Development of Medical Devices Based On Protein Marker For Animal and Human Diseases

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Abstract: The concept of medical devices based on immunochromatography, is a combination of chromatography technique (for separation of sample components based on differences of their movement through the sorbent) and the immunochemical reaction that has been implemented in various ways. In this review, we discuss the evolution of immunoblotting techniques during the last decades, and was followed by development of reverse/lateral flow immunochromatography technique, also its use in various fields of biomedical research, food technology and explore the future direction for continuous improvement. The aim of this review is to improve the understanding of reverse/lateral flow immunochromatography technology, enabling the production of better products to be made, and provide impetus for further advances in technology.

Lateral flow immunochromatography were first launched commercially in 1984, as a simple urine-based pregnancy test for home use. In the early 1980's, two new rapid immunodiagnostic platforms were invented, namely the lateral-flow test and flow-through test (or vertical-flow test), both completing in the healthcare market. We highlight some successfully commercialized rapid test for both human and animal. In order to have a rapid test product that have a high sensitivity and specificity, the point to note is the antigen or antibody used for the detection marker. We have developed several rapid diagnostic tests by reverse flow immunochromatography assay for metabolic disorders and infectious diseases. To be able to produce a good rapid test, should be developed based on the patho-mechanism of the disease, so it is important to choose a protein marker that appears in the early stages of disease.

Keyword: *Medical devices, Immunochromatography technique, Protein marker*

INTRODUCTION

Infectious disease is one of the important key factors to affect on the profitability of livestock industries. Therefore, the use of veterinary antibacterial drugs for treatment and/or prophylaxis has rapidly increased for the economical production of animal product, also require innovative development of a detection device that is simple, economical, and easy to apply. New, rapid point-of-need diagnostic methods for infectious disease ie . *Bacillus anthracis* detection can enhance civil and military responses to accidental or deliberate dispersal of anthrax as a biological weapon. Immunoassays have been the standard method for detecting biomolecules such as marker proteins, hormones, and environmental residues in the diagnosis of disease and in the assay of chemicals for environmental risk assessments. Detecting biomolecules, especially against the protein marker of diseases has become very important for a variety of purposes, including diagnosis of metabolic degenerative and infectious diseases.

Immunochromatography (IC) is an antigen/antibody-detection assay that plays an important role in the rapid diagnosis of diseases because of its rapid turn around and ease of use. Immunochromatography based on the interaction between the ligand to the receptor or antigen with its antibody is the most common method used to detect the protein marker that is the target protein to detect the development process of diseases using a sample such as serum, plasma, urine, sputum and tissue or body part. In this review, we discuss the evolution of immunoassay techniques during the last decades, and was followed by the development of immunochromatography technique, and highlights its use in various fields of biomedical research, and explore the future direction for continuous improvement. We hope this review will improve the understanding of the lateral/reverse flow immunoassay technology (LTFA), enabling the production of better products to be made, and provide impetus for further advances in technology. Rapidity and simplicity are other important factors in the development of lateral/reverse immunochromatography for practical use. Immunochromatography both Lateral/reverse are a well-established technique and widely used in kits for the initial screening of medical diagnoses. We hope this review will improve the understanding of the reverse or lateral flow immunochromatography technology, enabling the production of better products to be made, and provide impetus for further advances in technology.

Lateral Flow Immunoassay

The lateral flow immunoassay (LTFA) devices are compact and easily portable. The main reason for its

popularity is the simplicity of the test design. Results are quick and easy to interpret, the application without the need for sophisticated equipment. The technology is also powerful. Multiple analytes can be tested simultaneously with a single device. Manufacturing of the test is relatively easy and inexpensive. Lateral flow immunoassays have achieved broad penetration in a variety of markets.

