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Chapter

Lateral Flow Assay for Salmonella Detection and Potential Reagents

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Abstract

Salmonella is among the very important pathogens threatening human and animal health. It is a common food pathogen transmitted from animals to humans via contaminated food, drinking water, and air. It invades the intestinal tract of hosts and causes salmonellosis leading to death. S. enteritidis was the most common species accounted for all salmonellosis cases. S. typhimurium is also another significant species causing the serious cases worldwide. To ensure public health, early detection of pathogens is crucial. Lateral flow assay (LFA), immunochromatographic assay, is a simple and rapid diagnostic test kits used in various fields and can be developed by, aptamers, antibodies (Abs), and nucleic acids. They are also being continued to develop different capture reagents coming from the recombinant technology. It has many advantages such as having mature technology, market presence, low cost, easy to use for end users without education, and stable shelf life. Gold nanoparticles (GNPs) are the most commonly used labels in the LFAs for the naked-eye analysis. Therefore, Salmonella detection by LFA based on GNPs in a rapid and simple way is always open to be developed by new reagents and methods.

Keywords: Salmonella, gold nanoparticles, lateral flow, food pathogens, rapid detection

1. Introduction

Most of Salmonella infections are typically food-borne illness. It was reported that around 15% of salmonellosis cases is caused by pork [1], turkey products, and meat [2]. Early detection of pathogens which contaminated the foods or consumption products is a crucial issue especially for the government authorities to ensure public health. Thus, many kinds of identification methods are in use, and new detection platforms are also being tried to develop for improving the sensitivity and selectivity of detection with low cost as rapid tests. Traditionally, the Salmonella diagnosis in the laboratory is based on common cultural techniques [3], biochemical and serological confirmation tests. Along with immunomagnetic nanospheres as immunological tools [4], multiplex PCR [5] and real-time multiplex PCR [6–9] are other detection methods of Salmonella in chicken samples or other sources. However, some of those techniques require 5 or 7 days, skilled personnel, sterile working conditions, and sensitive and costly equipment, and they are inconvenient for food sector or industrial applications [10] and not...
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Figure 1. Preparation of LFA strip (a) and schematic representation of it in plastic case [21].

Figure 2. Schematic diagram of the immunochromatographic test strip principle for the dipstick assay. CSA Ab, common structural antigenic antibody; GNPs, gold nanoparticles [21].

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Bacteria isolated from the food samples were used. Dry soil bacteria, *E. coli*, *Bacillus*, and PBS were used as negative control. After culturing, test strips were dipped into the bacterial media without any treatment and showed positive results with target and nontarget samples. To highlight developed strip assays have high sensitivity and selectivity for the targets without non-specific interactions with the membrane and other samples.

The sensitivity and susceptibility of LFAs may also be improved by using high-affinity reagents including recombinant antibodies (Abs), one-step GNPs, or silver enhancement and integration of microfluidic papers with onboard electronics. Therefore, sensitive detection of *Salmonella* such as $10^2$ or $10^3$ cfu/mL is achieved for multiple recognition. Although cultural techniques associated with biochemical and serological confirmation tests and molecular methods are being developed for sensitive detection, they are time-consuming processes and not practical for end users. Thus, LFAs for *Salmonella* became attractive to make a rapid and sensitive detection for various species without nucleic acid isolation and advanced equipments. It is also open to improvement by integrating various detection systems for multiple recognition.

3. Antibodies for LFAs

Abs are more common reagents used for LFAs and available from a number of commercial sources. Various kinds of Abs generated by different ways includes recombinant protein technology, phage display technology, and hybridoma techniques. Although LFAs developed by monoclonal or polyclonal Ab are commonly in use, there are highly limited sources of LFA based on single-chain variable fragments (scFvs) [28]. Generally, commercial Abs used for sandwich assay in LFA might be obtained as prequalified by the vendor in pairs. These pairs are most readily available for relatively common and high-volume assays, such as tests for pregnancy, infectious disease, cardiac markers, and malignancies. Abs specific to various antigens of *Salmonella* species are in use for the development of LFAs [27, 29–31]. The common Ab-based LFAs for *Salmonella* recognition require these steps: (i) coating of GNPs with target specific Abs (detection Abs) via chemical or physical adsorption under the optimal pH value, (ii) immobilization of capture Abs on nitrocellulose membrane, and (iii) preparation of the pad and running buffers which has the optimal releasing effect through the membrane. The first use of Abs with colloidal gold reagent for a diagnostic immunoassay was reported in 1981 [32]. The optimal concentration of Abs to cover the GNPs and preventing them from agglomeration can be changeable. The specificity and selectivity of the strip assay depends on the affinity of used Abs. Thus, using high-affinity Abs will increase the sensitivity and decrease the limit of detection (LOD) and non-specific interactions with different antigens. To achieve this goal, engineered Abs are being continued to generate and adapt to LFAs, recently [33, 34]. As it is seen on Figure 3, all the requirements for LFAs mentioned above were achieved by Ab-based strip assay. Therefore, it makes possible to develop the strip assay for multiple *Salmonella* detection using both the monoclonal or polyclonal Abs on one assay.

