Chemiluminescence-based biosensor for fumonisins quantitative detection in maize samples

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A Chemiluminescence-based biosensor for fumonisins B quantitative detection in maize samples

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Abstract

A compact portable chemiluminescent (CL) biosensor for simple, rapid, and ultrasensitive on-site quantification of fumonisins B in maize has been developed. The biosensor integrates a competitive lateral flow immunoassay based on enzyme-catalyzed CL detection and a highly sensitive portable CCD camera, employed in a lensless “contact” imaging configuration. A limit of detection of 2.5 µg L^{-1} for fumonisin B1 was achieved, with an analytical working range of 2.5-500 µg L^{-1} (corresponding to 25-5000 µg kg^{-1} in maize flour samples, according to the extraction procedure). Total assay time was 25 min, including sample preparation. A simple and convenient extraction procedure, performed by suspending the sample in a buffered solution and rapidly heating to eliminate endogenous enzyme activity was employed for maize flour samples analysis, obtaining recoveries in the range 90-115%, when compared with HPLC-MS-MS analysis. The CL immunochromatography-based biosensor is a rapid, low cost portable test suitable for point-of-use applications.

Keywords

Chemiluminescence, Lens-less contact imaging, Lateral flow immunoassay, Fumoninis, Mycotoxins.

INTRODUCTION

Portable devices and biosensors useful for “point-of-use” applications are gaining wide consideration for performing rapid and low-cost analyses directly where the sample is obtained, thus reducing assay costs and turnaround times with respect to traditional laboratory-based analytical systems. Devices for on-field sample analysis have been developed for a wide range of applications in the clinical, environmental, forensic and agro-food fields. In the context of food safety control, where the ability to early detect any

contamination is of particular importance, cheap and rapid on-field tests are increasingly employed for as a first level screening purposes.

Fumonisins are toxins mainly produced by *Fusarium* mould species, which commonly infect corn and other agricultural products, and represent a remarkable problem which appears to be more and more relevant in the context of global market. Indeed, cereals and other agricultural products imported from foreign countries need to be extensively controlled to assure safety. Fumonisins have been shown to be neurotoxic, hepatotoxic, and nephrotoxic in animals [*Stockmann-Juvala H et al., Hum Exp Toxicol. 2008*] and the most prevalent, fumonisin B1 (FmB1) is classified as a group 2B human carcinogen according to the International Agency for Research on Cancer (IARC) [*IARC, 1993, pp. 301–366*]. The maximum permissible levels of fumonisins in corn-derived foodstuff have been established by the European Union, range from 200 µg kg^{-1} for baby food to 4000 µg kg^{-1} for raw maize [*(EC) No. 576/2006, (EC) No. 1126/2007*].

Analytical methods for fumonisins analysis have been recently reviewed by Maragos et al. [*C.M. Maragos et al., Food Addit Contam 2010, Krska R et al., Food Addit Contam 2008*]. Reference analytical techniques are HPLC methods with fluorescence or mass spectrometry detection, while immunoassays, particularly enzyme-linked immunosorbent assays (ELISA), are widely employed for screening purposes, due to their low cost and high analytical throughput.

Since contamination by fumonisins can occur at any stage of the food chain (e.g., on field, at harvest, during storage and transportation) frequent analyses are required to promptly detect any contamination, thus reducing risks for the consumer. Various rapid and simple “point-of-use” analytical methods have been developed for fumonisins detection, including immunological assays employing simple analytical formats such as Lateral Flow Immunoassays (LFIAs) [*Shiu C.M. et al., J. Sci. Food Agric. 2010; Wang S. et al., J. Agric. Food Chem. 2006*] and biosensors [*E. Sapsforda et al., Sensors and actuators. B 2006*]. In

LFIAs a porous membrane on which specific immunoreagents are immobilized in definite areas (test-line and control-line) is employed as a solid support, while other reagents are deposited in a solid form on a pad positioned at the beginning of the strip. A lateral flow along the membrane, which is established upon sample addition, solubilizes the reagents in the pad and drags them by capillary forces along the strip. The immunological reactions occurring in correspondence of the functionalized strip areas and separation of antibody-bound and free fractions are expedited by the flow, thus reducing overall assay time. Both non-competitive sandwich-type and competitive immunoassays have been developed using this analytical format and a number of them are also commercially available [*Posthuma-Trumpie GA et al. Anal Bioanal Chem. 2009*]. Conventional LFIA are usually based on the use of colloidal gold or latex particles as labels and the analyte is visually detected through color formation due to label accumulation in definite strip zones. This approach often suffers from poor quantitative discrimination and low assay sensitivity, thus might be not suitable to fulfill the regulatory requirements [*E. Reiter et al., Mol. Nutr. Food. Res. 2009*]. The main risk in the use of out-lab portable devices is a poor quality control assessment and method validation due to the uncontrolled variability and to the semi-quantitative approach of many methods previously developed.

