Ultrasensitive lateralflow immunoassay with chemiluminescence detection: new miniaturized and smartphone - based devices

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Point-of-care testing (POCT)

Developing an accurate and user-friendly diagnostic device for “point-of-care” (POC) applications is one of the most challenging objectives in the analytical field.
Devices for POC analysis should be portable, quick, easy to use should be able to perform the entire analytical process, from sample pre-treatment to measurement and data processing.

Advantages

- ✓ Eliminates transport issues
- ✓ Eliminates some biohazards
- ✓ Provides immediate results

Applications

Medical diagnostics

Food safety

Environmental pollution

Forensic science

Lateral Flow Immunoassay

Immunological methods are suitable for this because their high specificity and sensitivity makes it possible to detect analytes even at low concentrations and in complex matrices. Within this context, Lateral Flow Immunoassay (LFIA) represent an interesting format for a rapid and on site detection, alternative to conventional immunoassay tecnquiques.

Pros

LFIA techniques combine:

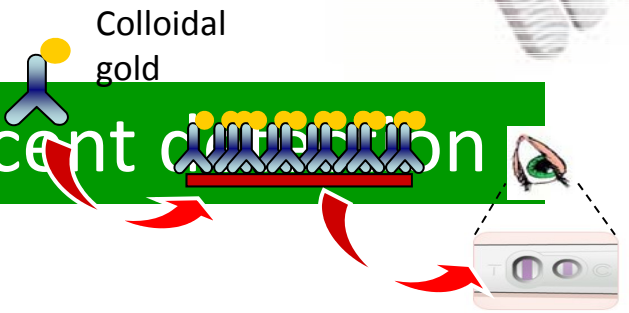
- ✓ Specificity of the antigen-antibody reaction
- ✓ Portable analytical device can be developed: On-site analyses

Cons

Conventional LFIAs are based on visually detection:

- ✗ Semiquantitative detection
- ✗ Relatively high detection limit

Alternative: Chemiluminescent detection

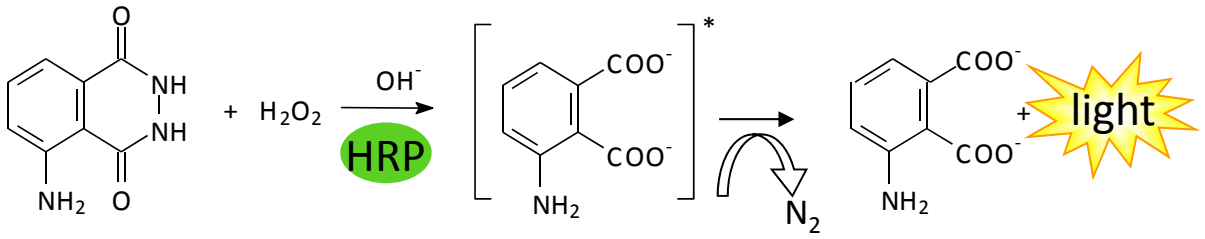


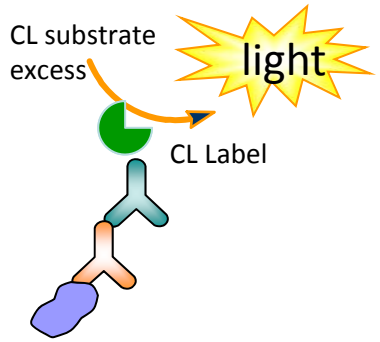
→ Not suitable for quantitative detection

Chemiluminescent label

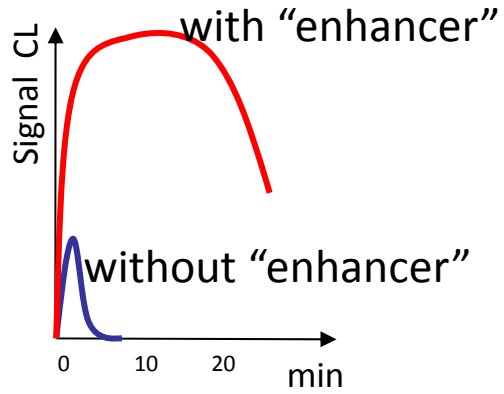
The amplification of the analytical signal is due to the presence of an excess of substrate, producing 10^4-10^5 chemiluminescent molecules.

Horseradish Peroxidase (HRP)/ luminol/ H_2O_2





A significant increase in light output was observed by the addition of nucleophilic acylation catalyst to the enhancer/luminol/oxidant substrate.

