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Rapid detection of fumonisin B_1 using a colloidal gold immunoassay strip test in corn samples

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ABSTRACT

Fumonisin B_1 (FB_1) is the most common and highest toxic of fumonisins species, exists frequently in corn and corn-based foods, leading to several animal and human diseases. Furthermore, FB_1 was reported that it was associated with the human esophageal cancer. In view of the harmful of FB_1, it is urgent to develop a feasible and accuracy method for rapid detection of FB_1. In this study, a competitive immunoassay for FB_1 detection was developed based on colloidal gold-antibody conjugate. The FB_1-keyhole limpet hemoeyanin (FB_1-KLH) conjugate was embedded in the test line, and goat anti-mouse IgG antibody embedded in the control line. The color density of the test line correlated with the concentration of FB_1 in the range from 2.5 to 10 ng/mL, and the visual limit detection of test for FB_1 was 2.5 ng/mL. The results indicated that the test strip is specific for FB_1, and no cross-reactivity to other toxins. The quantitative detection for FB_1 was simple, only needing one step without complicated assay performance and expensive equipment, and the total time of visual evaluation was less than 5 min. Hence, the developed colloidal gold-antibody assay can be used as a feasible method for FB_1 rapid and quantitative detection in corn samples.

1. Introduction

Fumonisins, exists frequently in corn and corn-based foods, are produced primarily by *Fusarium moniliforme* (Pagliuca et al., 2005; Mexiasalazar et al., 2008). FB_1 was first discovered in 1988, and it is the most common and highest toxic of fumonisins species, often leading to several animal and human diseases, such as encephalomalacia in horses, pulmonary edema in pigs, and hepatic and renal toxicity in several species (Tardieu et al., 2009). Furthermore, FB_1 was reported that it was associated with the human esophageal cancer. Due to its strong toxicity, several measures of regulation and prevention have been made by the Food and Drug Administration (FDA) and Commission Regulation of European Community (EC). For example, FDA has determined the limit of FB_1 in different foods, as 2000–4000 μg/kg total fumonisins ($FB_1 + FB_2 + FB_3$) for human foods, and 5000–10,0000 μg/kg for animal feeds (Wang et al., 2011), and the European Union maximum guidance level for fumonisins is 20 mg/kg for feeds (Antonissen et al., 2015), and 2–4 mg/kg for foods (Kadir and Tothill, 2010).

In naturally contaminated samples, approximately 70% of the total detected fumonisins was FB_1, especially in corn samples (Samapundo et al., 2006). Therefore, it is very important to develop effective methods to detect and quantify the contamination of FB_1 in samples. Recently, several assays have been used for detection FB_1 in different samples, such as high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), liquid chromatography (LC) assays and so on. The detection limit of Immunoaffinity Extraction combined with High-performance Liquid Chromatography (HPLC) with fluorescence for FB_1 was 0.013 μg FB_1/g in liver, kidney and muscle tissue. The minimum detection level of LC-MS method for FB_1 in bovine milk was 0.1 μg/kg (Tardieu et al., 2008; Gazzotti et al., 2009).

These methods are sensitive and reliable to determine the FB_1 in samples, but these methods need expensive equipment, trained operators, and complicated sample preparation, making them

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unsuitable for on-site inspection. Up to now, enzyme linked immunosorbent assay (ELISA) with high sensitivity and specificity based on monoclonal and polyclonal antibodies (mAb and pAb) have been widely used to detect FB_1 in foods. The Limit detection of enhanced chemiluminescent (ECL)-ELISA method for quantification of FB_1 was 0.09 μg/L (Quan et al., 2006). However, ELISA also has some drawback, including time-consuming, tedious washing and incubation steps. Therefore, the detection methods described above did not meet on-site testing.

From 1980, colloidal gold immunoassay has been developed to detect the toxins in different samples in one step without other treatment, using a cellulose membrane as the carrier. To solve the problem of rapid assay for FB_1 efficiently, a sensitive, specific and simple immunoassay for FB_1 detection in corn samples was urgently needed. Our group had successfully obtained one hybrid cell lines 4G5 excreting monoclonal antibody specific against FB_1, and then developed Ic-ELISA method for detection (Ling et al., 2014). In this study, colloidal gold immunoassay was established based on this McAb. The purified anti-FB_1 McAb was conjugated to the prepared colloidal gold particles, and the formed colloidal gold-labeled IgG antibody was used as the tracer to detect FB_1 on a test strip. The strip test can be used for rapid detection of FB_1 in corn samples with high specificity and sensitivity, and the process of detection was simple, without any equipment and complicated handling procedures. Hence, the strip test has good application prospect for mycotoxin detection in samples.

2. Materials and methods

2.1. Reagents and buffers

Nitrocellulose membranes were obtained from Whatman company (Middlesex, UK). Fumonisin B_1 (the purity ≥98% by HPLC), goat anti-mouse IgG, bovine serum albumin (BSA), Chloroauric acid ($HAuCl_4$), and trisodium citrate were purchased from Shanghai Chemical Reagents (Shanghai, China). All other chemicals were analytical grade and purchased from Beijing Chemical Reagent Co. (Beijing, China).