To facilitate the interpretation of results from LFIA and to obtain quantitative information, several hand-held devices capable of scanning the strip and estimating toxin content from the quantitative evaluation of color intensities of the lines have been proposed [*Anfossi L et al. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2011; L. Anfossi et al., Anal. Chim. Acta 2010; A. Molinelli et al., Anal Bioanal Chem 2009; R. Salter et al., J. AOAC Int. 2006*]. In addition, various detection systems have been applied to LFIA to increase sensitivity, relying on enzyme amplification and chromogenic susbtrates [*J-H Cho et al., Anal. Chem. 2006*], fluorescent nanoparticles [*Z. Li et al., Anal. Chem. 2010; Zou ZX et al., Anal. Chem. 2010; Xia XH et al., Clin. Chem. 2009*] or electrochemical

measurements [*Muhammad-Tahir Z et al., Biosens Bioelectron 2003; Fernandez-Sanchez C et al., Anal. Chem. 2004*].

Among the various possible detection systems employed in imunoassays, enzyme-catalyzed chemiluminescence (CL) has been widely demonstrated to provide superior analytical performance, providing several advantages, such as high detectability, amenability to miniaturization, shorter assay times and reduced sample and reagents consumption [*Magliulo M et al, J Agric Food Chem. 2005;Roda A et al., Anal Chem. 2003; Kricka LJ., Methods Enzymol. 2000*]. In enzyme-catalyzed CL an enzyme, usually horseradish peroxidase (HRP) or alkaline phosphatase, is employed as a label and detected with highly sensitive photomultiplier-based or CCD-based light measurement instruments upon addition of a suitable CL substrate. Its superior analytical performance derives from both the amplification factor offered by the cyclic enzyme reaction and the intrinsic characteristics of CL. Indeed, light emission is generated from dark by a chemical reaction, thus circumventing limitations typical of the spectrophotometric or fluorescence detection techniques [*Roda A et al. Anal Chem. 2003; Kricka LJ. Methods Enzymol. 2000*].

Recently, enzyme-catalyzed CL detection has been also proposed for LFIA, providing several advantages, such as the possibility to obtain quantitative information and to improve limits of detection with respect to color-formation-based LFIAs [*I-H Cho et al., Anal. Chim. Acta 2009*]. Nevertheless, only a few examples have been reported in the literature, and in most cases the described systems rely on bulky instrumentation for photon emission measurements, thus reducing the on-field applicability of the methods.

In this work we describe the development a quantitative CL competitive LFIA for fumonisins B (FmB1 and FmB2) characterized by high specificity and sensitivity, exploiting a HRP-labeled secondary antibody as a tracer, which is revealed by means of a CL substrate. The competitive immunological reaction and the enzyme-catalyzed CL reaction are conducted sequentially in the LFIA strip. To obtain a compact and portable biosensor,

the 2D measurement of CL emission from the LFIA strip was performed by “contact” imaging employing a compact light detection device equipped with an ultrasensitive cooled CCD sensor [*A. Roda et al., Anal. Chem. 2011*]. In this set-up, the LFIA membrane was placed in contact with a CCD imaging sensor through a tapered fiber optic faceplate without the use of a lens-based optical system, thus allowing localization and quantification of the emitted photons with high light collection efficiency in a very compact device. The tapered configuration of the faceplate increased the useful analytical surface with respect to the CCD sensor area, making it compatible with the size of the LFIA membrane.

The developed LFIA with CL detection was able to provide sensitive and quantitative information on fumonisins B content in corn flour samples, employing a simple pre-analytical sample clean-up, using a compact portable instrumentation and therefore enabling out-lab on-field application.

