Analytica Chimica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

A sensitive lateral flow biosensor for *Escherichia coli* O157:H7 detection based on aptamer mediated strand displacement amplification

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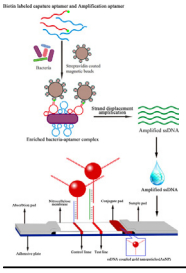
HIGHLIGHTS

- Limit of detection as low as 10 CFU mL^{-1} *Escherichia coli* O157:H7.
- No need of antibodies and substituted with aptamers.
- Isothermal strand displacement amplification for signal amplification.
- Results observed by the naked eye.
- Great potential application in the area of food control.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 May 2014
Received in revised form 15 December 2014
Accepted 17 December 2014
Available online xxx

Keywords:
Lateral flow biosensor
Escherichia coli O157:H7
Aptamer
Isothermal strand displacement amplification

ABSTRACT

Foodborne diseases caused by pathogens are one of the major problems in food safety. Convenient and sensitive point-of-care rapid diagnostic tests for food-borne pathogens have been a long-felt need of clinicians. Commonly used methods for pathogen detection rely on conventional culture-based tests, antibody-based assays and polymerase chain reaction (PCR)-based techniques. These methods are costly, laborious and time-consuming. Herein, we present a simple and sensitive aptamer based biosensor for rapid detection of *Escherichia coli* O157:H7 (*E. coli* O157:H7). In this assay, two different aptamers specific for the outmembrane of *E. coli* O157:H7 were used. One of the aptamers was used for magnetic bead enrichment, and the other was used as a signal reporter for this pathogen, which was amplified by isothermal strand displacement amplification (SDA) and further detected by a lateral flow biosensor. Only the captured aptamers on cell membrane were amplified, limitations of conventional DNA amplification based method such as false-positive can be largely reduced. The generated signals (red bands on the test zone of a lateral flow strip) can be unambiguously read out by the naked eye. As low as 10 colony forming units (CFU) of *E. coli* O157:H7 were detected in this study. Without DNA extraction, the reduced handling and simpler equipment requirement render this assay a simple and rapid alternative to conventional methods.

1. Introduction

Over the past decades, the global incidence of foodborne infections has markedly increased, which pose a great threat to public health. The World Health Organization estimates that

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http://dx.doi.org/10.1016/j.aca.2014.12.041
0003-2670/

foodborne diarrheal diseases together kill around 2.2 million people annually [1]. According to the Center for Disease Control and Prevention (CDC) in USA, a total of 19,531 infections, 4563 hospitalizations, and 68 deaths associated with foodborne diseases were reported in 2012 [2,3]. Among these infected foodborne pathogens, *Escherichia coli* O157:H7 (*E. coli* O157:H7) is one of the most harmful ones that can cause serious foodborne illnesses, even deaths. There have been several outbreaks of foodborne illnesses caused by consumption of food containing *E. coli* O157:H7 from 1996 to now [4,5]. Significant improvements of disinfection in food processes have been achieved, such as the rigorous adherence to good manufacturing practices and good agricultural practices, but the outcome of *E. coli* O157:H7 control is still not optimistic [6,7]. Thus, accurate and sensitive methods to detect *E. coli* O157:H7 in food products are extremely necessary to prevent catastrophic outbreaks caused by public consumption.

Conventional culture-based methods for *E. coli* detection, involving enrichment, isolation and detection steps, have been used due to their sensitivity, low cost and ability to monitor cell viability [8]. However, they require 3 days to obtain confirmatory results which cannot meet the needs of real-time detection [9]. Therefore, rapid methods, including polymerase chain reaction (PCR) based assays, isothermal amplification assays and antibody based immunoassays, have been developed for *E. coli* detection, which have good sensitivity and specificity [10]. However, PCR based methods require expensive equipment, skilled personnel and cannot differentiate viable cells from dead cells. DNA from dead cells can lead to false-positive PCR results, leading to unnecessary product recalls and economic losses [11]. Antibody based immunoassays such as enzyme-linked immunosorbent assay (ELISA) depends on antibody for *E. coli*, which is expensive, sensitive to matrix effect, and difficult to store and transport. Meanwhile, the low sensitivity is still a major limitation for ELISA because of the use of the colorimetric detection [12].

Magnetic beads (MBs) are an ideal platform candidate to eliminate the need for pretreatment steps and the matrix effects from samples. Recently, magnetic separation has enabled the development of new diagnostic platforms for the determination of bacteria, proteins, nucleic acids and drugs, allowing greater sensitivity and swifter detection [13–17].

