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Magnetic lateral flow immunoassay test strip development – Considerations for proof of concept evaluation

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ABSTRACT

Lateral flow immunoassays (LFIA) have grown to become the predominant test device format for the diagnostics and point-of-care industries. The demand for robust and reproducible LFIAs has been facilitated through scale-up production methods using specialized and automated instruments. However, the feasibility of a LFIA device can still be evaluated in a small-scale laboratory setting through controlled manual preparation methods. The advent of super-paramagnetic (SPMP) labels for use in lateral flow has heralded the possibility of highly sensitive and stable LFIAs. The methods used for the preparation of a magnetic LFIA prototype device using a reserved suite of laboratory equipment are described.

1. Introduction

The genesis of the lateral flow immunoassay (LFIA) in the 1950s was the result of a confluence of scientific activity, including the latex agglutination test first described by Singer and Plotz [1]. In the following decades, the LFIA concept was improved, with the human pregnancy test becoming the first major success of this assay format [2]. Since then, the LFIA has been adapted for use in a multitude of fields including medical, veterinary, food, agricultural and environmental settings. Large-scale clinical diagnostic use in infectious and cardiac diseases, cancer and drugs of abuse has followed [2,3]. The LFIA has grown to become the most popular commercial point-of-care (POC) device format with an estimated market size of US$18 billion predicted for 2016 [2,4]. The prevalence of infectious diseases, an aging population and the drive for more home-based and decentralized POC testing, have all been significant contributors to this growth. The LFIA is particularly well suited to this emerging requirement for decentralized testing due to numerous factors such as ease of use, small sample volume requirement, large-scale production within a short timeframe and the ability to be integrated with reader and central information systems [5]. The capacity to supply the large-scale requirement for LFIA devices has been met through the use of highly specialized instruments such as reagent dispenser systems and automated guillotine cutters for the rapid preparation of test strips. However, it is entirely possible to assess the feasibility of a LFIA test format using manual preparation methods on a small laboratory scale, with a high level of reproducibility, ensuring limited use of precious critical raw materials prior to considering the move to scale-up production. Here we consider the approach for the manual development of a magnetic lateral flow test strip.

1.1. Principle of a lateral flow immunoassay

Typically, a LFIA involves the migration of a liquid sample, containing the analyte of interest, by capillary action through a porous membrane where biological interactions occur from the binding of labeled particles or analyte at specific detection zones containing immobilized antibodies to produce a signal that can be visualized through the use of colored nanoparticles, including gold or latex [6] or by incorporation of fluorescent [7], chemiluminescent [8] or super-paramagnetic labels [9] for optical detection. These methods afford the opportunity for quantitative LFIAs and are particularly valuable for POC applications.

1.2. Components of lateral flow immunoassays

The simplicity of LFIA devices hides the complex assembly of many vital components which must be fully considered from an early stage. The biological reagents, such as antibodies, are of utmost importance to the generation of a sensitive immunoassay, however, the selection of the most appropriate device materials will ensure the consistency of the device. LFIAs are generally

Abbreviations: LFIA, lateral flow immunoassay; POC, point-of-care; SPMP, super-paramagnetic; NC, nitrocellulose; IgG, immunoglobulin.

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composed of a nitrocellulose membrane, sample pad, conjugate pad and absorbent pad which are assembled onto a backing card for support (Fig. 1) before housing in a plastic test cassette. Nitrocellulose (NC) membranes are porous polymers that are available with a variety of pore sizes that control the capillary flow rate characteristics and facilitate sample flow across the test strip to the detection zone. The material also effects the binding and immobilization of antibodies/antigens used in the assay, is generally treated with surfactant to make it hydrophilic for test use and is supported with a plastic layer for handling and cutting purposes. Several factors are taken into account for selection of the NC membrane. The pore size of the membrane in relation to label size and the capillary flow time are considered vitally important for an optimal reaction time. In reality, pore size is not an accurate description for a material where fluid and particles travel through a labyrinth or mesh of interweaving openings. The "wicking rate" is ultimately what affects the development time of the assay. Taking this into account can offer some control over assay performance and sensitivity, and thus improving test result reproducibility [10]. The sample pad, commonly composed of cellulose, is the loading point for the test sample and can act as a filter for undesired materials, such as red blood cells, from a plasma sample. This pad can also be pretreated to neutralize sample pH when testing with urine. For example, a buffer with high concentration of salts can be used to neutralize urine sample pH. Human urine can vary between pH 5 and 10 and high fluctuations can alter the specificity and sensitivity of capture antibodies by affecting changes in charge densities [11]. The addition of a surfactant to increase wettability of the sample pad can also be considered. The conjugate pad, generally composed of glass fiber, facilitates drying and stabilization of particle conjugates until test use. The inclusion of carbohydrates such as sucrose in the conjugate buffer can stabilize IgG antibody conjugates during drying and protect the conjugate activity until the sample enters the pad. An absorbent "wicking" pad, often composed of high-density cellulose or cotton, is located at the end of the test strip and terminates sample flow by having an absorption capacity greater than the sample volume [3]. Each of the lateral flow test strip components must be considered for sample flow characteristics, including rate of flow and the sample matrix to be assessed. The selection of optimal test strip components and the inclusion of a sample pad pre-treatment step from the outset assures suitability for future use with clinical samples, if warranted [12,6].