MATERIALS AND METHODS

Reagents

Bovine serum albumin (BSA), Tween-20, horseradish peroxidase (HRP), fumonisin B1 (FmB1, Oekanal standard solution), anti-HRP antibody, and HRP-labeled goat anti-rabbit immunoglobulin were purchased from Sigma Aldrich (St. Louis, MO, USA). Goat anti-rabbit antibody, the FmB1-BSA conjugate, and rabbit anti-fumonisin antibody were kindly supplied by Generon srl (Modena, Italy). The luminol-based CL substrate for HRP, Supersignal ELISA Femto, was purchased from Thermo Fisher Scientific Inc. (Rockford, IL).

Phosphate buffered saline (PBS) was prepared as follows: 10 mmol L^{-1} Na_2HPO_4, 2 mmol L^{-1} KH_2PO_4, 137 mmol L^{-1} NaCl, 2.7 mmol L^{-1} KCl, pH 7.4.

Standard FmB1 solutions, rabbit anti-fumonisin polyclonal antibody and assay strips for LFIA were prepared as previously described [*L. Anfossi et al., Anal. Chim. Acta 2010*]. In particular, assay strips were prepared from nitrocellulose membranes (Hi-flow plus 180 membrane cards from Millipore, Billerica, MA) employing a XYZ3050 platform (Biodot, Irvine, CA). In particular, the FmB1-BSA conjugate (0.2 g L^{-1}) and the goat anti-rabbit antibody (1 g L^{-1}) were dispended in correspondence of the test line (T-line) and of the control line (C-line), respectively. Reagents were deposited at the density of 1 µL cm^{-1}, keeping a distance of 7 mm between the two lines. The strips were dried at 37°C for 60 minutes, then blocked with 1% BSA (w/v) in PBS buffer (5 min at room temperature), washed with PBS added of 0.05% of Tween 20 and finally dried at 37°C for 60 min under vacuum. The membrane was assembled with an adsorbent pad, which was necessary to promote complete liquids migration upon sample addition, then cut into sections (5 mm width) and stored in a desiccator at room temperature.

The other reagents were of analytical grade and were employed as received.

Instrumentation

Chemiluminescence detection was performed employing a CCD-based imaging detector [*A. Roda et al., Anal. Chem. 2011*], which was built from a thermoelectrically cooled MZ-2PRO CCD camera (MagZero, Pordenone, Italy) equipped with a Sony ICX285 progressive scan monochrome CCD image sensor (1360 x 1024 pixels, pixel size 6.45 x 6.45 μm^2). The camera, which can be powered either by a 220V to 12V AC/DC power adapter or by a 12V battery, is computer-controlled via a USB 2.0 interface by a dedicated software.

The biosensor, shown in Figure 1, was assembled employing the CCD camera in a lensless “contact” imaging configuration. In particular, the LFIA strip was positioned on the larger surface of a round fiber optic taper (25/11 mm size, Edmund Optics, Barrington, NJ,

USA), which smaller surface was placed directly in contact with the CCD sensor. This assembly was enclosed in a dark box to provide shielding from ambient light. During the acquisition the CCD sensor temperature was kept at -10°C.

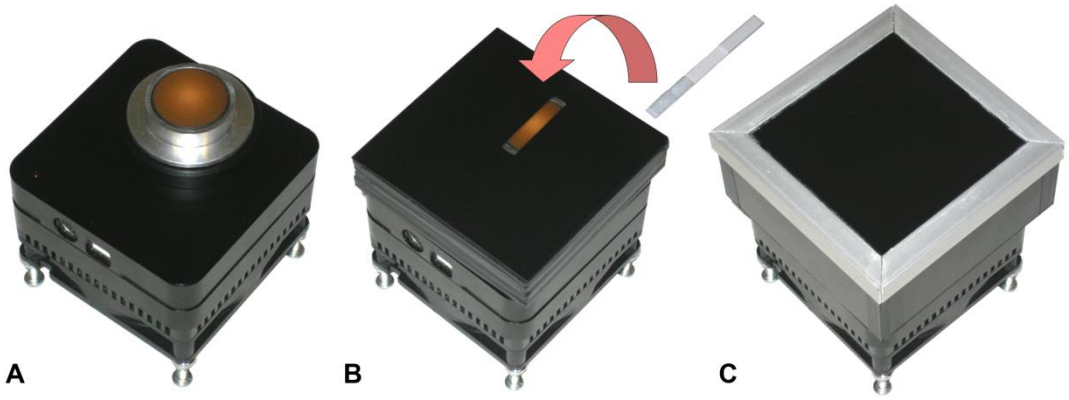

Figure 1: CCD-based device employed for CL detection from LFIA in a lensless “contact” imaging detection. (A) CCD sensor coupled with the fiber optics taper. The system was equipped with a mask to ensure reproducible LFIA strip positioning on the taper (B) and with a cover to shield the sensor from ambient light (C).

