Chemiluminescence-based biosensor for fumonisins quantitative detection in maize samples

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Supplementary information

MATERIALS AND METHODS

Reagents

Assay strips for LFIA were prepared as previously described (Anfossi et al., 2010). Briefly, they were prepared from nitrocellulose membranes (Hi-flow plus 180 membrane cards from Millipore, Billerica, MA) employing a XYZ3050 platform (Biodot, Irvine, CA). The FmB1-BSA conjugate (0.2 g L^{-1}) and the goat anti-rabbit antibody (1 g L^{-1}) were dispended in correspondence of the test line (T-line) and of the control line (C-line), respectively. Reagents were deposited at the density of 1 µL cm^{-1}, keeping a distance of 7 mm between the two lines. The strips were dried at 37°C for 60 minutes, 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 an adsorbent pad, which was necessary to promote complete liquids migration upon sample addition, then cut into sections (5 mm width) and stored in a desiccator at room temperature.

RESULTS AND DISCUSSIONS

Imaging resolution and CL signal detectability

To assess light detectability, model strips with anti-HRP antibody (200 mg L^{-1} in PBS) immobilized in one line were prepared employing the procedure described in the Materials and methods section. After saturation with BSA, 100 µL of HRP solutions at concentrations ranging from 4.0 to 2.5 × 10^4 ng L^{-1} was let flow through the membrane. Captured HRP was measured by acquiring images of the strips using both contact CCD imaging and the LB-981 luminograph (10 s acquisition time), upon addition of 80 µL of the CL substrate and 4-min substrate flow. As expected, changes in the lines CL intensity were linearly proportional to the concentration of HRP in the sample (Figure 1S) and a limit of detection (calculated as the HRP concentration corresponding to the signal of the blank plus three

times its standard deviation) of 35 ng L^{-1} (corresponding to approximately 0.1 fmol of HRP in the sample solution) was achieved.

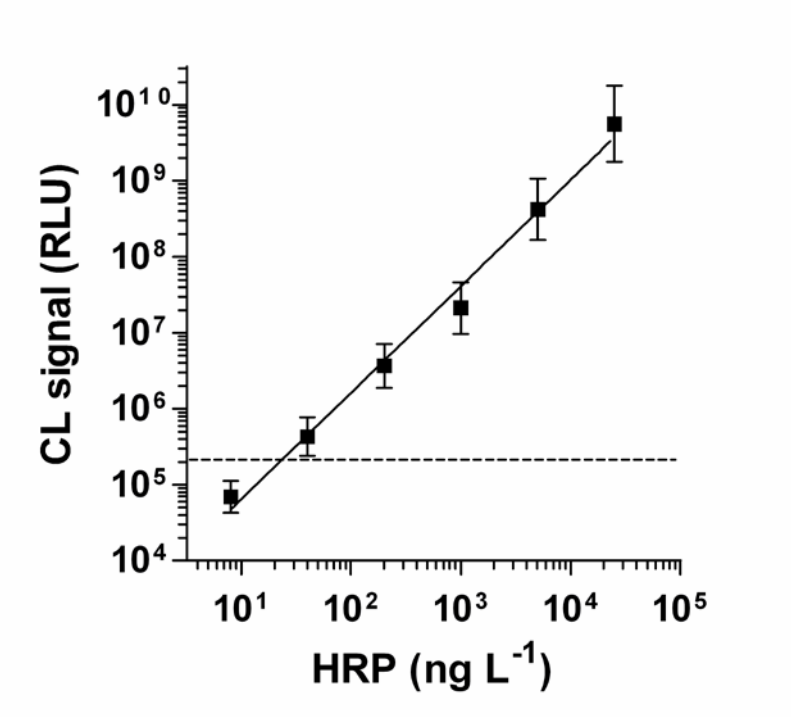

Figure 1S*. Calibration curve obtained by analyzing HRP standard solutions using LFIA membranes with immobilized anti-HRP antibody as a model. Photons emission was acquired using contact CCD imaging upon addition of the CL substrate. The dotted line indicates the blank signal plus three standard deviations.*

Optimization of experimental parameters

The main problem in the use of the LFIA format is the non-specific interaction of the immunoreagents with the solid support, which must be avoided by using appropriate saturation procedures. Preliminary experiments were performed by employing different saturation agents (BSA, non fat dry milk, soybean milk). The best performance was obtained by blocking the strips by incubation for 5 min at room temperature with 1% BSA

in PBS buffer, as previously described for the colloidal gold-based LFIA (Anfossi et al., 2010) (data not shown).

