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Rapid Determination of Fumonisin B_1 in Food Samples by Enzyme-Linked Immunosorbent Assay and Colloidal Gold Immunoassay

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A rapid enzyme-linked immunosorbent assay (ELISA) test (microwell plate) and a membrane-based colloidal gold immunoassay in flow-through and lateral-flow formats for the rapid detection of fumonisin B_1 (FB_1) were developed. The rapid microwell assay can be completed within 20 min with the detection limit of 0.5 ± 0.2 μg/L. Membrane-based colloidal gold immunoassays had a visual detection limit of 1.0 μg/L for FB_1 with the detection time of <10 min. Matrix interference was eliminated by 15-fold dilutions of methanol extracts with buffer. These immunoassays can be used as quantitative or qualitative tools for the rapid detection of FB_1 residues in 10−20 min on-site.

KEYWORDS: Fumonisin B_1; rapid microwell plate ELISA; colloidal gold immunoassay; on-site screening

INTRODUCTION

Fumonisins are a group of mycotoxins primarily produced by *Fusarium moniliforme* and are one of the most common fungi colonizing corn throughout the world (*1*−*3*). More than 11 structurally related fumonisins have been found since the discovery of fumonisin B_1 (FB_1) in 1988 (*4*). The most investigated fumonisin is FB_1, which can cause leukoencephalomalacia in horses, pulmonary edema in pigs, nephrotoxicity, liver cancer in rats, and esophageal cancer in humans (*5*). The U.S. Environmental Protection Agency classifies fumonisins as category 2B carcinogens. They are most frequently found in corn, corn-based foods and feeds, and other grains such as sorghum and rice. **Figure 1** shows the chemical structures of the fumonisins.

Although the presence and sometimes high contents of fumonisins in cereals are a great problem all over the world, only Switzerland has proposed legislation for FB_1, and the "acceptable" limit was determined as 1000 μg/kg (*6*). The U.S. FDA has issued maximum residue limits in corn and corn byproducts in food and animal feeds, which are 2000 and 5000−20000 μg/kg total fumonisins ($FB_1 + FB_2 + FB_3$) for humans and animals, respectively (*7*).

Fumonisins are typically analyzed by chromatographic methods such as TLC, LC and LC-MS, GC-MS, and HPLC, requiring expensive and time-consuming methods and needing appropriate instrumentation and trained personnel. Immunochemical methods for fumonisins using polyclonal or monoclonal antibodies have been developed in the past two decades because of their adaptability, simplicity, selectivity, and low cost (*8*−*14*). Commercial immunoassay kits (ELISA: microwell and affinity column) are now available in many counties, such as the Veratox (Neogen Corp., Lansing, MI) fumonisin quantitative test kit. However, a long analysis time is needed because of the required incubation time for enzyme−substrate reactions, which is not suitable for detection on-site. In this study, a rapid ELISA test (microwell plate) and two formats of colloidal gold immunoassay (membrane-based) were developed to analyze FB_1 in food and animal feeds. Membrane-based colloidal gold immunoassays had a visual detection limit of 1.0 μg/L for FB_1 with the detection time of <10 min. We discuss in detail the validation of these assays with spiked samples.

EXPERIMENTAL PROCEDURES

Materials. Horseradish peroxidase (HRP) was obtained from Roche (Basel, Switzerland). Fish skin gelatin (FG), 3,3′,5,5′-tetramethylbenzidine, ovalbumin (OA), and hydrogen peroxide were purchased from Sigma (St. Louis, MO). Bovine serum albumin (BSA) was obtained from Merck (Darmstadt, Germany). Purified water was obtained using a Millipore Milli-Q water system (Millipore, Bedford, MA). An anti-FB_1 polyclonal antibody was kindly provided by the University of Sydney. Nitrocellulose membranes were from Pierce (Rockford, IL). Immobilon-nylon membranes were from Millipore. All membranes had a pore size of 0.45 μm. All reagents were of analytical grade.

Fumonisins B_1 and B_2 were purchased from Sigma. Stock solution was prepared by dissolving 1.0 mg of FB_1 or FB_2 in 1.0 mL of methanol and then kept at −20 °C for further dilution. Fumonisins are suspected carcinogens and should be handled with care.