As a reference laboratory instrument, a Night OWL LB 981 luminograph (Berthold Technologies GmbH & Co KG, Bad Wildbad, Germany) equipped with a conventional lens-based optics and a highly sensitive, back-illuminated, double- Peltier-cooled CCD camera [*Roda A. et al., Clin. Chem.; Roda A. et al., Anal. Chem. 1996; Guardigli M. et al., Anal. Chim. Acta 2005*] was also used.

All CL images were processed and analyzed employing the WinLight software v. 1.2 (Berthold Technologies GmbH & Co KG, Bad Wildbad, Germany).

Assay procedure

The nitrocellulose strip was placed horizontally, then the LFIA assay was started by depositing on the bottom of the strip a volume of 100 µL of solution, containing 40 µL of PBS with 3% BSA (w/v), 5 µL of HRP-labeled goat anti-rabbit antibody diluted 1:10,000

(v/v) in PBS, 5 µL of rabbit anti-fumonisin antibody diluted 1:500 (v/v) in PBS, and 50 µL of maize sample extract (or blank maize sample extract for the blank). To produce calibration curves, FmB1 standard solutions ranging from 1 to 500 µg L^{-1} prepared in blank maize sample extract were added instead of maize sample extract. Upon complete migration of the solution (10 min), 80 µL of the CL substrate was added at the bottom of the strip and let flow through the membrane (4 min), which was kept at 25°C. Therefore, the CL signal was acquired with the "contact" CCD-based imaging device (10 s acquisition time) or with the Night OWL LB 981 luminograph (10 s acquisition time). Total analysis time was about 15 min. To obtain quantitative information, the mean photon emission was measured in the areas corresponding to C-line and T-line of each LFIA strips and subtracted of the mean background signal measured in two adjacent areas below and above each line. The T-line/C-line ratio was calculated for each strip and then converted into B/B_0 ratio by dividing it for the T-line/C-line ratio measured in the absence of the target analyte (maximum T-line/C-line value). Calibration curves were obtained by plotting B/B_0 values against the log of FmB1 concentration and fitting the experimental data with a four-parameter logistic equation.

Analysis of corn samples

Maize flour samples were bought from local food stores and their content in fumonisins (FmB1 + FmB2) was determined by LC-MS, as previously described [*L. Anfossi et al., Anal. Chim. Acta 2010*]. Maize flour samples were subjected to a previously described pre-analytical treatment [*L. Anfossi et al., Anal. Chim. Acta 2010*] with slight modification. Briefly, 1 g of corn flour was suspended in 10 mL of PBS buffer, hand-shaken for 3 minutes at RT and let settle for 5 min. A volume of 100 µL of the supernatant was heated for 3 min at 100°C to inactivate endogenous maize peroxidase, then cooled to room temperature and subjected to analysis by LFIA. To obtain the analyte concentration value

for each sample, its B/B_0 value was calculated as described above and interpolated on a stored calibration curve.

RESULTS AND DISCUSSIONS

Imaging resolution and CL signal detectability

To allow on-field analysis, a previously described portable CCD-based device designed for "contact" imaging detection [*A. Roda et al., Anal. Chem. 2011*] was employed to measure CL signals emitted from the nitrocellulose strips.

Since lensless "contact" imaging had not been previously employed for acquiring CL signals from LFIA membranes, the performance of the CCD device was compared with reference laboratory imaging instrumentation employing HRP enzyme as a model. To evaluate imaging resolution and assess the possibility to accurately measure the photons emission from a line on the strip without any significant interference from the other line, model strips containing the HRP enzyme immobilized in correspondence of the two lines at a distance of 7 mm were used. The model strips were imaged using both the "contact" CCD imaging device and the LB-981 luminograph.

