Chimica & Ricerca

CHEMILUMINESCENCE MULTIPLEX LATERAL FLOW IMMUNOASSAYS*

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LFIA is a technology currently widely applied out of laboratory environments. Enzymes can be used as tracers in LFIA, coupled with chemiluminescence detection, to obtain quantitative information, even in multiplex assays.

Lateral Flow Immunoassays e chemiluminescenza per analisi multiplex
LFIA è una tecnologia ampiamente utilizzata in ambienti al di fuori dei laboratori. Gli enzimi possono essere utilizzati come traccianti, accoppiati con una rivelazione in chemiluminescenza per ottenere un'informazione quantitativa, anche in analisi multiplex.

Immunoassays are well-established bioanalytical assays that are being employed since several decades for screening purposes, owing to their high specificity, low limits of detection and amenability for high trhoughput analyses. Nowadays, the enzyme-linked immunosorbent assay (ELISA) on microtiter plate formats or employing automated instrumentation are commonly employed for screening purposes in diagnostics, biomedical research, agrofood and environmental applications.
Nevertheless, the need for point-of-use applications (i.e., the analysis of samples directly at the site where they are obtained) has prompted the development of portable and simple-to-use formats. Immunochromatographic techniques (Lateral Flow Immunoassays, LFIA), in which the test sample flows by capillary forces along a prefabricated strip containing dry reagents that are activated by applying the fluid sample, are the most successful format for on-field immunoassays[1]. Lateral flow through the membrane strip expedites the immunological reaction and enables the *in situ* separation of bound and unbound immunoreagents, allowing for one-step analysis. These tests are mostly based on visual evaluation of the results and provide yes/no answer, exhibiting sensitivities lower than those associated with the traditional ELISA formats. As shown in Fig. 1, LFIA strips are composed of porous nitrocellulose membrane on which specific immunoreagents are immobilized in definite areas (lines), as well as an absorbent pad (generally made of cellulose) to drive sample flowing and a sample pad (generally made of glass fibre) on which immunoreagents are deposited and result to be flowed along the strip upon sample application (Fig. 1).

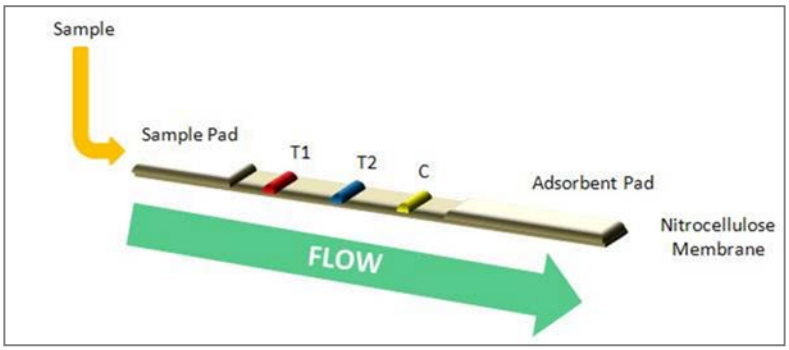

Fig. 1 - Multiplex LFIA strip for the quantitative detection of two analytes. Upon sample addition, immunoreagents deposited on the sample pad are flowed along the strip. Specific immunoassays occur in correspondence of each T-line (in this case two analytes are detected). C-line is used for confrmation of correct assay performance and for normalization purposes. The adsorbent pad drives fluids flow by capillary forces

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Several LFIA assays were developed for agrofood applications, such as detection of toxins and contaminants in food, for example mycotoxins in cereals[2]. Mycotoxins are low molecular weight natural compounds, produced as secondary metabolites by filamentous fungi. Many mycotoxins display overlapping toxicity to animals and humans and they can be found in agricultural commodities, such as corn, wheat and cereals products[3]. Higher incidences of severe pathologies in humans were found in populations chronically exposed to contaminated food. In particular, aflatoxins and fumonisins are two mycotoxins produced by *Aspergillus* and *Fusarium* species respectively; they are known for their acute toxic, immunosuppressive and carcinogenic effects. The ability to rapidly detect mycotoxins in feed and food is important to limit hazards for human health. The European Union has established maximum residue limits (MRLs) of these mycotoxins in corn-derived foodstuff[4]. Several methods for mycotoxins determination have been developed such as chromatography-based methods and immunochemical assays, which however are not suited for point-of-use applications[5]. Therefore, several LFIA assays have been developed for screening purposes.

