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Rapid detection of aflatoxin B_1 on membrane by dot-immunogold filtration assay

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ABSTRACT

Immunofiltration assay for mycotoxins in which nitrocellulose membrane (NCM) was used as a support and enzyme was used as the label has been developed since the late 1980s. As colloidal gold is a good labeling substance that can accelerate antibody–antigen reaction which result can be read directly by naked eyes, the colloidal gold particles could replace the enzyme to be labeled to antibody in aflatoxin B_1 (AFB_1) immunoassay. Dot-immunogold filtration assay (DIGFA) of AFB_1 on NCM was developed in this study. At first, the colloidal gold was synthesized and colloidal gold-monoclonal antibody (McAb) conjugates against AFB_1 were prepared at pH 7.0 of colloidal gold solution, 0.018 mg/mL of McAb. Then the colloidal gold-McAb conjugates were used to develop AFB_1 DIGFA, which detection time was only 15 min, six times less than that of ELISA. With this method to determine the standard AFB_1 solution, the results demonstrated a visual detection limit of approximately 2 ng/mL of AFB_1, which was similar to that of ELISA. This method had good specificities for AFG_1, AFG_2 and AFM_1 and a little cross-reactivity with AFB_2. 45 food samples collected from the markets were subjected to DIGFA and the results showed that one corn sample was positive and in agreement that of HPLC. It is suggested that DIGFA developed in current study has a potential use as a rapid and cost-effective screening tool for the determination of AFB_1 in foods in the field within 15 min without complicated steps.

1. Introduction

Aflatoxin B_1 (AFB_1) is a toxic metabolite produced mainly by *Aspergillus flavus* and *A. parasiticus* that contaminates a wide range of agricultural products. AFB_1 which was listed in Group I carcinogen by the International Agency for Research on Cancer (IARC) [1] is a potent of carcinogen, teratogen and mutagen [2,3]. In order to control AFB_1 levels in foods and feeds, rigorous programs have been established by governmental agencies [4,5] and a number of sensitive methods for the analysis of AFB_1 [6–10], such as high performance liquid chromatography (HPLC), enzyme linked immunosorbent assay (ELISA), near infrared spectroscopy (NIRS) [11], electrochemical immunosensor (ECIS) [12] and so on, have been developed.

Although ELISA, HPLC and ECIS have good detection limits of AFB_1 (0.01–0.1 ng/mL) and NIRS is an excellent candidate for a rapid and low-cost method for the detection of AFB_1, these methods are time consuming or require a laboratory equipped with proper instruments and trained personnel [5,9,11,12]. Now there is a need for more cost-effective, field portable assay systems that can be conducted and interpreted by users who are as close to the source of contamination as possible. Most of them are basically designed as visual tests that require only low-cost instrumentation and offer an advantage of speed. Thus, more and more on-site immunoassays such as immunochromatography (IC) [13–18] and immunofiltration assay (IFA) are gaining interest in the area of mycotoxin detection in foods and feeds [19]. A competitive IC assay system for AFB_1 standard solution detection was developed by Sun et al. [20] using colloidal gold-McAb conjugates.

Since the late 1980s, based on a principle of direct competitive ELISA several groups have developed IFA for mycotoxins in which nitrocellulose membrane (NCM) was used as a support, [19,21]. However, this IFA needs fussy incubation, washing and enzymatic reactions during signal generation [22–25]. As colloidal gold has been widely used in immunoassay for large molecular, the colloidal gold particles could replace the enzyme to be labeled to antibody in AFB_1 immunoassay.

Dot-immunogold filtration assay (DIGFA) is a new technique of solid phase labeled IFA in which NCM is used as a support and

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colloidal gold is used as the label. Colloidal gold is a good labeling substance and it has been used as probe for labeling antibody or antigen by non-covalent electrostatic adsorption [26]. Furthermore, nanoscale surfaces provided by colloidal gold particles can accelerate antibody–antigen reaction and provide an amplified signal for immunoassay [27,28]. Especially, the results can be read directly by naked eyes, which ensure the convenience of assay on-site [20]. This user-friendly format possesses several advantages, such as a very short time for obtaining test results, long-term stability over a wide range of climates and relative inexpensiveness. These characteristics make DIGFA ideally suit for on-site testing by untrained personnel.

Therefore, DIGFA has been developed and applied increasingly in various research fields such as for the detection of alpha fetoprotein [29], myoglobin [24], insecticide carbaryl [30], HIV [31], white spot syndrome virus of shrimp [22] and *Schistosoma japonicum* [23]. However, to our knowledge, there is a lack of studies on small molecular materials like AFB_1 determination by membrane-based competitive DIGFA.

