Chapter

Lateral Flow Assay for *Salmonella* Detection and Potential Reagents

Dilek ÇAM

Abstract

Salmonella is among the very important pathogens threating 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

portable to perform sensitive and rapid microbial analysis. To develop the fast and sensitive method for bacterial antigens, electrochemical [11], optical [12], microfluidic [13], and magnetoelastic biosensors are also being developed for the detection of *Salmonella* species. Among those techniques lateral flow assay (LFA) is still the most practical and easy to use test and multiple detection tool as an immunosensor for end users.

2. Lateral flow assay

LFA, immunochromatographic strip test, which can be developed by Abs, aptamer, and nucleic acids, was described in the 1960s [14] and become a popular platform for rapid immunoassays since the mid-1980s [15–17]. Depending on their formats, LFAs might be expressed as dipstick assay, lateral flow device (LFD), point of care (POC) to bedside test, and lateral flow immunochromatographic assay (LFIA). LFAs are used to detect the presence or absence of a target analyte in sample and allow naked-eye analyses based on accumulation concepts [18]. LFAs have many advantages compared to other detection methods. They are established mature technology, with processes already developed, relative ease of manufacture, and stable shelf lives of 12–24 months often without refrigeration; easily scalable to high-volume production; and integrated with various systems, having high sensitivity, specificity, relatively low cost, market presence, and minimal education required for users and regulators [19]. However, test-to-test reproducibility, unclear patent situation, sensitivity issues in some systems, and integration with onboard electronics are drawbacks of LFAs. To note LFA market is expected to reach USD 8.7 billion by 2023 from an estimated USD 6.0 billion in 2018, at a compound annual growth rate (CAGR) of 7.7% [20].

The production of typical strip assay includes the preparation of colloidal gold conjugates, application of reagents onto the membrane and pads, lamination of the strip membranes onto a support backing, cutting the prepared master cards into strips of defined length and width, and strip packaging (**Figure 1A** and **B**).

Three types of pads, a sample pad, conjugate pad, and absorbent pad, and nitrocellulose membrane are used for developing the strip assay. The test sample is applied onto the sample pad. Conjugate pad contains Abs, aptamers, or nucleic acids specific to the target analyte which are usually conjugated to colored particles, gold nanoparticles (GNPs), and latex beads. Capture reagents such as anti-target Abs or aptamers are immobilized in a line across the membrane which are nitrocellulose or cellulose acetate as a test line. It has also a control line containing capture reagents such as Abs or complementary nucleic acids specific for the conjugate Abs or aptamers present on the conjugate, respectively. The strip components are usually fixed to an inert backing material and may be placed in a plastic casing with a sample port, and reaction window showing the test and control line or strip can be prepared as a simple dipstick format [22]. After soaking of sample pad with analyte, it flows through the conjugate pad and nitrocellulose membrane via capillary action and ends on an absorbent pad. When the flow is continuing, the analyte bound by gold conjugate on conjugate pad is captured and accumulated on test line. The excess conjugate is also captured by a control line, and it should always be visible. If the test strip works correctly and it is positive, both the test and control lines are seen as red. If no colored capture lines or only a red color at the test line appears, the strip is invalid, and the test should be repeated [21, 23, 24] (**Figure 2**).

Although LFAs for *Salmonella* are commonly noncompetitive, the competitive format of LFA can also be developed for the smaller analytes [25, 26]. The principle of this

