

Development and in house validation of multiplex dipstick immunoassays for semi-quantitative determination of *Fusarium* mycotoxins in cereals

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WP overall objectives

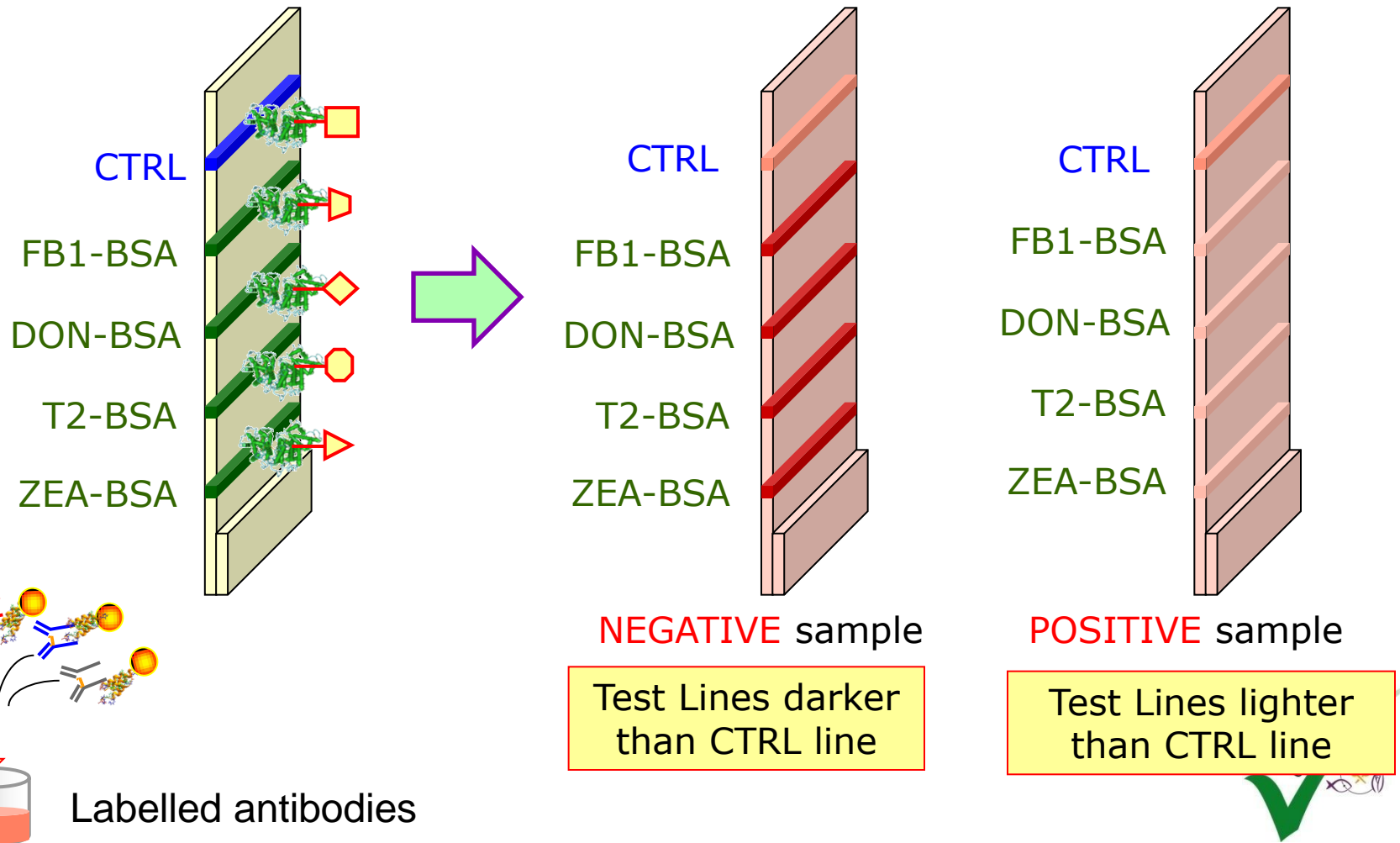
To develop commodity dedicated **multiplex dipstick tests** for the determination of the ***Fusarium* toxins** deoxynivalenol, zearalenone, T2/HT2 toxins, fumonisins

- Objective 1: prototype dipstick development
- Objective 2: development of simplified sample preparation protocols
- Objective 3: method validation
- Objective 4: impact demonstration