The goal of this study was to develop a rapid and simple detection method that was based on one-step membrane-based competitive DIGFA. Preparation and characterization of colloidal gold–McAb conjugates specific to AFB_1 and its use in developing a competitive DIGFA for on-site assessment of AFB_1 were studied. The AFB_1 DIGFA which can preliminarily analyze the AFB_1 levels in foods has been developed.

2. Materials and methods

2.1. Materials

Chloroauric acid ($HAuCl_4{\cdot}4H_2O$, AR) and tri-sodium citrate (AR) were obtained from Shanghai Chemical Reagent Company in China. AFB_1 (aflatoxin B_1), AFB_2, AFG_1, AFG_2 and AFM_1 were purchased from Alexis (Lausen, Switzerland). Bovine serum albumin (BSA) was obtained from Sigma (St. Louis, MO, USA). AFB_1-cBSA ($n_{AFB1}:n_{cBSA}$ = 5.2:1) and AFB_1-cOVA ($n_{AFB1}:n_{cOVA}$ = 3.2:1) was produced in our laboratory [32]. Monoclonal antibody to AFB_1 (anti-AFB_1 McAb) was obtained from Oil Crops Research Institute of Chinese Academy of Agricultural Sciences. High-flow nitrocellulose membrane (NCM, 0.8 μm) was obtained from Sierra Company (USA). All other chemicals used in the present study were either analytical pure or with higher quality.

2.2. Synthesis of colloidal gold

All glassware used in this preparation was thoroughly cleaned in lotion [$K_2Cr_2O_7/H_2SO_4$ (2m/5 v)], rinsed in deionized water and oven-dried prior to use. Colloidal gold particles were prepared by tri-sodium citrate recovery method according to the procedure described by Frens [33] and Wang [30]. The procedures for preparing colloidal gold were as follows: an aqueous solution containing chloroauric acid (50 mL of 0.01% [w/v] $HAuCl_4{\cdot}4H_2O$) and tri-sodium citrate (2 mL of 0.1% [w/v] $C_6H_5Na_3O_7{\cdot}2H_2O$) was heated to boiling under constant stirring. The color of the solution had changed from blue to dark red within 15 min. After cooling, deionized water was added till the initial volume. The colloidal gold solution was scanned between 400 nm and 700 nm by using a spectrophotometer (Shimadzu, model UV-1700, Japan). The colloidal gold suspensions supplemented with 0.05% (m/v) of sodium azide were stored at 4 °C for several months.

2.3. Preparation of colloidal gold-McAb conjugates

Anti-AFB_1 McAb was conjugated to colloidal gold particles (about 12 nm in diameter) in colloidal suspension, under variable conditions of pH and McAb concentration, to determine the optimal conditions [34].

2.3.1. Effect of pH

The pH of the gold suspension (two groups) was adjusted in the range 4–11 by adding 0.1 M HCl or 0.1 M Na_2CO_3 and the McAb solution dialyzed in distilled water, was diluted to 100 μg/mL with deionized water. Then 20 μL McAb diluted solution was mixed with 100 μL colloidal gold solution at various pH in wells of 96-well microplate. The mixtures were allowed to react for an additional 15 min at room temperature (20–30 °C). The pH optimum was noted from the final solution of Group 1 monitored by absorbance at 520 and 580 nm [35]. In Group 2, after the mixtures of McAb solution and colloidal gold solution were allowed to react for 15 min, 20 μL of 10% NaCl was added to each well and left for an additional 15 min to react at room temperature. The pH optimum was also noted from color change of the solution.

2.3.2. Effect of McAb concentration

A picture was constructed for colloidal gold-labeled McAb, to determine the optimum amount of protein needed to coat the surface of the gold particles. The solutions were prepared in various concentrations (0–300 μg/mL) of anti-AFB_1 McAb and 10 μL were added to each well in a 96-well microplate containing 100 μL colloidal gold adjusted to the optimal pH (7.0). The solutions left to react for 15 min at room temperature (20–30 °C), 20 μL of 10% NaCl was added to each well and the solutions left for an additional 15 min at room temperature. If the red color of the mixture did not change within 10 min, it indicated that the amount of McAb present was sufficient. In this way, the necessary amounts of McAb and of colloidal gold solution were calculated.

