Development and application of a quantitative lateral flow immunoassay for fumonisins in maize

UNIVERSITÀ DEGLI STUDI DI TORINO

DEVELOPMENT AND APPLICATION OF A QUANTITATIVE LATERAL FLOW IMMUNOASSAY FOR FUMONISINS IN MAIZE

LAURA ANFOSSI[†]*, MARIANNA CALDERARA[†], CLAUDIO BAGGIANI[†], CRISTINA GIOVANNOLI[†], ENRICO ARLETTI [‡], GIANFRANCO GIRAUDI[†]

ABSTRACT

A quantitative lateral flow immunoassay for measuring fumonisins in maize was developed. Strip preparation and assay parameters were optimized to obtain a dipstick usable outside the laboratory with different samples, and which shows performance comparable with that of other screening methods, as confirmed by the intra- and the inter-day precision of data (RSD 5-16%). Quantification was obtained by an external calibration curve, which can be stored and used for measurements made with strips of the same batch in different days and at varying temperatures (22-37°C). Limit of detection (120 μg l^{-1}) and dynamic range (200-5000 μg l^{-1}) allow the direct assessment of fumonisin contamination at all levels of regulatory relevance. Twenty-seven maize samples were analyzed after a simple sample preparation which avoids the use of organic solvent. Linear correlation was observed (y = 1.07 x - 0.2, r^2 = 0.990) when data was compared with that obtained through a reference LC-MS/MS method, across a wide range of fumonisin contamination.

KEYWORDS

Fumonisins, lateral flow immunoassay, quantitative assay, maize

*to whom correspondence should be addressed. Department of Analytical Chemistry, University of Turin. Via Giuria, 5 10125 Torino. Italy

Tel: +390116707846, fax: +390116707615. E-mail: laura.anfossi@unito.it

[†] Department of Analytical Chemistry, University of Turin. Torino. Italy

[‡] Generon srl. S. Maria di Mugnano, Modena, Italy

INTRODUCTION

The fumonisins are a group of mycotoxins produced by several *Fusarium* species that grow on agricultural commodities, in the field or during storage. There are many different forms of fumonisin; among these, Fumonisin B_1 (FmB1) is the most common and economically important form, followed by B_2 (FmB2) and B_3 (FmB3). Fumonisin contamination involves almost exclusively maize, for which is the major source of mycotoxin contamination and it is found throughout the world. Fumonisins are associated with a variety of adverse health effects in livestock and animals in experiments. The major compound, fumonisin B1, has been demonstrated to cause hepatotoxicity and carcinogenic effects in horses, pigs, and rats; and has been classified as a group 2B human carcinogen by the International Agency for Research on Cancer (IARC). [1] In order to reduce the risk associated with the consumption of contaminated corn, many countries have regulated the levels of fumonisins in corn and, in particular, the European Union has set up maximum permissible levels for fumonisins (intended as FmB1+FmB2) which vary from 200 μg kg^{-1} for baby food to 4000 μg kg^{-1} for raw maize [2-3].

Several analytical methods are currently available for fumonisins determination, including high-performance liquid chromatography coupled to fluorescence or mass spectrometric detection [4-5]. Generally, chromatographic methods are expensive and time-consuming. Enzyme-linked immunosorbent assay (ELISA)-methods have also been described [6-7] and are widely employed as screening methods in routine analysis, mainly because of their simplicity and rapidity.

To effectively monitor the occurrence of fumonisins in food and feed at low contamination levels sensitive, reliable, and simple analytical methods are required. In addition, the early detection of contaminated crops requires screening tests that can be carried out at all stages of food and feed production and that can be conducted by users who are close to the site of contamination, so that contaminated ingredients can be identified. For this reason, there is great interest in the development of point-of-test methods of analysis for the determination of mycotoxins. Several articles have appeared in the last few years that described the development of point-of-use methods, based on immunochemical techniques such as lateral flow dipstick, immunochromatography, and immunofiltration [8-11]. These techniques entail the use simple and minimal manipulations and to provide accurate results with little or no instrumentation. Most of them are basically designed as visual tests and, with few exceptions [12], use membranes as solid phases [8-14]. The first works reporting the quantitative determination of mycotoxins by using lateral flow dipsticks are beginning to be published [15-16]. In addition, some quantitative lateral flow devices are now commercially available. [17-18] The major advantage of being able to make a quantitative measurement rather than a visual detection is represented by the objectivity of the result which does not depend on the

interpretation of the operator. On the other hand, if the quantification requires the use of special equipment or carrying out complex operations, this frustrates the concept of on-site analysis.