Prototype multiplex dipstick

Indirect competitive immunoassay

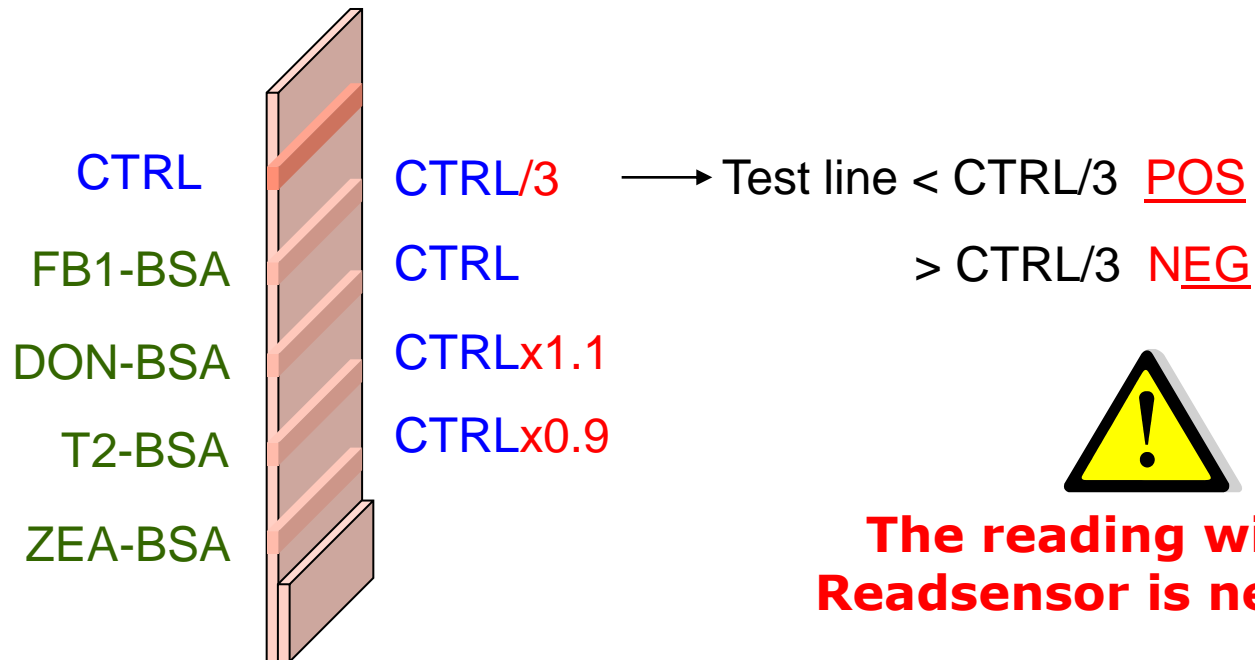


Prototype multiplex dipstick

The **CONTROL line** is used to compare all the test lines but also to **validate** the test.

No CTRL line = **Invalid**
CTRL line = **Valid**

In order to conclude if the test is POSITIVE or NEGATIVE (with respect to a cut off value) the reader takes the intensity value of the CTRL line and applies a calculation for each test line.



Target matrices and *desired* cut off

| | Target levels $\mu\text{g}/\text{kg}$ (80% EU ML) | | | |
|------------------|---|-------------------|--------------|--|
| | DON | T-2 + HT-2 | ZEA | FB₁ + FB₂ |
| Maize | 1400 (1750) | 400 (500) | 280 (350) | 3200 (4000) |
| Wheat/oat | 1400 (1750) | 400 (500) | 80 (100) | |

Cut off values in real matrices are determined through in house and interlaboratory validation



Simplified sample preparation protocols

Commodity dedicated **extraction procedures:**

wheat, oat
(DON, ZEA, T2/HT2)

Ground sample (10g)



{ Methanol /water 80/20, 50 mL
{ High speed blending, 2min



Extract dilution



Dipstick analysis

3 min

maize
(DON, ZEA, T2/HT2, FB₁/FB₂)

Ground sample (10g)



{ Water (40 mL)
{ High speed blending, 2min
{ Add Methanol (60 mL)
{ High speed blending, 2min



Extract dilution



Dipstick analysis

6 min



The final assay procedure



Methanol/water extraction



Dilution with buffer



Incubation at 40°C, 10 min
Migration, 10 min



Reading



Negative sample
positive ZEA
Positive ZEA/T2
Positive ZEA/T2/DON
Positive ZEA/T2/DON/FB1