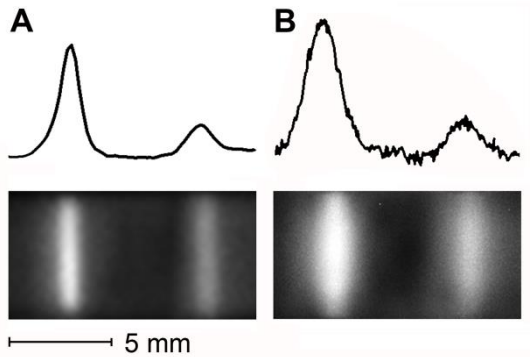

Figure 2. Chemiluminescence images and intensity profiles of nitrocellulose strips where HRP was immobilized in two lines at 7 mm distance. Images were acquired both with the LB-981 luminograph (left) and with the "contact" CCD device (right).

Figure 2 shows that, although contact imaging offers a slightly lower resolution as compared with the LB-981 luminograph based on an optical system, the CL signal

intensity of each line could be measured without a significant cross talk. Indeed, the CL intensity profiles measured across the lines show that the peaks are resolved at the baseline, even in case of different CL intensities between the two lines.

Despite reduction in resolution, we and others have previously shown that contact imaging offers a higher detectability with respect to optics-based imaging [*A. Roda et al., Anal. Chem. 2011; R. R. Singh et al., IEEE Trans. Circuits Syst. 2010; B. Filanoski et al., Luminescence 2008*]. This is mainly due to the possibility to collect a much larger fraction of emitted photons with respect to lens-based optical systems, in which light collection efficiency depends on the lens's numerical aperture.

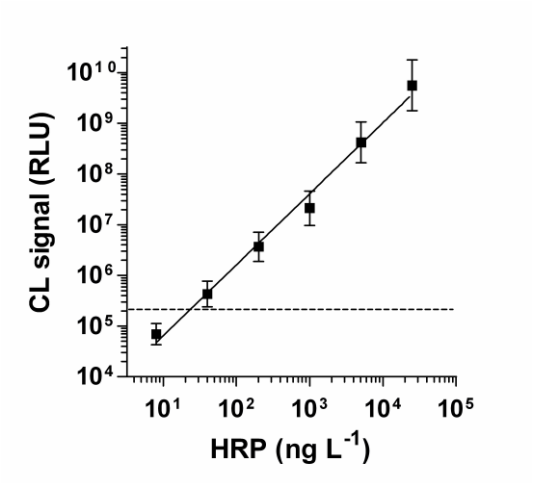

Figure 3. Calibration curve obtained by analyzing HRP standard solutions using LFIA membranes with immobilized anti-HRP antibody as a model. Photons emission was acquired using "contact" CCD imaging upon addition of the CL substrate. The dotted line indicates the blank signal plus three standard deviations.

To assess light detectability, model strips with anti-HRP antibody (200 mg L^{-1} in PBS) immobilized in one line were prepared employing the procedure described above. After saturation with BSA, 100 µL of HRP solutions at concentrations ranging from 4.0 to 2.5 × 10^4 ng L^{-1} was let flow through the membrane. Captured HRP was measured by acquiring images of the strips using both "contact" CCD imaging and the laboratory luminograph (10 s acquisition time), upon addition of 80 µL of the CL substrate and 4-min substrate flow. As

expected, changes in the lines CL intensity were linearly proportional to the concentration of HRP in the sample (Figure 3) and a limit of detection (calculated as the HRP concentration corresponding to the signal of the blank plus three times its standard deviation) of 35 ng L^{-1} (corresponding to approximately 0.1 fmol of HRP in the sample solution) was achieved. It was also observed that the “contact” CCD camera provided a CL signal detectability comparable with the reference laboratory instrumentation, in accordance with results previously reported for different analytical formats [*A. Roda et al., Anal. Chem. 2011*] (data not shown).

Optimization of experimental parameters

The main problem in the use of the LFIA format is the non-specific interaction of the immunoreagents with the solid support, which must be avoided by using appropriate saturation procedures. Preliminary experiments were performed by employing different saturation agents (BSA, non fat dry milk, soybean milk). The best performance was obtained by blocking the strips by incubation for 5 min at room temperature with 1% BSA in PBS buffer, as previously described for the colloidal gold-based LFIA [*L. Anfossi et al., Anal. Chim. Acta 2010*] (data not shown).