In the present work, a portable lateral flow dipstick for the rapid on-site quantification of fumonisin B1 and B2 in maize has been developed. The quantification has been obtained by reading results with a usual scanner, connected to a laptop, and by interpolating them onto a memorized standard curve. In addition, a rapid pre-treatment protocol using an aqueous extraction buffer has been applied to avoid hazardous waste disposal and to permit the widest possible application of the lateral flow immunoassay (LFIA) that could be run easily, and requiring the minimum amount of equipment.

A further aim of this study was to overcome the problems related to the variability of the point-of-use test with respect to the different matrices already highlighted in Molinelli et al. [15]. Indeed in their work, the authors observed that samples of different origin and or portion size perform very differently. This fact makes their quantification unreliable. However, this was overcome by preparing different calibration curves for each of the different sample types. Therefore, the dipstick architecture and the protocol for the sample treatment were studied to make the application of the developed LFIA as general as possible. Moreover, since it is proposed as a quantitative method, the accuracy and reproducibility of the data were comparable to those of other screening methods.

The developed assay was validated through comparison with an LC-MS/MS method and proven to be accurate enough to allow the quantitative determination of FmB1+FmB2 in different corn food and feed.

MATERIALS AND METHODS

Materials

Tetrachloroauric(III) acid, bovine serum albumin (BSA), fumonisin B1 (FmB1, Oekanal standard solution), polyethylene oxide (PEO, average mw 100 kDa), polyethylene glycol (PEG, average mw 10 kDa), polyvinylpyrrolidone (PVP, average mw 40 kDa), polyvinylpolypyrrolidone (PVPP, average mw 360 kDa), gelatine from pork skin, dextran (average mw 2000 KDa), methylcellulose (viscosity 4000 cP 2% in water at 20°C), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Fumonisin B1-BSA conjugate, the anti-fumonisin antibody (rabbit polyclonal antiserum raised against FmB1, relative cross-reactivity towards FMB2 97%) and the goat anti-rabbit immunoglobulin antibody were kindly supplied by Generon srl (Modena, Italy). Tween 20 and all other chemicals were obtained from VWR International (Milan, Italy).

Nitrocellulose membrane (Hi-flow plus membrane cards, 60x300 mm), cellulose fibre pads (sample and adsorbent pads) and glass fibre pads (conjugate pad) were purchased from Millipore (Billerica, MA, USA).

Standards of fumonisin B1 were prepared daily by dilution of the certified reference solutions in a phosphate buffer which contains 2% (w/v) PEG.

Preparation of colloidal gold and colloidal gold-labelled polyclonal antibodies

Gold colloids with an adsorption maximum of 525 nm were prepared using the sodium citrate method as previously described [19-20]. Briefly, 1 ml of sodium citrate (1% w/v) was added to 100 ml of a 0.01% solution of tetrachloroauric acid under vigorous stirring and warming (100°C).

The colloidal gold solution was adjusted to pH 8.5 with a 50mM carbonate buffer (pH 9.6) for coating with the antiserum. The optimum concentration of the polyclonal antibody towards fumonisin (pAb) for conjugation was determined prior to conjugation according to Horisberg [21]. For conjugation, 100 µl of a 1 mg ml^{-1} pAb solution in a borate buffer (BB, 20 mM, pH 8.0) was added to 10 mL of pH-adjusted colloidal gold solution. After 30 minutes' incubation at room temperature, 1 ml of the borate buffer, which contains 1% of BSA, was added. The mixture was centrifuged at 10000 rpm at 25°C for 30 min, and the pellet was washed twice by re-suspension in the borate buffer which contains 0.1% BSA. Finally, the pellet was re-suspended in the BB, with 1% BSA, 0.25% Tween 20, 2% sucrose, and 0.02% sodium azide as preservative and stored at 4°C until use. The absorption maximum shift was checked and was considered acceptable if lower than 10 nm.

Preparation of the test strips

Test and control lines were spotted on the membrane by means of a XYZ3050 platform (BioDot, Irvine CA, USA), which is equipped with two Biojet Quanti 3000 dispenser for non-contact dispensing. The distance between the lines was 7 mm. The BSA-fumonisin conjugate and the goat anti-rabbit IgG antibodies were separately diluted in the phosphate buffer 20 mM pH 7.4 (PBS) to the concentration of 0.2 and 1 mg ml^{-1} respectively and applied at 1 µl cm^{-1}. After drying at 37°C for 60 min, the membranes were blocked with PBS containing 1% BSA, at room temperature, for 5 min and washed with PBS containing 0.05% Tween 20. Then, the membranes were dried at 37°C under vacuum for 60 min.