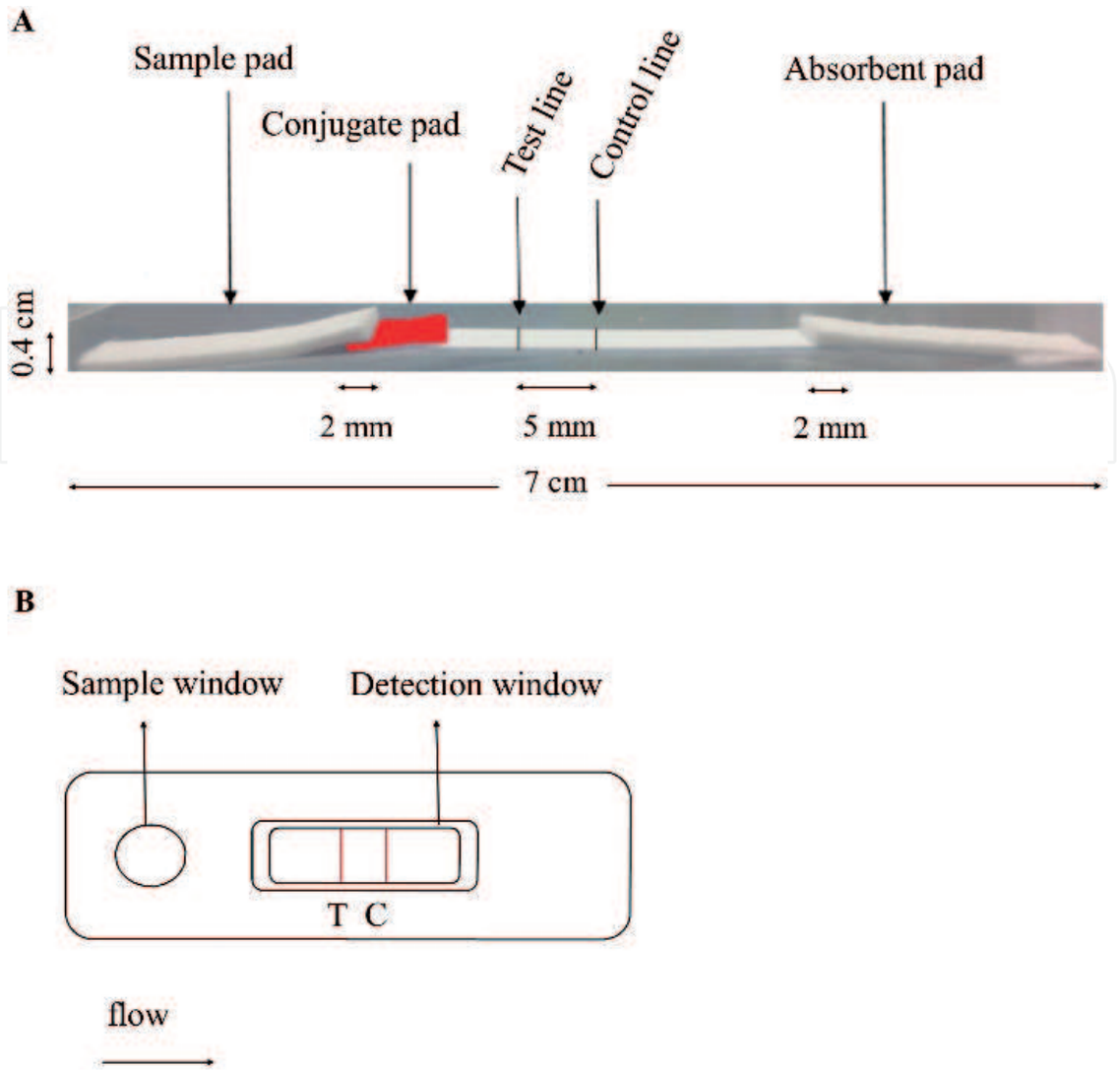

Figure 1.
Preparation of LFA strip (a) and schematic representation of it in plastic case [21].

