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A Review on Lateral Flow Test Strip for Food Safety

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Abstract

Background: Foodborne disease outbreaks from various food sources are a major health concern worldwide. Current methods for detection of foodborne pathogens are both expensive and time-consuming. **Purpose:** This review aims to present the current information available on the use of lateral flow test strips to detect pathogens in food products to enhance food safety. **Results:** Frequent foodborne disease outbreaks from various food sources have increased the need for rapid and easy methods for routine analysis of foodborne pathogens. Present detection methods for foodborne pathogens require expensive instruments, experts, and long time for sample analysis. Lateral flow test strips have drawn attention in recent years because of their ability to detect analytes quickly and easily. This review focuses on the principle of the lateral flow test, the various formats of lateral flow test strips, recognition elements, labeling tags, and reading instruments. In addition, this review also discusses the future prospects for the lateral flow test strips.

Keywords: Food safety, Lateral flow test strip, Nanoparticle

Introduction

In recent years, the increase in food borne disease outbreaks from various food sources have raised public awareness about food safety. In 2014 alone, several multistate foodborne disease outbreaks occurred including *Salmonella* from bean sprouts, nut butter, chicken, and cheese; *Listeria monocytogenes* from caramel apples, soy sprouts, and cheese; and *Escherichia coli* from clover sprouts and ground beef in the USA (CDC, 2015). WHO estimates that each year, about 2 million people die of foodborne disease in the world, particularly, in developing countries (WHO, 2015). In Korea, 7466 people were hospitalized and 349 incidents were reported in 2014 (KFDA, 2015). Conventional methods for foodborne pathogen detection require expensive instruments or a long analysis time. To protect and maintain public health, rapid methods to identify hazardous substances in food products are required.

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Recent advances in rapid detection technology including enzyme-linked immunoassay (ELISA), polymerase chain reaction (PCR) technology, and biosensors have made detection and identification of foodborne pathogens faster, more sensitive, and more specific than traditional methods. However, these methods require several steps, expensive instruments, and skilled operators (Hart et al., 2011; Hossain et al., 2012).

Lateral flow test strips (LFTSs) have shown great potential for rapid detection of foodborne pathogens. LFTSs are as well-known as home pregnancy tests and are a rapid test platform in clinical practices. The LFTS have many advantages compared to some laboratory tests in terms of ease of use, rapidity, portability, reliability, and cost. Because of these advantages, LFTSs have been widely applied as rapid tests for food contaminants including bacteria (Chua, 2011; Bruno, 2014), viruses (Hagström et al., 2015), pesticides (Wang, 2014), and toxins (Moon, 2011; Ching et al., 2015).

This review explains the principles, recognition elements, labeling tags, and reading instruments for lateral flow test strips. In addition, future directions for lateral flow

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test strips are discussed. Compared to previous studies (Posthuma-Trumpie et al., 2009; Sajid et al., 2014), this review focuses on the use of LFTS for food safety as well as the reading instruments used.

Lateral Flow Test Strip

The lateral flow test is also known as the lateral flow immunoassay or immunochromatographic assay because this test usually utilizes antibody-antigen reaction. Typically, an LFTS is constructed with four different parts: a sample application pad, a conjugate release pad, a nitrocellulose membrane, and an absorption pad. The basic structure of an LFTS is shown in Figure 1(a). To start the assay, a sample is applied on the sample application pad and the sample is transported to the conjugation pad in a continuous manner. The sample pad is made of cellulose or glass fiber that can transport the sample in a smooth and homogenous manner. The conjugation pad stores labeled reagents that recognize pathogens in the sample. The labeled reagents are usually antibody-labeling tag conjugates that interact with the pathogens in a moving liquid sample. The labeled reagents are stored in a dried form and released upon contact with the sample liquid. An automated dispenser is used to apply the reagents onto the surface of the conjugation pad for uniform distribution of accurate quantity (O'Farrell, 2013). The conjugation pad is made of glass fiber, cellulose, and polyesters that can store reagents stable over long periods and release most of the reagents at the time of reaction. The nitrocellulose membrane has test and control lines or spots onto which receptors are immobilized to capture the analyte. The wicking rate of the nitrocellulose membrane varies depending on the pore size and this affects the sensitivity of a LFTS. The nitrocellulose membrane should have low nonspecific binding affinity and high affinity for capturing ligands. Typically, a non-contact dispenser is used to ensure even distribution of ligands for capture onto the surface of the membrane. The absorption pad entraps and maintains flow rate of the sample liquid and is commonly made of the same material as the sample pad. The liquid holding capacity of the absorption pad should be large enough to be able to contain the whole sample liquid and provide wicking force throughout the assay (Sajid et al., 2014). Parts of the LFTS are usually pretreated for separation of components, easy release of reagents, removal of interferences, and reduction of non-specific binding.

