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Visual detection of nucleic acids based on lateral flow biosensor and hybridization chain reaction amplification

Na Ying[1], Chuanjing Ju[2], Zhongyi Li[1], Wensen Liu[1,*],Jiayu Wan[1,*]

1. Institute of Military Veterinary, Academy of Military Medical Sciences, Changchun, China
2. The Fourth Hospital of Jilin University

***Correspondence to**

Jiayu Wan; E-mail:wanjiayu@hotmail.com;

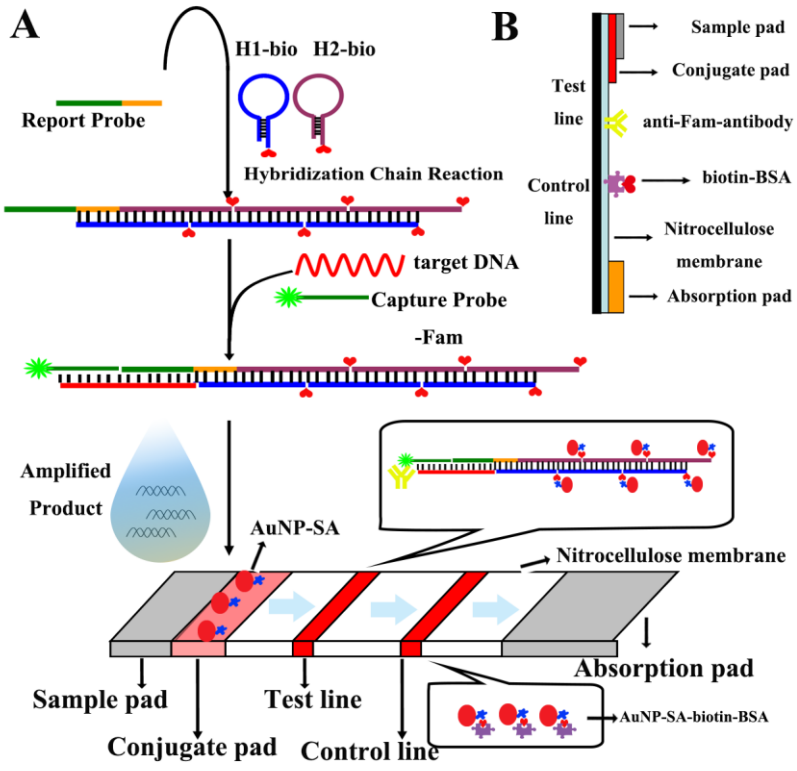
Wensen Liu; E-mail:Liuws85952@163.com

Abstract

In this study, a new lateral flow nucleic acid biosensor (LFNAB) using hybridization chain reaction (HCR) for signal amplification was developed for visual detection of nucleic acids with high sensitivity and low cost. A “sandwich-type” detection strategy was employed in our design. The sandwich system of capture probe (CP)/target DNA/reporter probe (RP)-HCR complexes was fabricated as the sensing platform. As the initiator strand, reporter probe propagated a chain reaction of hybridization events between the two hairpin probes modified with biotin, and determined whether long nicked DNA polymers were formed. The biotin-labeled double-strand

DNA polymers then introduced numerous Streptavidin (SA)-labeled gold nanoparticles (AuNPs) on the lateral flow device. The CP/target DNA/RP-HCR complexes were captured on the test zone by the specific reaction between anti-Fam monoclonal antibody (anti-Fam mAb) on the test zone and Fam of the complexes. The accumulation of AuNPs on the test zone of the biosensor enabled the visual detection of specific sequences. The detection limit of specific DNA was as low as 1.76 pM, which was about 2 orders lower than that of the LFNAB without HCR amplification. And the detection limit of *Salmonella* was 3×10^{3}cfu.mL^{-1} In conclusion, this visual detection system, HCR-LFNAB, is suitable for non-specialist personnel and point-of-care (POC) diagnosis in low-resource settings.

Graphical Abstract



In this study, a new lateral flow nucleic acid biosensor (LFNAB) using hybridization chain reaction (HCR) for signal amplification was developed for visual detection of nucleic acids with high sensitivity and low cost. A "sandwich-type" detection strategy was employed in our design. The sandwich system of capture probe (CP)/target DNA/reporter probe (RP)-HCR complexes was fabricated as the sensing platform. As the initiator strand, reporter probe propagated a chain reaction of hybridization events between the two hairpin probes modified with biotin, and determined whether long nicked DNA polymers were formed. The biotin-labeled double-strand DNA polymers then introduced numerous Streptavidin (SA)-labeled gold nanoparticles (AuNPs) on the lateral flow device.The accumulation of AuNPs on the test zone of the biosensor enabled the visual detection of specific sequences.

