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Short communication

Chemiluminescence-based biosensor for fumonisins quantitative detection in maize samples

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ABSTRACT

A compact portable chemiluminescent biosensor for simple, rapid, and ultrasensitive on-site quantification of fumonisins (fumonisin B1 + fumonisin B2) in maize has been developed. The biosensor integrates a competitive lateral flow immunoassay based on enzyme-catalyzed chemiluminescence detection and a highly sensitive portable charge-coupled device (CCD) camera, employed in a contact imaging configuration. The use of chemiluminescence detection allowed accurate and objective analyte quantification, rather than qualitative or semi-quantitative information usually obtained employing conventional lateral flow immunoassays based on colloidal gold labeling. A limit of detection of 2.5 $\mu g\,L^{-1}$ for fumonisins was achieved, with an analytical working range of 2.5–500 $\mu g\,L^{-1}$ (corresponding to 25–5000 $\mu g\,kg^{-1}$ 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 peroxidase enzyme activity was employed for maize flour samples analysis, obtaining recoveries in the range 90–115%, when compared with LC–MS/MS analysis. The chemiluminescence immunochromatography-based biosensor is a rapid, low cost portable test suitable for point-of-use applications.

1. 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 (Kaittanis et al., 2010), environmental (Spier et al., 2011), forensic (Hoile et al., 2011) and agri-food (Fernandez et al., 2010) fields. In the context of food safety control, where the ability for early detection of contamination is of particular importance, cheap and rapid on-field tests are increasingly employed for first level screening purposes.

Fumonisins, mainly produced by *Fusarium* mould species commonly infecting corn and other agricultural products, represent a remarkable problem which appears to be more and more relevant in the context of global market. Due to their adverse effects in animals (Stockmann-Juvala and Savolainen, 2008) and humans (International Agency for Research on Cancer, IARC, 1993, pp. 301–366), maximum residue limits (MRLs) of fumonisins (as the sum of FmB1 + FmB2) in corn-derived foodstuff have been established by the European Union, ranging from 200 $\mu g\,kg^{-1}$ for baby food to 4000 $\mu g\,kg^{-1}$ for raw maize (EC No. 1126/2007).

Analytical methods for fumonisins have been recently reviewed (Krska et al., 2008; Maragos and Busman, 2010). Reference analytical methods are based on HPLC techniques (AOAC Official Methods 995.15 and 2001.04), while enzyme-linked immunosorbent assays are widely employed for screening purposes.

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 reduce risks for the consumer. Various rapid and simple "point-of-use" analytical methods have been developed, including Lateral Flow Immunoassays (LFIAs) (Shiu et al., 2010; Wang et al., 2006) and biosensors (Sapsforda et al., 2006).

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In LFIAs a porous membrane with specific immunoreagents immobilized in definite areas is employed as a solid support, while other reagents are carried along the strip by the flow (driven by capillary forces) established upon sample addition. 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 LFIAs have been developed and a number of them are also commercially available (Posthuma-Trumpie et al., 2009). Conventionally LFIAs are based on the use of colloidal gold or latex particles labelling and the analyte is visually detected through color formation due to label accumulation in definite strip zones. This approach often suffers from poor and subjective quantitative discrimination and low assay sensitivity thus might be not suitable to fulfill the regulatory requirements (Reiter et al., 2009).

To obtain quantitative information, several hand-held devices capable of scanning the LFIA strip and estimating analyte content from the quantitative evaluation of the lines' color intensity have been proposed (Anfossi et al., 2010, 2011; Molinelli et al., 2009; Salter et al., 2006). In addition, detection systems based on enzyme amplification (Cho et al., 2006, 2009), fluorescent nanoparticles (Li et al., 2010; Xia et al., 2009; Zou et al., 2010) or electrochemical measurements (Fernandez-Sanchez et al., 2004; Muhammad-Tahir and Alocilja, 2003) have been developed to increase detectability.

Enzyme-catalyzed chemiluminescence (CL) relies in the use of an enzyme as a label, usually horseradish peroxidase (HRP) or alkaline phosphatase, which is detected with highly sensitive photomultiplier-based or CCD-based light measurement instruments upon addition of a suitable CL substrate. This approach has been widely demonstrated to provide superior analytical performance, yielding high detectability, amenability to miniaturization, short assay times and low sample and reagents consumption (Kricka, 2000; Magliulo et al., 2005; Roda et al., 2003). These features derive from both the amplification factor offered by the cyclic enzyme reaction and the intrinsic characteristics of CL. Indeed, since light emission is generated from dark by a chemical reaction, limitations typical of spectrophotometric or fluorescence detection techniques are circumvented (Kricka, 2000; Roda et al., 2003).