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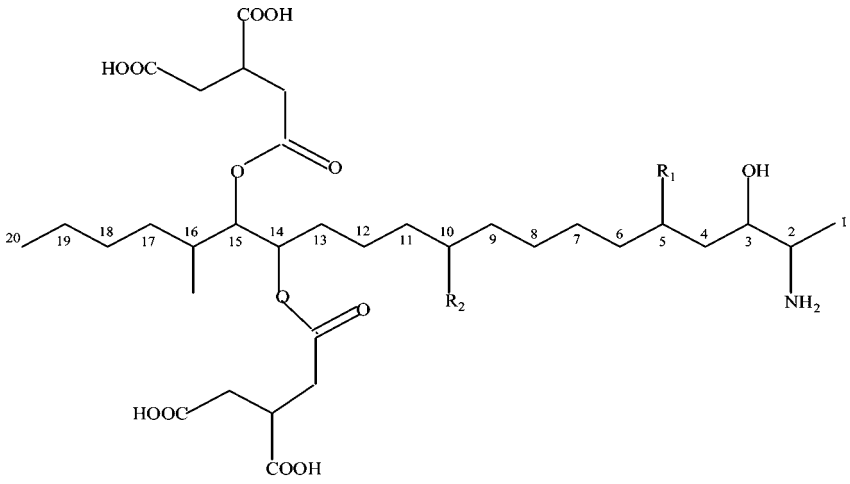

	R_1	R_2
FB_1	OH	OH
FB_2	OH	H
FB_3	H	OH
FB_4	H	H

Figure 1. Chemical structures of fumonisins B_1, B_2, B_3, and B_4.

Instrumentation. Maxisorp polystyrene 96-well plates were purchased from Nunc (Roskilde, Denmark), and the plates were washed with a 96PW microplate washer from Bio-Rad. Immunoassay absorbance was read with a Multiskan Spectrum purchased from Thermo (Labsystems, Vantaa, Finland) in dual-wavelength mode (450−650 nm). FB_1 was analyzed by a reversed-phase, isocratic HPLC system (Shimadzu LC-10 ATVP pump and RF-10AXL fluorescence detector), and a 4.6 mm × 150 mm i.d. Shim-pack GVP-ODS column (Shimadzu) was used.

Antibody Production. A polyclonal antibody was raised against FB_1−keyhole limpet hemocyanin (KLH) in New Zealand white rabbits at the University of Sydney. The immunization schedule was conducted using the procedure described in Lee et al. (*15*).

ELISA Protocols. Conjugation of FB_1 to HRP was achieved by the periodate method as used by Yu and Chu (*16*). Briefly, 2.0 mg of HRP was activated with sodium periodate for 20 min at room temperature and then dialyzed against 1 mM sodium acetate buffer (pH 4.4) overnight. The activated HRP was mixed with 1.0 mL of FB_1 solution (1 mg in 1 mL of carbonate buffer, pH 9.5) and incubated at room temperature for 2 h. After the reaction, 0.1 mL of sodium borohydride solution was added to the mixture and incubated at 4 °C for 1 h. The mixture was dialyzed against 0.01 mol/L phosphate-buffered saline (PBS) for 72 h.

Conjugation of FB1 to OA was achieved by the glutaraldehyde method as used by Yu and Chu (*16*). One milligram of FB_1 in 1 mL of 25% ethanol was mixed with 5 mg of OA, to which 0.08 mL of a 25% glutaraldehyde solution was added dropwise. The reaction was carried out at 4 °C overnight with constant stirring and then stopped by the addition of 0.1 mL of lysine (1 mol/L, pH 7.0). The mixture was dialyzed against PBS for 72 h.

Antibody Coating Protocol. Microwells were coated with anti-FB_1 at 1.0 μg per well in 100 μL of 50 mM carbonate buffer, pH 9.6. After overnight incubation at room temperature, the plates were washed three times with PBS/T washing solution [PBS with 0.05% (v/v) Tween 20], and unbound active sites were blocked with 200 μL of 1% BSA/PBS per well for 1 h. All incubations were performed at room temperature.

Conventional Competition Assay. FB_1 standard or diluted sample extract (100 μL) and HRP−FB_1 conjugate (100 μL, diluted in 1% BSA in PBS) were added to the antibody-coated wells and incubated for 1 h. Following washing of the plates with PBS/T washing buffer, 150 μL of substrate solution (1.25 mM 3,3′,5,5′-tetramethylbenzidine and 1.6 mM hydrogen peroxide in acetate buffer, pH 5.0) was added to each well. The reaction was stopped after 30 min at room temperature by adding 50 μL of 1.25 mol/L H_2SO_4, and absorbance was recorded in the microplate reader (450−650 nm).