The detection limit of specific DNA was as low as 1.76 pM, the detection limit of *Salmonella* was 3×10^3cfu.mL-1

Key words: Lateral flow nucleic acid biosensor; Hybridization chain reaction; point of care; visual detection; 16S rRNA; *Salmonella*

1 .Introduction

Nucleic acid molecular diagnostics are gaining more attention due to their important roles in medical diagnostics, food safety analysis and environmental monitoring. The application of nucleic acid molecular diagnostic techniques often relies on laboratories with required instruments, cutting-edge equipments, and experienced technical personnel. Moreover the diagnostics are also costly. In developing and low developed countries where resources are relatively deficient, the application of these techniques is limited[1, 2]. As more and more foodborne bacteria

events occur in these third-world countries, the development of convenient, low-cost, portable point-of-care (POC) molecular diagnostic tools is essential. In recent years, the application of lateral flow nucleic acid biosensor (LFNAB) in biological analysis and clinical diagnosis has significantly increased, and is considered the most promising POC nucleic acid molecular diagnostic technique [3]. LFNAB has the advantages of short detection time, high cost-effectiveness, long-term stable storage and portability[4, 5]. In addition, the operation of LFNAB is relatively simple and does not require highly-skilled operators. Complicated analytical processes and expensive instruments are also unnecessary. Detection results are easy to judge by observing color intensity in the red band using the naked eye. The quantitative data can be gathered by a portable "strip reader" which can record optical response[6]. Importantly, visible analysis of LFNAB for target DNA avoids complex procedures such as pipetting, incubation, washing and data analysis[7]; therefore, LFNAB is applicable in non-laboratory environments without sufficient resources [8].

The limit of LFNAB in the detection of *Escherichia coli* 16S rRNA, without signal amplification, is 5×10^4 CFU mL^{-1} [9]. However, the amount of target nucleic acids in pathogens in biological samples is too low to be directly detected by LFNAB and the target nucleic acids need to be amplified for analysis. Polymerase chain reaction (PCR) can amplify the target nucleic acids to the amount that LFNAB can detect thereby the sensitivity increases significantly [9, 10]. PCR is extensively used in the amplification of nucleic acid materials and plays a critical role in academic research and clinical diagnosis; however, PCR requires thermal cycling instruments,

is time-consuming and often results in false-positive findings, which limits its application in some environments, especially as a tool in POC[11].

Dirks and Pierce, for the first time, developed an isothermal nucleic acid amplification technique, HCR, which is suitable for POC[12]. A pair of probes hairpin 1 and hairpin 2, which have a hairpin structure, are designed to target the DNA/RNA sequence (initiation sequence) and the target DNA/RNA specifically initiates hairpin 1 and hairpin 2 to assemble long double-strand DNA; thus the signal of target DNA/RNA is indirectly amplified. Compared to thermal cycling techniques such as PCR, HCR does not require enzymes and can react under isothermal conditions. HCR has been used to amplify nucleic acid molecules on the surface or inside living cells [13, 14]. The long double-strand DNAs need to be analyzed by a combination of other techniques. To date, many studies have reported on the analytical methods used for the long double-strand DNAs produced by HCR including colorimetric nucleic acid assays[15, 16], fluorescent nucleic acid assays[17-19], electrochemical nucleic acid assays[20-22], chemiluminescent [23], electrochemiluminescent [24], and bioluminescent nucleic acid assays[25], and surface enhanced Raman spectroscopy (SERS)[26]. However, no studies have used LFNAB in combination with HCR to detect target DNA/RNA. In the present study, we combined LFNAB with HCR to achieve visible detection of a specific fragment of *Salmonella* 16S rRNA. Our results suggest that not only did the detection sensitivity increase significantly (target DNA detection with a limitation of 1.76 pM, *Salmonella* detection with a limitation of 3×10^{3} cfu.mL^{-1}), but also the whole procedure was convenient and rapid.

2. Materials and methods

2.1 Reagents

All chemicals were of analytical reagent grade without further purification and used as received. Water was purified using a Millipore Milli-XQ system (Millipore, Bedford, MA, USA). DNA ladders were obtained from Takara Biotechnology Co. (Dalian, China). SA was purchased from Sangon Biotech. (Changchun, China). Colloidal AuNPs was purchased from Hualan Chemical Co., Ltd.(Shanghai, China). The anti-Fam antibody, bovine serum albumin (BSA)-biotin conjugate was purchased from Ruiqi Biotech Co., Ltd.(Shanghai, China). The target sequence of 47-mer DNA is a copy of the partial region of the Salmonella 16S rRNA gene (position 422–469). The sequences adopted from the literature are listed below (from 5′ to 3′, Table 1). All synthetic oligonucleotides (HPLC purified) were obtained from Shanghai Sangon Biological Engineering Technology (Shanghai,China), and were dissolved in sodium phosphate sodium chloride buffer solution (SPSC;150 mM Na_2HPO_4, 0.75 M NaCl, pH 7.4) to a storage concentration of 10 μM.

Table1. The sequences used in this assay.

