CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

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, oat and barley).

II) Antibodies characterization

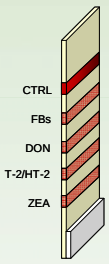
Antibodies raised against T-2/HT-2, DON, ZEA and Fumonisins were produced on rabbits. The sera were collected after injections of a mixture of modified-Hemocyanin immunogens and Freund's adjuvant.

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

Table 1 : IC_{50} of antibodies raised against the corresponding toxins

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

III) 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/barley	1400	80	400	-

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

VI) Conclusions

We have described in this poster the development of a multiplex dipstick test able to detect six mycotoxins in maize, wheat, oat and barley in 20 minutes. The immunoassay enables to detect DON, ZEA, T-2/HT-2 and FB1/FB2 at 80% of the EU maximum permitted levels after a short extraction step of a maximum of four minutes by high speed vortex. This test was validated inside the CONffIDENCE project and the analysis of the variance proved the ruggedness of this dipstick test since neither the matrix nor the day have important influence on the variabiltiy of results. Furthermore, the total variability is less than 15% for wheat and less than 20% for Maize which is acceptable for this kind of screening method. The 4Mycosensor is now available on the market.

IV) Protocol of the dipstick test

1) Extract 2 g of ground sample with H_2O (vortex 2 min) and then with MeOH (vortex 2 min) for maize or 2 minutes directly with a mixture MeOH/H_20 (80:20) for wheat, oat and barley
2) Dilute the extract 10 times (maize) or 20 times (wheat/Oat/barley) in buffer (supplied in the kit)
3) Mix 200 µl of the extract with the freeze-dried reagents
4) Start Incubation for 10 minutes at 40°C
5) Introduce dipsticks, read the test line intensities after 10 minutes

Figure 1: Examples 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 2).

V) In-house validation

The 4Mycosensor dipstick test has been validated in maize and in wheat following a nested design involving 7 different varieties of grains on three different days. On each day, in the aim of estimating the repeatability, three varieties have been analyzed in duplicate. Four different levels of contamination have been selected (blank, 25%, 50% and 100% of EU MRLs). Analysis of variance (ANOVA) was performed using MINITAB™ Statistical Software for Windows. The ANOVA method allows to determine the main variability sources of the total variability of results (+++ important; ++ significant; + low; - no effect) (Table 3).

	Repeatability	Matrix effect	Intraday effect	RSD (%)
Wheat				
ZEA	+++	-	+	< 15
T2/HT2	+++	+	++	
DON	+++	+	+	
Maize				
ZEA	+++	++	+	< 20
T2/HT2	+++	++	+	
DON	+++	++	+	
FB1/B2	+++	++	++	

Table 3 : Contribution of the main variability sources to the total variability

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