Aptamers are synthetic single-stranded oligonucleotides – either DNA or RNA – that can fold into a three-dimensional structure to bind specifically to a variety of targets, such as proteins, organic molecules, various cell surface receptors, and whole cells [18,19]. Due to their high specific affinity, they are similar to antibodies having low dissociation constants of nanomolar even picomolar level. Aptamers have several unique characteristics that make them more attractive in the fields of biosensor, diagnostics, therapeutics, and bio-analytical applications than antibodies [20–23]. They are inexpensive, stable, and can be chemically synthesized in high purity and modified with relative ease. Aptamers are also ideal tools for the detection of food-borne pathogens, especially bacteria in environmental or food samples [24–26]. A few studies have reported several aptamers for *E. coli* O157:H7, which can be used in *E. coli* O157:H7 detection [27].

Lateral flow biosensor (LFB), also called a dipstick test strip, offers a promising approach to realize point-of-care DNA detection. We have developed different kinds of LFB for the visual detection of nucleic acids, metal ions and stem cells [28–32]. These sensors are sensitive, specific, easy to use, and does not require the use of complex and expensive instrumentation. Most importantly, the results of LFB can be observed by the naked eye.

In this study, we developed an aptamer based biosensor for simple and rapid detection of viable *E. coli*. Two aptamers against different outermembrane proteins of *E. coli* were used in the detection [33]. Biotin modified capture-aptamer (c-aptamer) was used for the bacteria enrichment by using streptavidin modified MBs. Another unmodified amplification-aptamer (a-aptamer) against another outermembrane protein was used as template for strand displacement amplification (SDA). The amplified single strand DNA was further detected with a lateral flow biosensor (Fig. 1). Thus, a simple and reliable biosensor for pathogen diagnosis was established.

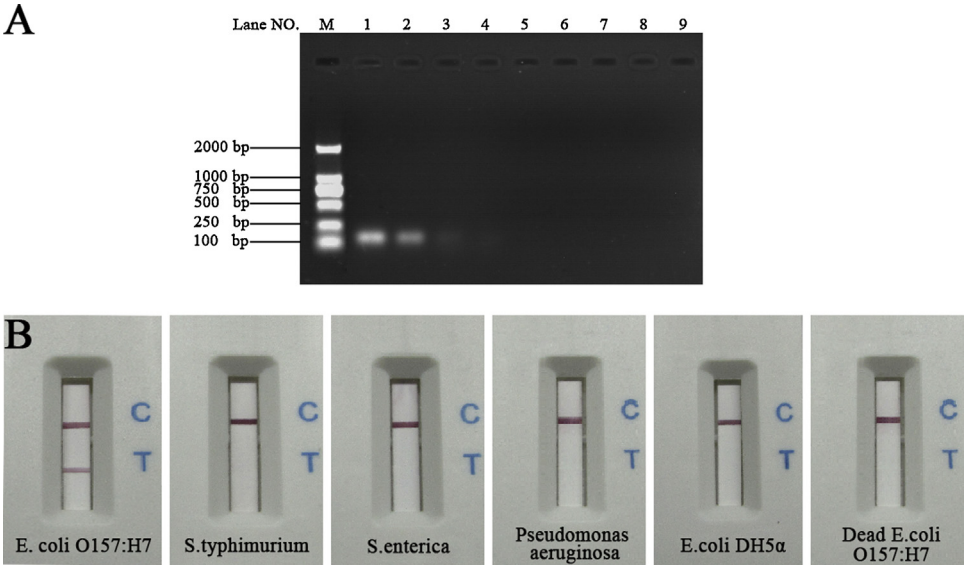

Fig. 1. Verification for *E. coli* O157:H7 detection. (A) Agarose gel electrophoresis detection of *E. coli* O157:H7. Lane M is the molecular marker; Lane 1–4 are 10 fold dilutions of *E. coli* O157:H7 ranging from 1×10^4 CFU mL^{-1} to 10 CFU mL^{-1}; Lane 5 is the negative control; Lanes 6–9 are different strains of 10^4 CFU mL^{-1}, *S. typhimurium*, *S. enterica*, *Pseudomonas aeruginosa*, *and E. coli* DH5α successively. (B) Typical images of the biosensor for the detection of different strains of pathogens and dead *E. coli* O157:H7 (10^4 CFU mL^{-1}). The viable *E. coli* in a water bath of 100 °C for 30 min to obtain dead bacterial.