Name	Abbreviation	Sequence (5′-3′)
Hairpin1-biotin	H1-bio	biotin-TTTTTTTTAACCCACGCCGAATCCTAGACT***CAAAGT***AGTCTAGGATTCGGCGTG
Hairpin2-biotin	H2-bio	AGTCTAGGATTCGGCGTG***GGTTAA***CACGCCGAATCCTAGACTACTTTGTTTTTT-biotin

Hairpin 1	H1	TTAACCCACGCCGAATCCTAGACT***CAAAGT***AGTCTAGGATTCGGCGTG
Hairpin 2	H2	AGTCTAGGATTCGGCGTG***GGTTAA***CACGCCGAATCCTAGACTACTTTG
Reporter probe	RP	ACCACAACACCTTCCTCCCCAGTCTAGGATTCGGCGTGGGTTAA
Detect probe	DP	ACCACAACACCTTCCTCCCCTTTTTT-biotin
Capture probe	CP	Fam-TTTTTTGGTAACGTCAATTGCTGCG
Target DNA	Target DNA	CGGGGAGGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACC

*In the hairpin sequences, loops are italicized and sticky ends are overlined

2.2 Native gel electrophoresis

H1-bio and H2-bio at the working concentration (diluted with SPSC buffer) of 1 μM were heated to 95℃ for 5 min and then allowed to cool to room temperature for 1 h before use. Ten microliters of different concentrations of RP were incubated with the equal volume of H1-bio/H2-bio at room temperature for 1 h. A gel stock solution (30%, m/v) was prepared from 29 g of acrylamide and 1 g of N, N-methylenebisacrylamide dissolved in 100 mL of purified water. The separating gel solution was prepared by mixing 3 mL of gel stock solution, 6.890 mL of 1×TBE buffer (45 mM Tris Borate, 1 mM EDTA, pH 8.3) , 100 μL of ammonium persulfate, and 10 mL of tetramethylethylenediamine. The native gel was run at 140 V for 1 hour with the load of 10 μL of each sample into the lanes, stained for 30 min with a solution containing 0.5 μg of ethidium bromide per mL, and viewed under UV light.

2.3 Test strip preparation

The lateral flow nucleic acid biosensor was designed, prepared and assembled

using the procedure described by the reference with some modifications[27]. SA was added to 20 nm AuNPs (0.01%, m/v) at a final concentration of 18 μg mL^{-1} to form colloidal gold particles-streptavidin (AuNPs-SA). The mixture was impregnated on the absorbent pad of the biosensor. The anti-Fam mAb (4 mg mL^{-1}) was coated on the surface of a nitrocellulose (NC) filter membrane at 1 μL/cm as the test line (T line) and BSA-biotin conjugate was coated on the surface of the nitrocellulose membrane at 1 μL/cm as the control line (C line). The concentration of BSA-biotin conjugate was 1.2 mg mL^{-1}. The LFNAB was assembled with the absorbent pad, the NC membrane (in the middle) and absorbent pad and was then cut by the dipstick into 0.5 cm wide, 5 cm long pieces. All the strips were sealed in a plastic bag and stored at room temperature until used.

2.4 Procedure for the detection of synthetic DNA

H1-bio and H2-bio were separately diluted to 1 μM, heated at 95℃ for 5 min, and then cooled to room temperature for 1 h. Then H1-bio/H2-bio probes were mixed with RP (0.2μM) at 1: 1:1 volume ratio and incubated at room temperature for 2 h for the HCR to form long nicked DNA polymers. Ten microliters of the above mixture was then mixed evenly with 10 μL of CP (0.3 μM) and 10 μL of target DNAs at different concentrations (0, 2.5pM, 25pM, 250pM, 2.5nM, 25nM, 250nM and 500 nM) to react at room temperature for 15 min to form the CP/target DNA/RP-HCR duplexes. In a typical DNA test using LFNAB, 100 μL of running buffer (25% saline sodium citrate (SSC) buffer containing 4% BSA) with the desired amount of CP/target DNA/RP-HCR duplexes was applied to the sample pad. During the assay,

the solution migrated up by capillary force. The results of LFNAB were read after 10 min. The appearance of visible reddish purple lines at both the control and test lines was considered positive detection of target DNA. A negative test result was indicated by the presence of a reddish purple line solely at the control line. For quantitative analysis, the signal strength of the test/control (T/C) lines (peak areas) of the LFNAB was measured using Image J software [28].

In the procedure of direct detection of target DNA without HCR,10 μL of DP, which is modified with biotin and the concentration of 1μM,was mixed with 10 μL of CP(0.3μM) and 10 μL of target DNA(the concentration gradient was as above : 0, 2.5pM, 25pM, 250pM, 2.5nM, 25nM, 250nM and 500 nM). Other steps were same to the amplified detection by HCR.

2.5. Bacterial culture and total RNA extraction

Salmonella enteritidis was cultivated for 18 h on a rotary shaker at 37 ℃ and 170 rpm in LB medium (5 g. L^{-1} NaCl, 10 g. L^{-1} peptone, 5 g .L^{-1} yeast extract, pH 7.0), For preparation of exponential growth phase cultures, overnight cultures were diluted in fresh growth medium and propagated as described above. Optical density at 600 nm (OD_{600}) was used for determination of colony forming units (cfu) according to a standardized growth curve, and cultures were diluted to the desired OD_{600} with the medium. *Salmonella* counts were determined by plating of decimal dilution series in 0.9% (w/v) NaCl on LB agar plates incubated for 24 h at 37 ℃.