Recently, enzyme-catalyzed CL detection has been also proposed for LFIA, providing quantitative information and improved limits of detection with respect to color-formation-based LFIAs (Cho et al., 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 biosensor exploiting a CL competitive LFIA for fumonisins (FmB1 and FmB2). The competitive immunological reaction and the enzyme-catalyzed CL reaction were conducted sequentially in the LFIA strip and the HRP-labeled tracer antibody was revealed by CL to achieve objective quantitative information. To produce a compact and portable biosensor, the CL signal measurement was performed by contact imaging employing a compact light detection device equipped with an ultrasensitive cooled CCD sensor (Roda et al., 2011a, 2011b). In this set-up, the LFIA membrane was placed in contact with the 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 content in corn flour samples, employing a simple pre-analytical sample clean-up, using a compact portable device and therefore enabling out-lab on-field application.

2. Materials and methods

2.1. Reagents

Bovine serum albumin (BSA), Tween-20, horseradish peroxidase (HRP), 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) and previously described (Anfossi et al., 2010). The Supersignal ELISA Femto CL substrate for HRP was purchased from Thermo Fisher Scientific Inc. (Rockford, IL).

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

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.

Assay strips for LFIA were prepared as previously described (Anfossi et al., 2010) by immobilizing the FmB1-BSA conjugate (T-line) and the goat anti-rabbit antibody (C-line) on nitrocellulose membranes, which were then assembled with an adsorbent pad and cut into sections (5 mm width). Details are available as Supplementary material.

2.2. Instrumentation

The biosensor, shown in Fig. 1, was assembled employing a previously described CCD-based contact imaging configuration (Roda et al., 2011b). In particular, the LFIA strip was positioned on the larger surface of a round fiber optic taper, which smaller surface was placed directly in contact with the CCD sensor. A mask was used to ensure reproducible strip positioning. 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.

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 (Guardigli et al., 2005; Roda et al., 1996, 2002) 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).

2.3. Assay procedure

The nitrocellulose strip was placed horizontally on the fiber optic taper surface, 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, or FmB1 standard solutions ranging from 1 to 500 μg L^{-1} prepared in blank maize sample extract to produce calibration curves). 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. 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

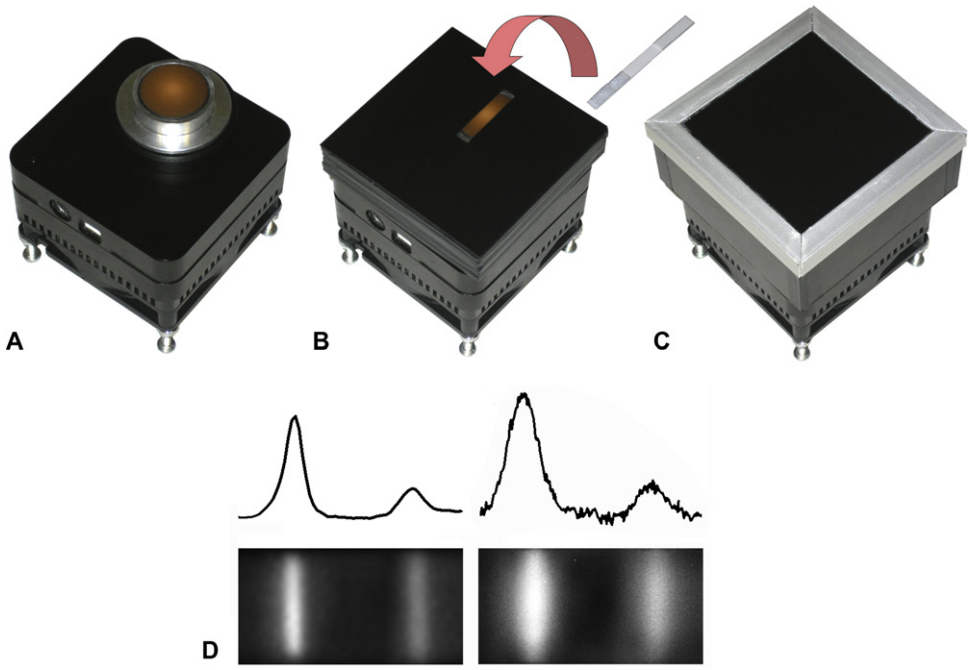

Fig. 1. Biosensor assembling employing a CCD-based device in a contact imaging detection format. CCD sensor coupled with the fiber optics taper (A). The system was equipped with a mask to ensure reproducible LFIA strip positioning on the taper (B) and with a cover to shield the sensor from ambient light (C). 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 (D) and with the contact CCD device (E).