Gold conjugate was dispensed onto the conjugate pad with the Biojet Quanti 3000 dispenser at 10 µl cm^{-1}. Conjugate pads were previously blocked with the BB used for the re-suspension of the gold conjugate and dried at 65°C for 60 min. After gold conjugate dispensing, pads were dried at room

temperature for 2 hours and immediately attached to the bottom of the membranes with 1-2 mm of overlap. The sample pads were attached to the bottom of the conjugate pads in the same manner. The adsorbent pads were attached to the top of the membranes with a 1-2 mm overlap on the membranes too. The prepared master card was cut by a CM4000 guillotine (BioDot, Irvine CA, USA) to obtain 5 mm test strips. Each strip was inserted into a rigid plastic cassette (Dima Diagnostics, Goettingen, Germany), which has a sample well and a reading window on the upper lid. These were stored in plastic bags containing silica at room temperature until being used. The stability of the prepared lateral flow devices was assessed by running strips after 1, 3 and 6 months of storage.

Effect of polymer addition to modulate viscosity

Two maize samples, one cornmeal and one ground corn grain, which were measured as being contaminated at the same level (about 2000 μg kg^{-1}) by the LC-MS/MS reference method, were extracted with PBS containing different polymers (at different w/v): PEG 1-2-4%, PVP 1-2%, PVPP 1-2%, pork skin gelatine 1-2%, dextran 0.1-1%,methylcellulose 0.1%, dextran 1% + PEG 1%. Each sub-sample (5g) was extracted with 50 ml of each buffers in duplicate and each extract was analyzed in triplicate. Contemporarily, standard solutions of fumonisins at the same presumed concentration levels were prepared by diluting the certified standard solution in the various buffers and were also analyzed in quadruplicate. Extracts and standards prepared in each of the listed buffers were run in parallel to compare flow rate, the sum of the line intensities and the ratio between test and control line intensities.

Various sample to buffer ratios were also considered for the extraction of fumonisins (1+1, 1+5, 1+10), using PBS with 2% (w/v) PEG added. In addition, further dilution of the extracts (1+1, 1+3) using the same buffer was also considered.

Sample and sample preparation

Corn samples were obtained directly from producers or mills. Grain maize was ground and homogenized, cornmeal was directly extracted. All samples used in comparative studies were analyzed without fortification.

Five grams of corn samples were extracted with 50 ml of PBS with 2% (w/v) PEG added. After a 2 minute manual shaking, the suspension was allowed to settle for 5 minutes and the supernatant was directly analyzed.

Test procedure

150 µl of sample extract were added in the sample well of the cassette and the test was allowed to develop colour for 10 minutes at room temperature. Results were read immediately after, even if results remained stable for at least 10 minutes. Line intensity was measured by scanning the image with a bench top scanner (CanoScan LiDE 200, Canon). Images were acquired and processed by the QuantiScan 3.0 software (Biosoft, Cambridge, UK). The density peaks obtained in the correspondence of coloured lines were fitted by means of the automated function of the software to calculate areas (Figure 1). A standard curve was obtained by plotting the ratio between the area of the test and the control line against the log of fumonisin concentrations by a nonlinear regression analysis of the data of the standards using the four-parameter logistic equation [22]. Quantification of fumonisins in samples was obtained by interpolation on a stored calibration curve.

LC-MS/MS analysis

Preparation, extraction and clean-up of samples for validation purposes was performed by an AOAC official method [23], with slight modification, as previously reported [24]. Ground samples were extracted with water (0.4% acetic acid):methanol:acetonitrile 50:25:25 by mechanical shaking for 60 minutes and in an ultrasound bath for a further 5 minutes. Filtered extracts (obtained by means of a 0.45µm cellulose membrane filter) were analyzed by HPLC-MS-MS (TSQ 7000, Thermo Scientific, Waltham, MA, USA) equipped with a XTerra MS C18 column 2.5 µm, 2.0 x 15 (Waters, Milford, MA, USA). Each extract was injected in triplicate. The mobile phase consisted of 0.1% of formic acid (A) and acetonitrile (B). Separations were carried out at a flow rate of 0.5 ml/min using a linear gradient from 0% to 56% solvent B over 15 min. Detection of fumonisins was carried out by means of a triple stage quadrupole mass spectrometer, equipped with an electrospray source operated in positive ionisation, multiple reaction monitoring. The transitions monitored were 722.8/ 334.2, 722.8/352.2, 722.8/704.1 for FmB1 and 706.3/318.3, 706.3/336.4, 706.3/354.1 for FmB2. Quantification was obtained by interpolation on an external calibration curve. Quantitative results were given as the sum of FmB1 and FmB2 (total fumonisins).