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2. Experimental

2.1. Materials

A portable strip reader (DT1030) was purchased from Shanghai Goldbio Technology Co. (Shanghai, China). Bovine serum albumin (BSA), trisodium citrate, and $HAuCl_4$ were purchased from Sigma–Aldrich (Steinheim, Germany). Streptavidin (SA)-modified magnetic beads and magnetic rack were purchased from Invitrogen (USA). The polymerase Klenow fragment exo$^-$ and dNTPs were purchased from New England Biolabs. Glass fiber, cellulose fiber sample pads, laminated cards, and nitrocellulose membranes were purchased from Millipore (Billerica, MA). All buffer solutions used in this study were prepared in our laboratory with ultrapure water (resistance of 18 MΩ/cm). All the oligonucleotides used in the present study were synthesized and purified with HPLC by Sangon Biotechnology Co. (Shanghai, China) and used without further purification. The oligonucleotide sequences used were listed in Table 1. Strains of *E. coli* O157:H7, *Salmonella typhimurium*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and DH5α were obtained from Guangzhou microbiology institute. All other reagents were purchased from standard commercial sources and were of analytical grade.

2.2. Bacterial culture

Strains were cultured aerobically for 16 h at 37 °C in 50 mL of Luria-Bertani (LB) medium (final OD > 1 for each sample). For colony formation test, bacteria were incubated at 37 °C for 30 min. 50 μL of the bacteria suspension were diluted and plated on dishes with LB agar in triplicate. The dishes were incubated at 37 °C for 24 h to allow bacterial growth. The colonies were then counted. Colony forming units (CFUs) were counted as 1.72×10^9 CFU mL^{-1} in 0.01 mol L^{-1} PBS buffer (pH 7.4) containing 0.1% NaN_3, and the colonies were stored at 4 °C until use.

2.3. Preparation of gold-nanoparticle (AuNP)–DNA conjugates

AuNPs (average of 15 nm) were prepared according to the literature with slight modification [34]. Briefly, in a 250-mL round-bottom flask, 4 mL of 1% trisodium citrate was added to 100 mL of rapidly stirred and boiling $HAuCl_4$ (10 mg). The color of the solution turned deep blue in 20 s and then turned wine red in 60 s. The solution was kept boiling for approximately 10 min and then was returned to room temperature by natural cooling to form AuNPs. The resulting AuNPs solution was stored at 4 °C and was used for the preparation of the AuNPs–DNA conjugates.

To prepare AuNP–DNA conjugate, 100 μL of ultra-pure water (18.2 MΩ cm^{-1}, Millipore, Billerica, MA, USA) containing 2.5 nmol of thiolated DNA was added to 1 mL of the AuNPs solution (4 times concentrated) and shaken gently overnight at 4 °C. The DNA coated AuNPs (1.1 mL) were subjected to "aging" by adding 110 μL 100 mM phosphate buffer (pH 7.0) with 1% sodium dodecyl sulfate and 1.5 M NaCl. The solution was kept at 4 °C for 12 h [35]. Particles were centrifuged (12×10^3 rpm, 20 min) and rinsed three times with rinsing buffer (20 mM Na_3PO_4, 5% BSA, 0.25% Tween-20 and 10% sucrose) to remove any unbound DNA. The red pellet was re-suspended in 150 μL of rinsing buffer and then stored in a refrigerator at 4 °C until use. The AuNP–DNA conjugate solution was dispensed onto fiberglass as conjugate pad.

2.4. Construction of lateral flow biosensor

The LFB for *E. coli* O157:H7 detection is fabricated as the schematic diagram shown in Scheme 1B. The biosensor consists of three components: sample pad, NC membrane, and absorbent pad. Then it was dried and stored in a low-humidity chamber at room temperature. Thirty microliters of 100 μM DNA probes (test line probe and control line probe) were dispensed onto the nitrocellulose membrane simultaneously with a lateral flow dispenser to form a test line and control line (Shanghai Kinbio, Shanghai, China), respectively. The distance between the test line and the control line was approximately 5 mm. The membrane was then dried at room temperature for 12 h and stored at 4 °C. Finally, the three components of the LFB were assembled on a plastic adhesive backing (60 mm × 30 cm). Each part overlapped 2 mm to ensure the migration of sample solution through the biosensor during the assay. Strips with a 3 mm width were cut by using a paper cutter (programmed high-speed cutter, Shanghai Kinbio, China).

2.5. Procedure for E. coli O157:H7 detection

Bacteria (10^4 CFU) were first mixed with excess amount of aptamers in a 500 μL of phosphate buffered saline (PBS) supplied with 0.1 mg mL^{-1} of salmon sperm DNA. The ultimate concentration of each aptamer was 1 μM. The mixture was incubated at room temperature for 30 min. Ten microliters of streptavidin coated magnetic beads (Invitrogen, M-270 Streptavidin) were then added to the mixture. The mixture was incubated at room temperature for another 10 min with intermittent shaking. After incubation, the biotin modified c-aptamer was bound to streptavidin coated magnetic beads (MNB-Ap-bacteria), and then collected by a magnet separator. The precipitant was transferred to another tube and washed three times with PBST (PBS supplied with 0.05% Tween-20). The collected MNB-Ap-bacteria complexes were suspended with 10 μL of ultrapure water.