Total RNA were extracted from the samples using the Bacteria Total RNA Purification Kit, obtained from Shanghai Sangon Biological Engineering Technology

(Shanghai, China). RNA quality and quantity was assessed using the Thermo Fisher Nanodrop UV–Vis Spectrophotometer and non-denaturing agarose gel electrophoresis. Samples were stored at -80℃ in RNase/ DNase-free water prior to use.

2.6 Procedure for the detection of 16S rRNA extracted from cell cultured *Salmonella enteritidis.*

Ten microliters of the target solution was mixed with 10 μL of CP (0.3 μM) at 95℃ for 2 min, then the mixture was mixed with 10 μL HCR products and incubated at room temperature for 15 min for hybridization. Other result readout steps were as the same as section 2.4.

3. Results and discussion

3.1 Principle of HCR-LFNAB measurement

The traditional sandwich assay model "capture/target/reporter" was employed in this study. As shown in Scheme 1(A), in the presence of target DNA, the sandwich DNA models were formed among CPs, target DNA and RPs. The carried initiator strands of RP pair with the sticky end of H1-bio underwent an unbiased strand-displacement interaction to open the hairpin. The newly exposed sticky end of H1-bio nucleated at the sticky end of H2-bio and opened the hairpin to expose the sticky end of H2-bio. This sticky end was identical in sequence to the initiator strands. Thus, each initiator strand on the sandwiched probes propagated a chain reaction of hybridization events between the alternating H1-bio and H2-bio hairpins to form a nicked double-helix. The CP/target DNA/RP-HCR complexes were finally formed.

Visual detection of the formed CP/target DNA/RP-HCR complexes was performed using the LFNAB. SA-AuNPs and anti-Fam mAb were pre-immobilized on the conjugate pad and test zone of the LFNAB (Scheme 1(B)), respectively. BSA-biotin was pre-immobilized on the control zone. The sample pad of LFNAB was dipped into the mixture of running buffer and the sample solution containing CP/target DNA/RP-HCR complexes. When the solution migrated by capillary action and passed the conjugate pad, it rehydrated the SA-AuNP conjugates. The numerous biotin-attached double-helix DNAs of the CP/target DNA/RP-HCR complexes reacted with SA on the AuNP surface to form CP/ target DNA/DP-HCR-biotin/SA-AuNP complexes and continued to migrate along the strip. The complexes were captured on the test zone by the specific reaction between anti-Fam mAb on the test zone and the Fam labeled capture probe of the complexes. The accumulation of AuNPs in the test zone was visualized as a characteristic red band. The excess SA-AuNP conjugates continued to move and were captured on the control zone by reactions between biotin and SA on the AuNP surface, thus forming a second red band. The presence of target DNA was determined by the color of the test line, and the semi-quantitative detection of target DNA was based on the color depth of the detection line. In the absence of target DNA and in the presence of mutant DNA and non-complementary DNA, there were no CP/target DNA/RP-HCR complexes in the sample solution; therefore, no SA-AuNP conjugates were captured on the test zone of the LFNAB, and no red band was observed in the test zone. A red band (control line) on the control zone showed that the LFNAB was working correctly.

Scheme 1

3.2 Optimization of experimental conditions

3.2.1 Optimal concentration of RP

Using this strategy, it was essential that the initiator domain on RP could propagate a chain reaction of hybridization events between H1-bio and H2-bio and long nicked DNA polymers could be formed. To investigate these issues, we also used gel electrophoresis to characterize two hairpin probes in the presence and absence of RPs. As shown in Fig. S1, there were only low molecular weight bands in lane 8 (H1-bio/H2-bio), this indicated that the mixture of H1-bio and H2-bio did not cause a self-hybridization reaction and no HCR occurred in the absence of RP. When RP were added to the H1-bio and H2-bio mixture, partial H1-bio and H2-bio were consumed as a result of the HCR reaction (lane 2–8). Smears of high molecular weight structures were observed. These results showed that the HCR could only be implemented in the presence of H1-bio/H2-bio/RPs. It was also observed from lane 2–8 (1, 0.6, 0.5, 0.4, 0.3, 0.2, and 0.1 μM of RP, respectively) that the molecular weight of the resulting polymers was inversely related to the concentration of RP given the fixed supply of hairpin probes (1 μM H1-bio/H2-bio). These results are similar to those in other reports on initiator sequence/(H1 and H2)[12]. The lower of the ratio of RP (initiator)/H1-bio and H2-bio, the longer the double strands of HCR products. Every complex was conjugated with more SA–AuNPs through numerous biotin-attached double-helix DNAs. However, overly decreasing the concentration of

RP reduced the efficiency of HCR in a certain period of time. As shown in lane 8, when the concentration of RP is 0.1 μM, there was little polymer formed. When the concentrations of target DNAs and CPs were fixed, detection by the LFNAB suggested that the best ratio of RP/H1-Bio and H2-Bio was 0.2.