RESULTS AND DISCUSSION

Development and optimization of the strip test

The principle of the competitive lateral flow assay relies on the competition for antibody binding sites on gold nanoparticles. Polyclonal rabbit antibodies that are raised to FMB1 are bound to gold particles, which are then applied onto a glass-fibre conjugate pad. Two lines of reagents are immobilized on the membrane. The test line comprises a FmB1-BSA conjugate. The control line is

a line of anti-species antibodies. The conjugate pad and membrane are assembled, together with a sample and an absorbent pad, in a plastic housing. Droplets of a sample liquid extract are added to the sample well, releasing the gold particles, which then begin to flow across the membrane. If some fumonisins are present in the sample, antibody will bind them. Any gold particles that fail to bind to fumonisins will be captured by the immobilized test line as they traverse the membrane; thus producing a visible line of deposited gold. The anti species antibody on the control line captures any excess gold particles, bound or unbound, to produce a control line as a visible confirmation of particle flow. The decrease of the test line is directly correlated to the increase of the amount of fumonisin in the sample extract.

As a preliminary measure, the optimal amount of FmB1-BSA conjugate and gold labelled-antibodies against FmB1 was optimized by comparing dose-response curves obtained using different combinations of antigen (concentrations: 0.1, 0.2, 0.5, and 1 mg ml^{-1}; dispensation rate: 0.5-1-2 µl cm^{-1}) and gold labelled-antibody (OD: 1-2-5-10; dispensation rate: 2-5-10 µl cm^{-1}). The lowest LOD (limit of detection) and the highest sensitivity (slope of the curve) were obtained by using a 0.2 mg ml^{-1} of FmB1-BSA solution dispensed at a flow rate of 1 µl cm^{-1} as a coating antigen and a gold-labelled antibody (5 OD) dispensed at 10 µl cm^{-1}.

The pore size of the nitrocellulose membrane was also optimized by comparing thickness of the test line and dose-response curves. A HiFlow180 membrane was finally chosen as the best compromise between the lower LOD and thickness of the test line and the time needed to develop the colour on the strip.

Since one of the objectives of this work was to minimize handling and provide a fully ready-to-use system gold-labelled antibodies were dispensed onto an appropriate pad, which do not bind proteins. The glass fibre pad used retains the gold nanoparticles by a simple mechanical action; a suspension of gold-labelled antibodies is dispensed or sprayed onto the pad and the latter is air dried. However, this procedure introduces wide non-reproducibility because some drops of the suspension can cross the fibre and disperse on a supporting surface or, in general, can diffuse in an irregular manner along and across the fibre. The consequence is that the dispensing of gold-labelled antibodies strongly contributes to the non-reproducibility from strip to strip. In addition, when the line of gold-labelled antibodies widens, owing to diffusion, the sensitivity of the assay is affected. In order to reduce diffusion phenomena, conjugate pads were treated differently before the deposition of the gold conjugate, and a significant reduction in the number of drawbacks could be obtained by pre-washing the pad in the buffer which contains sucrose and BSA.

Blocking of the nitrocellulose membrane was also considered, as several authors underline the benefits of such a treatment. [14-15,25] As previously observed, the coating of the membrane with

BSA has a number of effects: slowing of the flow which leads to increased signals, increasing the stability of the strips, but, above all, reducing the variability of the response as a function of the matrix.