Rapid Microwell Immunoassay. FB_1 standard or diluted sample extract (100 μL) and HRP−FB_1 conjugate (100 μL, diluted in 1% BSA in PBS) were premixed in a glass tube. The premixed solution (50 μL) was added to the antibody-coated wells and incubated for 10 min. Following washing plates with washing buffer, 150 μL of substrate was added to each well. The reaction was stopped after 10 min at room temperature by adding 50 μL of 1.25 mol/L H_2SO_4, and absorbance was recorded in the microplate reader (450−650 nm).

Colloidal Gold Immunoassay. *Conjugation of Colloidal Gold Solution to Anti-FB_1 Polyclonal Cntibody.* The pH of the colloidal gold solution for anti-FB_1 polyclonal antibody conjugation was adjusted to pH 9.0 with 0.1 M K_2CO_3 or 0.1 M HCl. Before conjugation, the optimal concentration of antibody for conjugation was determined. With gentle stirring, 0.6 mL of anti-FB_1 antibody (1.585 mg/mL) was added dropwise to 100 mL of pH-adjusted colloidal gold solution (particle size of 40 nm checked by transmission electron microscope). After overnight incubation at 4 °C, the mixture was centrifuged at 10000 rpm and 4 °C for 30 min, and the pellet was resuspended in 10 mL of conjugate storage buffer (2 mM sodium borate containing 0.1% BSA and 0.1% sodium azide, pH 7.2) and diluted for use.

The preparation of the membranes for flow-through assay and lateral-flow assay were the same as described by Wang et al. (*17*) except that 2.7 μg of FB_1 hapten−OA was coated on the membrane. Nitrocellulose membrane from Pierce was used for the flow-through assay, and Hi-flow plus membrane from Millipore was used for the lateral-flow assay.

Procedure of Colloidal Gold Flow-through Immunoassay. Sixty microliters of FB_1 standard in 5% methanol (prepared in PBS/T) was mixed with 30 μL of gold−antibody conjugate. After incubation for 5 min, 50 μL of mixture was added to the test strip that was coated with hapten−OA conjugate and anti-rabbit IgG. After the liquid reagent had passed through the test lines, different intensities of color on the test lines were observed visually. The color of the test line was compared with the test line of negative control strip (without FB_1).

Procedure for Colloidal Gold Lateral-Flow Immunoassay. The test strip was pasted onto a plastic backing. Dried filter paper acted as an absorbent pad. FB_1 standards (60 μL) in 5% methanol (prepared in PBS/T) were mixed with the gold−antibody conjugate (60 μL), and after 5 min of incubation, 100 μL of mixture of gold−antibody and sample containing FB_1 was pipetted into the bottom of the strip (sample application site). After the liquid reagent had migrated past the test line, different color intensities on the test line were observed by eye.

The color of the test line was compared with the test line of a negative control strip (without FB_1).

Extraction and HPLC Analysis of FB_1. *Sample Spiking.* Corn, barley, rice, oats, peanuts, and sorghum were purchased locally and shown to contain no toxin, as determined by HPLC. They were finely ground with a laboratory blender (IKA, Staufen, Germany) and were dried at 50 °C with air circulation for no longer than 20 h and then stored at −20 °C before analysis. For spike and recovery studies, 10 g samples were spiked with FB_1, which was dissolved in methanol at different levels. The samples were thoroughly mixed and then allowed to stand at room-temperature overnight.

Sample Extraction. The spiked samples were mixed with 40 mL of methanol/water (75:25, v/v) and then shaken using a rotary shaker (IKA Labortechnik, Staufen, Germany) at 250 rpm for 15 min. The mixture was kept for 10−20 min. The supernatant was divided for fumonisin analysis by immunoassay and HPLC. For detection by immunoassay, an aliquot of the extract was diluted with 0.5% fish skin gelatin (FG), or PBS/T was directly added to microwells or membranes without a cleanup procedure. For HPLC detection, extract (2 mL) was applied to a SAX cartridge (Phenomenex, Torrance, CA; 1 mg/mL), which had been conditioned with methanol followed by methanol/water (75:25, v/v), the cartridge was then washed with methanol/water (75:25, v/v) and methanol, and then the toxins were eluted with 1% acetic acid in methanol. The eluate was evaporated to dryness, and the residue was redissolved in 200 μL of methanol for derivatization.

