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

Veronica M.T. Lattanzio  
*National Research Council of Italy*  
*Institute of Sciences of Food Production*

C. von Holst, N. Nivarlet, B. Granier, A. Visconti

www.conffidence.eu
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

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

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

NEGATIVE sample
Test Lines darker than CTRL line

POSITIVE sample
Test Lines lighter than CTRL line

Labelled antibodies
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.

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

CTRL/3 \[\rightarrow\] Test line < CTRL/3  **POS**
CTRL \[\rightarrow\] Test line > CTRL/3  **NEG**
CTRLx1.1
CTRLx0.9

The reading with the **Readsensor** is necessary.
### Target matrices and desired cut off levels

<table>
<thead>
<tr>
<th></th>
<th>DON</th>
<th>T-2 + HT-2</th>
<th>ZEA</th>
<th>(\text{FB}_1 + \text{FB}_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>1400 (1750)</td>
<td>400 (500)</td>
<td>280 (350)</td>
<td>3200 (4000)</td>
</tr>
<tr>
<td>Wheat/oat</td>
<td>1400 (1750)</td>
<td>400 (500)</td>
<td>80 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Target levels µg/kg (80% EU ML)

**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

**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

3 min

6 min

*Lattanzio et al., Analitica Chimica Acta 2012, 718:99-108*
The final assay procedure

1. Methanol/water extraction
2. Dilution with buffer
3. Incubation at 40°C, 10 min
4. Migration, 10 min

Total analysis time: 30 min

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

Reading
The commercial kit

4 myco sensor
Multiple strip test detecting Deoxynivalenol, Zearalenone, Fumonisins FB1/FB2 and T-2/HT-2 mycotoxins in one single test

www.unisensor.be
## Analysis of naturally contaminated samples

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>ZEA</th>
<th>T-2+HT2</th>
<th>DON</th>
<th>FB1+FB2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dipstick</td>
<td>LC-MS/MS (µg/kg)</td>
<td>Dipstick</td>
<td>LC-MS/MS (µg/kg)</td>
</tr>
<tr>
<td>1 maize</td>
<td>3.0</td>
<td>NEG</td>
<td>n.d.</td>
<td>3.2</td>
</tr>
<tr>
<td>2 maize</td>
<td>2.5</td>
<td>NEG</td>
<td>n.d.</td>
<td>3.0</td>
</tr>
<tr>
<td>3 maize</td>
<td>0.6</td>
<td>POS</td>
<td>420</td>
<td>1.5</td>
</tr>
<tr>
<td>4 maize</td>
<td>2.0</td>
<td>NEG</td>
<td>13</td>
<td>2.4</td>
</tr>
<tr>
<td>5 wheat</td>
<td>2.0</td>
<td>NEG</td>
<td>n.d.</td>
<td>1.2</td>
</tr>
<tr>
<td>6 wheat</td>
<td>2.1</td>
<td>NEG</td>
<td>n.d.</td>
<td>0.9</td>
</tr>
<tr>
<td>7 wheat</td>
<td>1.9</td>
<td>NEG</td>
<td>n.d.</td>
<td>0.9</td>
</tr>
<tr>
<td>8 wheat</td>
<td>2.0</td>
<td>POS</td>
<td>n.d.</td>
<td>0.8</td>
</tr>
<tr>
<td>9 wheat</td>
<td>2.4</td>
<td>NEG</td>
<td>n.d.</td>
<td>0.9</td>
</tr>
<tr>
<td>10 oats</td>
<td>0.7</td>
<td>POS</td>
<td>614</td>
<td>1.3</td>
</tr>
<tr>
<td>11 oats</td>
<td>1.9</td>
<td>NEG</td>
<td>n.d.</td>
<td>0.9</td>
</tr>
<tr>
<td>12 oats</td>
<td>2.0</td>
<td>NEG</td>
<td>n.d.</td>
<td>0.5</td>
</tr>
<tr>
<td>13 oats</td>
<td>1.4</td>
<td>POS</td>
<td>55</td>
<td>0.7</td>
</tr>
<tr>
<td>14 oats</td>
<td>2.1</td>
<td>NEG</td>
<td>n.d.</td>
<td>0.8</td>
</tr>
<tr>
<td>15 oats</td>
<td>2.1</td>
<td>NEG</td>
<td>n.d.</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*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, FB_1, FB_2

- **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 for hard work and very fruitful collaboration!

Angelo Visconti
Vincenzo Lippolis
Noan Nivarlet
Benoit Granier
Christoph von Holst
Anne Chaterine Huet
Philippe Delahault
Hans van Egmond
Albert Swinkels
Karin Kraehenbuehl

Thank you for your attention!