Total analysis time: 30 min

The commercial kit



4 myco sensor

Multiple strip test detecting Deoxynivalenol, Zearalenone, Fumonisin FB1/FB2 and T-2/HT-2 mycotoxins in one single test



www.unisensor.be



Analysis of naturally contaminated samples

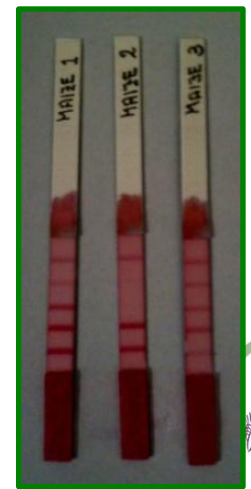
| SAMPLE | | ZEA | | | T-2+HT2 | | | DON | | | FB1+FB2 | | |
|--------|-------|------------|------------|------------------|------------|------------|------------------|------------|------------|------------------|----------|--------|------------------|
| | | Dipstick | | LC-MS/MS (µg/kg) | Dipstick | | LC-MS/MS (µg/kg) | Disptick | | LC-MS/MS (µg/kg) | Dipstick | | LC-MS/MS (µg/kg) |
| | | Ratio | Result | | Ratio | Result | | Ratio | Result | | Ratio | Result | |
| 1 | maize | 3.0 | NEG | n.d. | 3.2 | NEG | n.d. | 2.4 | NEG | n.d. | 0.7 | NEG | 725 |
| 2 | maize | 2.5 | NEG | n.d. | 3.0 | NEG | n.d. | 0.0 | POS | 24200 | 0.0 | POS | 8150 |
| 3 | maize | 0.6 | POS | 420 | 1.5 | NEG | 392 | 1.1 | POS | 298 | 0.8 | NEG | 725 |
| 4 | maize | 2.0 | NEG | 13 | 2.4 | NEG | n.d. | 1.5 | NEG | 198 | 3.2 | NEG | 47 |
| 5 | wheat | 2.0 | NEG | n.d. | 1.2 | POS | 324 | 2.3 | NEG | 15 | | | |
| 6 | wheat | 2.1 | NEG | n.d. | 0.9 | POS | 653 | 2.5 | NEG | 21 | | | |
| 7 | wheat | 1.9 | NEG | n.d. | 0.9 | POS | 824 | 2.5 | NEG | 74 | | | |
| 8 | wheat | 2.0 | NEG | n.d. | 0.8 | POS | 1198 | 1.2 | NEG | 1328 | | | |
| 9 | wheat | 2.4 | NEG | n.d. | 0.9 | POS | 1852 | 1.4 | NEG | 1066 | | | |
| 10 | oats | 0.7 | POS | 614 | 1.3 | POS | 1053 | 0.2 | POS | 640 | | | |
| 11 | oats | 1.9 | NEG | n.d. | 0.9 | POS | 508 | 2.0 | NEG | 96 | | | |
| 12 | oats | 2.0 | NEG | n.d. | 0.5 | POS | 2302 | 2.0 | NEG | 124 | | | |
| 13 | oats | 1.4 | POS | 55 | 0.7 | POS | 1160 | 1.8 | NEG | 188 | | | |
| 14 | oats | 2.1 | NEG | n.d. | 0.8 | POS | 794 | 1.7 | NEG | 210 | | | |
| 15 | oats | 2.1 | NEG | n.d. | 1.4 | POS | 159 | 2.6 | NEG | 4 | | | |

* False positive results are in bold characters

Analysis of naturally contaminated samples (maize, wheat and oats, n=15) resulted in a good agreement between dipstick and LC-MS/MS results:

- **few false positive results** (ZEA: 1 oats; T-2/HT-2: 1 oats and 1 wheat; DON: 1 maize and 1 oats; FBs: no false positive)
- **no false negative results**

Lattanzio et al., Analitica Chimica Acta 2012, 718:99-108



In house validation: main results

- By conducting of a validation with **30 samples** we were able to establish
 - Precision data
 - Ruggedness test
 - Cut off value with rate of false positive results
- We checked **fitness for purpose** by considering the **cost situation and expected frequency distribution** of target analytes
- The **test** presented here is considered **fit for purpose**



Follow up....

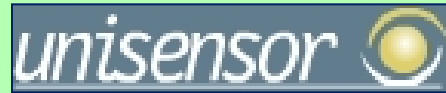
Large scale interlaboratory validation

- **Number of participants:** 13 Laboratories
- **Matrix/mycotoxin combinations:**
Wheat: DON, ZEA, T-2, HT-2
Maize: DON, ZEA, T-2, HT-2, FB₁, FB₂
- **Samples and contamination levels to be analyzed by each participant:**
Wheat/Maize
Blank, spiked at 50%, 100% EU maximum permitted levels
- **Expected results/information:**
Precision profile under reproducibility conditions
Cut off – rate of false positives

Study in progress – results expected by December 31, 2012



Aknowledgements



*Thanks to
all WP4c partners
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***Thank you
for your attention!***