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.

2.4. 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/MS, as previously described (Anfossi et al., 2010). Maize flour samples were subjected to a previously described pre-analytical treatment (Anfossi et al., 2010) with slight modification. Briefly, 1 g of corn flour was suspended in 10 mL of PBS buffer, hand-shaken for 3 min 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.

3. Results and discussions

3.1. Imaging resolution and CL signal detectability

To allow on-field analysis, a compact biosensor was assembled employing a portable CCD-based device designed for contact imaging detection (Roda et al., 2011a,b).

Since this approach 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 imaged using both the contact CCD imaging device and the LB-981 luminograph.

Fig. 1 shows that, although contact imaging offers a slightly lower resolution as compared with the LB-981 luminograph, 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 (Filanoski et al., 2008; Roda et al., 2011b; Singh et al., 2010). 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.

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 in the Section 2. 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 and captured HRP was measured as described in the Section 2. As expected, CL signals were linearly proportional to the concentration of HRP in the sample (calibration curve is available as Supplementary material) 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-based device provided a CL signal detectability comparable with the reference laboratory instrumentation, in accordance with results previously reported for different analytical formats (Roda et al., 2011b) (data not shown).

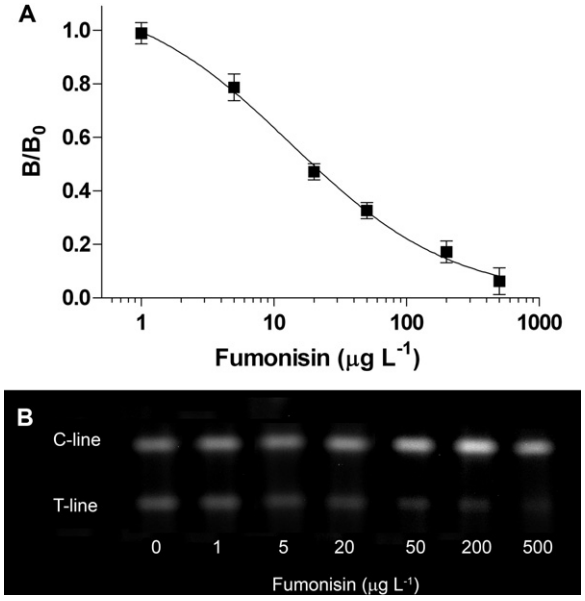

Fig. 2. (A) Calibration curve obtained for FmB1 employing the contact imaging detection device. The curve fitting was performed employing a four-parameter logistic equation. Error bars = ±SD, $n = 4$. (B) Chemiluminescence images of LFIA membranes acquired with the contact imaging detection device.

3.2. Optimization of experimental parameters

The experimental procedure was optimized by evaluating the conditions yielding the highest detectability for FmB1. Data referring to the antibodies concentrations and reduction of non-specific binding are reported in the Supplementary data.

3.3. 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 imaging device and the correspondent calibration curve are shown in Fig. 2. 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 (Anfossi et al., 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.

Table 1
Results obtained in the analysis of maize flour samples by CL–LFIA biosensor. (A) Fortified samples were produced by adding known amounts of fumonisin B1 to a blank maize sample extract. (B) Maize flour samples, previously analyzed by LC–MS/MS, were subjected to extraction and analyzed by CL–LFIA biosensor. CL–LFIA data are expressed as mean and CV% of three independent measurements.

(A) Maize flour extracts fortified with fumonisin B1

Expected concentration (μg L^{-1})[a]	CL–LFIA (μg L^{-1})	Recovery (%)
Within assay		
5,0	5,5 (CV% = 13)	110
20,0	19,0 (CV% = 9)	95
80,0	86,0 (CV% = 10)	108
Between assay		
5,0	5,8 (CV% = 19)	116
20,0	20,8 (CV% = 14)	104
80,0	75,0 (CV% = 12)	94

(B) Maize flour samples (fumonisin B1 + fumonisin B2)