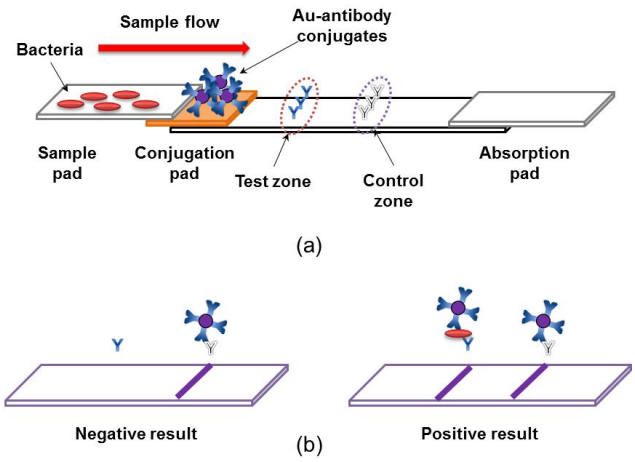

Figure 1. (a) A schematic representation of the lateral flow strip sensor. (b) Illustrations of the sandwich assay immunochromatographic test results (Kim et al., 2013).

The operating principle of the LFTS is based on the retention and formation of visual lines or spots of labeled probes in sensing zones on the membrane during sample flow through. The sample liquid is transported through the pores of the strip by capillary forces. The pads are arranged to overlap each other to bridge the pores between the pads. The pathogens in the sample are combined to the labeled reagents in the conjugate pad and they continuously flow to the nitrocellulose membrane. The pathogen-labeled probe conjugates react with the immobilized capture ligands in the sensing zone on the nitrocellulose membrane to generate signals and any remaining sample flows through to the absorption pad.

The type of the labeled probes stored in the conjugation pad and the capture ligands immobilized on the nitrocellulose can be varied according to the specific assay format. Frequently used LFTS assay formats are sandwich and competitive assay. Sandwich assay is used for relatively large pathogens that can have more than two different epitopes for an antibody pair, which include a labeled probe and a capture ligand. Kim et al. (2013) utilized the sandwich assay format to develop a strip sensor for *E. coli* O157:H7 detection. In this study, they used gold nanoparticles as the labeling tag and antibodies against the recognition elements. Gold nanoparticles were conjugated to anti-*E. coli* O157:H7 monoclonal antibodies to prepare color-labeled probes. The color-labeled antibodies were applied onto the conjugation pad and dried. As the liquid sample flowed through the

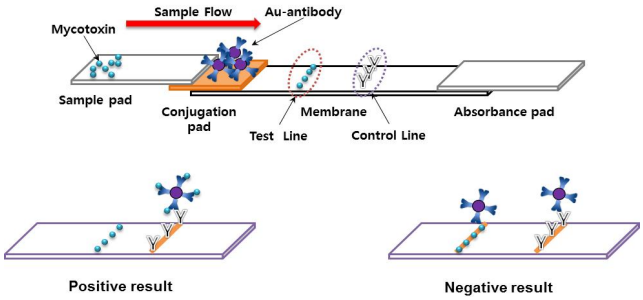

Figure 2. An illustration of the competitive assay LFTS test results.

conjugation pad, *E. coli* O157:H7 cells in the sample interacted with the conjugates and the bound conjugates flowed in a continuous manner onto the nitrocellulose membrane. These *E. coli*-antibody-gold particle conjugates interacted with the capture antibodies at the test zone and created a sandwich-like form. Any unbound antibody-gold particle conjugates interacted with the secondary antibodies in the control zone and formed another sandwich-like form. Thus, in the sandwich assay, positive samples created two red colored zones while negative samples created one (Figure 1(b)). The color of the test zone was more intense with increase in the number of *E. coli* cells in the sample.

The competitive assay is used to detect small analytes with one epitope, which can bind to one antibody. In contrast to the sandwich assay, no color is seen at the test zone in the competitive assay if analytes are present in the sample (Figure 2). The competitive assay uses standard analytes as a labeled probes or capture ligands at the test zone. When a labeled probe is used, the primary antibodies are immobilized at the test zone; however, if primary antibodies are labeled with labeling tags and stored in the conjugation pad, the standard analytes are immobilized at the test zone. Either way the analytes in the sample compete with the standard analytes. Moon et al. (2012) utilized the competitive assay to detect aflatoxin B1 (AFB1). In this study, colloidal gold particles were conjugated to the AFB1-bovine serum albumin (BSA) and dried on the conjugation pad to serve as labeled probes. The anti-AFB1 Pab was used as a capture ligand on the test zone to capture the AFB1s in the sample or the AFB1-BSA conjugated gold particles from the conjugation pad.