3.2.2 Best Ratio of Hairpin-Bio/Hairpin probes

Theoretically, the more biotin every polymer (product of HCR) carried, and the stronger the signal was amplified. However, modification of the hairpin structure by other chemicals possibly influenced the efficiency of HCR. We performed a comparative study using different concentration ratios of hairpin probe-biotin/hairpin probe to identify the best ratio of biotin-labeled and unlabeled hairpin probes. The four groups of probes (H1-bio, H2-bio, H1 and H2) were diluted to 1 μM, respectively, heated to 95℃ and cooled to room temperature to form the hairpin structure. Ten microliters of the reaction systems of H1-biotin/H1 and H2-biotin/H2 with different concentration ratios of biotin-labeled and unlabeled probes (10:0, 9:1, 8:2, 7:3, 6:4 and 5:5) were established. Ten microliters of RP (0.2 μM) was added to a final volume of 30 μL. The reaction mixtures were incubated at room temperature for 2 h. The bands of dsDNA polymers formed via HCR were observed using polyacrylamide gel electrophoresis (9%). As shown in Figure1(a), when the ratio of the hairpin probe-biotin/hairpin probe decreased, the reaction increased significantly; the efficiency of HCR using the biotin-labeled hairpin probe was significantly lower than that using the unlabeled probe which suggests that the modification of biotin affected the hybridization chain reaction. Ten microliters of HCR products was mixed with an

equal concentration of capture probe (0.3 μM) and target DNA (1 nM) in equal volumes. The reaction mixture was incubated at room temperature for 15 min and applied to the LFNAB (as shown in Figure 1(b) and 2(c)), Test line of the strip was brightest when the ratio was 9:1 and the peak area of T/C was highest. Therefore, the ratio of 9:1 was used in the following tests.

Figure1

3.2.3 Effect of the concentration of CP

To assess the optimal coverage for hybridization, the amount of CP versus signal intensity was selected and was shown in Figure2. As the amount of capture probe increased, signal intensity increased initially and reached a maximum at 0.3 μM of CP, and then decreased with increasing CP concentration. These results suggested that an insufficient amount of capture probe can affect the binding efficiency of CP/target DNA/RP-HCR on the test line of the strip. Excessive free capture probe may competitively inhibit the complex binding with mono antibody on the test line. Thus, the concentration of CP (0.3 μM) was used in subsequent studies.

Figure2

3.3 Assay performance of the HCR-LFNAB detection method

High specificity is essential for analytical methods. The specificity was tested by measuring and comparing the response of target DNA to non-target similar DNA (base mismatch DNA sequences). The DNA of 6 pathogen species (*Salmonella enteritidis, Citrobacter freundii, Escherichia coli, Staphylococcus aureus, Enterobacter sakazakii, Listeria monocytogenes*) was determined using the HCR-LFNAB detection method, and 47 nt-long DNA fragments (The concentration of every DNA was 500 nM, and the procedure was as above) were with high similarity to the target DNA (the sequences of the six fragments are shown in Table S1). As shown in Fig. 3, after repeated testing, positive results were only obtained with target DNA, while negative results were observed with other non-target DNA. These results demonstrated that it was difficult for base mismatch and non-complementary DNA to form CP/target DNA/RP-HCR conjugates. Only the perfect target DNA triggered the process efficiently. The high specificity of this DNA detection method was attributed to the requirement for full complementarity between the target DNA and substrate hairpin probes in the HCR-LFNAB system. Thus, the DNA assay was highly selective for completely complementary DNA.

Figure3

3.4 Sensitivity assay of the HCR-LFNAB detection method

The analytical performance of HCR-LFNAB in detecting different concentrations of target DNA ranging from 2.5pM to 500 nM under optimal experimental conditions

was assessed. Fig.4 shows typical images of the LFNAB in the presence of different concentrations of target DNA ranging from 2.5 pM to 500 nM. No red band was observed on the test zone of the LFNAB in the absence of target DNA (control), indicating negligible nonspecific adsorption under optimized experimental conditions. The detection limit (calculated as 3σ/slope of the calibration curve, where σ is the standard deviation of the blank samples) was 1.76 pM. The detection limit of target DNA by LFNAB without HCR was 0.31 nM. HCR-LFNAB for signal amplification improves both the limits of detection and sensitivity for DNA by an average of 176-fold relative to methods for direct detection by the LFNAB.

HCR for signal amplification in several assay approaches for specific targets showed a sensitivity of approximately 2~3 orders of magnitude higher than that of the one-step assay without HCR [29-31]. However, HCR for signal amplification in some assay was less than 1 orders of magnitude than that of the direct assay without HCR[32]. HCR amplification efficiency was influenced by many factors in different detection strategies. Firstly, to achieve high HCR signal gain, the best way is to have a good intrinsic hairpin sequence design [33, 34]. Secondly, some factors, such as the concentration of K^+, hairpin probes, and the reaction time of HCR process, would affect the performance of the HCR[34].