4. Aptamers for LFAs

Aptamers are single-stranded DNA or RNA molecules that bind to the specific targets. Usage of aptamers in biosensors and development of new diagnostic systems based on aptamers become popular since 2000. Because they have high
affinity to their targets, their generation is rapid and easy compared to the Abs, and conjugation with GNPs is chemical which is basically performed by thiol bonds. Besides, aptamer conjugates have long shelf life without degradation in comparison to Abs. Although they are used for developing LFAs, recently studies have still limited numbers in terms of the technical and application. While aptamers can be used together with Abs, they are commonly used as pairs for developing LFAs, and they should be decided carefully. Recognition aptamers present on GNPs and capture aptamers immobilized on the capture lines should have different binding sites to increase the sensitivity. Some LFAs for *Salmonella* detection based on aptamers are recorded in the literature with various reagents and techniques [35, 36]. While some aptamers show lower LOD such as 10^{2} cfu/mL of *S. enteritidis* [37], some of them show higher. Those variabilities can be caused by some reasons which are choosing the aptamer pairs, the distance of aptamers from the immobilization zone of membrane, affinity of aptamers [37, 38], and experimental assay conditions. Although large numbers of aptamers were recorded in the literature [39–41], there is still a lack of their adaptation to LFAs for the recognition of *Salmonella* species. Because the optimization of test parameters including immobilization procedure of aptamers on capture zones, optimal buffer ingredients, and membrane types, the exact size of GNPs has more complexity than Ab-based strip assays. Therefore, LFAs should be manufactured by high-affinity aptamers to detect whole *Salmonella* cells.

Nucleic acid-based LFAs using nucleic acid hybridization or amplification methods are also developed for *Salmonella*. However, further experimental steps including nucleic acid or genomic DNA isolation, primer design, and PCR are required. Due to the poorly suited point-of-care testing of PCR, new methods such as isothermal amplification become popular. The most common isothermal amplification methods are loop-mediated amplification (LAMP) [42, 43], nucleic acid sequence-based amplification (NASBA), rolling circle amplification (RCA), nicking enzyme-mediated amplification (NEMA), recombinase polymerase amplification (RPA), helicase-dependent amplification (HDA), multiple displacement amplification (MDA), and transcription-mediated amplification (TMA) depending on the detection techniques [44, 45]. Using this type of LFAs, lower detection limit of **Table 1. LFAs for *Salmonella* detection by Ab, aptamer, and nucleic acids.**
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Salmonella such as 20 fg of target DNA or 1.05 × 10^1 cfu of bacteria in pure culture [46] or 1.3–1.9 cfu/g or 1.3–1.9 cfu/mL of Salmonella in contaminated chicken products can be achieved after enrichment [47]. The assay sensitivity may also show variety according to the length of amplicon or target [48]. The commonly used reagents in this assay are biotin/fluorescein, biotin/digoxigenin tags for amplicons and gold/anti-digoxin Ab or gold/streptavidin conjugate on conjugate pad. Depending on the immobilized capture agents such as Abs, labeled nucleic acids, or aptamers on test and control line, assay is performed and results become visible for Salmonella [49].

Table 1 shows some LFAs for Salmonella detection by using reagents mentioned above.

5. Gold nanoparticles for LFAs

Currently the nanoscale properties of GNPs have attracted more attention, and they are used in different fields like electronics [53], optics [54], and biosensors [55]. A common way to synthesize the nearly monodisperse spherical GNPs is the aqueous reduction of HAuCl4 by sodium citrate at boiling point [56]. Other reducing agents such as borohydrides and amines have also been used [57]. The nature of the surface chemistry of GNPs promotes easy and controlled attachment of other molecules especially those with thiol functionalities. Following their biocompatibility, high stability, ease of characterization [58, 59], and the controllable morphology, GNP-based bioconjugates are found to be good candidates for biomedical applications because they are stable with their conjugated parts compared to the unbound forms. If sodium chloride is present in the solution, repulsive and attractive forces between the particles are imbalanced, due to the masking of negative charge of colloidal solution [60]. This resulted with collapsing of gold particles after adsorbing one particle onto another, and visualization of this phenomenon is seen as the color change of colloids. However, in the presence of coating molecules including proteins, nucleic acids, and aptamers, they adsorb onto gold particles and help in preventing them from aggregation by inhibiting the binding of other gold particles. To make GNP conjugates, physical interaction is the simple method, while chemical interaction is also another method including covalent conjugation [61] by using thiol derivatives and bifunctional linkers.

LFAs based on GNPs conjugates have become useful innovation in nanotechnology. Colloidal gold is the most widely used label today in commercial LFAs for many reasons. It is fairly easy and inexpensive to prepare in the laboratory. The color is intense, and no development process is needed for visualization. However, assays may have varying sensitivity with respect to their target agents [22] in LFAs. As a conclusion LFA based on GNPs is rapid and sensitive assay for Salmonella detection as point-of-care tests compared to other detection methods because it is a naked-eye analysis test and does not require the skilled personnel. Once it is developed, it can be used for 1 year by the end users without advanced equipments. Adapting different reagents including Abs, aptamers, or nucleic acids onto LFAs is another advantage because of their practical immobilization and binding steps in terms of the whole-cell detection and also their potential to be adopted to enhance LFAs.

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New Insight into Brucella Infection and Foodborne Diseases

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