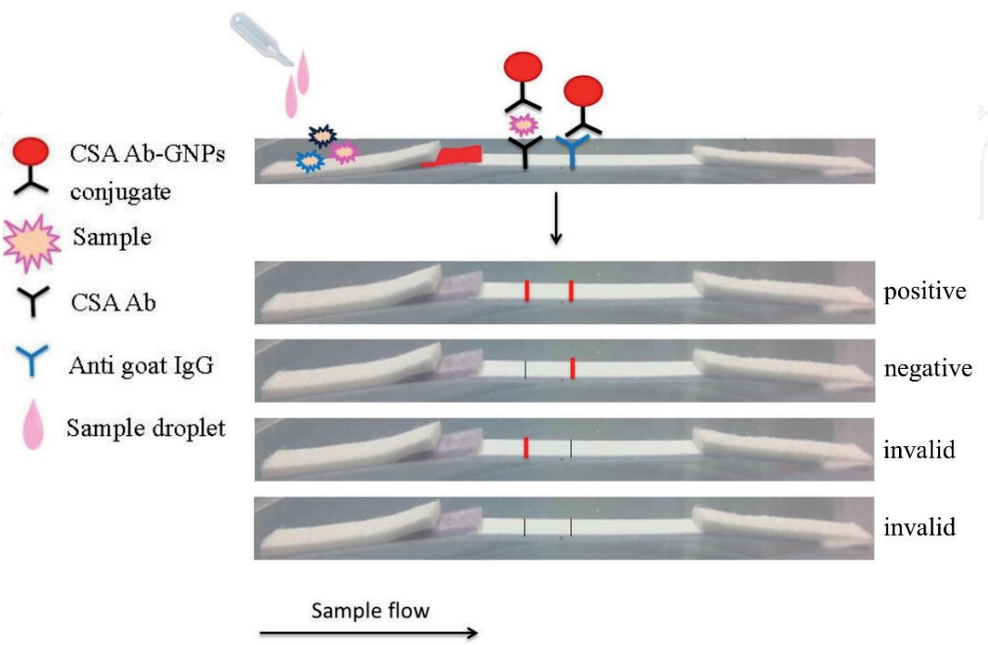

Figure 2.
Schematic diagram of the immunochromatographic test strip principle for the dipstick assay. CSA Ab, common structural antigenic antibody; GNPs, gold nanoparticles [21].

format is that sample extract is applied onto the sample pad and it flows through the absorbent pad. If the analyte is absent, all of the detection reagent would be trapped by capture reagent to form an easily visible test line. However, when the analyte is present, it competes with the immobilized capture reagent for the limited amount of competitive detection reagent. Thus, it means that the more analyte in the sample, the weaker the test line color.

Manufacturing of LFAs developed for *Salmonella* depends on some significant factors. First is the membrane type having suitable flow rate because it plays significant role for recognizing the whole bacteria cell. For instance, nitrocellulose Millipore membrane, Hi-Flow™ Plus 180 Membrane Card, shows good assay performance in terms of the whole-cell detection, analysis time, no background signal, and immobilization of capture reagents such as Abs and aptamers. If the flow rate of membrane is very slow, it takes a long time to see the results with the naked eye. However, if flow is very fast, capturing process cannot be completed and line intensities become unclear. Second is the application of analyte on sample pad. Both the dipstick assay and loading of sample as droplets can be preferred. The disadvantage of dipping the strip into bacterial media directly is that flow can be retarded on conjugate pad because of the media components. Thus, using the suitable running buffer, simple phosphate buffer saline (PBS), may enable conjugate to flow through the strip test, efficiently [21, 27]. Third is the immobilization of capture reagents. Generally streptavidin-biotin interaction is used for aptamer- or nucleic acid-based LFAs, and it sometimes may be required as multiple loading with consistent durations, while Abs can be directly immobilized once. The last one is the size of GNPs used for labeling. Although various studies showed that the size of GNPs between 15 and 40 nm can be used in LFAs, 35 nm GNPs are preferable. **Figure 3** displays the Ab-based LFAs for *Salmonella* when they are alone and present in bacterial mixture after optimal conditions are performed [27]. Strips were prepared by common structural Ab specific to *Salmonella enterica* species (*S. enteritidis*, *S. typhimurium*,