2.3.3. Preparation of colloidal gold-McAb conjugates under the optimal conditions

The colloidal gold-McAb conjugates were prepared following the procedure described by Horisberger and Clerr [36], with minor modifications. The colloidal gold-McAb conjugates were prepared under the optimal conditions. Colloidal gold (5 mL), adjusted to pH 7.0, was mixed with McAb (1 mg/mL, 120 μL) and allowed to react for 30 min. The residual surface of the gold particles was blocked by adding 1.28 mL of 5% BSA (instead of 1% carbowax described by Horisberger and Clerr) in deionized water and stirring for 30 min, then stored at 4 °C for 2 h. The conjugates were then centrifuged at 12,000 rpm at 4 °C for 30 min and the supernatant was checked its titer by indirect ELISA. The pellet was dispersed in 5 mL 0.01 M PBS (KCl 0.2 g, NaCl 8.0 g, KH_2PO_4 0.2 g, $Na_2HPO_4{\cdot}12H_2O$ 2.9 g, distilled water1000 mL, pH 7.4) containing 0.1% polyethylene glycol (PEG-10000), 0.05% NaN_3 (instead of 0.15 M NaC1-0.02 M Tris, pH 7.4, containing 0.5 mg/ml carbowax described by Horisberger and Clerr) and centrifuged once again at 12,000 rpm at 4 °C for 30 min. After removing the supernatant, the final volume was adjusted to 1 mL and the conjugates solutions were stored at 4 °C until used.

2.4. Characterization of colloidal gold-McAb conjugates

2.4.1. UV–vis spectroscopy studies

The colloidal gold and colloidal gold-McAb conjugates were monitored by UV–vis spectroscopy (400–700 nm) using a double-beam spectrophotometer, (Shimadzu, model UV-1700, Japan) equipped with quartz cuvettes with a path-length of 10 mm at 12 nm/s scanning speed and 1 nm bandwidth. The gold solutions should be monitored immediately after centrifugation and resuspension of the conjugates in appropriate buffers.

2.4.2. Titers determination of anti-AFB_1 McAb, colloidal gold-McAb conjugates and supernatant by indirect ELISA

The procedure of indirect ELISA was the same as described previously by Zhou et al [32]. Briefly, 100 μL of coating antigen (2 μg/mL) AFB_1-cOVA in bicarbonate buffer (pH 9.6) were added to each plate hole and incubated at 4 °C overnight with relative humidity. The plate was washed three times with PBST (0.1 M PBS, pH7.4, containing 0.05% Tween 20) for each well and decanted. To eliminate non-specific binding, the wells were blocked with 5% skim milk solution in PBST for 2 h at 37 °C. After the same washing procedures as described above, 100 μL of anti-AFB_1 McAb, colloidal gold-McAb conjugates or supernatant (doubling dilution) were added to each well and incubated at 37 °C for 1 h. After washing the plate, 100 μL of goat anti-mouse Ig G-horseradish peroxidase conjugate (1/4000) in PBST was added into each well and incubated at 37 °C for 1 h. Then 100 μL of peroxidase substrate (40 mg of 1,2-diamino-benzene and 150 μL H_2O_2 in 100 mL of citrate buffer, pH 4.5) were added after washing the plate five times, and incubated for 15 min at 37 °C. The reaction was terminated by adding 50 μL of 2 mol/L sulfuric acid. Absorbance at 492 nm was determined in a Multiskan MK3 ELISA reader (Thermo, China).

2.4.3. Detection of the sensitivity of anti-AFB_1 McAb by indirect competitive ELISA

The procedure of indirect competitive ELISA (icELISA) was similar to that mentioned in Section 2.4.2 with some modifications. Briefly, after the polystyrene plates were coated and blocked as described in Section 2.4.2 except with 90 μL of suitable diluted anti-AFB_1 McAb and 10 μL of serial concentrations (0.5, 1, 2.5, 5, 10 ng/mL) of AFB_1 in methanol solution represent of 100 μL of antibody.

2.5. DIGFA procedure

Immune filtration device used in this study was the same as described previously by Wang [22] and Wen [23]. Briefly, there were four components in the kit: (1) a small square-plastic box filled with water-absorbing material and NCM with 0.8 μm pore side laid on the top of it; (2) colloidal gold-McAb conjugates; (3) blocking agent (0.01 M PBS containing 2% BSA, pH 7.4) and (4) lotion (0.01 M PBST).