HPLC Determination of FB_1. The HPLC analysis of fumonisin B_1 was conducted according to the method of Shephard et al. (*18*) with modification. Excitation and emission wavelengths were 335 and 450 nm, respectively, and the mobile phase was methanol/0.05 M citric acid buffer (pH 4.1) (75:25, v/v) at a flow rate of 1.0 mL/min. After derivatization with *o*-phthalaldehyde (OPA) reagent [10 mg of OPA in 1 mL of methanol diluted with 2 mL of 0.1 M sodium borate (pH 9.1) and 14 μL of 2-mercaptoethanol], 20 μL of sample was injected into the HPLC for analysis.

RESULTS AND DISCUSSION

Features of the Rapid Microwell Immunoassay. The standard curves for the conventional competition assay and rapid assay are shown in **Figure 2**. The standard curve of the conventional assay was prepared in 5% methanol to reflect a 1 in 15 dilution of sample extract with 0.5% FG/PBS (0.5% fish skin gelatin in PBS, m/v) proportions to reduce solvent and matrix effects. By contrast, the standard curve of the rapid assay was prepared in 10% methanol with a 7.5-fold dilution to reduce the matrix effects. The IC_{50} value (concentration of analyte giving 50% inhibition of color development) was increased about >3-fold from 1.1 ± 0.4 μg/L in the conventional competition assay to 3.6 ± 0.2 μg/L in the rapid assay. The limits of detection (LOD), which was calculated as a concentration that gives 15% inhibition of color development (IC_{15}), for the conventional assay and rapid assay were 0.2 ± 0.1 and 0.5 ± 0.2 μg/L, respectively. However, the rapid assay can be completed within 20 min, compared with 2.5 h for the conventional assay.

The intra-assay reproducibility and interassay reproducibility were determined to study the precision of the rapid assay. The variations of percent inhibition for 100, 33.3, 11.1, 3.7, 1.2, and 0.41 μg/mL of FB_1 in 10% methanol tested three times on the same day were 0.4, 1.6, 5.5, 9.8, 8.4, and 4.6%, respectively. Assay of the same material run on 12 different days gave deviations from the mean values of 1.6, 3.9, 3.1, 5.4, 12.6, and 23.8% for each of the respective concentrations. A cross-reaction was observed with FB_2, which commonly coexists in food, and it cross-reacted at 36.5% relative to FB_1.

Analytical Characteristics of Colloidal Gold Immunoassay. The colloidal gold immunoassay was studied as a rapid visual qualitative assay that produces a simple presence/absence

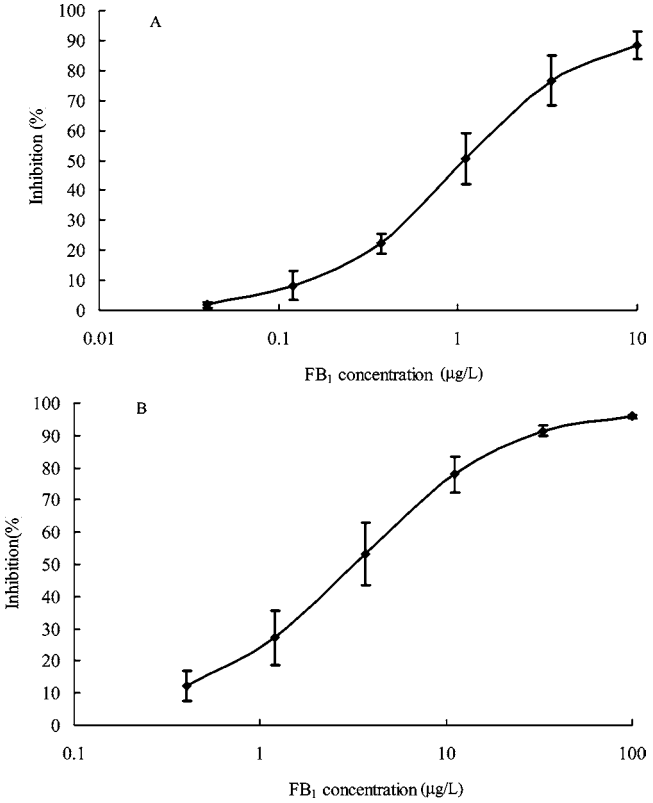

Figure 2. Standard curve of (**A**) the conventional competition assay of FB_1 and (**B**) the rapid competition assay of FB_1.

