Supplementary material

A SIMPLE AND COMPACT SMARTPHONE ACCESSORY FOR QUANTITATIVE CHEMILUMINESCENCE-BASED LATERAL FLOW IMMUNOASSAY FOR SALIVARY CORTISOL DETECTION

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Materials and methods

Preparation of immunochromatographic test strip

Assay strips were prepared from nitrocellulose membranes (Hi-flow plus 180 membrane cards from Merck Millipore, Billerica, MA) employing a XYZ3050 platform (Biodot, Irvine, CA), equipped with two BioJet Quanti™ 3000 Line Dispenser for non-contact dispensing. In particular, from bottom to top of the strip, the anti-cortisol antibody (1:50 v/v) and the anti-HRP antibody (1:500 v/v) diluted in PBS (20 mM phosphate buffer, pH 7.4), were dispensed to form the T-line and the C-line, respectively. Reagents were deposited at 1µL cm-1, keeping a distance of 5 mm among the lines. Membranes were dried at 37°C for 60 minutes under vacuum, then the nitrocellulose surface was saturated with 1% BSA (w/v in

PBS), washed with PBS supplemented with 0.05% of Tween 20, and finally dried at 37°C for 120 min under vacuum. Membranes were assembled with a glass fiber pad as the sample pad (Whatman Standard 14® from GE Healthcare Europe, Milan, Italy) and a cellulose pad as the adsorbent pad (Merck Millipore, Billerica, MA), with 1-2 mm of overlap between one and the other. Assembled membranes were cut into strips (5 mm width) by means of a CM4000 guillotine (Biodot, Irvine, CA) and stored in a desiccator at room temperature.

Results and discussion

Concentration of immunoreagent

To optimize the immunoassay, the concentration of HRP-cortisol conjugate yielding the highest detectability and a suitable sensitivity for salivary cortisol was evaluated. Blank samples and samples containing 1 and 10 ng/ml were assayed in presence of different dilutions of HRP-cortisol conjugate. It was observed that a decrease in the concentration of the HRP-cortisol conjugate employed in the assay in the range between 1:50 and 1:1000 (v/v) caused a decrease in the CL emission of the T-line. This was associated to an increase in the ability of cortisol to displace the HRP-cortisol conjugate from the immobilized anti-cortisol antibody, as shown by a decrease in the ratio T-line/C-line obtained in the presence and in the absence of cortisol in the sample. An higher dilution of the HRP-cortisol conjugate results in a significant decrease in the CL signal for the concentration of 10 ng/mL cortisol, shortening the dynamic range and making not possible to quantify higher cortisol levels (Figure 1S). The dilution that provides the best compromise between these effects was 1:100 v/v.

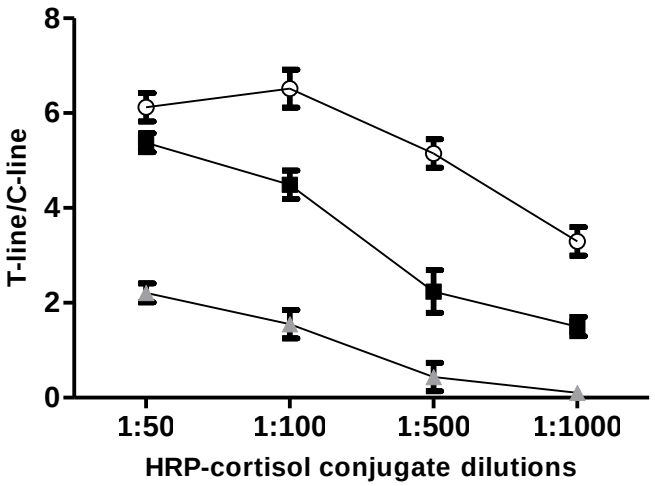

Figure 1S *Chemiluminescence signals obtained employing different dilutions of HRP-cortisol conjugate. Assays were performed in the absence of cortisol (white symbols) or in the presence of cortisol 1 ng/mL (black symbols) and 10 ng/mL (grey symbols).*

Chemiluminescent signal kinetics

To optimize the acquisition time we measured the CL signal obtained from LFIA membranes using different integration times (from 2 to 20s). It was observed that an integration time of 10s is sufficient to achieve the maximum CL signal. In Figure 2S the kinetics of the CL signals obtained in correspondence of the T-lines in presence of different concentrations of cortisol are reported. The steady state emission is achieved in a short time and it lasts 1min.

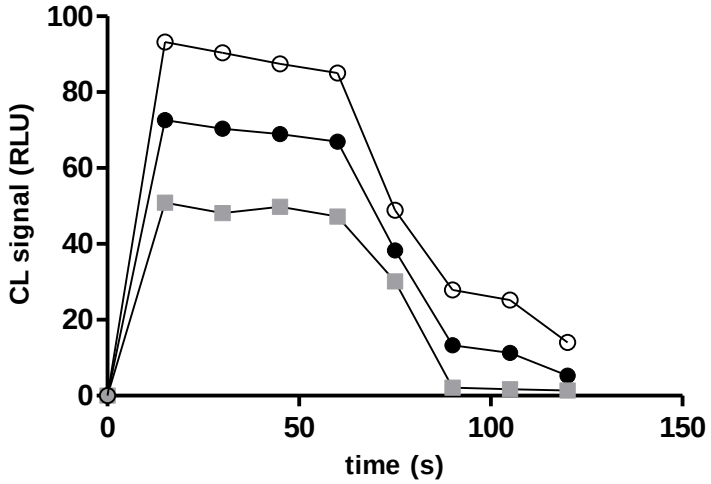

Figure 2S *Kinetics of the chemiluminescence signals obtained in correspondence of the T-line obtained in the absence of cortisol (white symbols) and in the presence of cortisol 1 ng/mL (black symbols) and 10 ng/mL (grey symbols).*