The tests were done as follows. At first, 0.8 μm NCM was dipped in distilled water for 2 h, then took out and dried in air. After dropped 10 μL AFB_1-cBSA solution (200 μg/mL, dissolved in 0.01 M PBS containing 3% methanol), the membrane placed for 15 min at room temperature. Secondly, NCM was dropped 2% BSA (50 μL) be blocked for 15 min, and washed three times by 0.01 M PBST. Then the dried membrane was put in filtration equipment. Thirdly, NCM was dropped 10 μL colloidal gold-McAb conjugates at a suitable concentration (dissolved in 0.01 M PBS) and reacted for 15 min at 37 °C. Finally, the appearance of a reddish dot indicated the colloidal gold-McAb conjugates were effective.

2.6. Detection of the sensitivity and specificity of colloidal gold-McAb conjugates by DIGFA

The procedure to detect the sensitivity of colloidal gold-McAb conjugates was similar to that mentioned in Section 2.5 with some modifications. Briefly, after NCM was coated and blocked, 10 μL mixture of colloidal gold-McAb conjugates and AFB_1 standard solution (v/v = 3/100, AFB_1 of different concentration dissolved in 0.01 M PBS containing 4% methanol) were added to NCM. The subsequent steps were as described in Section 2.5. The results can be read with the naked eye comparing the resulting color with those of the controls. When the color of the dot is only pale compared with a pink background that the concentration of AFB_1 is considered as the sensitivity of colloidal gold-McAb conjugates. The procedure to detect the specificity of colloidal gold-McAb conjugates was same as above except with AFB_2, AFG_1, AFG_2, AFM_1 represent of AFB_1.

2.7. Analytical recovery of AFB_1 added to rice sample by DIGFA

Analytical recovery of AFB_1 added to rice sample was extracted following the procedure as follows: samples of 2 kg were thoroughly mixed and 100 g sub-samples were ground to powder by grinder. Twenty grams of powder (added to 1, 2, 4, 10, 20 ng/g of AFB_1 respectively) was mixed with 60 mL of $CHCl_3$ by blending in aware blender. Extraction was followed by shaking for 30 min and filtered through Whatman 4 filter paper. 6 mL filtrate (equally 2 g rice) was dried at 65 °C and dissolved in 2 mL 0.01 M PBS (containing 20% methanol). Then the quantitative analysis of AFB_1 in rice samples was performed by DIGFA. The protocol was similar to that for determining the sensitivity of colloidal gold-McAb conjugates except that AFB_1 standards concentration was 1, 2, 4, 10, 20 ng/mL were prepared in AFB_1-free rice samples extract.

2.8. Analysis of AFB_1 in food samples

To test the efficacy of the DIGFA of AFB_1 in food samples, studies were carried out as follows. 45 samples (21 rice, 13 corn and 11 wheat flour samples) markets. 20 g of the finely ground sample were blended with 60 mL of $CHCl_3$ in a blender for 30 min and followed by filtration through Whatman 4 filter paper. 6 mL filtrate (equally 2 g sample) was dried at 65 °C and dissolved in 1 mL 0.01 M PBS (containing 20% methanol). Then the quantitative analysis of AFB_1 in samples was performed by DIGFA. The AFB_1 positive samples were determined by HPLC (Waters 2695-2475, USA) for corroboration.

2.9. Test of the stability of DIGFA

The kit was stored at 4 °C and was used to assay the AFB_1 standard solution at 15-day intervals in order to test the stability of DIGFA.

3. Results

3.1. Synthesis of colloidal gold

The UV–vis spectrum colloidal gold suspensions displayed a sharp peak at ~520 nm (Fig. 1) indicating the uniformity and

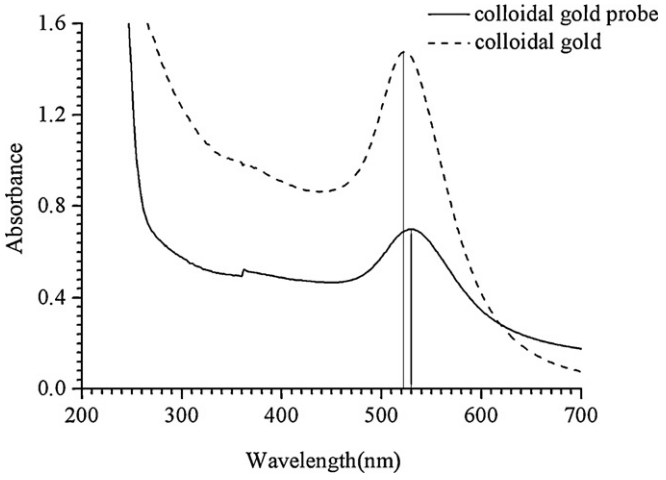

Fig. 1. UV–vis spectra of colloidal gold and colloidal gold-McAb conjugates.