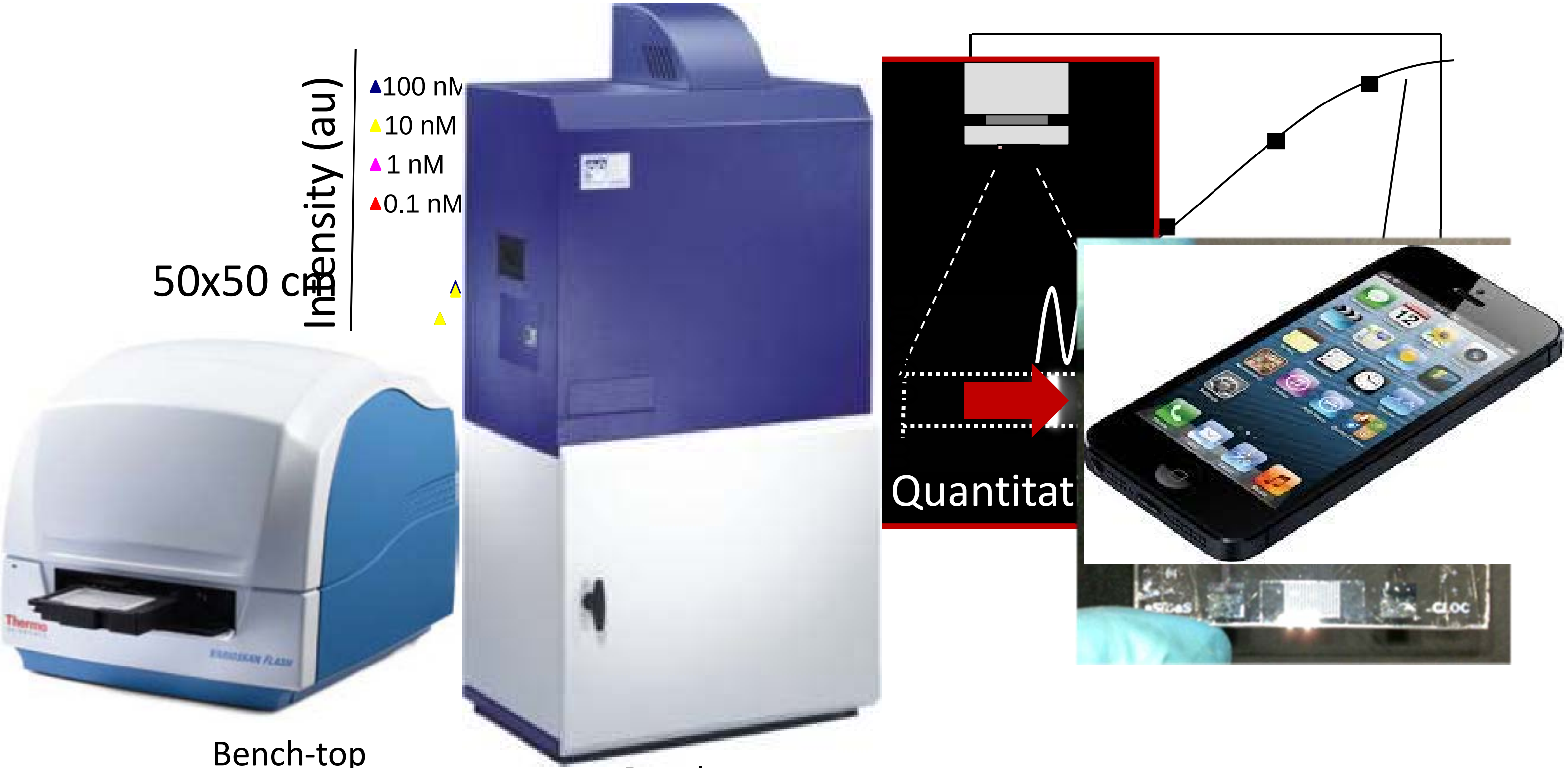


E.Marzocchi, S. Grilli, L. Della Ciana, L. Prodi, M. Mirasoli, A. Roda
Analytical Biochemistry 377 (2008) 189–194

Why chemiluminescence?

Chemiluminescence is particular suited for the development of miniaturized ultrasensitive analytical devices:

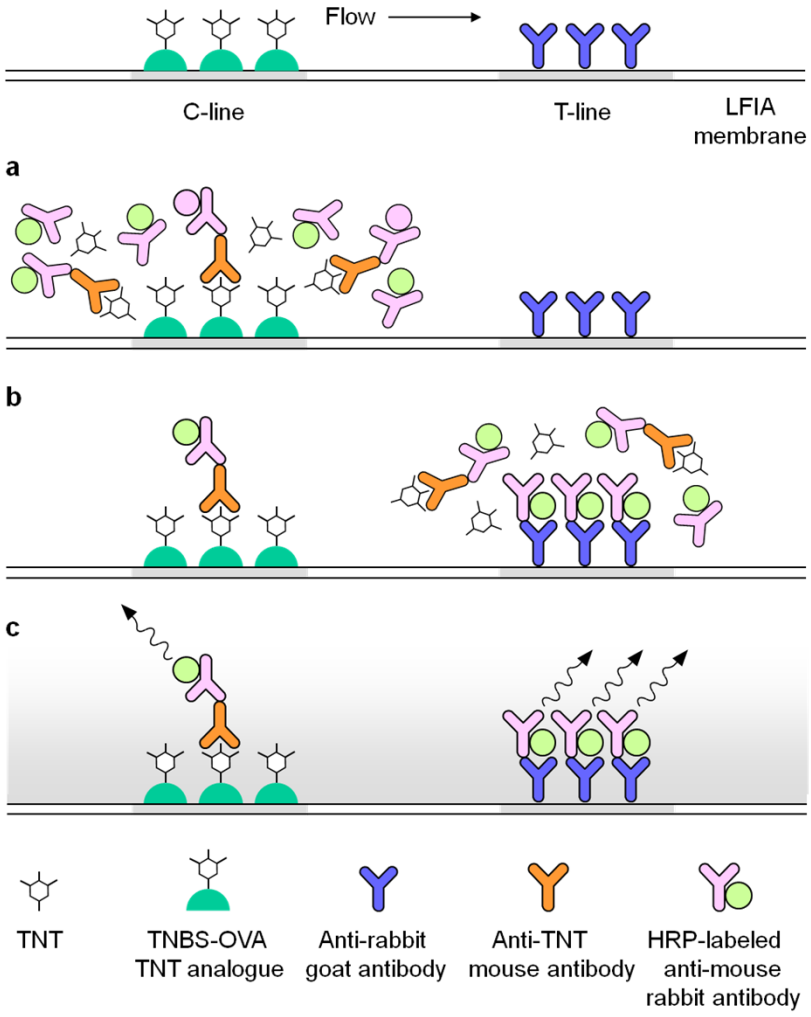
- ✓ [illegible] sample geometry problems