In addition to the amplification efficiency of HCR, signal analysis technology also has an important impact on the sensitivity of analytical methods. HCR in combination with highly-sensitive analytical techniques can significantly increase its

sensitivity. For example, Ye et al analyzed miR-203 using SERS combined with HCR, and the sensitivity reached 0.15 fM [26]. As signal transformation tools, SERS and electrochemistry significantly improve the sensitivity of HCR; however, these techniques require specific instruments and the test procedures are complicated and are not applicable for POC tests. In this study, we utilized LFNAB in combination with HCR to detect target DNAs, and although the sensitivity was lower than the above methods, the technique is suitable for POC tests. HCR can combine isothermal amplification techniques such as catalytic hairpin assembly[35], which is suitable for POC tests, to amplify the signal of target DNAs and increase the sensitivity of LFNAB. Moreover, nanogold-labeled the horseradish peroxidase can also be used with LFNAB to increase the sensitivity of LFNAB[36].

Figure4

3.5 *Salmonella* detection assay

To further test this assay for *Salmonella* detection, RNA was purified from the *Salmonella* culture as described in Section 2. In Fig. 5 (a), the red color intensity is faint but visible when the *Salmonella* amount is 10^4cfu mL^{-1}. Fig.5 (b) shows the corresponding color intensity relative value. And the detection limit of this assay for *Salmonella* detection is 3×10^3cful.mL^{-1} (calculated as 3σ/slope of the calibration curve, where σ is the standard deviation of the blank samples),which is consistent with above expectations.

Figure5

4. Conclusion

A visualized enzyme-free and isothermal amplification detection assay, coupled with HCR and a lateral flow biosensor, was developed. The detection limit of this process for detecting synthetic DNA was 1.76 pM, for detecting *Salmonella is* 3×10^3cful.mL^{-1}, without the need for enzymes, labels or sophisticated equipment. In addition, this novel technique could be extended to detect other nucleic targets. This technique provides a novel method for simple, fast, low-cost and convenient point-of-care diagnostics in the field of food-borne pathogen monitoring. In view of these advantages, this method shows great potential in *Salmonella* diagnostics and clinical analysis.

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Captions

Scheme1. (A) Schematic representation of lateral flow amplified detection for nucleic acid based on HCR. (B) Illustration of the test strip assembly.

Figure1. Effect of the ratio of Hairpin-bio/Hairpin on HCR reaction and LFNAB signal. (A). Electrophoresis of HCR when different ratios of H1-bio/H1(H2-bio/H2) (10:0, 9:1, 8:2, 7:3, 6:4, 5:5) were applied; (B). HCR-LFNAB test results when different ratios of H1-bio/H1(H2-bio/H2)(10:0, 9:1, 8:2, 7:3, 6:4, 5:5) were applied and target DNA was 1nM; (C). Detection curve of LFNAB test signal under different ratios of H1-bio/H1(H2-bio/H2)(10:0, 9:1, 8:2, 7:3, 6:4, 5:5).

Figure2. Effect of different concentrations of capture probe on the performance of the LFNAB. Calibration plots represent peak area of T/C corresponding to capture probe concentrations of 0.1, 0.3, 0.6, 0.9, and 1.2 μM, respectively. Error bars represent the standard deviation of three independent measurements.

Figure3. Selectivity assay of the strip sensor for detection of target DNA Strips 1−6 represent synthetic single-stranded DNA of *Salmonella enteritidis*, *Citrobacter freundii*, *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter sakazakii*, and *Listeria monocytogenes*, respectively.(For convenience. We abbreviate the full name of every bacteria to first three letters. And '***sal***' represent target DNA.)

Figure4. Sensitivity assay of the strip sensor for detection of target DNA. Photographic images and corresponding optical responses of performance of direct detection without HCR (A) and signal amplified detection (B) for different concentrations of target DNA. Strips 1−8 represent target DNA concentrations of 0, 2.5pM, 25pM, 250pM, 2.5nM, 25nM, 250nM and 500 nM respectively. (C) The peak area ratio of the test line to control line (T/C) in response to different concentrations of target DNA. Red curve represent the signals amplified by HCR and the black curve represent signals without amplification. Error bars represent the standard deviation of three independent measurements.

Figure5. Sensitivity assay of the strip sensor for detection of *Salmonella*. (A) Photographic images and corresponding optical responses of detection of *Salmonella.* Strips 1−8 represent *Salmonella* concentrations of 10^7, 10^6, 10^5, 10^4, 10^3, 10^2, 10^1cfu.mL^{-1} and negative control respectively. (B) The peak area ratio of the test line to control line (T/C) in response to different concentrations of *Salmonella*. Error bars represent the standard deviation of three independent measurements.