Quantitative measurements and optimization of a stored calibration curve

A developed strip shows two coloured lines: the test line and the control line. The first, being due to the binding of gold-labelled anti-fumonisin antibodies to the immobilized fumonisin, is the analytical signal which varies as a function of fumonisin concentration. However, there is certain variability from strip to strip, mainly due to the amount of gold conjugate, which can hardly be standardized. In fact, even qualitative tests never recommend the observation of the absolute intensity of the test line, but always the ratio between the test and control line intensities. This suggestion not only arises from the convenience of comparing two lines on the same strip instead of comparing a line on the strip and some pre-defined coloured line, but also depends on the fact that the more gold conjugate present, the more coloured the test line would be. A possible way to overcome this problem has for example been proposed by Molinelli et al. [15]; the gold labelled-antibodies are not pre-adsorbed onto a conjugate pad but mixed with the sample before carrying out the test. In this way the amount of gold labelled antibodies can be defined as a certain OD of the gold suspension and the system becomes more reproducible. Nevertheless, this approach presents some limitations. In detail, it introduces an additional operation (i.e. mixing a measured volume of the sample extract with a measured volume of the gold conjugate solution), requests the stabilization of the gold-labelled antibody solution (as far as both antibodies and gold colloid are concerned), and prevent the use of sample pads made of materials which can interact with proteins (for example cellulose pads) and as a consequence contribute to reduce matrix interference.

An alternative way to deal with the strip-to-strip variation due to the variability of the amount of gold conjugate in each strip is the use of some data corrections. The intensity of the control line or the total intensity of the test plus the control line may be both appropriate factors to normalize data and reduce variability between strips. The intensity of the control line depends on how much gold conjugate has not been captured by the test line and thus should be linearly correlated to the analyte concentration. However, the use of a polyclonal antiserum, without affinity purification, implies that the majority of antibodies are not directed towards the target. [26] Consequently, the control line is primarily due to non-target antibodies and it is minimally affected by changes of target concentrations. The variation of the control line can thus be associated with variability of the amount of the gold conjugate or to any other phenomena affecting the assay in general (bad flow, different temperature, etc...). This makes it possible to use the control line intensity as an

appropriate normalizing factor. Therefore, results were expressed as the test line area (T) divided by the control line area (C).

Accordingly, the calibration curve was obtained by plotting the measured T/C ratio versus fumonisin standard concentration. The curve was plotted by measuring each standard in five replicates and fitting them to a four-parameter logistic equation (Figure 2).

The developed competitive lateral flow immunoassay has a dynamic range of 20-500 $\mu g\ l^{-1}$, with an IC_{50} of 56 $\mu g\ l^{-1}$. The LOD, calculated as the concentration corresponding to the T/C of the blank minus three standard deviations of the blank, was 12 $\mu g\ l^{-1}$.

Since lateral flow immunoassays are intended to be point-of-use tests, applicable outside the laboratory for very rapid screening, the ultimate goal should be to supply a system capable of reading strips and obtaining quantitative results without any additional operations. To achieve this, the calibration curve should be stored and held at least for each batch of strips produced. This implies that the curve should be usable to interpolate data that could have been obtained in different experimental conditions, except from the strip belonging to the same preparation.

To confirm this point the parameters of the calibration curve were stored. Intra-day and inter-day precision and accuracy were measured using standards of fumonisins and two maize samples naturally contaminated at a low (540 $\mu g\ kg^{-1}$) and high level (2930 $\mu g\ kg^{-1}$) as controls. Each of them was quantified in five replicates on the same day (intra-day) and on five different days (day 1-3-5-8-15) for the inter-day experiment. Results are summarized in Table 1. The measured accuracy and precision prove that the results are sufficiently reproducible to allow the use of a stored calibration curve. In detail, the intra and inter-day precision does not vary significantly in the range 4.3 to 16.4%.

On the contrary, calibration curves obtained from different batches of strips gave non-reproducible results (data not shown). Each batch was formed using different membrane cards and pads, but the same gold-labelled antibody and FmB1-BSA preparation. Within the batch, the same calibration curve was used to calculate fumonisin concentrations in samples.

Another variable that can strongly influence the outcome of the measurement is the temperature at which the strip is developed, thus standardization of this parameter is needed. Calibration curves in triplicate were carried out at 18, 24, 30 and 37°C, and compared. The increase of temperature led to an increase in the intensity of the lines. However, the T/C ratio remains approximately constant, within experimental error, when the temperature varied from 24 to 37°C. Contrarily, T/C varied significantly at the lowest temperature investigated (18°C) and generally we observed reproducible performances at relatively high room temperatures, and, vice versa, discordant results when room

temperature dropped below 20-22°C. Ultimately, a range of 22-30°C was assigned for using the strips and within this range a unique calibration curve could be established.