TABLE 1. Market segments for LTFA

No.	Applications for	No.	Applications for
1.	Medical Devices	7.	Blood Banking
2.	Forensic Science	8.	Agriculture
3.	Therapeutic Monitoring	9.	Aquaculture
4.	Animal Health	10.	Enviromental
5.	Food Safety	11.	Military/ Biodefense
6.	Military/ Biodefense	12.	Industrial

Test Strip

The LTFA strips are designed to detect the presence of an analyte (antigen or antibody) by specific labelled-antigen or labelled antibody. The test strip consist of four sections; sampel pad (cellulose), conjugate pad (glass fiber), membrane (nitrocellulose) and absorbent pad (cellulose) which are laminated onto a sheet of plastic backing orderly to allow cutting and handling. The pad overlap the membrane to allow a continuous flow path for the sample. The sample pad allows the diffusion of the sample into the conjugate pad that is impregnated with detector reagent (labeled-antigen or labeled-antibody) depending on the application. If the sample contains an analyte, it will bind to the detector reagent and the complex will continue to flow and then irreversibly binds capture reagent (antigen or antibody) at the test line on the membrane and forms a colored line. A continuous flow of the sample through the strip toward the control line will always form a colored line an indicator for a proper function of the test device. The absorbent pad at the end of the strip wicks the fluid through the membrane to ensure a continuous flow and thus maintains a clear background. Strips can be housed in a plastic holder (cassette), where only the sample application window and a reading window are exposed, for protection and easier handling (Millipore, 2008; Ngom *et al.*,2010).

Antibodies : Key to Robust Lateral Flow Immunoassay

Antibodies suitable for lateral flow immunoassays are available from a number of commercial sources. Specific antibody mostly influences the sensitivity of the test. Antibodies used in LTFA is a polyclonal or monoclonal antibody. Lateral flow immunoassays are particularly demanding in terms of the affinity required in the interactions between antibodies and antigens. Consider a typical lateral flow strip with antibody immobilized on a test zone of 0.5–1.0 mm wide. Antigen flowing up the strip has a flow rate in the range of 0.66–0.16 mm per second depending on the flow rate of the nitrocellulose membrane selected . Several investigators used either monoclonal antibodies or polyclonal antibodies both in the conjugate pad and on the test line (Kawatsu *et al.*, 2008; Jiang *et al.*, 2011). Others used both monoclonal and polyclonal antibodies on the same strip (Wang *et al.*, 2011;). The control line is coated with primary or secondary (anti-IgG) antibody depending on the application to capture excess detector reagents regardless of the presence or absence of target analyte (Posthuma-Trumpie *et al.*, 2009; Ngom *et al.*, 2010). The control line and the clear background that appear on the reading window are the indicators of an internal positive and internal negative procedural control.

Label Particles and Preparation of Colloidal Gold

The majority of the available commercial LFIA strips (around 94%) use colloidal gold particles (red–pink color) for labeling, while the rest uses colored latex particles. There are various ways for the production of colloidal gold in the laboratory depending on the particle size desired. asically, all methods use a reducing agent to convert ionic gold into metallic gold in a controlled manner. The reducing agents used include sodium borohydride, white phosphorus, ethyl alcohol, ascorbic acid, sodium citrate, and citrate plus tannic acid. According to the recent review articles many investigators also used colloidal gold particles for labeling (Posthuma-Trumpie *et al.*, 2009; Ngom *et al.*, 2010) while few others used colored latex particles (Greenwald *et al.*, 2003).

Gold nanoparticles (GNPs) are widely used for various applications, such as their use as markers mainly for immunochromatographic test strips, as well as their use in immunoelectrophoresis, in electrochemical biosensors. The size of the gold particles varies between 2 and 150 nm, but generally 15–25 nm particles were used (Zhao *et al.*, 2008; Omidfar *et al.*, 2010). The advantages of gold particles are being stable, easy to use and have convenient surfaces to accelerate the antibody–antigen recognitions, which increases the immunoassay signals and allows a smooth flow through the membrane (Aulanni'am, 2013).

LFIA Strips For The Diagnosis Diseases

Several LFIA strips are currently commercially available for the diagnosis of infectious agent or protein marker of diseaases. This is a qualitative test that is used either to detect antibodies againt to marker protein or infectious agents in blood samples (whole blood, serum and/or plasma) or to detect antigens in stool samples.

