Validation of a biochip chemiluminescent immunoassay for multi-mycotoxins screening in maize

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INTRODUCTION

Mycotoxins are secondary metabolites of relatively small molecular weight (MW around 700) which can pre- or post-harvest contaminate a wide range of commodities from animal or plant origins (1).

The aim of this study was to validate a biochip chemiluminescent immunoassay for multi-mycotoxins screening in maize. Screened mycotoxins were aflatoxins B1 (AFB1) and G1 (AFG1) ochratoxin A (OTA), zearalenone (ZEA), toxin T2 (T2), fumonisins (FUM, sum of FB1 and FB2) and deoxynivalenol (DON) (Figure 1).

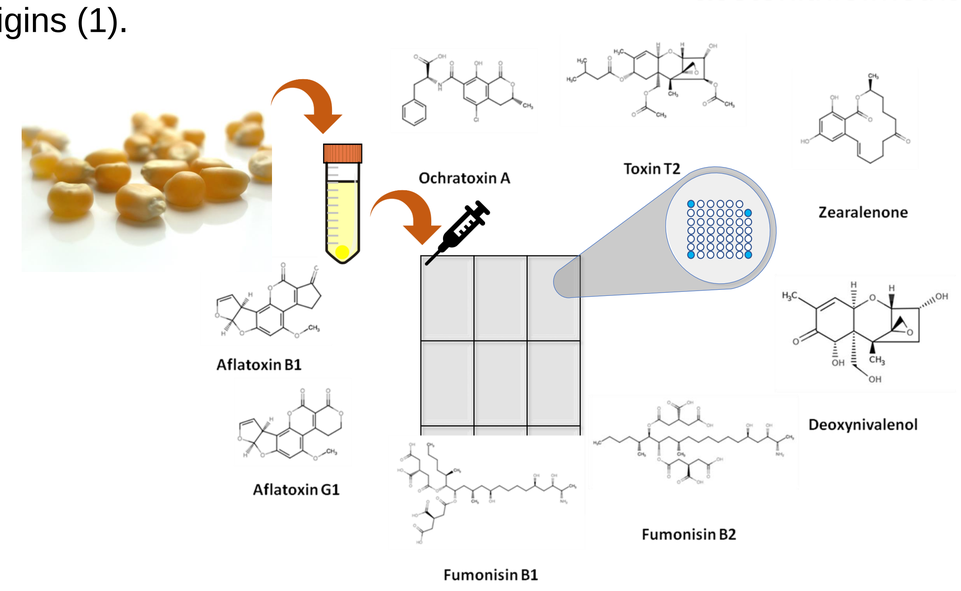

Figure 1: Scheme of the biochip chemiluminescent immunoassay for multi-mycotoxins screening in maize (*Zea mays* L.) and its target mycotoxins.

MATERIAL AND METHODS

Extraction: 5 ± 0.05 g of homogenised maize was extracted with 25 ml acetonitrile:methanol:water (50:40:10, v/v/v).

Competitive chemiluminescent immunoassay: Investigador™ EV 4065, Evidence Investigator Myco 7

Threshold value (T) and the cut-off (Fm) were calculated:

$T = B + 1.64 \times SD$

Where B is the mean and SD is the standard deviation of the signal in RLU of the blank samples.

$Fm = M + 1.6 \times SD$

Where: M is the mean and SD standard deviation of the signal in RLU of the spiked samples.

RESULTS AND DISCUSSION

Table 1. Linearity, limit of detection, regulatory limit, precision and recovery of biochip chemiluminescent immunoassay for the simultaneous determination of multimycotoxins.

Mycotoxin	Linear range (µg/kg)	LoD (µg/kg)	Regulatory Limit[2] (µg/kg)	r	Precision (%)	Recovery (%)
AFB1	0-9.5	0.33	5	0.9993	7.37	83.3
AFG1	0-75	0.28		0.9947	9.97	73.6
DON	0-7500	216.4	1750	0.9994	7.96	108.4
FB1 + FB2	0-300*	18	4000	0.9960*	21.2	85.6
OTA	0-60	0.64	5	0.9992	7.19	83.4
T2	0-300	7.68		0.9970	10.1	103.6
ZEA	0-150	1.1	350	0.9995	6.98	87.2

*For FB1

The calibration curves presented r values that met the acceptance criterion of r>0.95. (Table 1). Precision data (CVs) and recovery are in agreement with performance criteria analysis according to Regulation EC no. 401/2006 (2) and its amendments.

Table 2. Threshold value (T) and Cut-off value (Fm) of the chemiluminescent immunoassay for the different mycotoxins.

	FB1+FB2		AFG1		Zea		OTA		AFB1		T2		DON	
	Blank	Spiked	Blank	Spiked	Blank	Spiked	Blank	Spiked	Blank	Spiked	Blank	Spiked	Blank	Spiked
Spiking level (µg/kg)	-	250	-	1	-	50	-	1.5	-	1	-	25	-	375
Mean (RLU)	2441.3	139.6	5698.7	3175.4	3770.5	548.6	20753.6	9897.3	8125.2	3420.1	6570.8	1622.3	12538.3	5695.3
SD (RLU)	285.5	29.6	214.3	316.6	183.5	38.3	1981.8	2037.7	401.1	252.2	633.3	163.0	1002.6	453.1
T (Threshold value) (RLU)	1973.1		5347.2		3469.5		17503.4		7467.3		5532.1		10894.1	
Fm (Cut-off value) (RLU)	188.1		3694.5		611.3		13239.1		3833.6		1889.6		6438.4	

Table 3. Samples content on the sum of fumonisines B1 and B2.