Maize sample analysis

The presence of organic solvent strongly affects LFIA performance, as far as both the flow properties and the immunochemistry are concerned. Besides, as previously observed by Molinelli at al. [15] and confirmed by our experience, the use of organic solvent, typically aqueous methanol, determines the co-extraction of fatty materials which may interfere in the assay. Thus, the extraction protocol based on the use of PBS described and validated by Kulisek and Hazebroek [27] was preferred.

Despite the use of all aqueous solutions, the flow rate observed with sample extracts and with aqueous standards of fumonisins was quite different. Moreover, results strongly depended on the type of sample analyzed, due both to the size of the minced material and to the treatment received (raw maize, ground corn grain or cornmeal), as previously emphasized. [13] These phenomena were partially reduced by blocking membranes after line deposition by means of a buffer containing BSA. [14] A further improvement was achieved by using a cellulose sample pad, which in addition allows us to avoid filtration of sample extracts before the analysis. On the other hand, it increased the total time of the assay, because liquid needs about one or two minutes to soak the pad before starting to run across the nitrocellulose membrane. It was considered acceptable, however, since the development of the colour was completed within 10 minutes and the total time needed for the assay, including sample preparation, remained lower than 20 minutes.

Generally, samples flow more slowly than aqueous standards and cornmeal flows more slowly than ground corn grain. Accordingly, line intensities (both test and control lines) were higher for cornmeal, intermediate for ground corn grain and minimal for aqueous standards. The colour developed by cornmeal samples would allow us to reduce reagents (gold conjugate and immobilized FmB1-BSA), thus improving the sensitivity of the assay. Consequently, various polymers were tested with the aim of increasing the viscosity of the aqueous standard and to balance the variability of viscosity of extracts obtained from different kinds of samples: PEG, PVP, PVPP, pork skin gelatine, dextran, methylcellulose, and combinations of dextran with PEG. All tested polymers reduced the flow rate, but most of them caused the decrease of line intensities instead of the increase hoped for. For example, pork skin gelatine completely inhibited binding of gold-labelled antibodies with the immobilized reagents. On the other hand, polyethylenglycole caused an increase of the line intensity which continues to increase in intensity when the PEG amount was raised from 1% to 2% and from 2% to 4% (w/v). More interestingly, PEG allowed us

to level results obtained from different matrices and from sample extracts and aqueous standards. From this point of view, 4% (w/v) PEG performed better than all the others. However, the flow became irregular and too slow to permit complete colour development in ten minutes. Thus, the addition of 2% (w/v) PEG to the PBS was preferred both to dilute standard solutions and to extract samples. Finally, a 1:10 sample to buffer ratio without further dilution was selected for fumonisin extraction, as matrix interference and variability were substantially compensated. In this way, the dynamic range of the developed LFIA becomes 200-5000 μg kg^{-1}, which allows direct measurement of samples at all levels prescribed by EU legislation. [2,3]

Comparison of LFIA and LC-MS/MS determination

As previously observed, [14] fortification experiments gave controversial results, thus the accuracy of the developed method was assessed by comparing results with a validated LC-MS/MS method.

A total of 27 naturally contaminated samples, which included raw corn grain, corn feed, maize starch and cornmeal were analyzed with the developed lateral flow immunoassay. All samples resulted to be contaminated by detectable levels of fumonisins. Three of them gave values exceeding the upper limit of the curve and were confirmed as being very highly contaminated via the LC-MS/MS reference method. Results of the remaining twenty-four samples were compared with those obtained using the reference LC-MS/MS method [23], which has a limit of detection of 10 μg kg^{-1} and a RSD% of 25%. Agreeing results were obtained via the two methods; the linear regression analysis (Figure 3) yielded a good correlation between the methods (y = 1.07 x -0.2, r^2 = 0.990, n=24).

These results proved that the developed lateral flow immunoassay can be applied for the quantitative measurement of total fumonisins (FmB1+FmB2) in corn samples of different kinds up to 4000 μg kg^{-1}, with accuracy and precision comparable with those obtained with the reference method. Therefore, the developed quantitative LFIA method proved to be applicable for first level screening or routine quality control of various corn products.