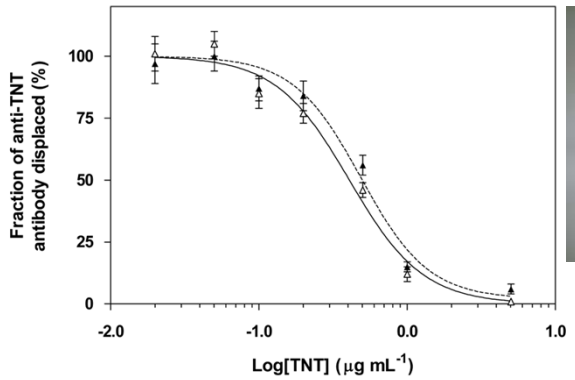


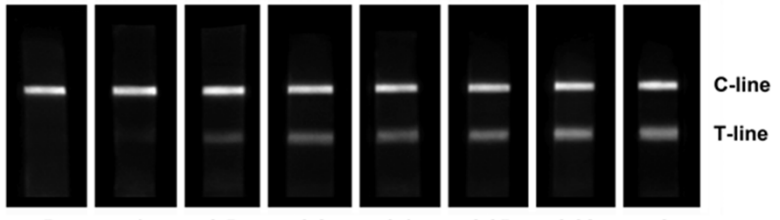
Bench-top luminometer

Bench-top luminograph

Chemiluminescence Lateral Flow ImmunoAssay: state of art





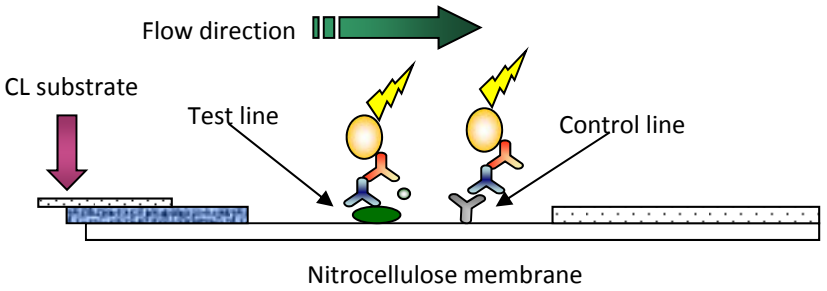


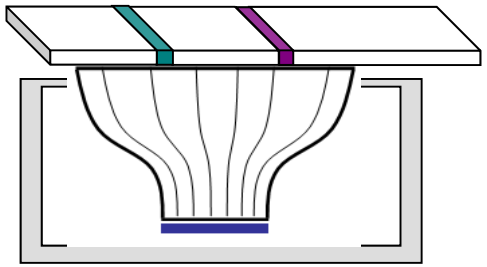
M. Mirasoli, A. Buragina, L.S. Dolci, M. Guardigli, P. Simoni, A. Montoya, E. Maiolini, S. Girotti, A. Roda , Analytica Chimica Acta 721 (2012) 167– 172

Chemiluminescence Lateral Flow ImmunoAssay: state of art

We recently developed a compact and portable biosensor based on Chemiluminescent Lateral Flow ImmunoAssay for simple, rapid, and ultrasensitive on-site quantification of type-B fumonisins in maize samples. The biosensor integrates:

- a competitive immunoassay based on enzyme-catalyzed chemiluminescence detection
- a higly sensitive portable charge-coupled device (CCD) camera employed in contact imaging configuration