For the aptamer based detection, 5 μL of previously enriched bacteria suspension was added to 15 μL of reaction solution (1 μM primer, 1 U BbvCI, 5 U polymerase Klenow fragment exo$^-$, 50 μM dNTPs, and 1 × polymerase Klenow fragment exo$^-$ reaction buffer). It was incubated at 50 °C for 30 min. After amplification, 10 μL of the product was diluted in 50 μL of 4 × saline-sodium citrate (SSC) and loaded onto the sample pad in the biosensor. Test line and control line in biosensor were visually determined in 5 min.

Table 1
Sequence used in this work.[a,b]

Oligonucleotide	Sequence (5′-3′)
a-Aptamer	ATCAAATGTGCAGATATCAAGACGATTTGTACAAGATCCATGCTGAGG**TGGTCATAGCTGATCCTACC**
c-Aptamer	TTTCCGGACGCTTATGCCTTGCCATCTACAGAGCAGGTGTGACGG-Biotin
Primer	GGTAGGATCAGCTATGACC
T-line	CGATTTGTACAAGATCCATGCTGACGATTTGTACAAGATCCATGCTGA
C-line	CTTGATATCTGCACATTTGATCTTGATATCTGCACATTTGAT
AuNP probe	SH-TTTTATCAAATGTGCAGATATCAAG
Forward primer	CGGACATCCATGTGATATGG
Reverse primer	TTGCCTATGTACAGCTAATCC

[a] Solid blue underlines indicate the recognition sequence of Nt.BbvCI.
[b] Bold characters indicate the additional primer sequence.

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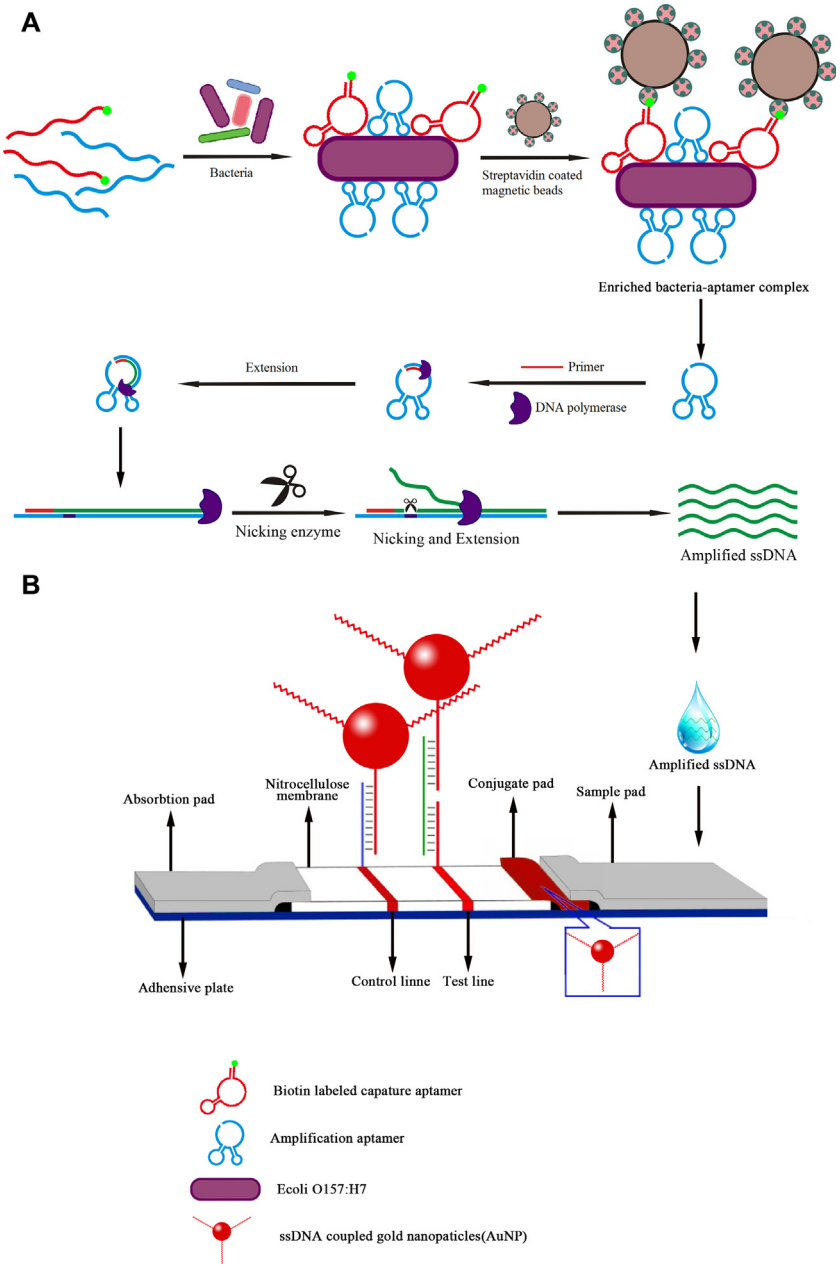