Recognition elements

Most of the current LFTSs are based on the use of antibodies as recognition elements and have been used in clinical diagnostics for several decades (Sajid et al., 2014). Antibodies are produced by injecting animals such as mice, rabbits, or goats with antigens that bind to analytes through immunoreactions. LFTSs based on antibodies have been used for common foodborne pathogens. LFTSs have been used for the detection of various pathogenic bacteria including, *Salmonella* Typhimurium (Kim et al., 2011), *Vibrio cholerae* (Chua et al., 2011), and *Staphylococcus aureus* (Park and Kim, 2011).

The use of antibodies as recognition elements has several limitations such as low stability, high production cost, long production time, and high batch-to-batch variation. In addition, producing antibodies for non-immunogenic or toxic targets is difficult (Chen and Yang, 2015). Because of these limitations, many researchers are trying to find alternatives to antibodies. Some researchers used nucleic acids as recognition elements for LFTSs. These LFTSs detect amplified nucleic acid products of foodborne pathogens by hybridization with complementary DNA sequences (Singh et al., 2015). Blazkova et al. (2009) developed a nucleic acid based LFTS for rapid detection of *L. monocytogenes*. They showed that nucleic acid based LFTSs were feasible for performing electrophoresis that is commonly used to interpret the results of PCR analysis in a much shorter time. However, the nucleic acid based LFTSs require additional procedures for extraction of genomic DNA and amplification of that DNA prior to the use of the LFTS.

Recently, aptamers have drawn much attention as a potential substitute for antibodies because they have high affinity and specificity (Luo et al., 2015). Aptamers are specific single stranded nucleic acid sequences that can reform their 3D structures to bind analytes. Aptamers can be identified by systematic evolution of ligands by the exponential enrichment (SELEX) procedure from nucleotide libraries of random-sequences (Meyer et al., 2013; Dong et al., 2014). They have a high reproducibility and low production cost because they can be chemically synthesized if the specific sequences are known (Stoltenburg, et al., 2007). Aptamers are stable over various conditions of pH and temperature. In addition, they can be easily modified to have active groups or probes (Moon et al, 2014; Chen and Yang, 2015). Bruno (2014) developed the sandwich format LFTSs for *E. coli*, *L. monocytogenes*, and *Salmonella enterica* by the use of DNA aptamers. Shim et al. (2014) developed a LFTS for aflatoxin B1 (AFB1) detection based on an indirect competitive assay. A biotin-modified

aptamer specific to AFB1 competes with a cy5-modified DNA probe to bind AFB1 in a sample in this assay.

Labeling Tags

Traditional LFTSs use colloidal gold particle conjugates to generate visual signals (Zhao et al., 2014). Using colloidal gold-monoclonal antibody conjugates as signal probes Wiriyachaiporn et al. (2013) developed a LFTS for *S. aureus* detection. Huang (2006) also developed a LFTS for *S. aureus* detection using a similar sandwich assay format. Chua et al. (2011) developed a glass fiber-based lateral flow DNA biosensor that uses capture reagents coupled to carrier beads and detector reagents bioconjugated to gold nanoparticles for the detection of food-borne pathogen, *V. cholerae*.

Even though colloidal gold particles are the most preferred labeling tag, the LFTSs based on colloidal gold have limited sensitivity that does not fulfill the food safety regulation requirements (Bruno, 2014). To overcome this limitation, researchers have worked on different labeling tags, including silver or gold enhancement (Anfossi et al., 2013; Hu et al., 2013), chemiluminescent tags or fluorescent tags (Zou et al., 2010; Berlina et al., 2013; Bruno, 2014; Taranova et al., 2015), magnetic nanoparticles (Liu et al., 2011; Wang et al., 2013), and carbon nanoparticles (Noguera et al., 2011). Anfossi et al. (2013) amplified the detection signal of an ochratoxin by using a detection LFTS that utilizes silver nucleation on gold nanoparticles. The signal enhancement based on the silver deposition on gold nanoparticles improved the sensitivity by 10-fold compared to the gold-based LFTS. Further, fluorescent nanoparticles or quantum dots have been successfully applied to improve the performance of LFTSs. Fluorescent nanoparticles or quantum dots (QDs) have high quantum yield and brightness, photostability, and resistance to chemical degradation. Several groups have reported QD-based LFTSs for food safety diagnosis including *Listeria monocytogenes* (Bruno, 2014), pesticide metabolites (Zou et al., 2010), antibiotics (Taranova et al., 2015), and chloramphenicol (Berlina et al., 2013). Taranova et al. (2015) demonstrated qualitative and quantitative analysis capability of the LFTSs by using different-color emission QDs. The LFTSs consisted of three lines of different colors to detect three different antibiotics, namely, ofloxacin, chloramphenicol, and streptomycin in milk.