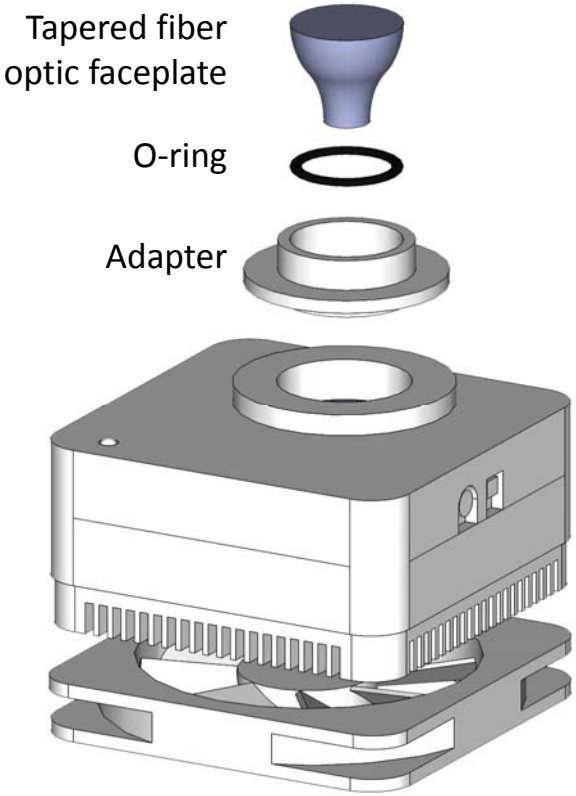


Contact imaging of LFIA membrane

Mirasoli M, Buragina A, Dolci LS, Simoni P, Anfossi L, Giraudi G, Roda A. Biosens Bioelectron. 2012 Feb 15;32(1):283-7

CCD camera for contact CL imaging

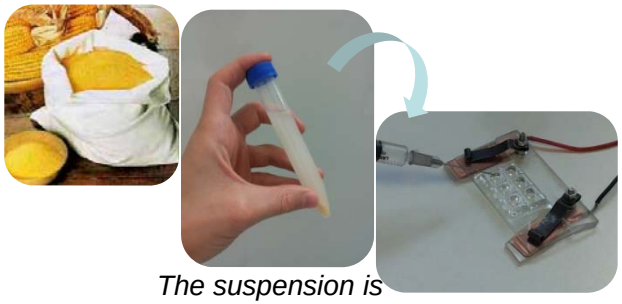
To produce a compact and portable biosensor, the CL signal measurement was performed by contact imaging employing a compact light detection device equipped with an ultrasensitive cooled CCD sensor



Chemiluminescence Lateral Flow ImmunoAssay: state of art

Recently we developed a portable ultrasensitive biosensor for a multiplex CL-LFIA, in which two competitive immunoassays are simultaneously performed on the same strip for detecting type-B fumonisins and B1 aflatoxin.

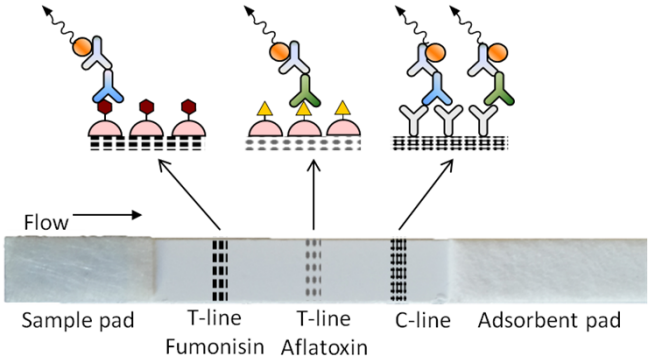
Extraction procedure



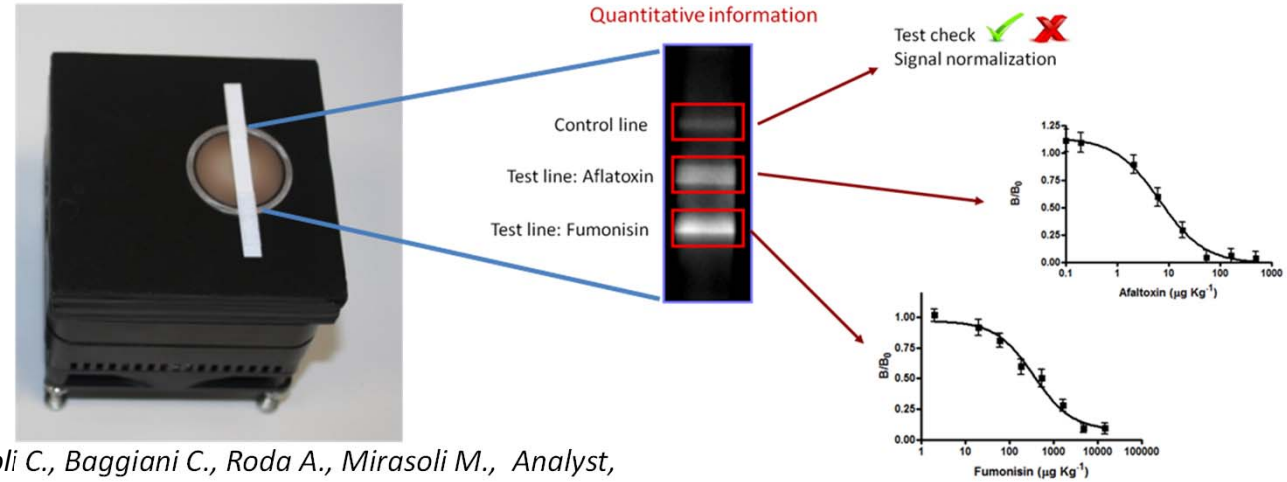
The suspension is hand-shaked…

…and heated at 100°.

Analytical procedure



Chemiluminescent detection



Zangheri M, Di Nardo F., Anfossi L., Giovannoli C., Baggiani C., Roda A., Mirasoli M., Analyst, DOI: 10.1039/c4an01613k

An alternative detector: smartphone’s camera

To date the measurements required the use of a portable CCD camera for the acquisition of the CL signal. The CCD camera must be connected to a personal computer for the storage and the elaboration of the images.