Scheme 1. Schematic illustration of the aptamer-based lateral flow biosensor for *E. coli* O157:H7 detection.

For quantitative detection, the biosensor was scanned using a handheld strip reader (Shanghai Kinbio). The optical intensity of the test line and control line could be recorded simultaneously by using the Shanghai Kinbio strip reader software. To define a positive result, we used the signal/noise (S/N) ratio (S/N $\geq$ 3) as reference. Signal is the optical intensity of the test line with detected sample, and noise is the intensity of the test line with PBS.

The performance of this method was also tested using bacteria spiked food products. Milk powder obtained from local supermarket was analyzed. Ten grams of milk powder was mixed with 100 mL of PBS. The confirmed *E. coli* O157:H7 negative milk was spiked with target bacteria and analyzed immediately. Briefly, 50 μL of target bacteria cell suspension was added into 950 μL of milk, mixed thoroughly, and centrifuged at 500 × *g* for 5 min to remove insoluble particles. Target bacteria in the supernatant were detected as described above. The recovery of this assay was equal to the ratio between the number of detected bacteria and the number of actually added bacteria.

2.6. PCR/electrophoresis

E. coli O157:H7 DNA was detected using a 5334 PCR system (Eppendorf, Germany) in a 25.0 μL mixture containing 2.0 μL of *E. coli* bacterial DNA (0.2 nM), 2.5 μL of 10× PCR buffer, 2.0 μL of dNTP (0.2 mM), 0.5 μL of both forward and reverse primers (0.2 μM), 0.5 μL of Taq DNA polymerase (2 units), and 17.0 μL of double-distilled water. The samples were subjected to 30 PCR cycles, each consisting of 5 min of initial denaturation at 95 °C, 30 s of denaturation at 95 °C, 40 s of annealing at 60 °C, and 20 s of elongation at 72 °C. The samples were stabilized at 72 °C for 8 min. Finally, 5 μL of PCR products were diluted with 6 × loading buffer (Takara) and applied to pre-made 1% (w/v) agarose gel containing ethidium bromide. Each gel was electrophoresed for 20 min at a constant 100 V with running buffer (1 × TAE). Marker (5 μL) was included in the first lane of the gel. The results of electrophoretogram were visualized and photographed by a UV transillumination (Tanon 1600).

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2.7. Statistical analysis

SPSS 10.0 software (SPSS Inc., IL, US) was used for statistical analysis. The results were expressed as means ± SD of three independent experiments. Individual comparisons were made by Student's *t*-test for paired data and *P*-values less than 0.05 were considered to be significant.

3. Results and discussion

3.1. Principle of LFB for E. coli O157:H7 detection

Two aptamers, capture-aptamer (c-aptamer) and amplification-aptamer (a-aptamer), were used in this assay. The capture-aptamer was pre-labeled with biotin moiety, which was captured by streptavidin coated magnetic beads for enrichment. The two aptamers were first incubated with pathogens. Then streptavidin coated magnetic beads were added into the mixture. The magnetic beads were washed for three times, then the enriched a-aptamer on magnetic beads that anchored on the outermembrane of *E. coli* O157:H7 was used as template for single direction strand displacement amplification (SDA). An SDA reaction, comprising primer, DNA polymerase and Nicking enzyme, is initiated by the enriched a-aptamer. The primer anneals with the a-aptamer and triggers a polymerization reaction in the presence of dNTPs and polymerase Klenow exo$^-$. During primer extension, the synthesized complementary strand is cut by Nicking enzyme at the Nt.BbvCI recognition site of 3′ of a-aptamer. The cut strand is then dissociated and the annealed primer initiates another round of polymerization. Through this cyclical process, a large number of free amplified single strand DNA (ssDNA) are produced and further detected with a lateral flow biosensor (Scheme 1A).

