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Development of a one-step test strip for rapid screening of fumonisins B1, B2 and B3 in maize

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ABSTRACT

Fumonisins (FBs), possible carcinogen to humans, are known to occur as a natural contaminant of corn worldwide. A monoclonal antibody (McAb) against FB1, which has high cross reactivity with FB2 and FB3 was produced and a nanoparticle-McAb probe was synthesized. Based on the probe, the one-step competitive immunochromatographic assay test strip for the rapid detection of total FBs (FB1, FB2 and FB3) was developed and applied to maize samples. The colour density of the test line is proportional to FBs mixture (FB1:FB2:FB3, 12:4:1) concentration in the range 2.5–40 ng mL^{-1}. The visual detection limit of FBs mixture spiked maize samples was found to be 2.5 ng mL^{-1}. The qualitative test based on the visual evaluation of results for FBs detection can be completed in 10 min. The performance of the assay is easy and convenient without the need of any instrumentation. The results demonstrated that the gold-McAb probe based strip could be used as a qualitative tool for rapid screening technique of FBs on-site.

1. Introduction

Fumonisins (FBs) are produced by *fusarium* species. They contaminate wheat, maize, maize-based foods and other grains worldwide (Shephard, Thiel, Stockenstrom, & Sydenham, 1996). Naturally contaminated maize grains contained FB1, FB2 and FB3 in a ratio of 12:4:1 (Weidenborner, 2001). FB1 is the most toxic and common naturally occurring FBs, which is possibly related to oesophageal cancer in humans consuming contaminated corns. It has been classified by the International Agency for Research on Cancer (IARC) as a possible carcinogen to humans (Wild & Gong, 2010). The maximum residue limit issued by US Food and Drug Administration (FDA) for total FBs in maize products for human foods is 2 mg kg^{-1} (FDA, 2001). While Codex Coordinating Committee for Latin America and the Caribbean (CCLAC) has proposed legislation that maximum level for FBs for unprocessed maize grain would be 5 mg kg^{-1} (FAO/WHO CCLAC, 2010).

Taking into account its toxicity, the detection of FBs in human foods is clearly important. Up to now, several analytical methods have been reported for FBs determination in food samples, including high performance liquid chromatography (HPLC) (Ghali, Ghorbel, & Hedilli, 2009; Lino, Silva, Pena, & Silveira, 2006; Ono, Kawamura, Ono, Ueno, & Hirooka, 2000; Sydenham, Shephard, Thiel, Bird, & Miller, 1996; Tardieu, Auby, Bluteau, Bailly, & Guerre, 2008), liquid chromatography with tandem mass detection (LC-MS-MS) (Silva, Pena, Lino, Fernandez, & Manes, 2010), fluorometric and chromatographic methods (Duncan, Kruger, Zabe, Kohn, & Prioli, 1998), TLC-Laser scanning densitometric (TLC-LSD) method (Karuna & Sashidhar, 1999), liquid chromatography/electrospray ionisation mass spectrometry (LC/ESI-MS) method (Vekiru et al., 2003), monoclonal antibody or polyclonal antibody based enzyme-linked immunosorbent assay (ELISA) (Abouzied, Askegard, Bird, & Miller, 1996; Bird et al., 2002; FAO/WHO CCLAC, 2010; Ghali et al., 2009; Quan, Zhang, Wang, Lee, & Kennedy, 2006; Sydenham et al., 1996; Yeung, Prelusky, Savard, Dang, & Robinson, 1996) and surface plasmon resonance biosensor based immunoassay (Mullett, Lai, & Yeung, 1998). However, these approaches require expensive equipment and skilled analysts. Gold nanoparticle-labelled antibody probe based immunochromatography assays (Shiu, Wang, & Yu, 2010; Sun, Zhao, Tang, Zhou, & Chu, 2005; Wang, Quan, Lee, & Kennedy, 2006) which do not require any equipment and skilled analysts, are suitable for rapid screening on-site for FBs. In this report, we present the

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synthesis of the gold nanoparticle-McAb probe and one-step test strip for the detection of FBs in maize samples.

2. Materials and methods

2.1. Materials and reagents

FB1, FB2 and FB3 were purchased from Sigma Aldrich (Milwaukee, USA). T-2 Toxin, Patulin, Deoxynivalenol, Citrinin and Zearalenone were supplied by Fermentek Ltd (Jerusalem, Israel). Chloroauric acid ($HAuCl_4$), trisodium citrate, glutaraldehyde and glycine were obtained from Shanghai Chemical Reagents (Shanghai, China). Other chemicals were of analytical grade and obtained from Beijing Chemical Reagent Co. (Beijing, China). The Nitrocellulose (NC) membranes were supplied by Sartorius (Göttingen, Germany). FBs purification columns TC-F120 were purchased from Beijing Rapidbio Co. Ltd (Beijing, China).