Previously described lateral flow dipsticks and commercially available devices for measuring fumonisins require the use of some organic solvents as the extracting medium and involve manipulations of sample extracts before running the LFIA [15-18]. On the contrary, in this work we developed a very simple preparation of the sample, which does not involve any instrumentation or apparatus, as no centrifugation/filtration are required. Minimum possible operations (dilution, addition of reagents, incubations) are needed. Besides, the extraction does not involve the use of organic solvents or other such hazardous chemicals, thus further simplifying its application outside the laboratory. Strips were read by means of a scanner connected to a PC and the assay could be

carried out without temperature control (in the range between 22 and 30°C), thus allowing its application almost everywhere, as would be required to qualify as a point-of-use test. Besides, the use of a stored calibration curve would allow us to further improve the system by completely automating the assay. A reading device able to process strips and to automatically calculate results from the stored calibration could also be created.

Fumonisins have been detected in all analyzed samples, thus confirming the potential risk associated with the presence of this toxic compound in food and feed widely consumed by humans and animals.

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TABLES

Table 1. Intra and inter-day precision for six fumonisin standard solutions and two naturally contaminated maize samples. Quantification of fumonisins was obtained on different days by the same stored calibration curve.

	Intra-day			Inter-day		
FmB1 theoretical concentration (μg kg^{-1})	average recalculated concentration	RSD (n=5)	Accuracy%	average recalculated concentration	RSD (n=5)	Accuracy%
100	89	4.5	89	91	12.0	91
250	259	8.3	104	272	6.9	109
500	469	4.3	94	490	16.4	98
1000	1064	9.5	106	1186	5.2	119
2500	2651	10.7	106	2439	6.8	98
5000	5108	4.6	102	5240	11.2	105
540	549	12.1	102	506	15.5	94
2430	2160	10.7	89	2303	14.3	95