As shown in Scheme 1B, the SDA products ssDNA are mixed with running buffer and migrate along the LFB by capillary action to the conjugate pad, where probe–AuNP conjugates have been deposited. The ssDNA are captured by probe–AuNP conjugates through the complementary reaction between the probe on the AuNP surface and ssDNA to form a product–probe–AuNP complex. Nucleic acid in test zone was complementary to the amplified products ssDNA. The complexes are captured at the test line by complementary reaction between the complex and DNA immobilized on the test line. The accumulated AuNPs in the test zone are visualized as a characteristic red band. As probe on AuNP is complementary to DNA immobilized on the control line, excess probe–AuNP conjugates continue to migrate and are captured at the control line by the specific reaction between the complex and DNA on the control line, thus forming a second red band. In the absence of target *E. coli* O157:H7, no a-aptamer (SDA template) is enriched by magnetic beads; therefore, no free ssDNA is synthesized, and no probe–AuNP conjugate is captured, and no red band is observed at the test zone. In this case, the observation of a red band at the control zone indicated that the LFB is working correctly. Thus, a simple, reliable, point-of-care rapid diagnostic tests for pathogen diagnosis was established.

3.2. Validation of LFB for E. coli O157:H7 detection

Firstly, we verified the bacterial strains by PCR. Fig. 1A shows the corresponding electrophoresis images of the PCR products. The amplified band was 250 bp of the IS200 insertion sequence which is specific for *E. coli* O157:H7 [36]. No positive band was observed in the absence of *E. coli* O157:H7. In order to validate this LFB for pathogen detection, we challenged it with 10^4 CFU mL^{-1} of *E. coli* O157:H7 and other types of pathogen strains and dead *E. coli* O157:H7. After amplification, the sample was loaded onto the sample pad. Fig. 1B shows the typical photo images of the biosensor. The test zones observed for *S. typhimurium*, *S. enterica*, *P. aeruginosa*, *E. coli* DH5α, and dead *E. coli* O157:H7 were similar with blank

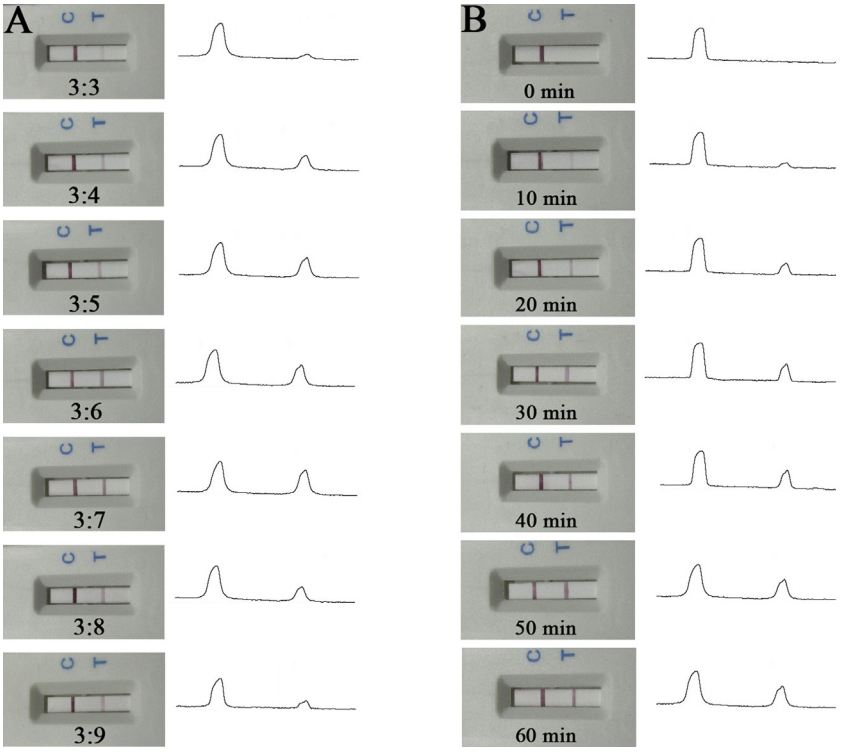

Fig. 2. Optimization of experimental conditions. (A) Typical images of biosensor for *E. coli* detection with different concentrations of c-aptamer, with a-aptamer of 1 μM. From top down, the ratios of a-aptamer/c-aptamer are 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:9. (B) Typical images of biosensor for *E. coli* detection with different amplification time. From top down, the amplification time is from 0 to 60 min.

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negative control. The distinctive red test line could only be observed in the presence of *E. coli* O157:H7. To further confirm the test line signal was obtained from SDA of a-aptamer, *E. coli* O157:H7 without a-aptamer was tested with this LFB. As expected, no test line was observed in the absence of template a-aptamer. The PCR result was consistent with that of LFB, only a unique positive band was produced in the presence of viable *E. coli* O157:H7. The results suggested that this biosensor works in *E. coli* O157:H7detection with high specificity.