1.3. Super-paramagnetic particles in lateral flow immunoassays

Magnetic particles have been described for use in medical areas including as MRI contrast agents, for cell labeling and separation, drug delivery and, more recently, as immunoassay labels in LFIAs (Fig. 2). Super-paramagnetic particles (SPMPs) are magnetic iron oxide or magnetite (Fe_3O_4) particles produced by various wet chemistry techniques where external conditions such as pH and temperature are carefully controlled. These techniques control the size and uniformity of the resulting particles with nanoparticles of diameter <100 nm and >200 nm used for *in vivo* and *in vitro* applications, respectively [13]. SPMPs used in LFIAs offer many advantages over the traditional and most widely used LFIA labels of colloidal gold and latex. SPMPs have low background noise due to biological materials lacking magnetism and are inactive until required, becoming magnetic only when placed in a strong magnetic field and subsequently maintaining a stable signal. The signal does not degrade for a long period of time, and thus magnetic LFIA strips can be retained for verifying results, if desired [14]. The surface of SPMPs can also be extensively modified with various coating chemistries to facilitate bio-conjugation. For example, EDC-NHS coupling chemistry can be utilized for the conjugation of SPMPs with carboxyl-functionalized surfaces to the amine groups of target analytes or antibodies (Fig. 3). The resultant magnetic signal at a detection zone can be measured quantitatively through the entire volume of the membrane whereas alternative methods, such as chemiluminescent detection, only account for the surface reaction observed [15–17].

1.4. Detection systems for magnetic lateral flow immunoassays

SPMPs possess a single magnetic domain that can be manipulated by an external magnetic field to produce uniform magnetic excitation that can be quantified using a magnetic detection sensor [16]. Several commercial and academic research groups have developed sensors for the detection and quantification of magnetic particles including MagnaBioSciences, LLC, Quantum Design, San Diego, CA, USA [18], LifeAssays AB, Lund, Sweden [19], Senova Immunoassay Systems, Jena, Germany [20], Magnotech, Philips, Amsterdam, The Netherlands [21], Magnisense, Paris, France [22] and MagArray Inc., Sunnyvale, CA, USA [23] and Magnasense, Vantaa, Finland [24]. The magnetic immunochromatographic test (MICT®) system from MagnaBioSciences, LLC has been evaluated for the development of magnetic LFIAs in an extensive number of fields including bacterial infection [18], parasitic infection [25], viral infection leading to cervical cancer [26], detection of fish allergens [27] and acute myocardial infarction [28]. Additionally, the MICT® instrument and a commercial thyrotropin-releasing hormone (TSH) test received clearance from the US Food and Drug Administration (FDA) governing body in March 2012, confirming the safety and efficacy of the MICT® system [29]. With the MICT® platform, the LFIA test strip is placed inside a C-shaped electromagnet that houses several coils between its poles. A voltage is applied from a parallel capacitor to activate the coils and generate a magnetic field. The subsequent magnetization of the SPMPs is detected by magnetic field sensors and the signal is amplified and processed to quantify the number of magnetic particles at both the test and control line locations only. This signal is quantified as

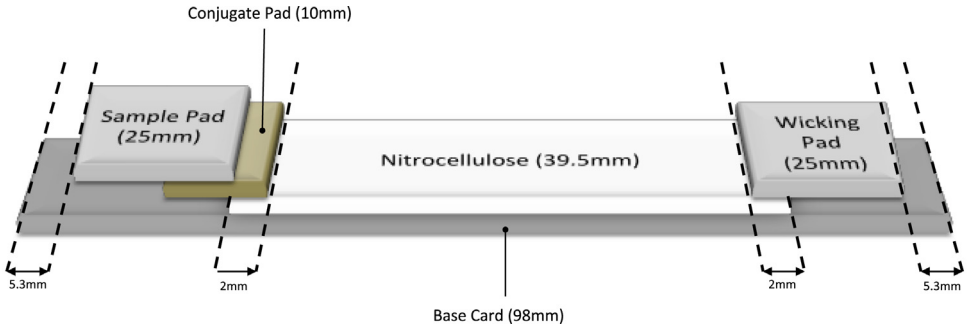

Fig. 1. Schematic diagram of lateral flow test strip assembly.