This system composed by CCD and PC can be replaced by a smartphone: the sensitivity of the back-side illuminated CMOS (BSI-CMOS) used in smartphone camera mean it can be used to detect chemiluminescence. Moreover specific applications can be used for data acquisition and signal processing.

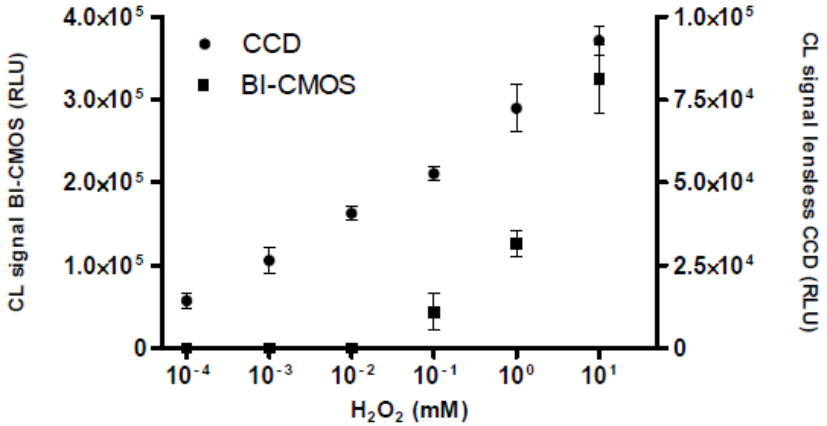
- ✓ Large diffusion
- ✓ Ease of use
- ✓ Connectivity

→ *These characteristics can be exploited to perform analysis at the point of need with simple procedures*

This would decrease costs and increase healthcare availability and accessibility.

Thermoelectrically cooled CCD vs BI-CMOS smartphone sensor

New generation smartphones use BI-CMOS photodiodes as light sensors to increase light collection with reduced size. Compared to cooled CCD camera, BI-CMOS is less sensitive but still adequate to measure the photons produced by BL and CL reactions.



VS

Resolution: smartphone's camera shows better performance thanks to the inclusion of a planoconvex lens to focus the image. This could be particularly useful to implement multiplexed assays into smartphone-based devices.

BSI-CMOS could offer adequate analytical performance with the advantage of being integrated in a smartphone

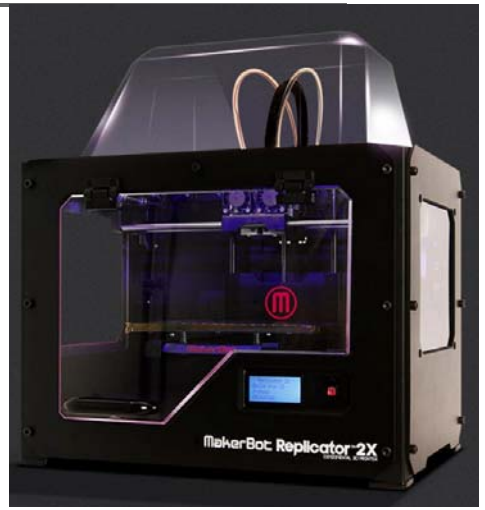
Detectability: the cooled CCD is able to quantify a concentration of H_2O_2 three decades lower but the BI-CMOS detector is suitable for detecting analytes present in biological fluids at micromolar levels, as the majority of common biomarkers of clinical interest.

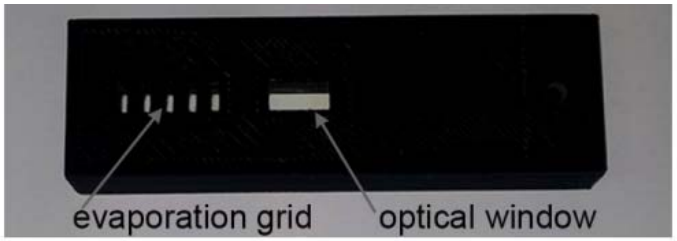
Roda A., Michelini E., Cevenini L., Calabria D., Calabretta MM., Simoni P., 2014, Anal Chem, 86, 7299-7304

Accessories for a portable analytical device

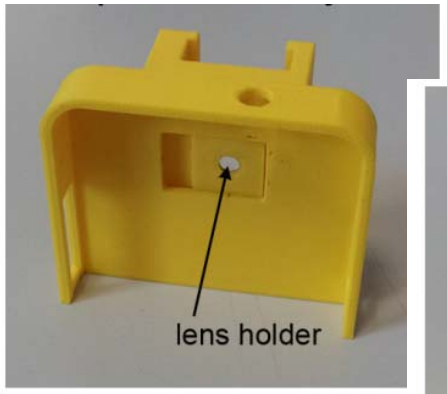
We developed a portable analytical device that transforms a smartphone into a chemiluminescence detector for quantitative LFIA analysis.