3.3. Optimization of experimental conditions

The a-aptamer acts as template for signal amplification, and the c-aptamer is used for the enrichment of the a-aptamer. Therefore, the molar ratio of a-aptamer/c-aptamer in the system determines the initial number of template and therefore the sensitivity of the assay. In the present study, the two aptamers with different proportions were tested for the optimal performance of the assay. The brightest test line was obtained when the ratio of a-aptamer/c-aptamer reached 3:7 (Fig. 2A). The amount of a-aptamer bonded to the *E. coli* O157:H7 decreased with the increased proportion of c-aptamer. Therefore, less target DNA was produced due to the fewer amount of template. On the other hand, the lower proportion of c-aptamer could not provide enough magnetic force to pull down the bacteria efficiently. Hence, the inefficient enrichment caused weaker signal on the test line. Accordingly, a-aptamer/c-aptamer at 3:7 was used in the following experiments.

The reaction time of SDA determines the total amount of SDA product ssDNA and further the sensitivity of the assay. The SDA mixture was incubated in water bath of 37 °C for different amount of time, including 10, 20, 30, 40, 50, 60 min. The performances of different amplification time are shown in Fig. 2B. It was found that the intensity of test lines increased gradually at the first half hour, and remained stable at the second half hour. This may due to that the amplification of SDA reached a plateau or the run out of amplification materials for 30 min. Accordingly, 30 min for SDA was chosen for sensitivity experiments.

3.4. Sensitivity of LFB for E. coli O157:H7 detection

The sensitivity of the LFB was examined by using *E. coli* O157:H7 at different concentrations. Firstly, the *E. coli* O157:H7 was dissolved in PBS at seven concentrations as follows: 10^8 CFU mL^{-1}, 10^6 CFU mL^{-1}, 10^4 CFU mL^{-1}, 10^3 CFU mL^{-1}, 10^2 CFU mL^{-1}, 50 CFU mL^{-1}, 10 CFU mL^{-1}, 0 CFU mL^{-1}. For each test, magnetic beads with different enriched pathogens were added to 20 μL of SDA reaction solution. After incubation at 37 °C for 30 min, the SDA products were loaded onto the LFB. Fig. 3A presents typical images together with the corresponding optical responses of the biosensors when loaded with various amounts of *E. coli* O157:H7. No red band was observed at the test zone of the LFB in the absence of *E. coli* O157:H7 (negative control). Red bands were observed at the test zone when using concentrations of *E. coli* O157:H7 as low as 10 CFU mL^{-1}, which can be considered as the limit of detection. This result suggested that the developed lateral flow biosensor had higher sensitivity and was superior to the previously reported methods for the *E. coli* O157:H7 measurement (1.2×10^3 CFU mL^{-1} in chemiluminescence immunoassay, 136 cells in flow-through DNA microarray assay) [12,36–38]. Well-defined peaks were observed, and the peak areas increased as the *E. coli* O157:H7 concentration increased. The resulting calibration plot of the peak areas versus the logarithm of pathogen number is linear over the 10 to 1×10^6 range, with an equation of $y = 65.233x + 10.408$ (Fig. 3B).

