Journal of Saudi Chemical Society (2015) **19**, 689–705

King Saud University

Journal of Saudi Chemical Society

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ORIGINAL ARTICLE

Designs, formats and applications of lateral flow assay: A literature review

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Received 4 May 2014; revised 24 August 2014; accepted 2 September 2014
Available online 16 September 2014

KEYWORDS

Point of care testing;
Lateral flow immunoassay;
Dry reagent strip biosensor;
Aptamer

Abstract This manuscript provides a brief overview of latest research involving the use of lateral flow assay for qualitative and quantitative analysis in different areas. The excellent features and versatility of detection formats make these strips an ideal choice for point of care applications. We outline and critically discuss detection formats, molecular recognition probes, labels, and detection systems used in lateral flow assay. Applications in different fields along with selected examples from the literature have been included to show analytical performance of these devices. At the end, we summarize accomplishments, weaknesses and future challenges in the area of lateral flow strips.

1. Introduction

Point of care (POC) testing has become the most famous way of diagnosis in clinical analysis, food safety and environment. Compared to centralized labs, POC provides prompt results in shorter times. Lateral flow assay (LFA) based POC devices are among very rapidly growing strategies for qualitative and quantitative analysis. LFA is performed over a strip, different parts of which are assembled on a plastic backing. These parts are sample application pad, conjugate pad, nitrocellulose membrane and adsorption pad. Nitrocellulose membrane is further divided into test and control lines. Pre-immobilized reagents at different parts of the strip become active upon flow of liquid sample. LFA combines unique advantages of biorecognition probes and chromatography. LFA based strips have different detection formats. Drawbacks associated with conventional clinical technique, enzyme linked immunosorbent assay (ELISA), were flabbergasted by LFA. Rapidity and one step analysis, low operational cost, simple instrumentation, user friendly format, less or no interferences due to chromatographic separation, high specificity, better sensitivity, long term stability under different set of environmental conditions and portability of the device are unique advantages related to LFA strips [1] (See Table 1). Rather than changing different physical parts, mathematical models are being used to optimize and improve quantitation ability of LFA [2–5].

Lateral flow assay basically combines a number of variants such as formats, biorecognition molecules, labels, detection systems and applications. Several review articles have been published which highlight different aspects of lateral flow

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2014.09.001

Table 1 Advantages and pitfalls of LFA.

Advantages	Pitfalls
• Ease of device preparation • Low cost • Stability over a wide range of environmental conditions and very long shelf life. • Simple and user friendly operation • Requirement of small sample volume • Most of the time, allows sample application without pretreatment • Versatility of formats, biorecognition molecules, labels and detection systems. • Less time of analysis • Comparable or better sensitivity and specificity than other well established methods • High potential of commercialization • Easy integration with electronics • Wide range of applications • No or very little energy consumption	• Mostly qualitative or semi-quantitative • Reproducibility varies from lot to lot • Most of the devices can detect more than one or two analytes simultaneously • Suffers from low biomolecules affinity toward analytes and tendency of cross-reactivity • Sometimes, pretreatment of sample is required which is time consuming • Once sample is applied to the strip, capillary action cannot be decreased or speeded up. • Analysis time is also dependent on nature of sample itself i.e. viscosity, surface tension.

assay. A recently published review emphasizes on principles of immunochromatography, its advantages and special use of lateral flow immunoassay in determination of toxic contaminants in agricultural and food products. Different methodologies for developing immunochromatographic systems have been considered in detail [6]. Labels used in LFA play an important role in determining sensitivity of analysis and a variety of labels are being employed in LFA. Description of labels along with advantages and pitfalls is given in a review [7]. A brief review of the LFA based test strips for detection of mycotoxins in food and feed is given by Rudolf and Alexandra [8]. LFA based strips can be an alternate and cheapest diagnostic tools in clinical areas particularly in the developing world and thus have been discussed in a review article of microfluidic diagnostic devices [9]. Another review article focuses on applications of lateral flow assay for detection of biological infectious agents and chemical contaminants. Bacteria, viruses, toxins, veterinary drugs and pesticides are considered in detail [10]. Paper based biosensors come in different configurations and formats and they employ nanomaterials for detection of proteins, cells and nucleic acids in diagnostic applications. LFA represent a special type of paper based biosensors and they have been discussed as a major component in reviews of nanomaterial based paper biosensors [11,12].

In this review, we intend to overview recent advances in different variants of LFA. We have summarized different formats, biorecognition molecules, labels, detection systems and applications in some specific areas.

2. Formats

Different formats are adopted in LFA. Strips used for LFA contain four main components. Brief description of each is given before describing format types.

Sample application pad: It is made of cellulose and/or glass fiber and sample is applied on this pad to start assay. Its function is to transport the sample to other components of lateral flow test strip (LFTS). Sample pad should be capable of transportation of the sample in a smooth, continuous and homogenous manner. Sample application pads are sometimes designed to pretreat the sample before its transportation. This pretreatment may include separation of sample components, removal of interferences, adjustment of pH, etc.

Conjugate pad: It is the place where labeled biorecognition molecules are dispensed. Material of conjugate pad should immediately release labeled conjugate upon contact with moving liquid sample. Labeled conjugate should stay stable over entire life span of lateral flow strip. Any variations in dispensing, drying or release of conjugate can change results of assay significantly. Poor preparation of labeled conjugate can adversely affect sensitivity of assay. Glass fiber, cellulose, polyesters and some other materials are used to make conjugate pad for LFA. Nature of conjugate pad material has an effect on release of labeled conjugate and sensitivity of assay.

Nitrocellulose membrane: It is highly critical in determining sensitivity of LFA. Nitrocellulose membranes are available in different grades. Test and control lines are drawn over this piece of membrane. So an ideal membrane should provide support and good binding to capture probes (antibodies, aptamers etc.). Nonspecific adsorption over test and control lines may affect results of assay significantly, thus a good membrane will be characterized by lesser non-specific adsorption in the regions of test and control lines. Wicking rate of nitrocellulose membrane can influence assay sensitivity. These membranes are easy to use, inexpensive, and offer high affinity for proteins and other biomolecules. Proper dispensing of bioreagents, drying and blocking play a role in improving sensitivity of assay.

Adsorbent pad: It works as sink at the end of the strip. It also helps in maintaining flow rate of the liquid over the membrane and stops back flow of the sample. Adsorbent capacity to hold liquid can play an important role in results of assay.

All these components are fixed or mounted over a backing card. Materials for backing card are highly flexible because they have nothing to do with LFA except providing a platform for proper assembling of all the components. Thus backing card serves as a support and it makes easy to handle the strip.

Major steps in LFA are (i) preparation of antibody against target analyte (ii) preparation of label (iii) labeling of biorecognition molecules (iv) assembling of all components onto a