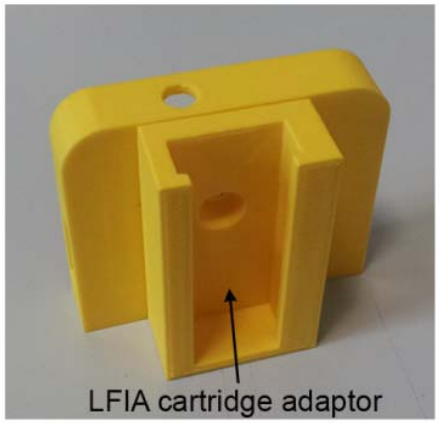
The device comprises a smartphone equipped with custom-designed accessories made using a low-cost desktop 3D printer:





- A cartridge hosting the LFIA membrane



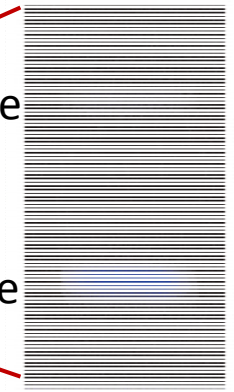


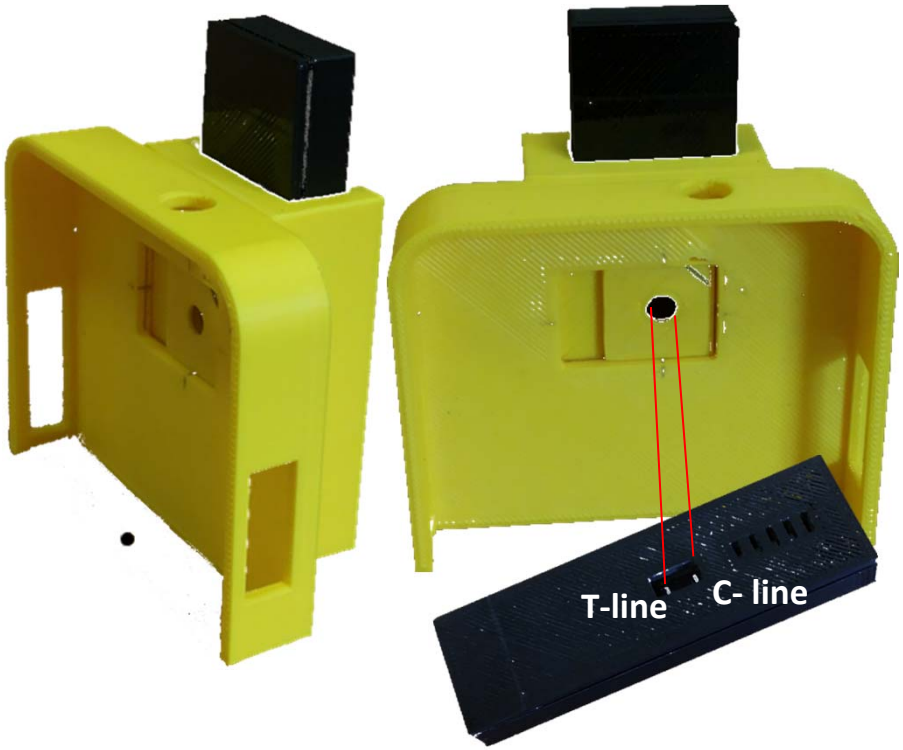
- *A smartphone adaptor, containing a plano-convex lens aligned with the camera and a slot for inserting the cartridge*

Zangheri M., Cevenini L., Anfossi L., Baggiani C., Simoni P., Di Nardo F., Roda A., Biosensors and Bioelectronics 64(2015)63–68

CL smartphone's camera detection

Once the operator has carried out the assay on the LFIA strip, both the smartphone and the cartridge are inserted into the assembled cradle creating a mini-darkbox to perform the measurement of the CL signal.

