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MULTIPLEX CHEMILUMINESCENT BIOSENSOR FOR TYPE B-FUMONISINS AND AFLATOXIN B1 QUANTITATIVE DETECTION IN MAIZE FLOUR

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Materials and methods

Reagents

Strips were prepared from nitrocellulose membranes (Hi-flow plus 180 membrane cards from Millipore, Billerica, MA) employing a XYZ3050 platform (Biodot, Irvine, CA) as previously described [22, 23]. Briefly, from bottom to top of the strip, the FmB1-BSA conjugate (0.2 g L^{-1}), the AfB1-BSA conjugate (0.1 g L^{-1}) and the goat anti-rabbit antibody (0.5 g L^{-1}) were dispended to form the two test lines (T-lines) and the control line (C-line), respectively. Reagents were deposited at the density of 1 µL cm^{-1}, keeping a distance of 4 mm among the lines. The strips were dried at 37°C for 60 minutes under vacuum, then blocked with 1% BSA (w/v) in PBS buffer (5 min at room temperature), washed with PBS added of 0.05% of Tween 20 and finally dried at 37°C for 60 min under vacuum. The membrane was assembled with a fiber glass pad as the sample pad and a cellulose pad as the adsorbent pad (Millipore, Billerica, MA), then cut into sections (5 mm width) by means of a CM4000 guillotine (Biodot, Irvine, CA) and stored in a desiccator at room temperature.

Preparation of mycotoxin-BSA conjugates

The FmB1-BSA conjugate was synthesized according to Christensen et al. [H.R. Christensen, F.Y. Yu and F.S. Chu, J Agric Food Chem, 2000, 48, 1977-84], with slight modifications. Briefly, 6.5 mg of FmB1 dissolved in 3 mL of MES-NaCl buffer (MES 0.1M, NaCl 0.9M, pH 4.7) were mixed with 3 mg of BSA dissolved in 0.6 mL of water. 0.7 mL of a freshly prepared water solution of EDC (10 mg mL^{-1}) was added drop wise to the mixture and reacted 2h at room temperature. By-products were removed by gel-filtration on a Sephadex G-25 cartridge (GE Healthcare Bio-Science, Sweden, eluent buffer: PBS, pH

7.4) and the concentration of the FmB1-BSA conjugate was determined by UV absorption at 280 nm.

Results and discussion

Optimization of experimental parameters

The concentrations of primary antibodies yielding the highest detectability for FmB1 and AfB1 were evaluated. Blank samples, as well as samples containing 50 µg L^{-1} FmB1 (or 2 µg L^{-1} AfB1), were assayed in the presence of different dilutions of rabbit anti-fumonisin (or rabbit anti-aflatoxin) antibodies and a fixed amount of HRP-labeled goat anti-rabbit antibody. As shown in Figure 1S, the optimal concentration was selected to be 1:500 (v/v) for both anti-fumonisin and anti-aflatoxin antibody, providing the best compromise between high CL signal intensities and good ability of the analyte of interest to displace its specific antibody from the corresponding immobilized hapten.

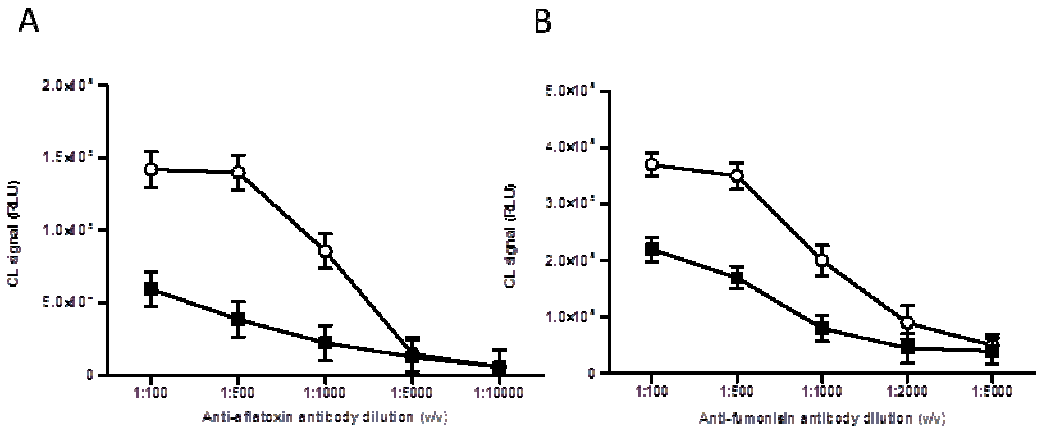

Fig. 1S. *(A, B) Chemiluminescence signals obtained in correspondence of the T-line employing different dilutions of rabbit anti-aflatoxin (a) and of rabbit anti-fumonisin (b) antibodies. Assays were performed in the absence of aflatoxins and fumonisins (white symbols) or in the presence of AfB1 2 µg L^{-1} and FmB1 50 µg L^{-1} (black symbols). The dilution of HRP-labeled goat anti-rabbit antibody was kept constant at 1:500 v/v.*

Second, the HRP-labelled anti-rabbit antibody concentration was optimized by assaying different dilutions of the antibody (ranging from 1:100 to 1:10,000 v/v) in the absence of analytes (maximum expected signal on T-lines) employing the optimized dilution (1:500 v/v) of anti-fumonisin and anti-aflatoxin antibodies. As shown in Figures 3, the highest signal/noise ratio for both T-lines and the C-line were obtained employing the HRP-labelled secondary antibody at 1:500 v/v dilution, while higher antibody amounts did not provide further improvements.

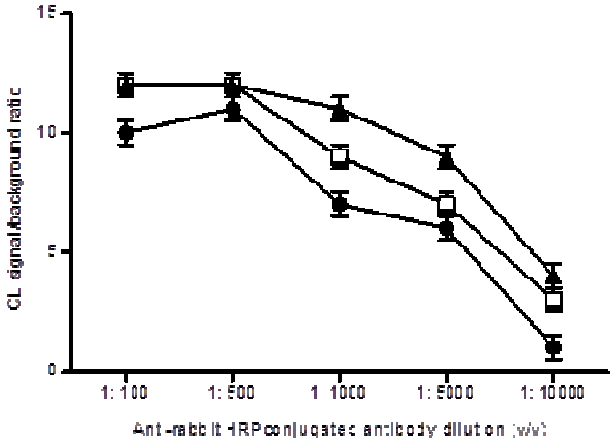

Fig. 2S. Selection of the optimal dilution of HRP-labelled anti-rabbit antibody. Data are reported as ratios between the CL signals of the T-lines of Aflatoxin (black symbols), Fumonisins (white symbols), C-line (grey symbols) and the background signals of the membrane.