test. The color intensity of the test lines must be high enough to be seen and to distinguish if there is a difference in color intensity between the negative control and a sample. As this developed assay is a competitive assay, a lower amount of coated FB_1 hapten−OA conjugate and antibody-gold conjugate should yield a higher sensitivity within certain limits. Optimization experiments (three factors, three levels), which included the optimal immobilization concentration of FB_1 hapten−OA, the optimal ratio of gold−antibody conjugate and FB_1, and optimal incubation time. Determined conditions for flow-through and lateral-flow colloidal gold immunoassay were as follows: FB_1 hapten−OA coated on the membrane at 2.7 μg/strip; gold−antibody conjugate and FB_1 in the ratio of 1:2 (flow-through, v/v) and 1:1 (lateral-flow, v/v); and 5 min of incubation time. As shown in **Figure 3**, 1.0 μg/L of FB_1 caused a slight but distinguishable difference compared to the negative control. It can be concluded that the colloidal gold immunoassay developed in this study had a visual detection limit of 1.0 μg/L for FB_1 in both flow-through and lateral-flow formats.

With regard to cross-reactivity, the two formats were re-evaluated using corn sample spiked with a $FB_1 + FB_2$ standard mixture at concentrations of 500 + 400 and 500 + 200 μg/kg. The presence of FB_2 at 400 μg/kg led to an increased response and correspondingly less color development compared to the samples spiked only with 500 μg/kg of FB_1. However, 200 μg/kg of FB_2 had little effect on color development compared to the samples spiked only with 500 μg/kg of FB_1.

Matrix Interference and Its Removal. One of the common challenges of immunoassay for food analysis is matrix interference. These interferences can be reduced in a number of ways, and dilution is a commonly used procedure. Corn, barley, peanuts, oats, rice, and sorghum were chosen as test samples to study the matrix interferences. Different dilution buffers including purified water (Milli-Q), 10 mM PBS (pH 7.2), PBS/T

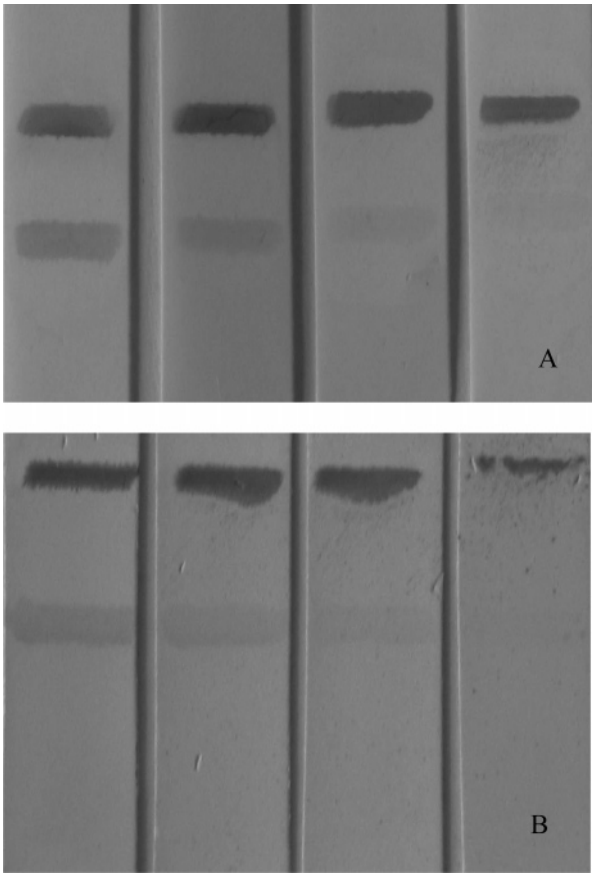

Figure 3. (**A**) Colloidal gold flow-through immunoassay assay of FB_1: upper line, control line (goat anti-rabbit IgG); lower line, test line (FB_1–OA); FB_1 concentrations, from left to right, 0, 1.0, 10, and 100 μg/L. The color intensity of 1.0 μg/L was clearly distinguishable from that of the negative control. (**B**) Colloidal gold lateral flow immunoassay of FB_1: upper line, control line (goat anti-rabbit IgG); lower line, test line (FB_1–OA); FB_1 concentrations, from left to right, 0, 1.0, 10, and 100 μg/L. The color intensity of 1.0 μg/L was clearly distinguishable from that of the negative control.