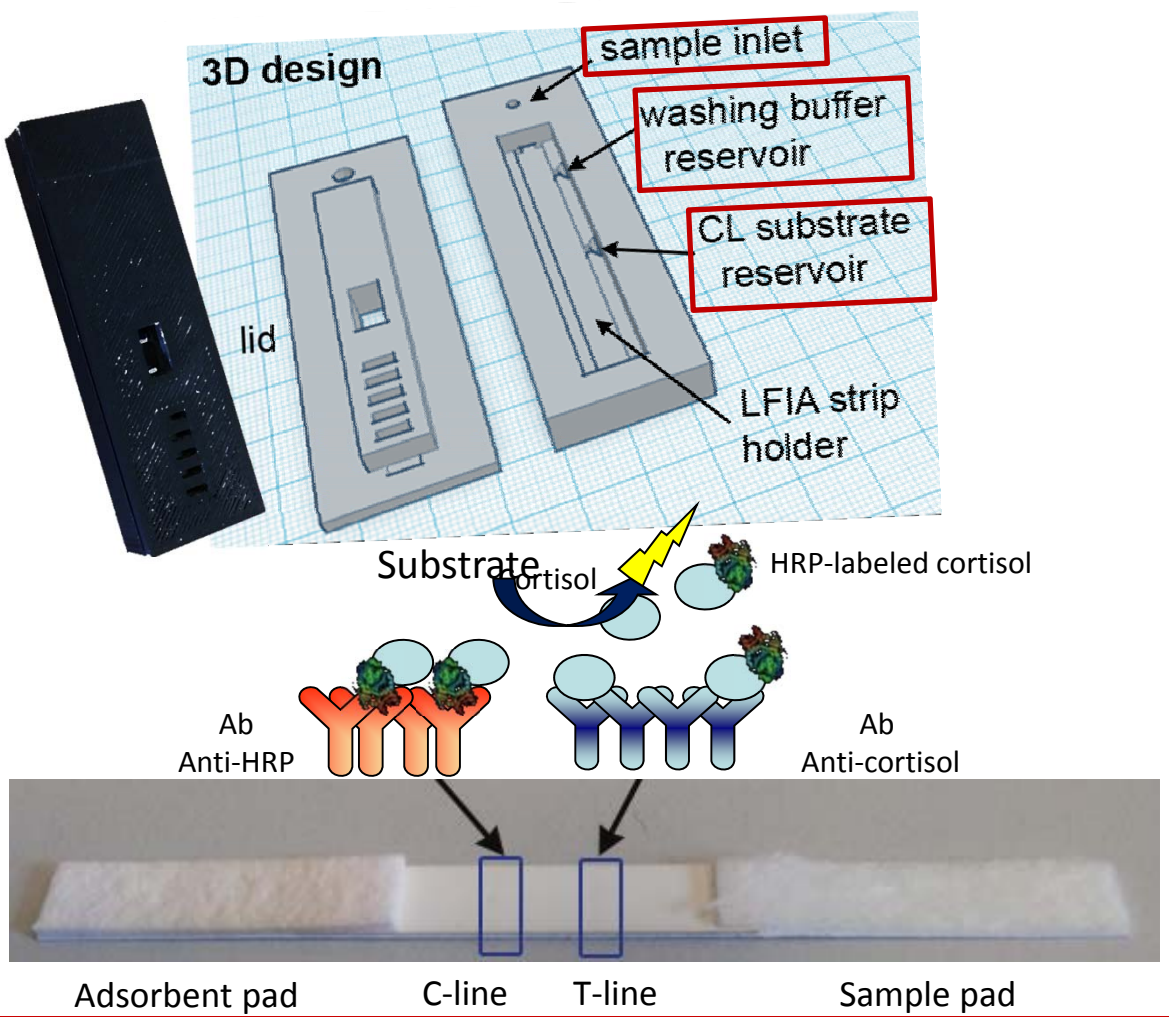




A built-in smartphone photography application and the camera's autofocus system were used to obtain an optimized image of the sensing surface.

Procedure and mechanism

We demonstrated the performance of the system by quantitative detection of salivary cortisol.



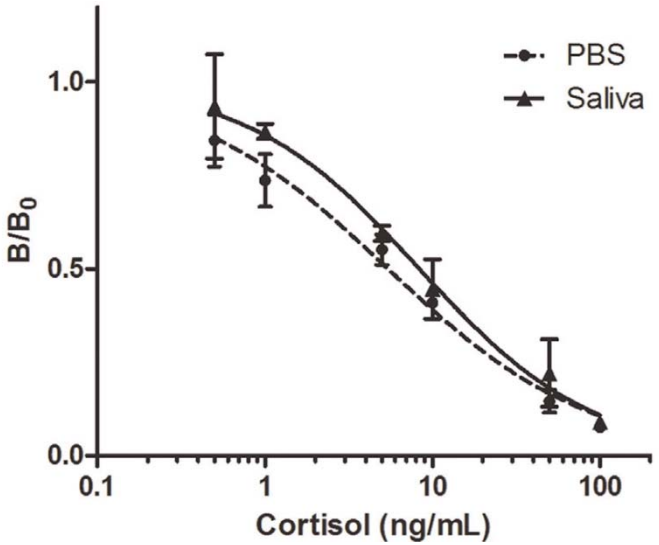
1) Saliva sample is transferred into a prefilled well positioned near to the sample pad and mixed up with the prefilled solution of HRP-cortisol conjugate. The solution flow across the membrane.

2) 50 μL of PBS buffer are squeezed from the washing buffer reservoir .

3) Strip is wetted by 100 μL of CL substrate positioned in the CL substrate reservoir. The cartdridge is inserted into the slot on the smartphone's cover and the CL signal is imaged.

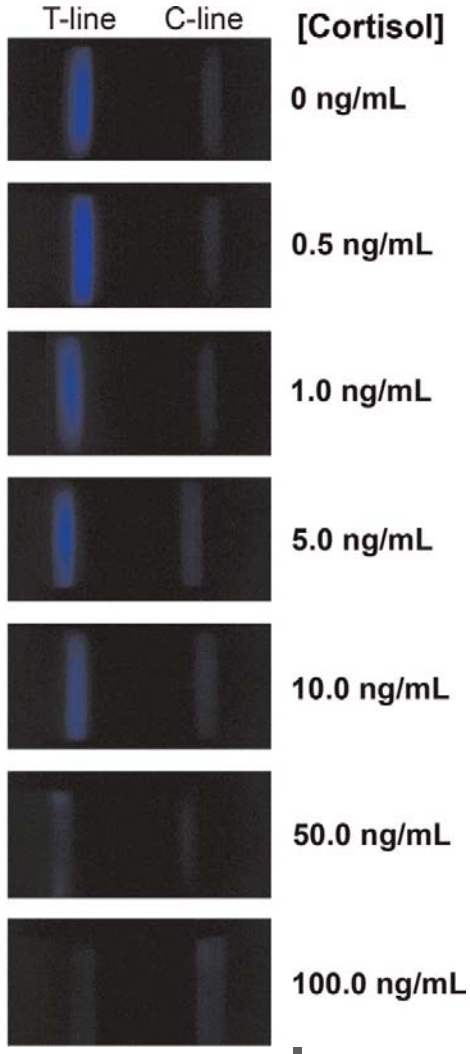
Calibration curve

Calibration curves were generated using cortisol standard solutions in the range of 0.5 - 100 ng/mL. Two calibration curve were constructed by adding known amounts of cortisol standard solutions to saliva cortisol free and to PBS to evaluate the matrix effect.



