Multiplex lateral flow immunoassay for Fusarium toxins in cereals

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I) Introduction

Fusarium species are plant pathogens commonly associated with cereals that, under favourable environmental conditions, can produce several secondary toxic metabolites. *Fusarium* toxins are widely distributed in the food chain in the EU and the major sources for their dietary intake are cereal products, mainly based on wheat and maize. The major *Fusarium* toxins found in cereals and cereal-based products that can be harmful to both human and animal health are deoxynivalenol (DON), T-2 toxin (T-2), HT-2 toxin (HT-2), zearalenone (ZEA) and fumonisins (FB1, FB2). In order to protect human health from exposure to these mycotoxins, the European Commission has recently established regulatory limits for DON, ZEA and fumonisins (sum of FB1 and FB2) in cereals and cereal-based foods and feeds, while permissible levels of T-2 and HT-2 are under discussion (EC Regulations No 1881/2006 and 1126/2007). The aim of mycotoxin research within CONffIDENCE project is to develop multiplex dipstick tests for the determination of the *Fusarium* toxins DON, ZEA, T-2/HT-2 and FBs toxins more particularly in cereals (wheat, maize and oat).

II) Antibodies characterization

Antibodies raised against T-2/HT-2, DON, ZEA and Fumonisins were produced on rabbits. The sera were collected after injections of a mixture of modified-Hemocyanin immunogens and Freund's adjuvant.

ELISA	ZEA	DON	T-2	HT-2	FB1	FB2	FB3
IC_{50} (ng/ml)	0.10	3.0	2.5	7.1	0.19	0.20	0.35

Those polyclonal antibodies have been used for the development of the multiplex lateral flow immunoassay.

III) Extraction of mycotoxins

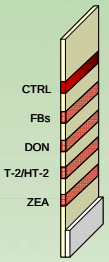
A simplified sample preparation protocol has been developed for raw wheat, oat and maize cereals for recovering the six mycotoxins of interest. In each kind of matrices, no more than 4 minutes of high speed blending in alcoholic medium are necessary to recover more than 70% of each mycotoxin (Table 1).

	Recovery %* (RSD %, n=3)			
	FB1	T-2/HT-2	DON	ZEA
Spiking level	-	500 µg/kg	1400 µg/kg	80 µg/kg
Wheat	-	73(6)	97(8)	103(6)
Oat	-	73(6)	93(4)	107(9)
Spiking level	3200 µg/kg	500 µg/kg	1400 µg/kg	280 µg/kg
Maize	109(3)	107(4)	105(3)	105(6)

Table 1 : Mycotoxin recoveries using the described extraction procedure
*Analysis made by LC-MS/MS

After extracting the 6 mycotoxins from cereals, the supernatant is recovered and is diluted in buffer 10 times for maize and 20 times for wheat and oat.

IV) Multiplex dipstick design



The multiplex dipstick is made up of four test lines and one dynamic control line. Each test line is constituted of a protein conjugate of the corresponding toxin. This dipstick assay is therefore a competitive test where the freeze-dried gold-labeled antibodies are in competition between the mycotoxins potentially present in the cereal and the mycotoxins immobilized on the strip.

The multiplex disptick assay makes it possible to detect six mycotoxins at 80% of the european regulations (Table 2).

LOD (µg/kg)	DON	ZEA	T-2/HT-2	FB1/FB2
Maize	1400	280	400	3200
Durum wheat/oat	1400	80	400	-

Table 2 : Detection limits of the multiplex lateral flow dipstick test

Protocol of the immunoassay

1) Dilute the extract 10 times (maize) or 20 times (wheat/Oat) in buffer
2) Mix 200 µl of the extract with the freeze-dried reagents
3) Start Incubation for 10 minutes at 40°C
4) Introduce dipsticks, read the test line intensities after 10 minutes

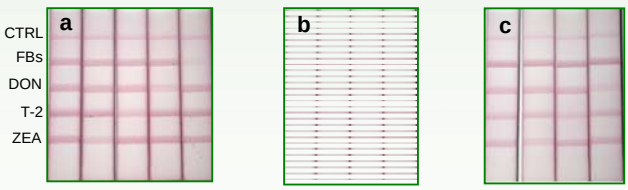


Figure 1: Example of dipstick tests run in spiked extracts of Maize (a), wheat (b) and oat (c) at the targeted level (i.e. 80% of the EC maximum permitted levels, see Table 1).

V) Conclusions

We have described in this work the development of a multiplex dipstick test able to detect six mycotoxins in maize, wheat and in oat in 20 minutes. The immunoassay enables to detect DON, ZEA, T-2/HT-2 and FB1/FB2 at 80% of the EC maximum permitted levels after a short extraction step of a maximum of four minutes by high speed blending. This test will be validated inside the CONffIDENCE project (FP7) and will be available for commercial purpose from June 2011.

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