To optimize the experimental procedure, the concentrations of antibodies yielding the highest detectability for FmB1 were evaluated. Blank samples and samples containing a fixed concentration of FmB1 (50 μg L^{-1}) were therefore assayed in the presence of different dilutions of rabbit anti-fumonisin antibody and HRP-labeled goat anti-rabbit antibody. As expected, it was observed that a decrease in the amount of rabbit anti-fumonisin antibody employed in the assay in the range between 1:500 and 1:2000 v/v caused a decrease in the CL emission of the T-line, as well as an increase in the ability of FmB1 present in the sample to displace the anti-fumonisin antibody from the immobilized FmB1-BSA conjugate (evidenced by a decrease of the ratio of T-line intensities obtained in

the absence and in the presence of FmB1, respectively). The optimal concentration of the rabbit anti-fumonisin antibody (1:500 v/v) was selected as the one providing the best compromise between the two effects described above (Figure 4A and 4B).

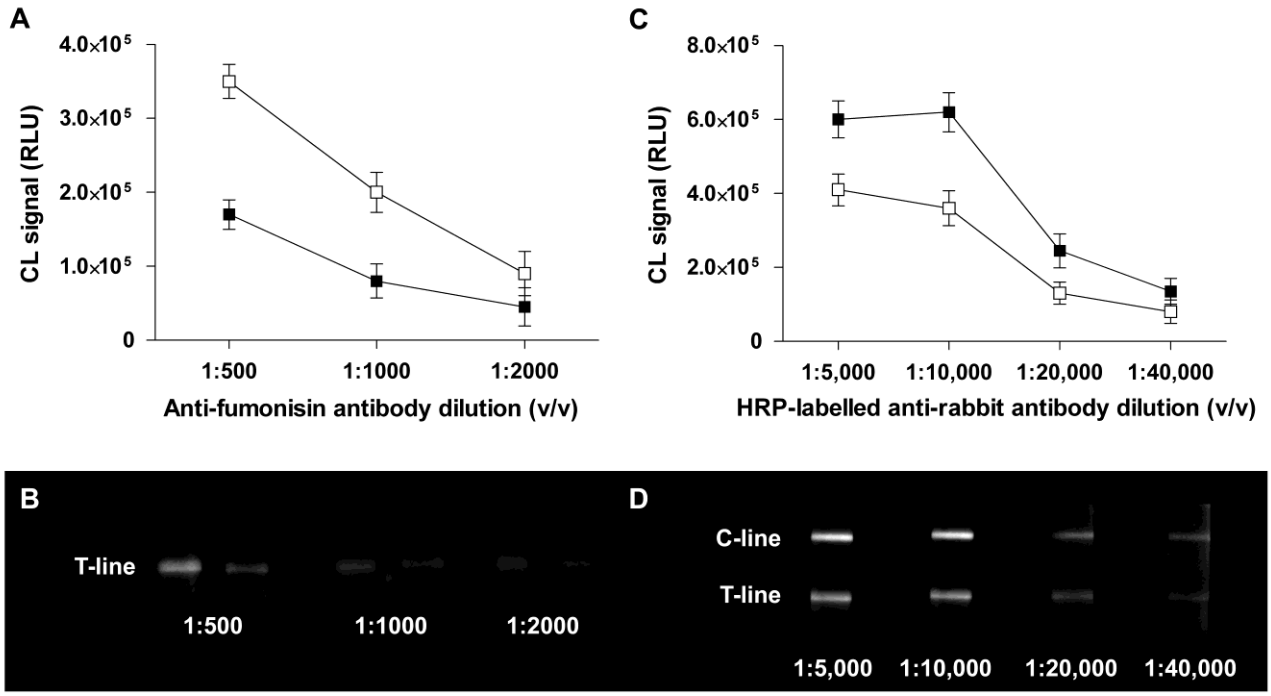

Figure 4. (A, B) Chemiluminescence signals obtained in correspondence of the T-line employing different dilutions of rabbit anti-fumonisin antibody. Assays were performed in the absence of fumonisins (white symbols) or in the presence of FmB1 50 ng mL^{-1} (black symbols). The dilution of HRP-labeled goat anti-rabbit antibody was kept constant at 1:10000 v/v. (C, D) Chemiluminescence signals obtained in correspondence of the C-line (white symbols) and T-line (black symbols) employing different dilutions of HRP-labeled goat anti-rabbit antibody. Assays were performed in the absence of fumonisins and the dilution of rabbit anti-fumonisin antibody was kept constant at 1:500 v/v. Data represent the mean of three assays.

