Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Chemiluminescence lateral flow immunoassay cartridge with integrated amorphous silicon photosensors array for human serum albumin detection in urine samples

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Results and discussion

Optimization of experimental parameters

The concentrations of primary antibody yielding the highest detectability for HSA in the relevant clinical range was evaluated. Blank samples, as well as samples containing 100 mg L^{-1} and 200 mg L^{-1} HSA, were assayed in the presence of different dilutions of rabbit anti-HSA antibody and a fixed amount of HRP-labeled goat anti-rabbit antibody.

It was observed that a decrease in the concentration of the primary antibody employed in the assay caused a decrease in the CL emission of the T-line. This was associated to an increase in the ability of the analyte to displace the primary antibody from the immobilized HSA. An higher dilution of the primary antibody results in a significant decrease in the CL signal for the concentration of 200 mg L^{-1} HSA, shortening the dynamic range and making not possible to quantify higher HSA levels (Fig.1). The dilution that provides the best compromise between these effects was 1:2000 v/v.

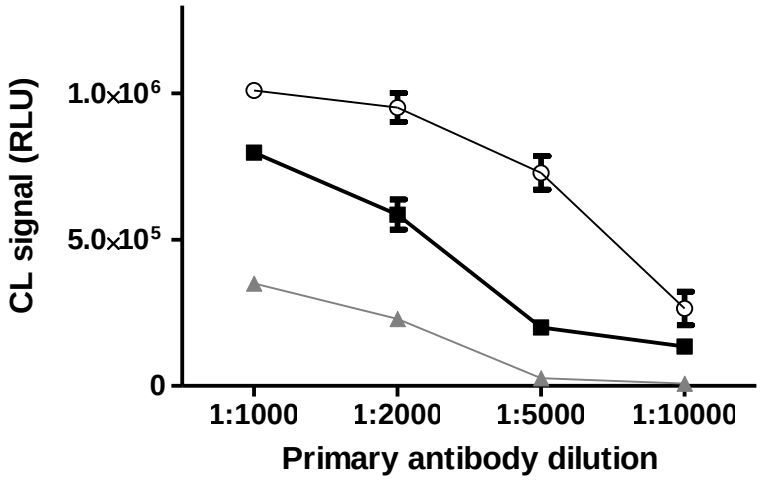

Fig. S1 Chemiluminescence signals obtained employing different dilutions of primary antibody Assays were performed in the absence of HSA (white symbols) or in the presence of HSA 100 mg L^{-1}(black symbols) and 200 mg L^{-1} (grey symbols)

Second, the HRP-labelled anti-rabbit antibody concentration was optimized by assaying different dilutions of the antibody (ranging from 1:1000 to 1:50,000 v/v) in the absence of analytes (maximum expected signal on T-lines) employing the optimized dilution (1:2000 v/v) of anti-HSA. As shown in Figures 2, the best compromise was obtained employing the HRP-labelled secondary antibody at 1:5000 v/v dilution, while higher antibody amounts did not provide further improvements.

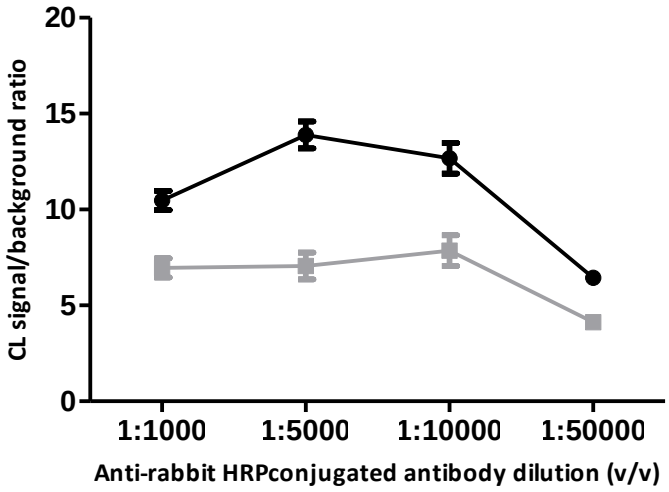

Fig. S2 Selection of the optimal dilution of HRP-labelled anti-rabbit antibody. Data are reported as ratios between the CL signals of the T-lines of HSA (black symbols), C-line (grey symbols) and the background signals of the membrane

Signal detection

Since photosensors array had never been used coupled with CL-LFIA, light measurements were also performed by imaging with a portable, thermoelectrically-cooled CCD camera. The results showed that the array of photosensors allowed to measure CL intensity of each interest area without significant crosstalk and that its performance was similar to that obtained using the CCD camera. In Figure 3S CL signals obtained in correspondence of the T-line and C-line of various LFIA strips using photosensors are plotted against the same signals acquired with CCD camera in a “contact” imaging configuration. There is a clear correspondence between the signals measured by the two different platforms,

showing that, as the CCD camera, even photosensors are suitable for the coupling with the CL-LFIA technique.

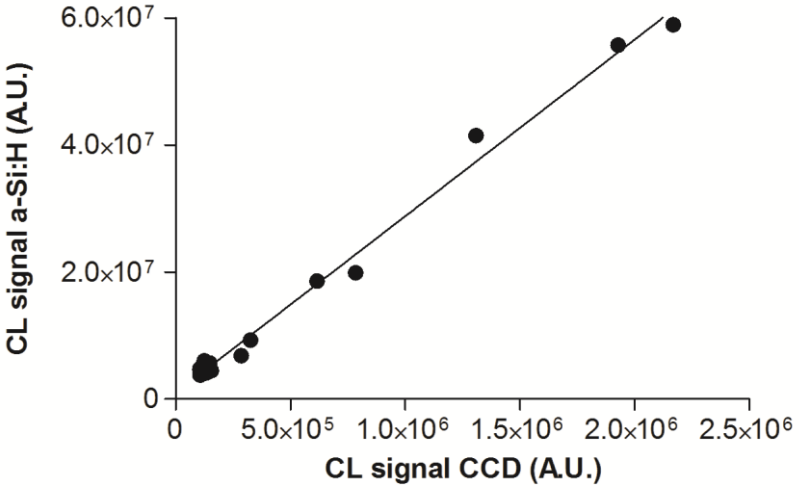

Fig. S3 Chemiluminescence signals obtained in correspondence of the T-line and C-line of various LFIA strips. The signals were simultaneously measured employed the integrated a-Si:H photosensors and the reference CCD in a “contact” imaging configuration.
A.U.: arbitrary units