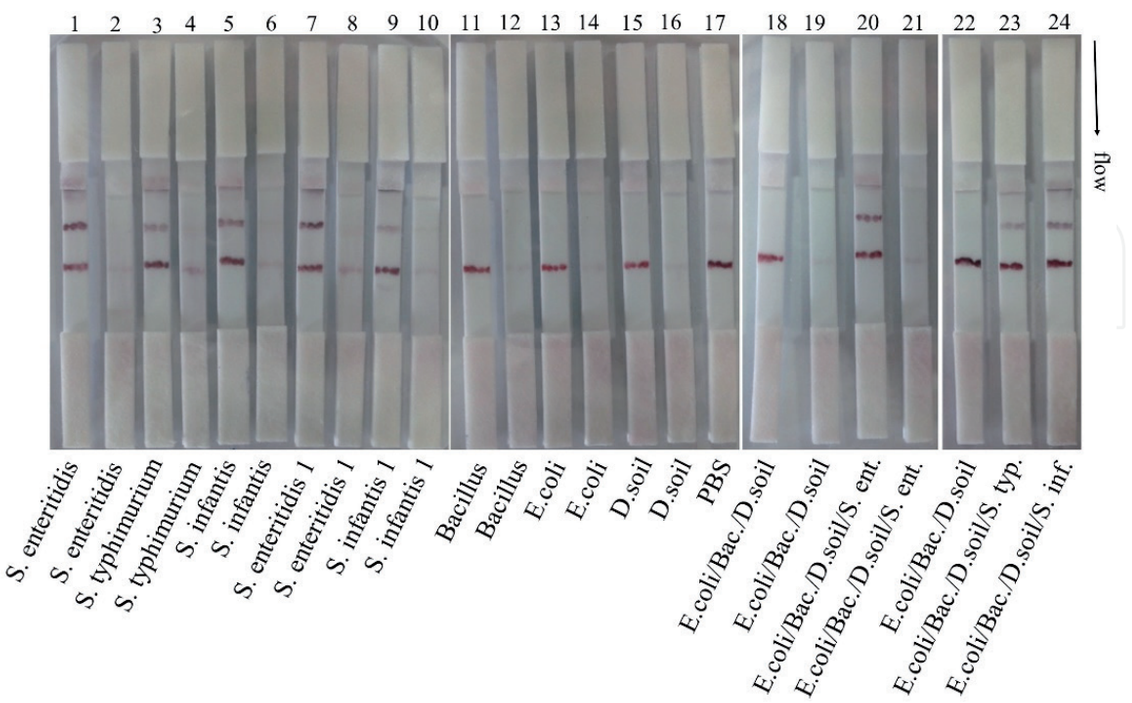

Figure 3.
Dipstick assay for live Salmonella *strains alone and in the mixture using M180 membrane. Test line:* Salmonella *Ab. Control line: Antigoat IgG. The strips were dipped into 200 μL of total bacteria with PBS. The strips 2–4–6–8–10–12–14–16–19–21 were prepared by naked GNPs. D. soil, dry soil bacteria; Bac,* B. cereus*; S. ent,* S. enteritidis*; S. typ,* S. typhimurium *[27].*

S. infantis). 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 102 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 10^1 colony forming unit (cfu) 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

	Detection	Detection reagent	Detection limit	Detection type	Refs.
S. typhimurium	LPS	Ab	-	Multiple	[29]
S. typhimurium	Whole cell	Ab	10^2 cfu/mL^{-1}	Single	[50]
Salmonella typhi	Whole cell	Ab	3×10^8 cfu	Single	[30]
S. typhimurium	16S ribosomal RNA and DNA	Nucleic acid-Ab	10^4 cells	Single	[51]
S. pullorum	Salmonella invA gene	Nucleic acid-Ab	89 fg/μl	Single	[52]
S. typhimurium	*Salmonella enterica* yfiR(375 bp) gene	Nucleic acid-Ab	0.75 pgμL^{-1}	Single	[49]
S. enteriditis	Out membrane of *S. enteritidis*	Aptamer	10 cfu mL^{-1}	Single	[37]
S. typhimurium	Whole cell	Aptamer	85 cfu mL^{-1}	Multiplex	[36]
S. typhimurium	Whole cell	Aptamer	-	Single	[35]

Table 1.
LFAs for Salmonella *detection by Ab, aptamer, and nucleic acids.*

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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Conflict of interest

The author has read and approved the paper. The author agrees to the contents of the paper and has no conflict of interest.

Author details

Dilek ÇAM
Department of Biology, Faculty of Science, Çankırı Karatekin University, Çankırı, Turkey

*Address all correspondence to: cam.dilek@gmail.com

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