To optimize the experimental procedure, the concentrations of antibodies yielding the highest detectability for FmB1 were evaluated. Blank samples and samples containing a fixed concentration of FmB1 (50 μg L^{-1}) were therefore assayed in the presence of different dilutions of rabbit anti-fumonisin antibody and HRP-labeled goat anti-rabbit antibody. As expected, it was observed that a decrease in the amount of rabbit anti-fumonisin antibody employed in the assay in the range between 1:500 and 1:2000 v/v caused a decrease in the CL emission of the T-line, as well as an increase in the ability of FmB1 present in the sample to displace the anti-fumonisin antibody from the immobilized FmB1-BSA conjugate (evidenced by a decrease of the ratio of T-line intensities obtained in the presence and in the absence of FmB1, respectively). The optimal concentration of the rabbit anti-fumonisin antibody (1:500 v/v) was selected as the one providing the best compromise between the two effects described above (Figures 2S.A and 2S.B).

The HRP-labelled anti-rabbit antibody must be added in excess to completely reveal the anti-fumonisin antibody captured on the T-line. However, a large excess of the labelled antibody could determine stronger non-specific CL signals and increases reagents consumption. Different dilutions of the HRP-labelled anti-rabbit antibody, ranging from 1:5,000 to 1:20,000 v/v, were assayed in the absence of FmB1 employing a fixed dilution of rabbit anti-fumonisin antibody (1:500, v/v). As shown in Figures 2S.C and 2S.D, the highest signals for both T-line and C-line were obtained at 1:10,000 v/v dilution, while higher antibody amounts did not provide further improvements. The HRP-labelled anti-rabbit antibody 1:10,000 v/v dilution was selected as the optimal one.

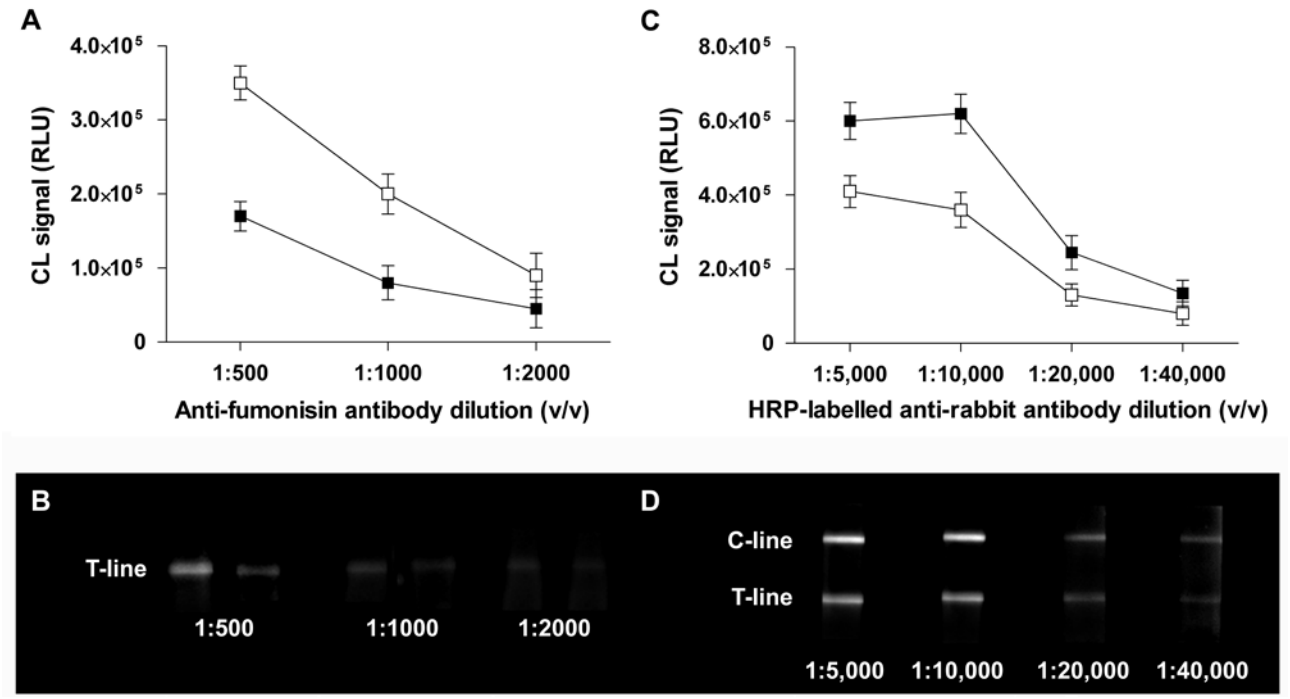

Figure 2S. *(A, B) Chemiluminescence signals obtained in correspondence of the T-line employing different dilutions of rabbit anti-fumonisin antibody. Assays were performed in the absence of fumonisins (white symbols) or in the presence of FmB1 50 ng mL^{-1} (black symbols). The dilution of HRP-labeled goat anti-rabbit antibody was kept constant at 1:10,000 v/v. (C, D) Chemiluminescence signals obtained in correspondence of the C-line (black symbols) and T-line (white symbols) employing different dilutions of HRP-labeled goat anti-rabbit antibody. Assays were performed in the absence of fumonisins and the dilution of rabbit anti-fumonisin antibody was kept constant at 1:500 v/v. Data represent the mean of three assays.*