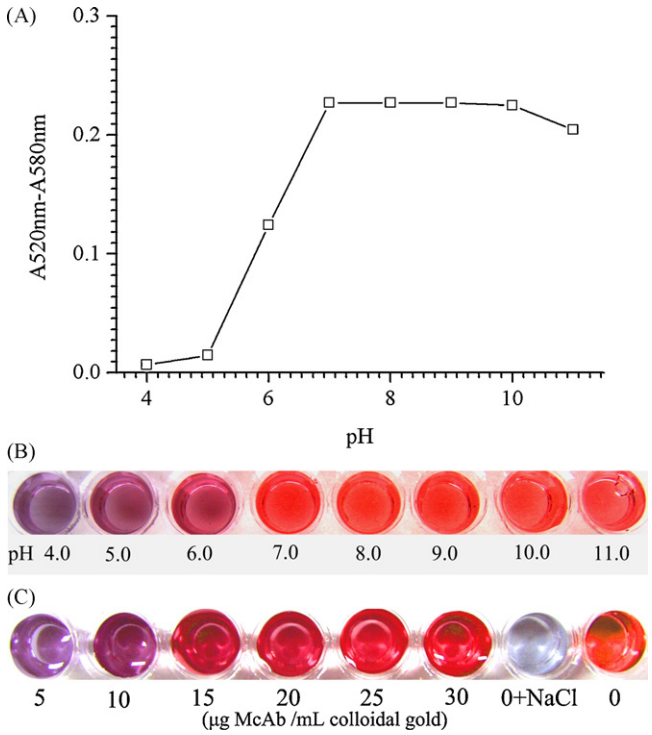

Fig. 2. Optimization of experimental conditions for preparation of colloidal gold-McAb conjugates. (A), (B) Influence of pH solution in the degree of conjugation. (C) Effect of the antibody concentration in the conjugation yields, under optimal pH conditions.

excellent dispersion of colloidal gold particles. According to the regression equation $y = 0.4271x + 514.56$ ($R = 0.974$, y represents the maximum wavelength of colloidal gold and x represents its particle diameter) [37], the average diameter of the colloidal gold obtained in this study was about 12 nm [20,26] indicating that the colloid gold met the experimental requirements.

3.2. Optimal condition studies for preparation of colloidal gold-McAb conjugates

The colloidal gold was formed in solution by virtue of a balance between electrostatic repulsion and London-van der Waals attraction among the particles. However, on addition of ionic substance, the attracting force becomes greater than the counteraction, which leads to an aggregation accompanying a color change from red ($\lambda_{max} \approx 520$ nm, A520) to blue ($\lambda_{max} \approx 580$ nm, A580) [38]. This instability can be prevented by coating the colloidal surfaces with protein molecules such as antibody.

Colloidal gold-McAb conjugates to detect AFB_1 were prepared by addition of McAb to the colloidal gold solution to form conjugates. Optimal conditions of pH and antibody concentration for conjugation can be determined by comparing the absorption between 520 and 580 nm (A520–A580) and color shown.

3.2.1. Effect of pH

To study the effect of the pH, a reference experiment was carried out with gold solutions adjusted to pH 4.0 and 11.0, respectively, before adding anti-AFB_1 McAb. If antibody molecules are adsorbed physically on the gold surface, the particles are stabilized against aggregation. From the A520–A580 and color shown, optimal conditions of the pH for the conjugation were determined. For the variation of pH, a maximum value of A520–A580 was reached at pH 7, but no significant change occurred from pH 7 to 11 (Fig. 2A). When the pH was over 7.0, the red color shown also remained approximately constant (Fig. 2B). It could be interpreted that colloidal gold being coated by McAb and NaCl could not make them aggregate. A pH 7.0 of colloidal gold was considered to be suitable by comparing the absorption between 520 and 580 nm (A520–A580) and color shown.

3.2.2. Effect of antibody concentration

To study the effect of antibody concentration, a reference experiment was also carried out with various concentrations (0–300 μg/mL) of anti-AFB_1 McAb after adding 100 μL colloidal gold adjusted to the optimal pH. Results shown that 15.0 μg antibody was the minimum content for stabilizing 1.0 mL colloidal gold (Fig. 2C), and was chosen for use. For conjugation, each McAb solution was used in 20% excess of the minimal amount to ensure complete reaction with the colloidal gold particles.