Dynamic range in saliva: 0.3-60 ng mL^{-1}

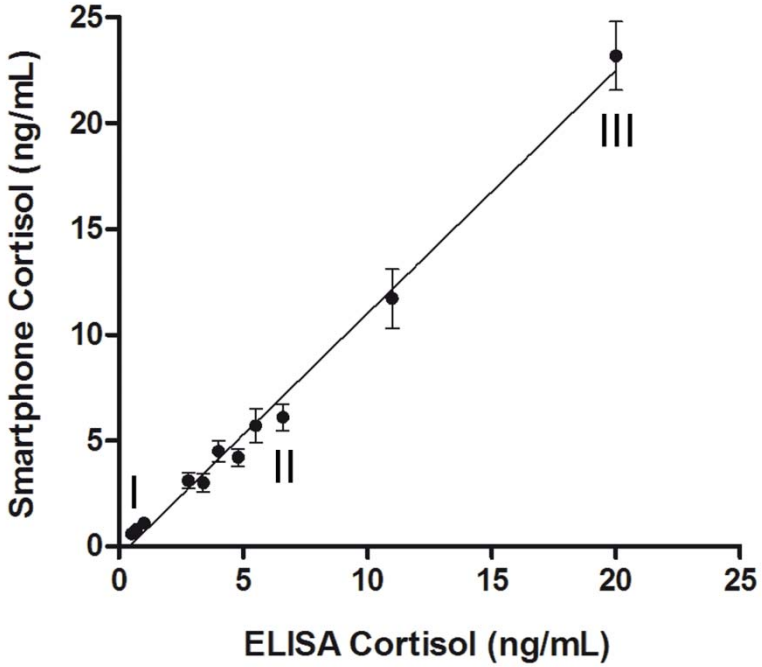
Dynamic range in PBS: 0.1-60 ng mL^{-1}

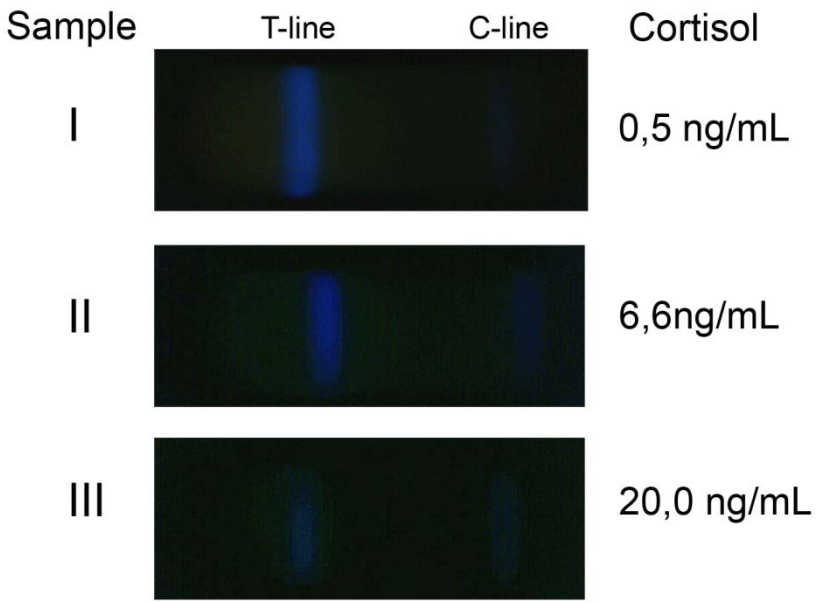


Suitable for evaluating cortisol concentrations in human saliva in both normal and pathological conditions.

Real samples

Saliva samples belonging to 11 subjects were analysed founding a good agreement between CL- LFIA and commercial ELISA kit results for all samples (Recovery values were in the range from 88% to 116%).





Conclusion

The developed assays based on Chemiluminescent-LateralFlow Immunoassay technique using different detecting platform allows to combine:

✓**Sensitivity:** chemiluminescece allows high detectability

✓**Rapidity:** the developed assay can be performed in only 30 minutes

✓ **Simplicity:** these methods could be performed without specialized personnel, allowing point-of-care analysis with reductions in cost and response time.

...Next steps...

➢The use of the 3D printing technology will allow to further improve these devices and to design different analytical formats even based on multiplex capability.

➢The concept thus paves the way for a new generation of analytical devices in the clinical diagnostic field thanks to the ideal combination of sensibility a simplicity of the CL with the day-by-day increase in the performance of the new generation smartphone camera.

Acknowledgements

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