LC–MS/MS (μg kg^{-1})[b]	CL–LFIA (μg kg^{-1})[c]	Recovery (%)
<LOD	<25[d]	–
<LOD	<25[d]	–
1920	1700 (CV% = 15)	89
2540	2300 (CV% = 10)	91
2930	3000 (CV% = 9)	102
2950	2700 (CV% = 14)	92
3640	3300 (CV% = 11)	91
4370	4900 (CV% = 13)	112
5580	6100 (CV% = 14)	109
9100	8800 (CV% = 11)	97
9800	9200 (CV% = 12)	94
11,100	11,600 (CV% = 12)	104

[a] Which would correspond to 0.1 times the μg kg^{-1} fumonisin content in maize flour samples according to the extraction procedure.
[b] From Ref. (Anfossi et al., 2010).
[c] Maize sample extracts were further diluted 1:10 with blank maize extract before analysis, to span over the dynamic range of the assay.
[d] Not detectable (below assay LOD 25 μg kg^{-1}).

3.4. Maize flour samples analysis

Maize flour samples were subjected to a pre-analytical extraction procedure similar to that described by Anfossi et al. (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 cannot 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 (Roda et al., 1996).

The assay analytical performance was evaluated by analyzing extracts from blank maize flour samples (previously analyzed by LC–MS/MS) fortified with FmB1. Since only two blank samples were available (Table 1), a pooled extract was prepared and fortified at the desired FmB1 concentration. The detection capability (CCβ) as defined by the European Commission (EC No. 657/2002) for the validation of screening methods, was determined taking into account the lower MRL for fumonisins in foodstuffs set by the European Community (EC No. 1126/2007), which is 200 μg kg^{-1}

(corresponding to 20 $\mu g\,L^{-1}$ in the extract, taking into account the dilution factor due to the extraction procedure). The blank pooled extract was analyzed ($n = 20$) to establish the cut-off value necessary to define whether a sample was suspected to contain fumonisins or not. Subsequently, the blank pooled extract was fortified at a value corresponding to 0.5 × MRL (10 $\mu g\,L^{-1}$, corresponding to 100 $\mu g\,kg^{-1}$ in the maize flour sample) and analyzed ($n = 20$). The 20 fortified samples were all found suspected, which resulted in a CCβ of <100 $\mu g\,kg^{-1}$.

The assay accuracy and precision (both intra- and inter-assay) were evaluated on spiked samples, obtained by adding known amounts of fumonisin B1 (5, 20 and 80 $\mu g\,L^{-1}$, corresponding approximately to 0.8, 0.5 and 0.25 B/B_0 values on the calibration curve) to the pooled blank extract. As shown in Table 1, recovery ranged from 90 to 116%, while coefficients of variation below 15% (intra-assay) and below 20% (inter-assay) were obtained.

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 Section 2. 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/MS (Anfossi et al., 2010).

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

4. Conclusions

In conclusion, in this work, an immunosensor based on a competitive LFIA format 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 involve 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 et al., 2008), to reduce variability on the rate of the enzyme-catalyzed CL reaction employed to obtain quantitative information.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.11.039.