3.3. Characterization of colloidal gold-McAb conjugates

3.3.1. UV–vis spectra

As can be seen from Fig. 1, the colloidal gold and colloidal gold-McAb conjugates exhibited strong and narrow absorption peak at 520 and 525 nm. A peak at ~520 nm is due to the surface plasmon resonance of colloidal gold particles. After addition of McAb, the surface plasmon band broadened, the absorption decreased and red shifted due to interaction of McAb with colloidal gold particles.

3.3.2. Titers determination of anti-AFB_1 McAb, colloidal gold-McAb conjugates and supernatant by indirect ELISA

Prior to constructing the immunofiltration device by using colloidal gold-McAb conjugates, we employed indirect imcompetitive ELISA demonstrated the stability of immunoreactivity compared with free antibody in solution (Fig. 3). Results showed that there was no obvious change in antibody titer after labeled by colloidal gold. It showed that the active site of the antibody molecules was not blocked by conjugation with colloidal gold particles. Whereas the removing supernatant showing very low antibody titer, it showed that free antibody was completely labeled by colloidal gold and colloidal gold-McAb conjugates was successfully obtained.

3.3.3. Detection of the sensitivity of anti-AFB_1 McAb by indirect competitive ELISA

According to the results of the square titration, select the corresponding coating antigen and the concentration of McAb, AFB_1 was used as competitive antigen, making indirect competitive ELISA of the AFB_1 standard curve, see Fig. 4. The result showed that the concentration of AFB_1 and competitive inhibition rate present

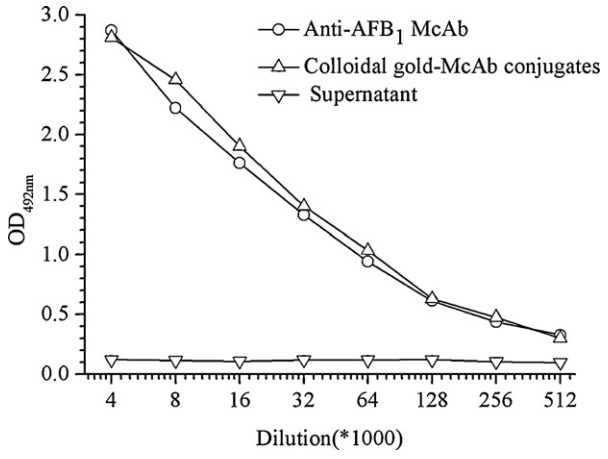

Fig. 3. Indirect imcompetitive ELISA titer curves of anti-AFB_1 McAb, colloidal gold-McAb conjugates and supernatant.

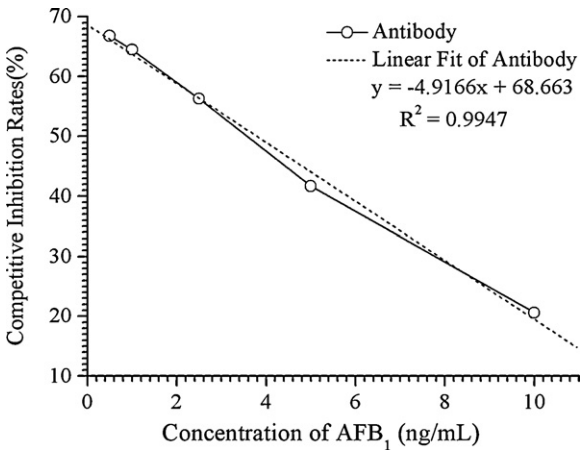

Fig. 4. Indirect competitive ELISA inhibitory curves of anti-AFB_1 McAb.

good correlation when the concentration of AFB_1 between the range of 0.5 ng/mL and 10 ng/mL. Its detection sensitivity were 3.8 ng/mL (IC_{50}) and the linear equation was $y = -4.9166x + 68.663$ ($R^2 = 0.9947$).

3.4. Analytical recovery of AFB_1 in standards solution and added to rice samples by DIGFA

The DIGFA was developed for AFB_1 analysis. In DIGFA system, colloidal gold-McAb conjugates served as probe, AFB_1-cBSA acted as the coating antigen and AFB_1 acted as the competitive antigen. AFB_1 and AFB_1-cBSA compete for the limited colloidal gold-McAb conjugates binding sites. For a negative sample, i.e., AFB_1 level less than the assay cut-off level, there will be a visible color spot in the center of the membrane. For a positive sample, i.e., AFB_1 level greater than/equal to the cut-off level, there will be no color spot on the membrane. When the concentration of the coating antigen was 200 μg/mL, colloidal gold-McAb conjugates were in its 34-fold dilute solution, the sensitivity for AFB_1 detection was 2 ng/mL (Fig. 5A) by visual observation. The assay can be completed in 15 min to give semi-quantitative estimation by visual observation and the device was stable and user friendly. Detection time was reduced 6–10 times comparative with icELISA.