The HRP-labelled anti-rabbit antibody must be added in excess to completely reveal the anti-fumonisin antibody captured on the T-line. However, a large excess of the labelled antibody could determine stronger non-specific CL signals and increases reagents consumption. Different dilutions of the HRP-labelled anti-rabbit antibody, ranging from 1:5000 to 1:20,000 v/v, were assayed in the absence of FmB1 employing a fixed dilution of

rabbit anti-fumonisin antibody (1:500, v/v). As shown in Figure 4C and 4D, the highest signals for both T-line and C-line were obtained at 1:10,000 v/v dilution, while higher antibody amounts did not provide further improvements. The HRP-labelled anti-rabbit antibody 1:10,000 v/v dilution was thus employed for subsequent experiments.

Calibration curve for fumonisins

Calibration curves were generated by using FmB1 standard solutions in the range between 1 and 500 µg L^{-1}. Being a competitive type format, the decrease of T-line/C-line ratio was directly proportional to the amount of the analyte in the sample (the T-line completely disappeared at the highest fumonisin concentration).

A representative series of CL images obtained employing the "contact" CCD detection device and the correspondent calibration curve are shown in Figure 5. The limit of detection was estimated as the concentration corresponding to the blank T-line/C-line value minus three times the blank standard deviation. The obtained value (2.5 µg L^{-1} FmB1, corresponding to 25 µg kg^{-1} in maize flour samples according to the extraction procedure employed in this work) is five times lower than the limit of detection obtained in a colloidal gold-based LFIA previously developed employing the same immunoreagents [*L. Anfossi et al., Anal. Chim. Acta 2010*]. The dynamic range of the method extended to 500 µg L^{-1} (5000 µg kg^{-1} in maize flour samples), with a midpoint value at 20 µg L^{-1}. The intra-assay CVs across the entire range were <12% (n=4), while inter-assay CVs, evaluated by comparing three calibration curves obtained in different days using the same batch of LFIA strips, was below 18 %.The possibility to obtain a fairly good reproducibility avoided the necessity to produce a calibration curve in each analytical session. Indeed, a calibration curve can be produced for a given batch of LFIA strips, then stored and employed to interpolate results obtained in successive sample analyses, which greatly simplified point-

of-use applications of this assay. For quality control purposes, the analysis of a 20 µg L^{-1} FmB1 standard solution could be performed in parallel with maize extract analysis.

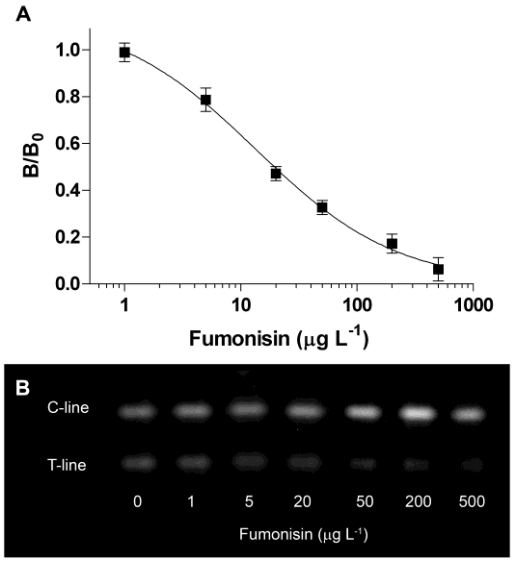

Figure 5. (A) Calibration curve obtained for FmB1 employing the "contact" imaging detection device. The curve fitting was performed employing a four-parameter logistic equation. Error bars = ± SD, *n* = 6. (B) Chemiluminescence images of LFIA membranes acquired with the "contact" imaging detection device.

Real samples analysis

Maize flour samples were subjected to a pre-analytical extraction procedure similar to that described by Anfossi et al. [*L. Anfossi et al., Anal. Chim. Acta 2010*]. However, an additional sample treatment step has been included to deactivate the endogenous peroxidase enzymes present in maize, which would cause high background signals in the presence of the CL substrate (this aspect is not relevant in conventional ELISA assays employing HRP as a tracer because the extensive washing steps prior to the addition of the enzyme substrate allow eliminating endogenous peroxidases). In this work, heating of the sample extracts at about 100°C for 3 min was employed to inactivate endogenous peroxidases. Due to the very small sample volumes required for the assay, sample heating and subsequent cooling were very fast and simple to perform, even on field. Specific HRP inhibitors can not be used because they would also inactivate the HRP label. Alternatively,

alkaline phosphatase (AP) could be employed as an enzyme label instead of HRP, and its activity detected by CL obtained upon addition of a 1,2-dioxetane phosphate substrate. However, in this case overall assay time would be significantly increased, being the kinetic of CL emission catalyzed by AP much slower [*A. Roda et al., Anal Chem, 1996*].