(pH 7.2), 0.5% FG−PBS, 2 mM sodium tetraborate buffer (pH 7.2, conjugate storage buffer), and 5 mM borate buffer (pH 9.0) were tested for reducing matrix effects.

The matrices were extracted with 75% methanol, and it was found that minimum 1:15 and 1:7.5 dilutions with 0.5% FG were adequate to remove the matrix interference for the conventional assay and the rapid assay, respectively. Filtration using a filter paper and centrifugation at 5000 rpm for 15 min were not effective in reducing matrix interference for the rapid assay (data not shown).

For colloidal gold immunoassay, under identical experimental conditions, 75% methanol extract diluted 15-fold with PBS/T showed lower background color in unspotted areas and high sensitivity, producing strong color development with the same amount of coated FB_1 hapten−OA conjugate and gold−antibody compound. PBS/T was therefore used as diluent buffer in all subsequent experiments. The FB_1 standard solutions were also prepared in PBS/T containing 5% methanol for the two formats. The matrix effects were determined by comparing the FB_1 standard (0, 1.0, 10, and 100 μg/L) prepared in a particular matrix (such as food methanol extract) and FB_1 prepared in matrix-free solution. Matrix interference was eliminated by 15-fold dilution of sample extracts with PBS/T.

Recovery Studies. Blank samples (determined by HPLC) were spiked with FB_1 concentrations at 0, 250, and 500 μg/kg and then analyzed by immunoassays and were confirmed by HPLC analyses. Each sample was evaluated three times in duplicate to verify the repeatability. The results are shown in **Table 1**. Good recoveries were obtained from these spiked samples.

Table 1. Comparison of Results Obtained by Microwell Immunoassay, Flow-through Immunoassay, and HPLC

matrix	spike level (μg/kg)	rapid assay results ($n = 3$)	visual results of flow-through assay[a] ($n = 3$)	HPLC confirmation (μg/kg)
corn	0	0	−, −, −	0
	250	262	±, +, +	270
	500	381	+, +, +	400
rice	0	0	−, −, −	0
	250	380	+, +, +	252
	500	440	+, +, +	320
barley	0	0	−, −, −	0
	250	183	±, +, +	263
	500	423	+, +, +	340
oats	0	0	−, −, −	0
	250	220	+, ±, +	240
	500	480	+, +, +	370
peanut	0	0	−, −, −	0
	250	265	+, +, +	242
	500	410	+, +, +	391
sorghum	0	0	−, −, −	0
	250	210	+, +, +	254
	500	394	+, +, +	310

[a] −, absence of FB_1; +, presence of FB_1.

Analysis of Real Food Samples. Cereal samples (corn, barley, peanuts, oats, rice, and sorghum) purchased in local markets were analyzed using the rapid assay and the two colloidal gold-based immunoassays. Results for all samples were negative. The confirmation step with HPLC analysis also resulted in negative samples. This means that the developed assays can be used for real sample analysis.

A rapid ELISA test (microwell plate) and two formats of colloidal gold immunoassay (membrane-based) were developed to detect FB_1 in grain samples. These immunoassays can be used as quantitative or qualitative tools for the rapid screening of FB_1 residues in 10−20 min on-site. The advantages of the rapid microwell assay compared to the conventional assay are the simplicity of the protocol and lower matrix interferences. Membrane-based colloidal gold immunoassays had a visual detection limit of 1.0 μg/L for FB_1 with the detection time of <10 min with 15-fold dilutions of methanol extracts. The colloidal gold assay developed in this paper is sufficiently sensitive for on-site toxin detection. The sensitivities of the current assay methods were sufficient to detect FB_1 at the maximum residue limit of 1000 μg/kg proposed for legislation in Switzerland and are suitable for use as rapid screening tests for fumonisin B_1.