This method had good specificities for AFG_1, AFG_2 and AFM_1 and a little cross-reactivity with AFB_2 (Fig. 5B–E). It was also showed that system certainly can be used widely for the determination in food.

AFB_1 was added to the rice samples without AFB_1 which were confirmed by HPLC and the concentration is 1, 2, 4, 10, 20 ng/mL, respectively. The method of extracting AFB_1 was the same as the procedure of 2.7 and the sensitivity for AFB_1 in rice samples detection was 4 ng/mL (Fig. 5F) by visual observation.

3.5. Analysis of AFB_1 in food samples

45 food samples, including rice, corn and wheat were subjected to DIGFA and results as shown in Fig. 6. The results of the food samples tests showed that one corn sample (sample 38) was positive and in agreement with that of HPLC (Fig. 7).

3.6. Stability of DIGFA

DIGFA was used to test the AFB_1 standard solution. After the kits had been stored at 4 °C, the above samples were assayed at 15-day intervals. The results showed that the kit was stable for at least 3 months at 4 °C.

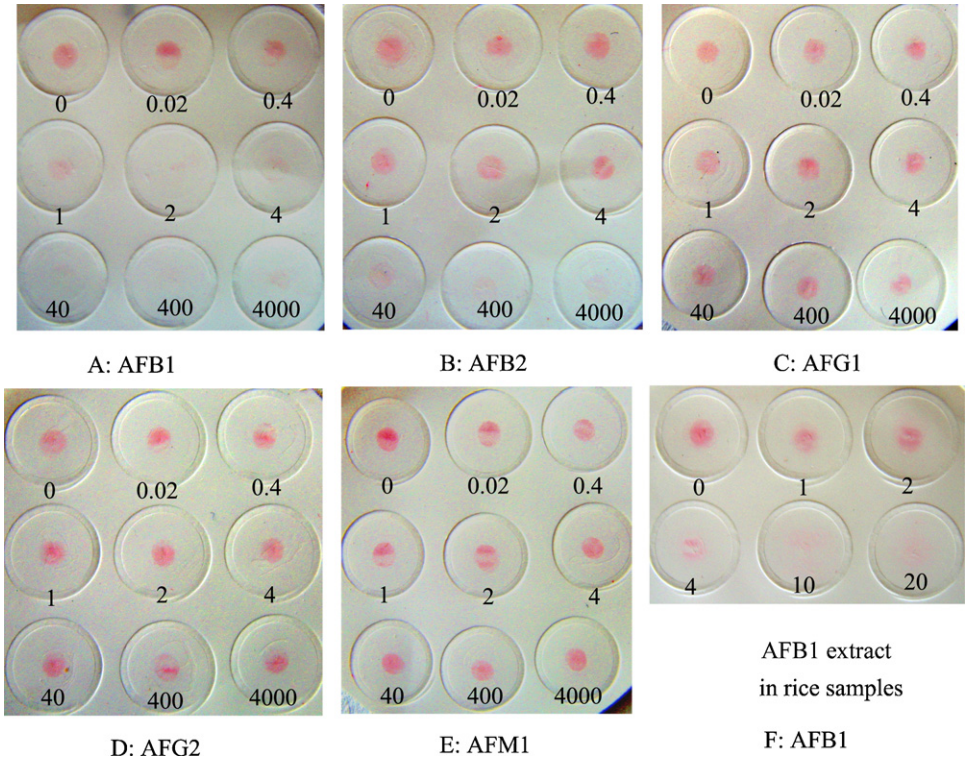

Fig. 5. Sensitivity, specificity and recovery assay of DIGFA method for detection of AFB_1. Concentration of antigens: 0–4000 ng/mL.