References

Anfossi, L., Calderara, M., Baggiani, C., Giovannoli, C., Arletti, E., Giraudi, G., 2010. Anal. Chim. Acta 682, 104–109.
Anfossi, L., D'Arco, G., Calderara, M., Baggiani, C., Giovannoli, C., Giraudi, G., 2011. Food Addit. Contam. A 28, 226–234.
AOAC Official Method 995.15, AOAC International 2002.
AOAC Official Method 2001.04, AOAC International 2002.
Bridle, H., Millingen, M., Jesorka, A., 2008. Lab. Chip 8, 480–483.
Cho, I-H., Paek, E-H., Kim, Y-K., Kim, J-H., Paek, S-H., 2009. Anal. Chim. Acta 632, 247–255.
Cho, J.-H., Han, S.-M., Paek, E.-H., Cho, I.-H., Paek, S.-H., 2006. Anal. Chem. 78, 793–800.
European Commission, 2002, Commission Regulation (EC) No. 657/2002, Off. J. Eur. Community, L 221, 8–36.
European Commission, 2007, Commission Regulation (EC) No. 1126/2007, Off. J. Eur. Community, L 255, 14–17.
Fernandez, F., Hegnerova, K., Piliarik, M., Sanchez-Baeza, F., Homola, J., Marco, M.P., 2010. Biosens. Bioelectron. 26, 1231–1238.
Fernandez-Sanchez, C., McNeil, C.J., Rawson, K., Nilsson, O., 2004. Anal. Chem. 76, 5649–5656.
Filanoski, B., Rastogi, S.K., Cameron, E., Mishra, N.N., Maki, W., Maki, G., 2008. Luminescence 23, 22–27.
Guardigli, M., Pasini, P., Mirasoli, M., Leoni, A., Andreani, A., Roda, A., 2005. Anal. Chim. Acta 535, 139–144.
Hoile, R., Yuen, M., James, G., Gilbert, G.L., 2011. Clin. Chim. Acta 412, 1749–1761.
International Agency for Research on Cancer (IARC), 1993. Monographs on the evaluation of the carcinogenic risk of chemicals to humans, Vol. 82. IARC, Lyon, France, pp. 301–366.
Kaittanis, C., Santra, S., Perez, J.M., 2010. Adv. Drug Deliver. Rev. 62, 408–423.
Kricka, L.J., 2000. Methods Enzymol. 305, 333–345.
Krska, R., Schubert-Ullrich, P., Molinelli, A., Sulyok, M., Macdonald, S., Crews, C., 2008. Food Addit. Contam. A 25, 152–163.
Li, Z., Wang, Y., Wang, J., Tang, Z., Pounds, J.G., Lin, Y., 2010. Anal. Chem. 82, 7008–7014.
Magliulo, M., Mirasoli, M., Simoni, P., Lelli, R., Portanti, O., Roda, A., 2005. J. Agric. Food Chem. 53, 3300–3305.
Maragos, C.M., Busman, M., 2010. Food Addit. Contam. A 27, 688–700.
Molinelli, A., Grossalber, K., Krska, R., 2009. Anal. Bioanal. Chem. 395, 1309–1316.
Muhammad-Tahir, Z., Alocilja, E.C., 2003. Biosens. Bioelectron. 18, 813–819.
Posthuma-Trumpie, G.A., Korf, J., van Amerongen, A., 2009. Anal. Bioanal. Chem. 393, 569–582.
Reiter, E., Zentek, J., Razzazi, E., 2009. Mol. Nutr. Food. Res. 53, 508–524.
Roda, A., Pasini, P., Musiani, M., Girotti, S., Baraldini, M., Carrea, G., Suozzi, A., 1996. Anal. Chem. 68, 1073–1080.
Roda, A., Mirasoli, M., Venturoli, S., Cricca, M., Bonvicini, F., Baraldini, M., Pasini, P., Zerbini, M., Musiani, M., 2002. Clin. Chem. 48, 1654–1660.
Roda, A., Guardigli, M., Michelini, E., Mirasoli, M., Pasini, P., 2003. Anal. Chem. 75, 463A–470A.
Roda, A., Cevenini, L., Michelini, E., Branchini, B.R., 2011a. Biosens. Bioelectron. 26, 3647–3653.
Roda, A., Mirasoli, M., Dolci, L.S., Buragina, A., Bonvicini, F., Simoni, P., Guardigli, M., 2011b. Anal. Chem. 83, 3178–3185.
Salter, R., Douglas, D., Tess, M., Markovsky, B., Saul, S.J., 2006. J. AOAC Int. 89, 1327–1334.
Sapsforda, E., Ngundib, M.M., Mooreb, M.H., Lassmanb, M.E., Shriver-Lakeb, L.C., Taittb, C.R., Liglerb, F.S., 2006. Sensor Actuat. B-Chem. 113, 599–607.
Shiu, C.M., Wang, J.J., Yu, F.Y., 2010. J. Sci. Food Agric. 90, 1020–1026.
Singh, R.R., Ho, D., Nilchi, A., Gulak, G., Yau, P., Genov, R., 2010. IEEE Trans. Circuits Syst. 57, 1029–1038.
Spier, C.R., Vadas, G.G., Kaattari, S.L., Unger, M.A., 2011. Environ. Toxicol. Chem. 30, 1557–1563.
Stockmann-Juvala, H., Savolainen, K., 2008. Hum. Exp. Toxicol. 27, 799–809.
Wang, S., Quan, Y., Lee, N., Kennedy, I.R., 2006. J. Agric. Food Chem. 54, 2491–2495.
Xia, X.H., Xu, Y., Zhao, X.L., Li, Q.G., 2009. Clin. Chem. 55, 179–182.
Zou, Z.X., Du, D., Wang, J., Smith, J.N., Timchalk, C., Li, Y.Q., Lin, Y.H., 2010. Anal. Chem. 82, 5125–5133.