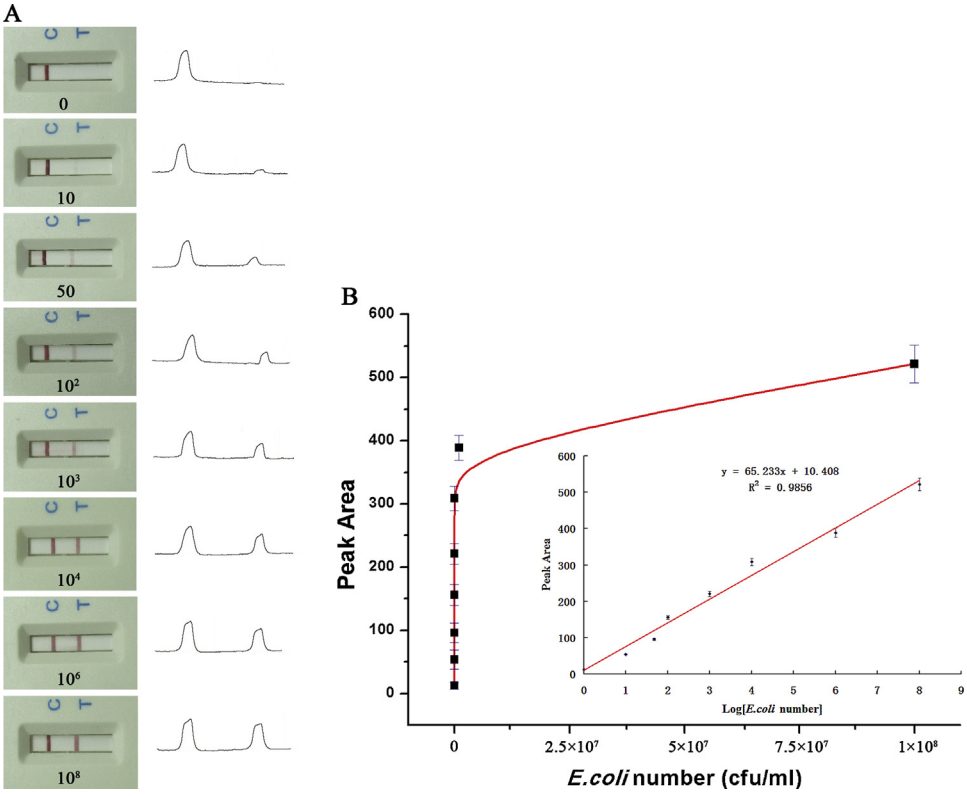

Fig. 3. Sensitivity of LFB for *E. coli* O157:H7 detection. (A) Typical images of biosensor for *E. coli* detection with different concentrations of *E. coli* O157:H7, ranging from 0 to 10^8 CFU mL^{-1}. (B) Calibration curve of the assay with different amounts of *E. coli* O157:H7. The peak areas were averages for three measurements.

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Table 2
Recoveries for several *E. coli* O157:H7-spiked real samples. Every sample was detected for 3 times with LFB, and recovery was equal to the ratio of found number/added number.

Sample number	Added (CFU mL^{-1})	Found (CFU mL^{-1})	RSD (%)	Recovery (%)
Milk 1	1.0×10^2	1.12×10^2	2.18	112
Milk 2	2.5×10^3	2.16×10^3	3.23	86.4
Milk 3	5.0×10^4	4.83×10^4	2.95	96.6
Water 1	1.0×10^2	1.01×10^2	3.15	101
Water 2	1.0×10^3	9.09×10^2	2.78	90.9
Water 3	1.0×10^4	1.08×10^4	2.49	108
Apple juice 1	1.0×10^2	1.11×10^2	3.24	111
Apple juice 2	2.5×10^3	2.38×10^3	2.96	94
Apple juice 3	5.0×10^4	4.813×10^4	2.74	96.2

RSD: relative standard deviation.

3.5. Real sample analysis

Commercially obtained milk, water and apple juice, which confirmed to be *E. coli* O157:H7-negative, was spiked with *E. coli* O157:H7 for recovery analysis using the LFB. All the *E. coli* O157:H7 spiked samples were detected as positive results and the blank sample had non-detectable *E. coli* O157:H7. Reliability was further evaluated by recovery experiments in real samples using the standard spiking experiment. The proposed method was used to detect *E. coli* O157:H7 in spiked samples (water, apple juice, and milk). *E. coli* O157:H7 concentrations were determined using the method described above. *E. coli* O157:H7 concentration in the samples was then analyzed using the proposed method (Table 2) and found to range from 86.4% to 112%, which was better than that of chemiluminescence assay (90–120%) [37]. The good recovery can be explained by the efficient separation by MBs, which can reduce loss of a-aptamer on MBs during the process. These results show that this method is applicable for the detection of *E. coli* O157:H7 in real food samples.

4. Conclusions

In conclusion, we developed an aptamer based biosensor for simple and sensitive detection of food-borne bacteria. It combined different rapid methods, including antibody-free enrichment of specific bacteria, and lateral flow biosensor based DNA detection. Furthermore, tedious DNA extraction and purification was avoided in this SDA-based isothermal amplification. Therefore, only the captured amplification-aptamer on cell membrane is amplified, limitations of conventional DNA amplification based method such as false-positive can be largely reduced. With reduced handling and simpler equipment, this assay provided simple and rapid alternatives to conventional methods. Moreover, this method could be applied to detect other bacteria by using different bacterium specific aptamers.

Acknowledgments

This work was supported by the Strategic Cooperation Project of the Chinese Academy of Sciences and Guangdong Province (2012B091100267) and Ministry of Science and Technology 973 Project (2013CB967100).

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Please cite this article in press as: W. Wu, et al., A sensitive lateral flow biosensor for *Escherichia coli* O157:H7 detection based on aptamer mediated strand displacement amplification, Anal. Chim. Acta (2014), http://dx.doi.org/10.1016/j.aca.2014.12.041