Format of LFIA Strips

Sandwich format: In this format rec protein marker antigens coated on gold particles were placed into the conjugate pad, rec protein marker unlabeled antigens were immobilized on the test line and similarly antibody against to Marker protein antibodies were immobilized on the control line. The addition of a sample drop onto the sample pad leads to a lateral flow of the sample fluid containing anti-protein marker antibodies toward the conjugate pad where it binds to the antigen coated on gold particles. The complex then flows to the test line where it binds to the immobilized rec protein marker unlabeled antigen and results in a red color line. The flow of the sample fluid will continue toward the control line where the remaining antigen coated gold particles will bind to the immobilized anti- rec protein marker antibodies and give a red color line.

The Principle of Immunochromatography and Its Main Benefits

The concept of immunochromatography as a combination of chromatography (separation of components of a sample based on differences in their movement through a sorbent) and immunochemical reactions emerged a long time ago, and it has been implemented in many different ways. Today, the most widespread immunochromatographic system is the test strip – an assembly of several plain porous carriers impregnated with immunoreagents. On contact with the test strip, a liquid sample flows along the carriers, and detectable immune complexes are formed in certain zones of the test strip. Test strips are widely used for the early detection of pregnancy, for drug screening, to identify markers for various diseases, and for a number of other analytical tasks. However, a few decades ago, the term ‘‘immunochromatography’’ was used to describe a different type of analysis (i.e., separation of samples on a column containing a sorbent with covalently-bound antibodies specific to a target substance).

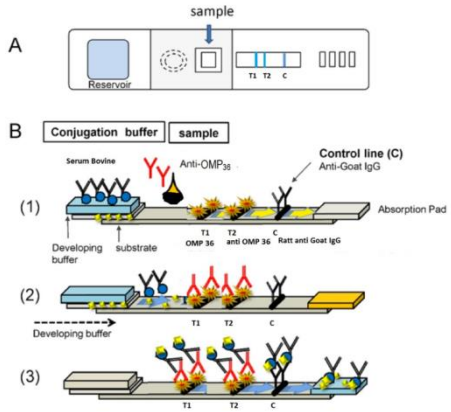

FIGURE 1. Design of Brucellosis Rapid Test for Detect Fragment of Brucella sp and its antibodies (Aulanni’am, 2016)

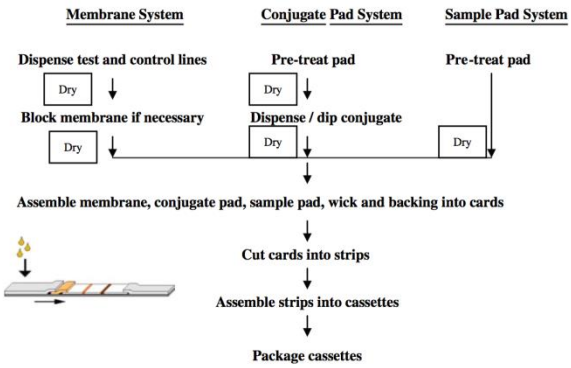

FIGURE 2. Outline of typical Lateral Flow Immunoassay Manufacturing Process (O’Farrell and Bauer, 2006)

Applications in Various Field of Biomedical and Food Technology Researches

In industry, immunoassays are used to detect contaminants in food and water, and in quality control to monitor specific molecules used during product processing. Immunoassays are performed in central laboratories, using a variety of instrument-based technologies or on-site via rapid test techniques. Over the last decades since immunoblotting developed, many studies have been published that have used "immunoblotting" as a keyword. Immunoblotting has evolved from a research method for routine laboratory techniques.

Lateral flow immunoassay tests, also known as immunochromatographic strip tests, have been a popular platform for rapid immunoassays. Novel approaches driven by market needs are leading to improvements in performance and utility to a vast array of new application areas. Lateral flow (LF) tests are used in veterinary medicine to test commercial livestock (large animals – cows, poultry, sheep, pigs, etc.) and household pets (cats, dogs, etc.) for a variety of medical conditions including: bacterial and viral infections, allergies, fertility issues, and diabetes.

Conclusion

Lateral/Reverse flow immunoassay technology is evolving rapidly. We developed a novel R/LTFA device using antibody-conjugated nanoparticle reporters to exploit the well-characterized marker protein or its antibodies that is as simple rapid test, a small sample volume, easy to apply, have a high level of sensitivity and specificity. In the development of lateral flow immunoassay, most attention has been paid to finding a good detection method or seeking the best antibody or antigen..

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