To evaluate the performance of the LFIA method on real samples, maize flour samples bought from local food stores were analyzed employing the extraction procedure and LFIA analysis described in the Materials and methods section. Since previous studies have demonstrated that the antibody employed in this work presents a cross reactivity for FmB2 close to 100%, and thus the developed LFIA assay would provide information about total fumonisins concentration in the sample, the obtained results were compared with the sum of FmB1 and FmB2 fumonisins as measured by LC-MS. [*L. Anfossi et al., Anal. Chim. Acta 2010*].

Table 1. Comparison of results obtained in the analysis of real samples by CL-LFIA and LC-MS

	Fumonisins (FmB1 + FmB2) (μg kg^{-1})		
Sample	**CL-LFIA**[a]	**LC-MS**[b]	**Recovery (%)**
1	< 25[c]	< LOD	-
2	< 25[c]	< LOD	-
3	1700 (CV% = 15)	1920 (1860 μg kg^{-1} FmB1 + 60 μg kg^{-1} FmB2)	109%
4	2300 (CV% = 10)	2540 (??? μg kg^{-1} FmB1 + ??? μg kg^{-1} FmB2)	91%
5	3000 (CV% = 9)	2930 (2160 μg kg^{-1} FmB1 + 770 μg kg^{-1} FmB2)	102%
6	2700 (CV% = 14)	2950 (??? μg kg^{-1} FmB1 + ??? μg kg^{-1} FmB2)	92%

7	3300 (CV% = 11)	3640 (3080 µg kg^{-1} FmB1 + 560 µg kg^{-1} FmB2)	91%
8	4900 (CV% = 13)[d]	4370 (3900 µg kg^{-1} FmB1 + 470 µg kg^{-1} FmB2)	112%
9	6100 (CV% = 14) [d]	5580 (4700 µg kg^{-1} FmB1 + 880 µg kg^{-1} FmB2)	109%
10	8800 (CV% = 11) [e]	9100 (7700 µg kg^{-1} FmB1 + 1400 µg kg^{-1} FmB2)	97%
11	9200 (CV% = 12) [e]	9800 (8000 µg kg^{-1} FmB1 + 1800 µg kg^{-1} FmB2)	94%
12	11600 (CV% = 12) [e]	11100 (9100 µg kg^{-1} FmB1 + 2000 µg kg^{-1} FmB2)	104%

a) Data are expressed as mean ± SD of three independent measurements.

b) From Ref. [L. Anfossi et al., Anal. Chim. Acta 2010]

c) Not detectable (below assay LOD 2.5 µg L^{-1})

d) Maize sample extracts were further diluted 1:2 with blank maize extract before analysis, to be included in the dynamic range of the assay.

e) Maize sample extracts were further diluted 1:4 with blank maize extract before analysis, to be included in the dynamic range of the assay.

As shown in Table 1, good accuracy was observed, with recovery values in the range from 90 to 115 %.

In conclusion, in this work, a competitive LFIA assay exploiting enzyme-catalyzed CL detection was developed to allow on-field simple and rapid quantitative detection of type-B fumonisins (FmB1 and FmB2) in maize flour with a LOD well below regulatory limits. The LFIA assay is simple and rapid (total assay time was 25 min, including sample preparation) and requires minimal labor when compared with conventional ELISAs, which requires multiple incubation and washing steps. To perform ultrasensitive and objective detection of the CL signal, a portable, low-cost and battery-operated device equipped with a thermoelectrically-cooled CCD sensor was employed, with performance similar to that of conventional laboratory instrumentation. The developed portable biosensor is therefore

suitable for rapid and accurate measurements of fumonisins in corn at any stage of production, allowing early detection of contamination in food and feed.

In the future, to increase assay robustness, especially for on-field applications, temperature control elements should be integrated in the portable CCD-based device [Bridle,H. et al., Lab Chip 2008], since the rate of the enzyme-catalyzed CL reaction employed to obtain quantitative information is significantly affected by temperature.

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