Samples #	Fumonisins B1 + B2 (µg/kg)
1	125.9
2	56.6
3	105.1
4	25.0
5	>300*
6	41.2
7	8.1
8	3.1
9	190.5
10	46.7

The chemiluminescent signal of discrete test regions on the biochip is expressed in Relative Light Unit, and this value differs according to the mycotoxins concentration.
Threshold value and the cut-off were calculated (Table 2). Low false results rate was achieved (<5%) (Figure 2) and the obtained precision data is in agreement with EU legislation performance criteria.

All the samples were negative for the other mycotoxins under study. (Table 3) Moreover, any of the samples exceeded EU maximum permitted levels for maize, except one sample which presented a concentration higher than 300 µg/kg for fumonisins and should be further analysed by LC-MS or LC-MS/MS.

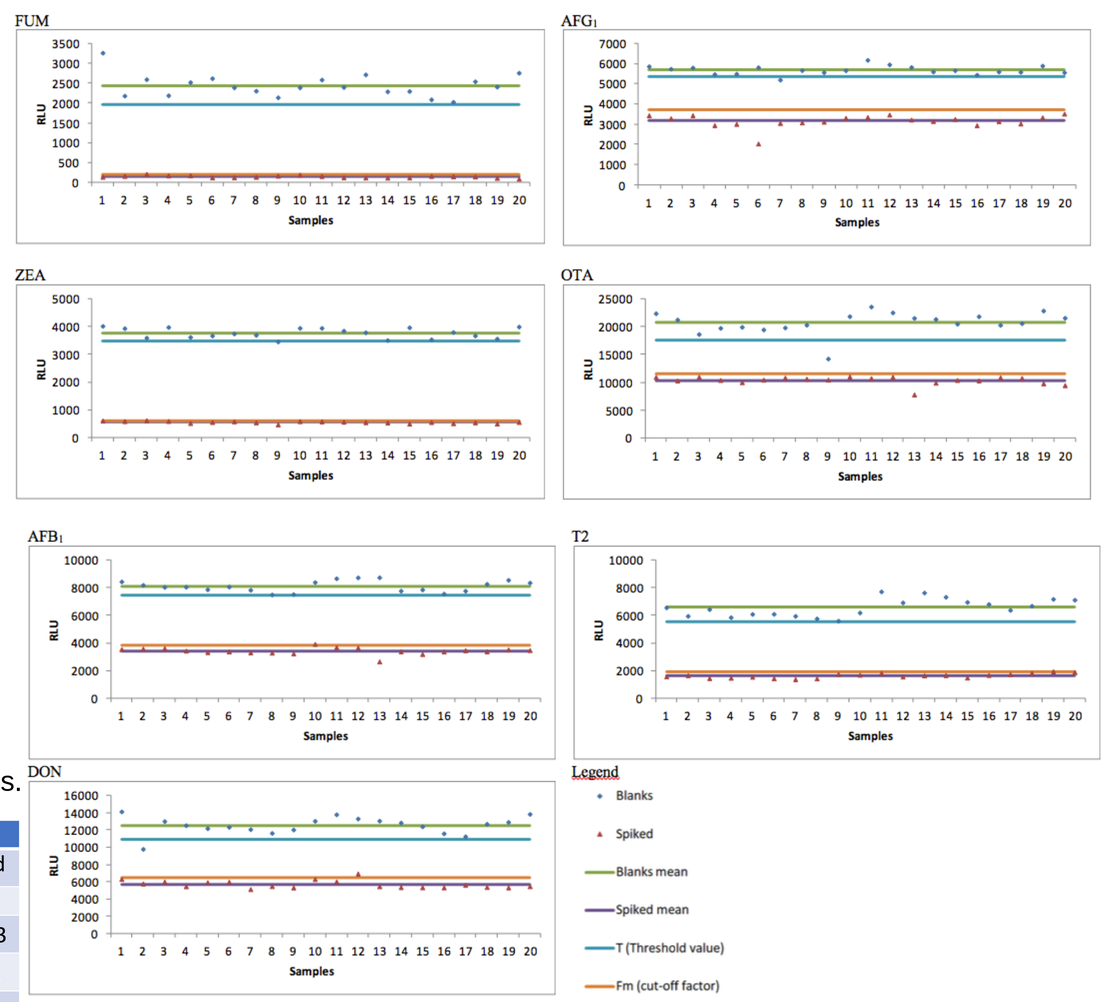

Figure 2. Threshold value (T) and Cut-off value (Fm) of each of the mycotoxins analysed by the biochip chemiluminescent immunoassay expressed in RLU (Relative Light Unit), for the 20 blank maize samples and for the 20 spiked maize samples at the level of interest.

CONCLUSIONS

The worldwide climatic changes (increase of global temperature and rainfall) caused by emission of greenhouse gases, will most probably be responsible for an increasing contamination of mycotoxins in food chain.
In this regard, it is very important to implement capable control programs as well as an established policy of risk assessment and management. These programs can be assured by immunoassays.
The validated immunoassay is reliable, cost effective, rapid, semiquantitative and environmentally friendly and covers the regulated mycotoxins.

References

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Acknowledgements

Authors are grateful to for the financial support of Rural Development Program through the Operational Group QUALIMILHO- New sustainable integration strategies that guarantee quality and safety in the national maize, PDR2020 nº 101-031295 (2017-2020) and Tiago Pinto from Anpromis for the management of experimental field trials and supply of the maize samples.