Figure 1

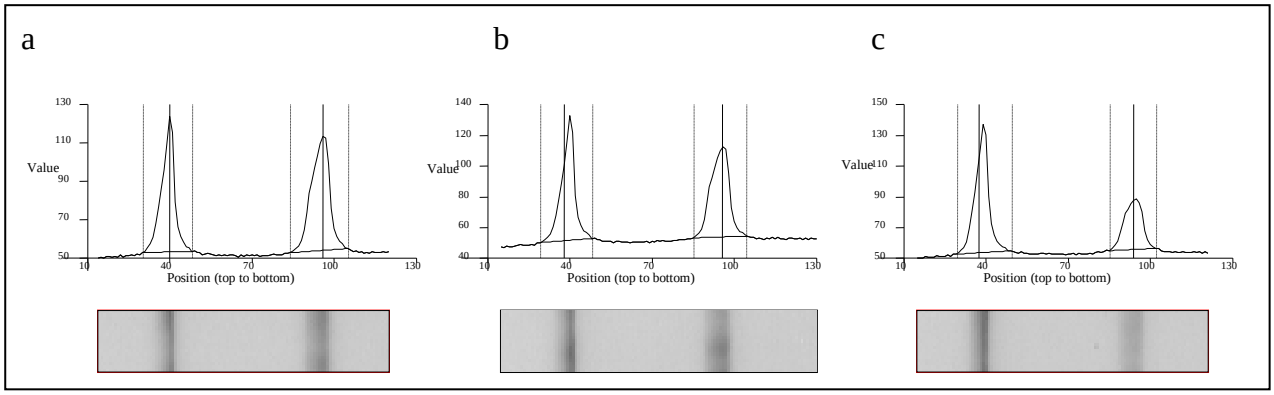

Figure 2

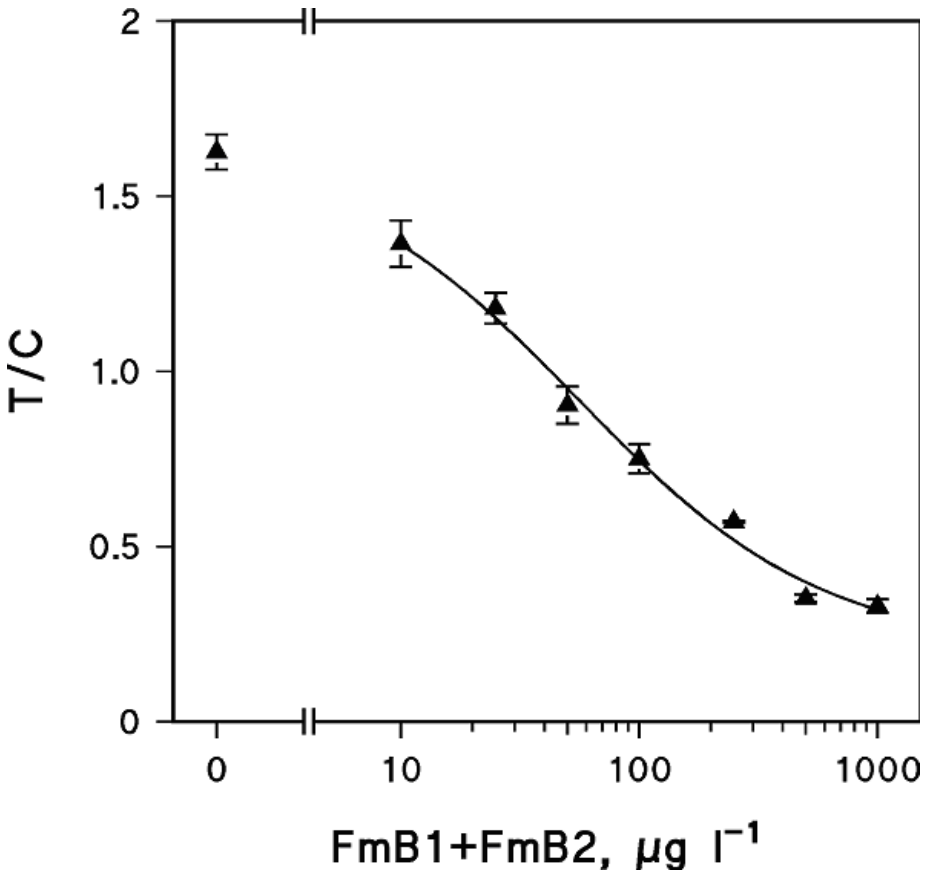

Figure 3

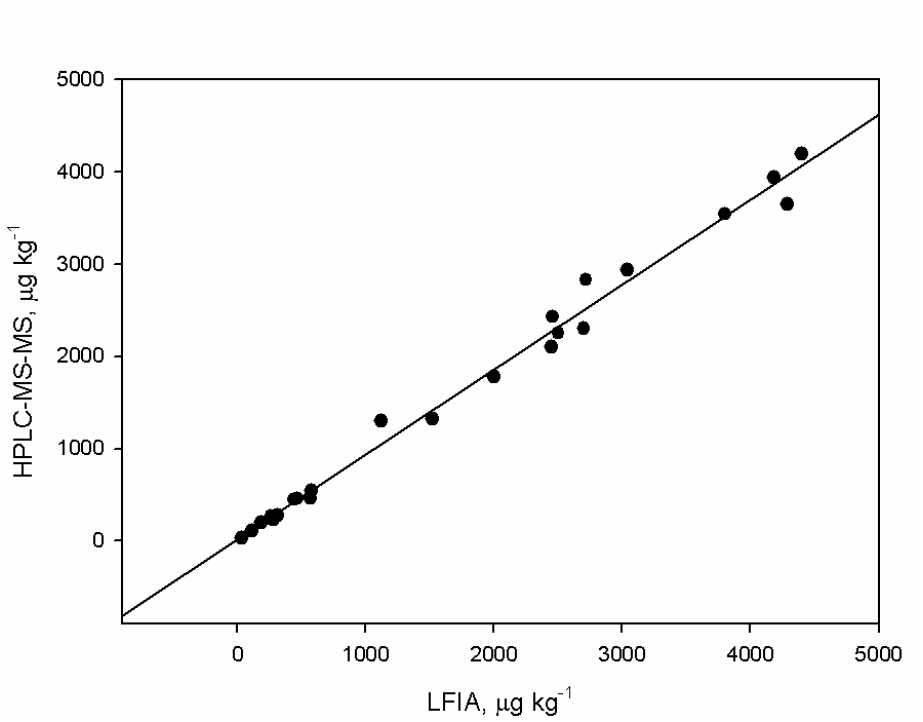
5000
4000
3000
2000
1000
0
HPLC-MS-MS, µg kg-1
0
1000
2000
3000
4000
5000
LFIA, µg kg-1

FIGURE CAPTIONS

Figure 1: Strip images processed by Quantiscan software for different concentrations of fumonisin standards: a) 0, b) 400, and c) 4000 $\mu g\ l^{-1}$

Figure 2: A typical inhibition curve, obtained under optimized conditions, for the developed quantitative lateral flow immunoassay

Figure 3: Correlation of results obtained by both LFIA and reference LC-MS/MS method for fumonisin detection on raw corn grain, corn feed, maize starch and cornmeal. The linear regression analysis yielded a good correlation between methods (y = 1.07 x -0.2, r^2 = 0.990, n=24)