2.2. Preparation of FB1-protein conjugates

FB1-BSA conjugate (immunogen) was prepared as follows. First, 350 μL of PBS solution containing 500 μg BSA, 150 μL of PBS solution containing 300 μg FB1 and 1500 μL of glutaraldehyde were added into a tube. After stirring for 2 h at room temperature, 125 μL of aminoacetic acid (1 M, pH7.0) was added to the mixture and incubated for 30 min at room temperature. Then the conjugation was dialyzed against 1000 mL of 0.01 M PBS (pH 7.4) at 4 °C for 24 h with 4 changes of PBS to remove residual free FB1. FB1-OVA conjugate (coating antigen) was prepared using the same methods as that of FB1-BSA.

2.3. Production of monoclonal antibody (McAb)

McAbs were prepared according to the procedure described by Zhou et al. (2009). Briefly, male Balb/C mice (8-week old) were immunized by subcutaneous injection in a hind footpad with FB1-BSA (50 μg). The popliteal lymph nodes harvested from immunized mice were mixed with murine myeloma cells SP2/0 at a ratio of 5–10:1 in the presence of PEG. The fused cells were cultured at 37 °C in an atmosphere of 5% CO_2, and the hybridomas secreting anti-FB1 antibody were cloned three times by limiting dilution. The hybridoma (2×10^6 cells for each mice) were injected into abdomens of the 10-week-old Balb/C mice for 7 days after liquid olefin was injected. The ascites were obtained through the needle of a 20 mL injector about seven days later. The subtyping of the McAb was identified by an ELISA commercial kit "mouse monoclonal antibody isotyping reagents" (Sigma). The affinity of the McAb was measured according to the method of Zhou et al. (2010).

2.4. Preparation of gold nanoparticle-McAb probe

Colloidal gold nanoparticles (20 nm mean diameter) were synthesized according to the procedures described by Wang, Zhang, Wang, and Zhang (2005). Briefly, 100 mL of 0.01% $HAuCl_4$ was boiled thoroughly, and then 2.0 mL of freshly made 1% trisodium citrate solution was added rapidly under stirring. After the colour of the solution changed to wine-red, the mixture was boiled

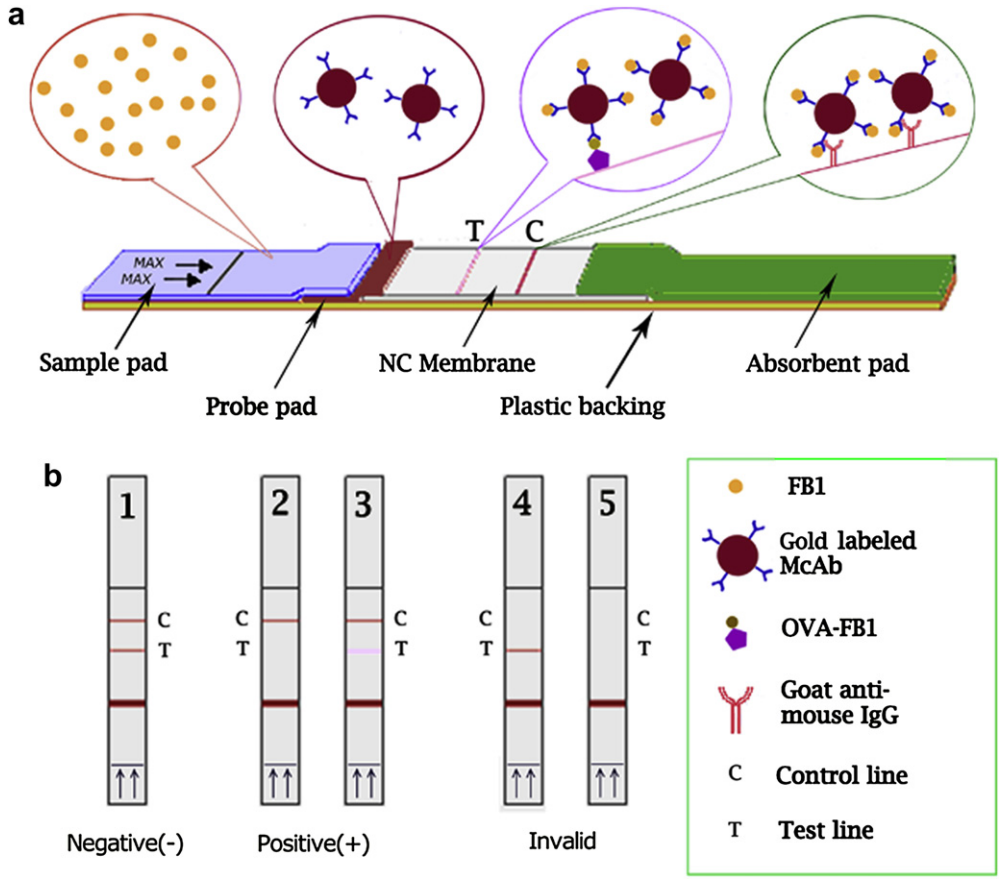

Fig. 1. (a) Schematic diagram of the immunochromatographic strip for FB1 detection. (b) Visual results assessment of the gold nanoparticle probe-based immunochromatographic strip. Details were described in text.