As mycotoxins are low-molecular-mass compounds, immunoassays in competitive formats should be conceived to measure them. In the indirect competitive LFIAs, a mycotoxin-carrier protein (e.g., bovine serum albumin, BSA) conjugate is immobilized on the nitrocellulose strip in the area called "Test line" or "T-line", while a labelled anti-analyte antibody is deposited on the sample pad. Upon application of the sample, the antibody is resuspended and flowed along the strip. When reaching the T-line, a competition occurs between immobilized and free analyte for binding to the labeled antibody, thus producing a typical calibration curve in which the analytical signal decreases with increasing amount of analyte in the sample. It is possible to develop multiplexed assays by depositing different BSA-labelled mycotoxins in consecutive T-lines and a mixture of their respective labelled anti-analyte antibodies on the sample pad. Finally, a secondary anti-species antibody, able to capture any excess of the anti-analyte labeled antibody, is immobilized on an area called "Control line" (C-line) (Fig. 1). The C-line signal is employed both as a check, providing confirmation of the correct development of the assay (reagents and materials integrity), and as a normalization factor.

As conventional LFIA based on colloidal gold tracers that generate colour signals perceptible to the naked eye are available mostly for qualitative analyses and exhibiting rather low sensitivity, alternative and more sensitive detection systems have been employed, such as photoluminescence or electrochemical measurements[6]. As in enzyme-linked immunosorbent assays (ELISA), enzymes can be used as tracers in LFIA, coupled with chemiluminescence (CL) detection[7,8]. Chemiluminescence, a phenomenon in which photons are produced by a chemical reaction, offers undoubted advantages over other optical detection methods, such as photoluminescence: relatively simple instrumentation required, low detection limits (due to high signal-to-noise ratio) and high specificity[9]. Since a particular sample geometry is not a critical requirement and no excitation source is needed, CL detection is particularly suited to point-of-care and miniaturized analytical tools. One of the most frequently used enzyme labels in CL detection is Horseradish Peroxidase (HRP), which catalyzes the oxidation of luminol in presence of H_2O_2, leading to photons emission. It is possible to ameliorate the analytical performance of HRP-catalized reaction by using enhancers, such as substituted phenols and boronic acids, and N-alkyl phenothiazines as nucleophylic acylation catalysts, greatly increasing the light output and extending the duration of the reaction kinetics.

The use of CL detection enables obtainment of quantitative LFIA assays, since digital images of the LFIA strip can be acquired upon CL cocktail addition (employing in this work a portable ultrasensitive CCD-based "contact" imaging device) and elaborated to obtain signal intensities in correspondence of each line (Fig. 2).

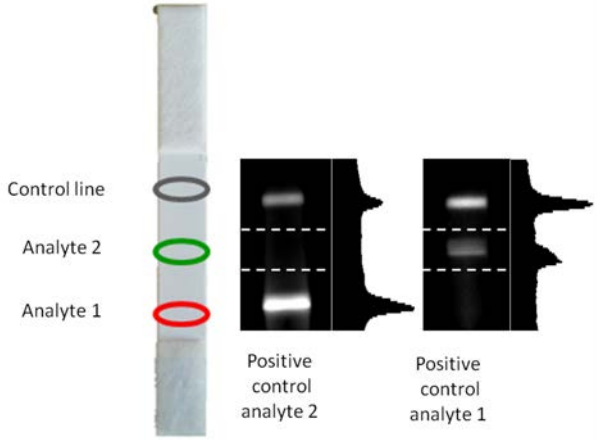

Fig. 2 - Left: layout of a multiplex LFIA strip for the quantitative detection of two mycotoxins employing CL detection. Being the immunoassays in competitive format, the signal intensity in correspondence of the T-line decreases at increasing amounts of analyte in the sample, as shown in right panels

The assay involved a simple extraction of mycotoxins from maize flour samples followed by their detection by a multiplex competitive immunoassay with chemiluminescent (CL) detection employing ready-to-use analytical cartridges.

References

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