Scheme 1

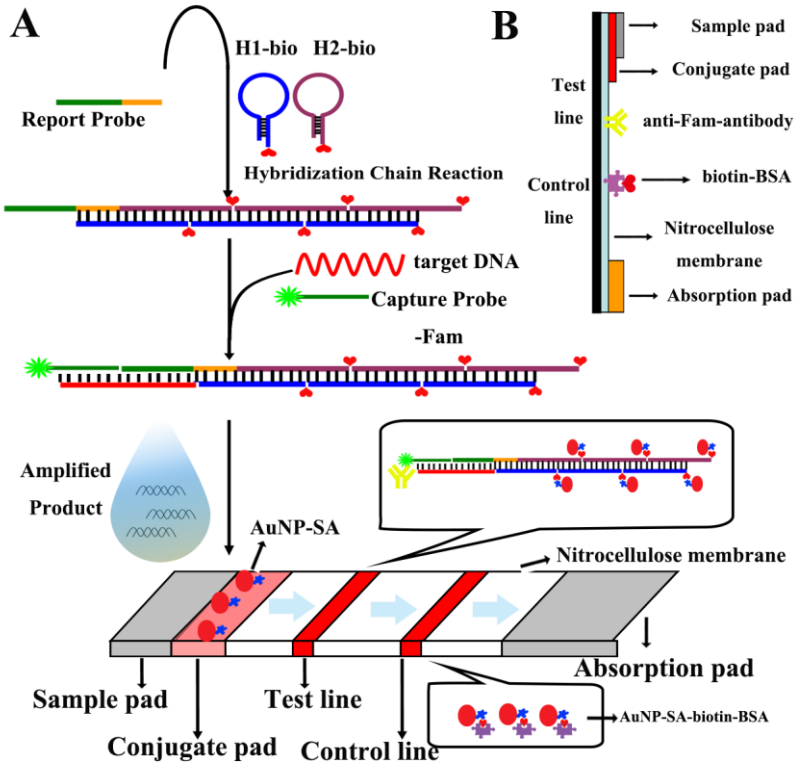


Figure 1

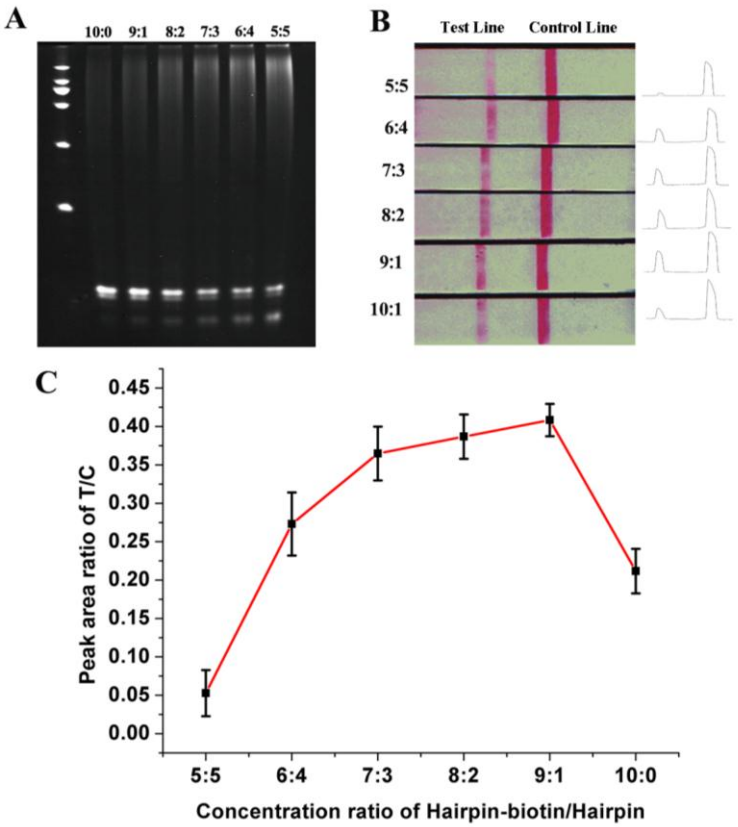


Figure 2