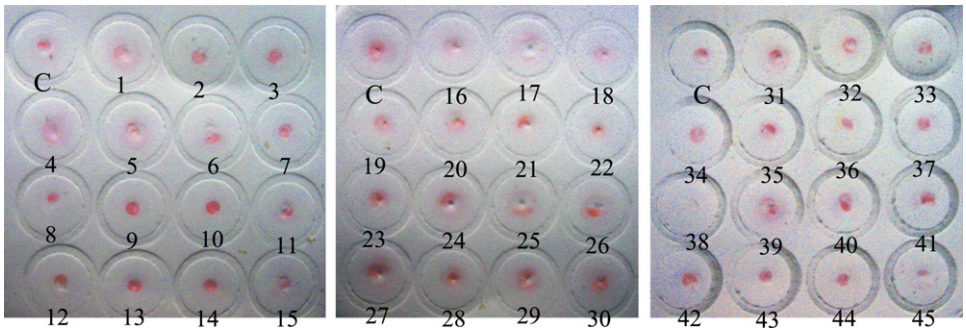

Fig. 6. DIGFA method detection results of 45 food samples. C: negative control; 21 rice samples: 1–21; 11 wheat flour samples: 22–32; 13 corn samples: 33–45.

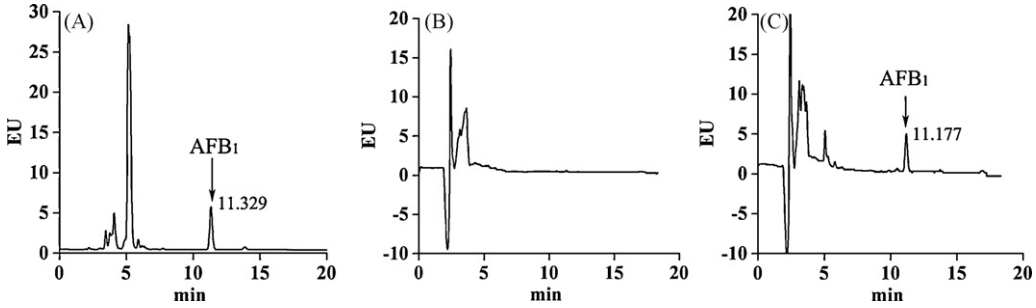

Fig. 7. HPLC analysis results of positive food sample. A: standard of AFB_1 (5ng/mL); B: negative sample; C: positive sample (No. 38). Column: Inertsil® ODS-3 (250 mm × 4.6 mm, 5 μm); mobile phase: water/aceonitrile/phosphonic acid (60/40/0.03); λ_{ex} = 360 nm; λ_{em} = 440 nm; flow rate: 0.8 mL/min; temperature: 30 °C.

4. Discussion

Nowadays, more and more on-site immunological techniques such as IC and IFA are gaining interest in the area of mycotoxin detection in foods and feeds [19]. We chose the IFA instead of IC in the present study for the following reasons: (i) manufacturing of the IC strip requires expensive equipment; (ii) the IFA kit is cheaper and easier to manufacture by hand when compared with IC kit.

DIGFA is a new technique of solid phase labeled immunoassay in which NCM is used as a support as well as colloidal gold is used as the label. The data (not shown) done in our laboratory showed that the performance of the DIGFA depends on external chemical properties of gold particles in addition to the affinity of the detection antibody. The intensity of signal as a response to an analyte concentration can be especially controlled by the characteristics of colloidal gold-McAb conjugates. Usually the strength of color demonstration is closely related to result judging, whereas the color of colloidal gold particle is related to the size of the colloidal gold particle, which is directly dependent on the amount of tri-sodium citrate used in its preparation process. When all other prepared conditions including procedures and reagents are kept stable, changes to the amount of tri-sodium citrate will result in different colloidal gold particles in diameter and in color. In this study, 2.0 mL of tri-sodium citrate (1.0%) was added into 50 mL (0.01%) of chloroauric acid solution. The formed colloidal gold particles were red with a diameter of around 12 nm. After the particles keeping it in steady state for two more months, the colloidal gold particles combined with McAb were found to be stable. The indicating color was obvious and easy to distinguish. Therefore, the prepared colloidal gold particle and colloidal gold- McAb conjugates in this study were suitable for DIGFA of AFB_1.

In the present study, the sensitivities of DIGFA and icELISA were similar although many studies have demonstrated that the sensitivity of flow-through assays was lower than those of conventional ELISA procedure [7]. This was probably due to the icELISA detecting only IgG molecules while the DIGFA test may also detect other Ig classes, e.g., IgA, IgE or IgM [39]. However, since the method is semi-quantitative, interpretation of results may be difficult when the mycotoxin concentration of the test sample is close to the method cut-off level.

In conclusion, DIGFA holds considerable promise for rapid and accurate detection of AFB_1 in the field, as it does not require any specific instruments and can be applied with ease.

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