LITERATURE CITED

(1) Dutton, M. F. Fumonisins, mycotoxins of increasing importance: their nature and their effects. *Pharmacol. Ther.* **1996**, *70*, 137−161.

(2) Nelson, P. E.; Plattner, R. D.; Shackelford, D. D.; Desjardins, A. E. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. *Appl. Environ. Microbiol.* **1991**, *57*, 2410−2412.

(3) Riley, R. T.; Richard, J. L. Fumonisins: a current perspective and view to the future. *Mycopathologia* **1992**, *117*, 1−124.

(4) Gelderblom, W. C. A.; Jasiewicz, K.; Marasas, W. F. O.; Thiel, P. G.; Horak, R. M.; Vleggar, R.; Kriek, N. P. J. Fumonisins—novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol*. **1988**, *54*, 1806−1811.

(5) Rheeder, J. P.; Marasas, W. F. O.; Thiel, P. G.; Sydenham, E. W.; Shephard, G. S.; Van Schalkwyk, D. J. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology* **1992**, *82*, 353−357.

(6) Boutrif, E.; Canet, C. Mycotoxin prevention and control: FAO programmes. *Rev. Med. Vet.* **1998**, *149*, 681−694.

(7) U.S. Food and Drug Administration. Draft guidance for industry: fumonisin levels in human foods and animal feeds; availability. *Fed. Regist.* **2002**, 65, 35945; http://www.cfsan.fda.gov/~dms/fumongui.html (accessed June 20, 2002).

(8) Azcona-Olivera, J. I.; Abouzied, M. M.; Plattner, R. D.; Pestka, J. J. Production of monoclonal antibodies to the mycotoxins fumonisins B_1, B_2 and B_3. *J. Agric. Food Chem.* **1992**, *40*, 531−534.

(9) Yu, F. Y.; Chu, F. S. Production and characterization of a monoclonal anti-anti-idiotype antibody against fumonisin B_1. *J. Agric. Food Chem.* **1999**, *47*, 4815−4820.

(10) Hanne, R. C.; Yu, F. Y.; Chu, F. S. Development of a polyclonal antibody-based sensitive enzyme-linked immunosorbent assay for fumonisin B_4. *J. Agric. Food Chem.* **2000**, *48*, 1977−1984.

(11) Paepens C.; Saeger, S. D.; Sibanda, L.; Barna-Vetro, I.; Leglise, I.; Van Hove, F.; Van Peteghem, C. A flow-through enzyme immunoassay for the screening of fumonisins in maize. *Anal. Chim. Acta* **2004**, *523*, 229−235.

(12) Savard, M. E.; Sinha, R. C.; Lau, R.; Seguin, C.; Buffam, S. Monoclonal antibodies for fumonisins B_1, B_2 and B_3. *Food Agric. Immunol.* **2004**, *15*, 127−134.

(13) Lauer, B.; Ottleben, I.; Jacobsen, H. J.; Reinard, T. Production of a single-chain variable fragment antibody against fumonisin B_1. *J. Agric. Food Chem.* **2005**, *53*, 899−904.

(14) Maragos, C. M.; Jolley, M. E.; Plattner, R. D.; Nasir, M. S. Fluorescence polarization as a means for determination of fumonisins in maize. *J. Agric. Food Chem.* **2001**, *49*, 596−602.

(15) Lee, N. A.; Wang, S.; Allan, R. D.; Kennedy, I. R. A rapid aflatoxin B_1 ELISA: development and validation with reduced matrix effects for peanuts, corn, pistachio, and soybeans. *J. Agric. Food Chem*. **2004**, *52*, 2746−2755.

(16) Yu, F. Y.; Chu, F. S. Production and characterization of monoclonal antibodies against fumonisin B_1. *Food Agric. Immunol*. **1999**, *11*, 297−306.

(17) Wang, S.; Zhang, C.; Zhang, Y. Development of a flow-through enzyme-linked immunosorbent assay and a dipstick assay for the rapid detection of insecticide carbaryl. *Anal. Chem. Acta* **2005**, *515*, 205−219.

(18) Shephard, G. S.; Sydenham, E. W.; Thiel, P. G.; Gelderblom, W. C. A. Quantitative determination of fumonisins B1 and B2 by high-performance liquid chromatography with fluorescence detection. *J. Liq. Chromatogr*. **1990**, *13*, 2077−2087.

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