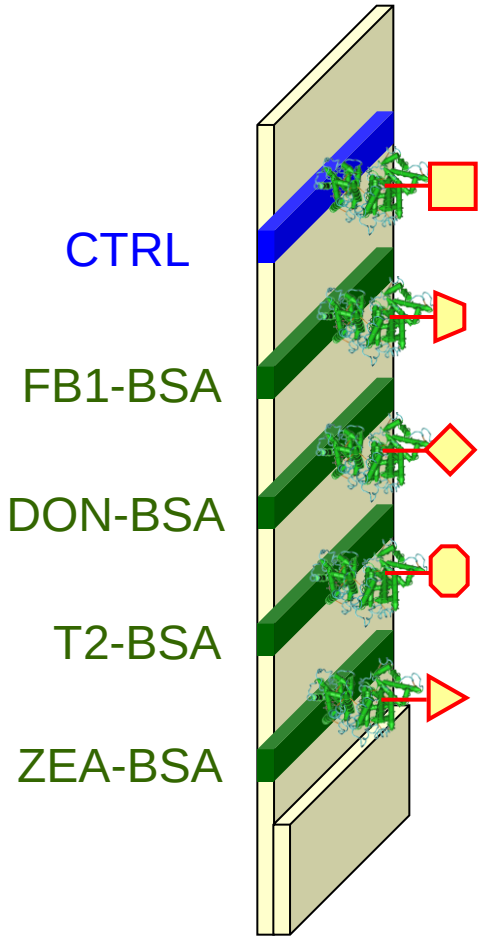


POSITIVE sample

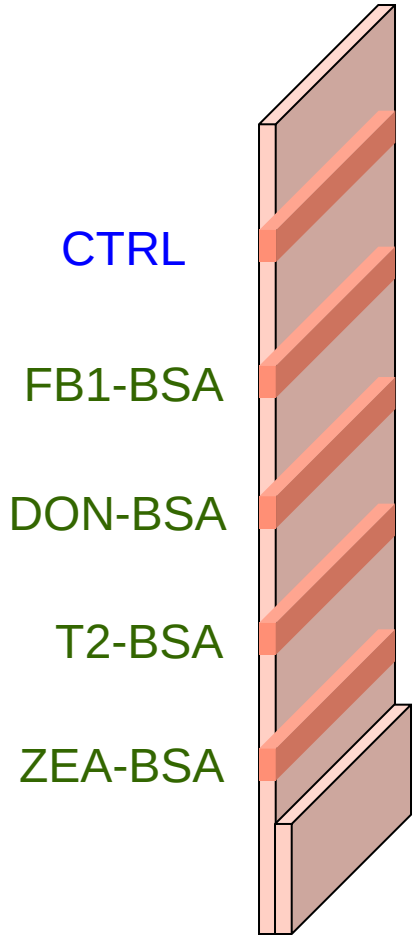
Test Lines lighter than CTRL line

Dipstick Tests

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



MULTIPLEX DIPSTICK



Test Lines equal to CTRL line

LOW POSITIVE sample

Dipstick Tests

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

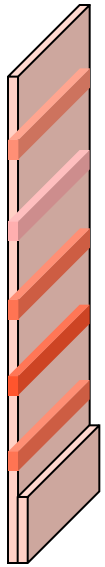
No CTRL line = Invalid

CTRL line = Valid

In order to conclude if the test is POSITIVE or NEGATIVE, the reader will compare each test line to the CTRL line.

Since each test line doesn't give positive result for the same ratio (usually 1), the reading with the readsensor is a must

ReadSensor



	Low POS
CTRL	≤ ratio 0.5
FB1	≤ ratio 1.3
DON	≤ ratio 1.6
T2	≤ ratio 1.3
ZEA	

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Multiplex dipstick tests in MAIZE

EXTRACTION

10g of ground sample

+ 40ml of Water

Blending 2 minutes

+ 60ml of Methanol

Blending 2 minutes

Dilution 10 times in Buffer 1

Run the dipstick test

Total time 4min.

Multiplex dipstick tests in MAIZE

PROTOCOL

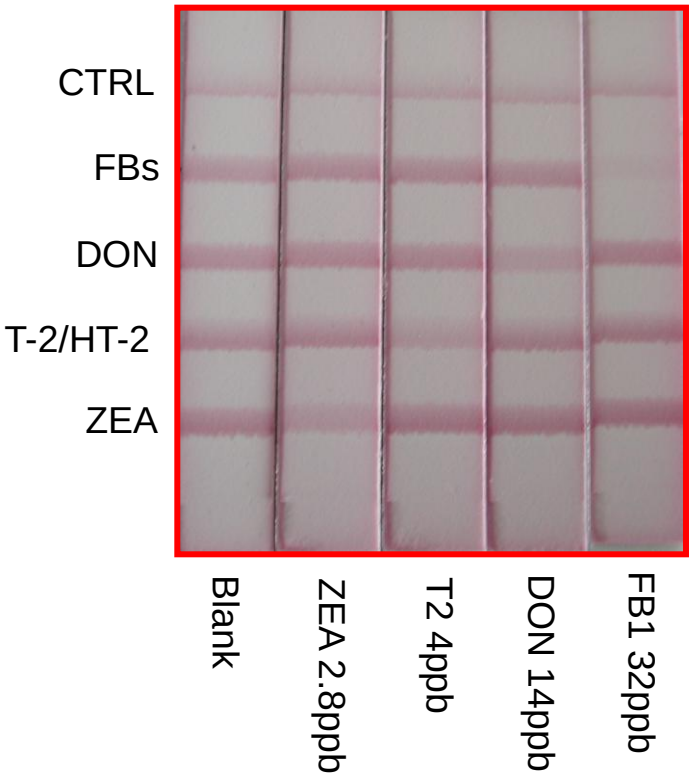
1) Dilute the extract 10 times in Buffer 1

2) Add 200 µl of the diluted extract in the well, mix 20 times to homogenize

3) Let Incubate 10 minutes @ 40°C

4) Add the strips in the wells and wait for another 10 minutes

5) Remove the filter and read the strips with the Readsensor



CUT OFF

	POS	LPOS	NEG
ZEA	0	1.0	1.3
T2	0	1.3	1.6
DON	0	1.0	1.3
FB1	0	0.3	0.5

Limits of Detection

- 80% of EU levels

Desired LOD (µg/kg)	**DON**	**ZEA**	**T2+HT-2**	**FB1+FB2**
Maize	1400	280	400	3200
Durum Wheat/Oats	1400	80	400	-
Maize feed (corn gluten)	9600	2400	400	4000
Maize food	400	80	80	640
Oat/Wheat food	400	40	80	-

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Future Plans

- Validation : Scheduled for **JULY 2011** (inside the conffidence)
- Launch of the Product : “MYCOSENSOR”
  Scheduled for the **beginning of 2011**
- Price : to be determined
- Distributors : Distributors of UNISENSOR’s Products
- General Informations : info@unisensor.be
- Commercial Purposes : contact olivier.heynen@unisensor.be

….That’s time for the
demonstration in Lab….

Thank you !