2.2. Preparation of anti-FB_1 IgG antibody

Hybridoma cells named 4G5 that secreted anti-FB_1 IgG antibody were previously prepared in our laboratory (Ling et al., 2014). After injection of 4G5 hybridoma cells into mice for 2–3 weeks, the produced ascites was collected every other day. The specific IgG antibody was precipitated by ammonium sulfate, then, further purified by affinity chromatography with an immobilized protein G column. The titer of anti-FB_1 McAb was determined by ELISA, and the absorbance was measured at 450 nm by Micro-plate reader. The concentration of purified IgG antibody was determined by the instruction of Enhanced BCA Protein Assay Kit.

2.3. Preparation of colloidal gold

Colloidal gold particles were produced as described with minor modification (Chen et al., 2012; Biagini et al., 2006). Briefly, 100 mL of 0.01% chloroauric acid solution ($HAuCl_4$) in distilled water was heated to the boiling point, and then 2.0 mL of 1% trisodium citrate solution was quickly added into this solution. After boiling for 5 min, the color of solution was changed from yellow to wine-red, then, allowed to cool the solution gradually to room temperature. Finally, the purified anti-FB_1 IgG antibody was added into the solution for conjugation at 4 °C for overnight. The conjugates were identified by a transmission electron microscope (TEM), and the absorbance peak of the conjugates was characterized by UV–visible spectra at 400–660 nm.

2.4. Optimization of test strips

One milliliter of colloidal gold solution was added separately to the series of 1.5 mL tubes. The pH of colloidal gold solution was adjusted with potassium carbonate. The same concentration of the anti-FB_1 IgG antibody diluted with phosphate buffer was added into the solution. After incubating the mixture for 5 min, 0.02 mL of 10% NaCl was added into the tube, and the minimum pH that keep the red color in tube without change was considered as the optimal pH. Different volume of purified anti-FB_1 IgG antibody solution (from 0 to 9.5 μL) was added separately to 1 mL of the colloidal gold solution. After incubating the mixture for 5 min, 0.02 mL of 10% NaCl was added to the solution, and the absorbance of solution was measured at 525 nm. The optimal concentration of purified anti-FB_1 IgG antibody was determined according to the standard curve drawn from the concentration and the absorbance.

2.5. Preparation of colloidal gold-antibody conjugates

Colloidal gold solution was adjusted to optimal pH with potassium carbonate. Then, 1 mL of purified anti-FB_1 IgG antibody was diluted with phosphate buffer to the optimal concentration, and then added into the 100 mL colloidal gold solution with gentle stirring for 60 min. After reaction, 1 mL of 1% (w/v) BSA solution was dropped into the mixture with gentle stirring for 30 min. The solution was incubated at 4 °C for overnight, then, centrifuged at 2000 *g* for 30 min. The supernatant was centrifuged at 10,000 *g* for 60 min, and the precipitated colloidal gold-antibody conjugates was re-suspended with conjugate dilution buffer (0.01 M Tris, 5% BSA, 2% sucrose, 0.87% NaCl and 0.1 M sodium azide) and stored at 4 °C for further use.

2.6. Preparation of colloidal gold immunoassay test strips

Colloidal gold immunoassay test strip consisted of three pads (sample, conjugate, and absorbent pads) and one nitrocellulose (NC) membrane with test and control zones (Liu et al., 2014). The colloid gold strip test device was constructed as follows: As FB_1 is a small molecular hapten, it cannot bind directly on the surface of nitrocellulose membrane. So the FB_1-KLH conjugate and rabbit anti-mouse IgG antibody were embedded onto nitrocellulose (NC) membrane, respectively. The distance between the two lines was about 5 mm. The treated nitrocellulose membrane was dried at room temperature for 10 min. Subsequently, the absorption pad, nitrocellulose (NC) membrane, conjugation pad, and sample application pad were assembled into a laminated sheet. Finally, the sheet was cut into 5 mm wide strips for further use. If both the test and control lines turn red, the sample is recorded as negative. When the control line but not the test line was colored, it is considered as positive. If no visible red line is present in the control area, the test strip is considered invalid regardless of whether an red line appears in the test area or not.

2.7. Determination of cross-reactivity and sensitivity of the test strips

To evaluate the cross-reactivity of the test strips, different toxins including Aflatoxin B_1 (AFB_1), HT-2 toxin (HT-2), Ochratoxin A (OTA), Ochratoxin B (OTB), T-2 toxin (T-2), Citreoviridin (CIT), were allowed to react with the colloidal gold–FB_1 McAb conjugate which was pipetted into glass fiber paper (Wang et al., 2014a). The mixture then moves upward on the nitrocellulose membrane. After incubation at room temperature for 5 min, the detection results