Carbon nanoparticles have been used as an alternative labeling tag because of their high sensitivity, low cost, high stability, and ease of preparation (Posthuma-Trumpie et al., 2012). Noguera et al. (2011) developed a carbon nanoparticle-based LFTS for the detection of Shiga toxin-producing *E. coli*. The LFTS detected nucleic acids for virulence factors of the pathogenic *E. coli.* Detection results of these LFTSs were similar to the results of q-PCR.

Magnetic beads have been usually used to separate and concentrate analytes from a liquid sample. Because of this additional function, magnetic beads have been utilized as a labeling tag for LFTS. Wang et al. (2013) developed a super-paramagnetic lateral-flow immunological detection system for *B. anthracis* spores. In this study, a sandwich assay format LFTS was used to detect the *B. anthracis* spores. A portable magnetic assay reader was used to measure the magnetic signal from the super-paramagnetic nano beads. Liu (2011) used magnetic Fe_3O_4 particle aggregates as color amplifying labels and these methods increased the detection limit of LFTSs for pesticide residue of paraoxon methyl by 40-fold.

Reading Instruments

Since the majority of LFTSs are based on gold nanoparticles, the results of the LFTSs are been commonly interpreted by bare eyes. Image analysis systems have recently been developed for objective and quantitative interpretation of the LFTSs. Kim et al. (2013) developed an image analysis system for LFTSs. The system acquired LFTS images with a CCD camera and analyzed the images to measure the peak area values of the test and the control zone. By using this image analysis system, the detection limit improved 10-fold compared to visual interpretation., Lee et al. (2013) developed a smartphone-based LFTS reading system for aflatoxin B1 detection. This was intended for field use. The smartphone-based reading system consists of a smartphone, an LFTS reader, and a smartphone application for image acquisition and data analysis. This kind of handheld communication device-based LFTS reading system can be a valuable clinical device in resource-limited countries. Mudanyali et al. (2012) developed a cellphone-based LFTS reader to assist health-care professionals in tracking epidemics. They tested the reader using malaria, tuber-

culosis, and human immunodeficiency virus (HIV). Feng et al. (2014) demonstrated a wearable LFTS reader that can be operated in hands free mode with voice-controlled interface. The reader is based on Google Glass that has a built-in camera. Individual LFTSs were identified by the Quick Response (QR) code and the images were wirelessly transferred to an external server for further processing. The wearable LFTS reader was evaluated using HIV and quantitative prostate-specific antigen (PSA) tests.

Conclusions

This review aims at providing information on lateral flow immunoassays to enhance food safety. Since most existing food safety detection methods rely on laboratory instruments and skilled persons, these methods are difficult to use on-site. The use of LFTSs is feasible to detect various pathogens in field faster than conventional methods. The LFTSs have comparable sensitivity and selectivity to enzyme immunoassays. In addition, they are simple, easy to use and store, and economical (Posthuma-Trumpie et al., 2009). LFTSs have shown great potential in detection of food borne pathogens because of these reasons. To further increase the usability of the LFTSs, several limitations of the LFTSs such as low sensitivity, lack of multianalyte detection capability, and low reproducibility due to batch-to-batch variation of antibody performance should be solved. In this context, aptamers are introduced in the LFTSs since they have several advantages over antibodies. In addition, many researchers have evaluated the use of alternative labeling tags. The detection sensitivity of LFTSs can be improved by using new labeling tags such as QDs, magnetic nanoparticles, and carbon nanoparticles. The detection sensitivity of LFTSs is also affected by recognition elements. Better recognition elements could improve the detection sensitivity as well as the reproducibility of LFTSs. Some multianalyte LFTSs have also been developed recently. Kolosova et al. (2007) developed a LFTS for simultaneous detection of two mycotoxins in wheat, deoxynivalenol (DON) and zearalenone (ZEA). Song et al. (2014) detected three mycotoxins simultaneously with a multianalyte LFTS. The LFTSs could detect AFB1, ZEA, and DON in cereal samples with result almost similar to those of the LC–MS/MS analysis.

Paper-based microfluidic biosensors have drawn much attention recently due to their ability to integrate the multiplex characteristics of microfluidics with the advantages of the LFTSs (Ge et al., 2014). Paper microfluidics are easy to fabricate, low-cost, and suitable for disposable applications since they have fewer components than conventional LFTSs. Park et al. (2013) demonstrated a paper microfluidics for *Salmonella* detection. In this study, pre-loaded *Salmonella* antibodies on the paper microfluidic channel agglutinated with *Salmonella* cells in a sample solution. The immunoagglutination induces Mie scattering and the scattering was measured with a smartphone to calculate and display the bacterial concentration.

Notably, several commercialized LFTSs for monitoring food safety are already in the market. However, their widespread acceptance is hindered by the shortcomings described above especially by their low sensitivity. To ensure successful evaluation of food safety by LFTSs, many efforts are needed to improve their sensitivity, reproducibility, and multi-analyte analysis capability.

Conflict of Interest

The authors have no conflicting financial or other interests.

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