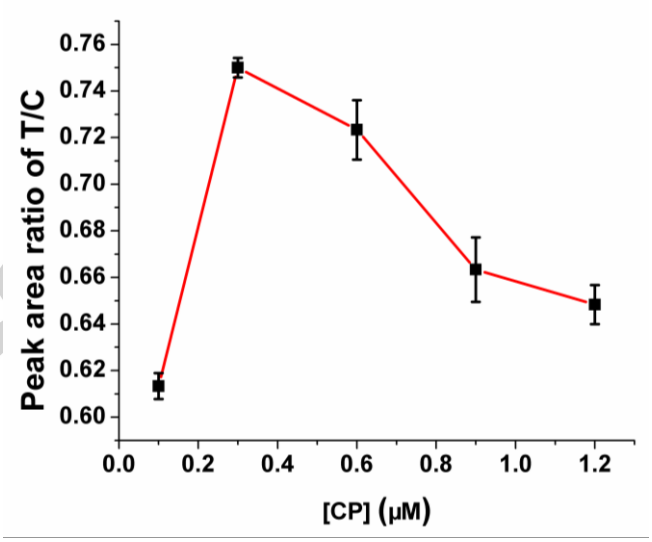


Figure 3

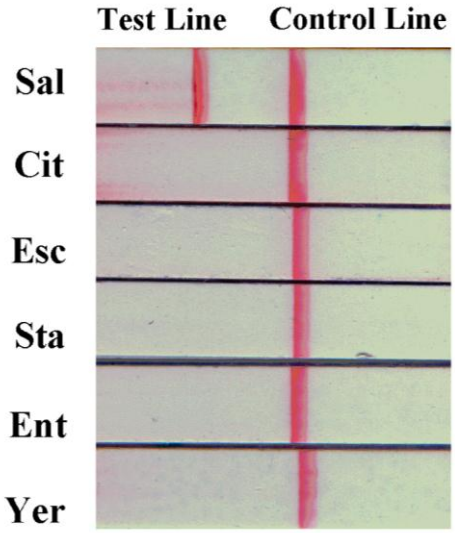


Figure 4

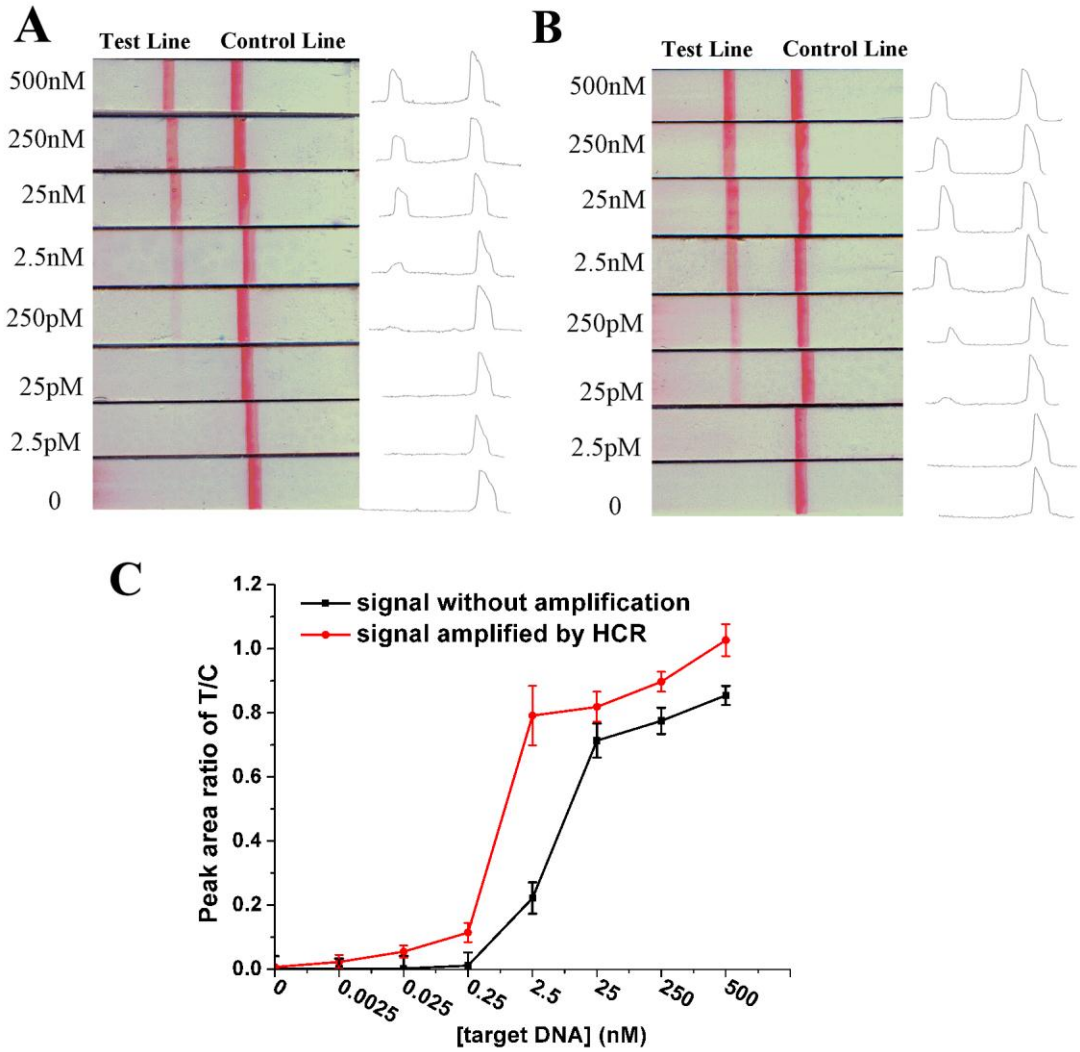
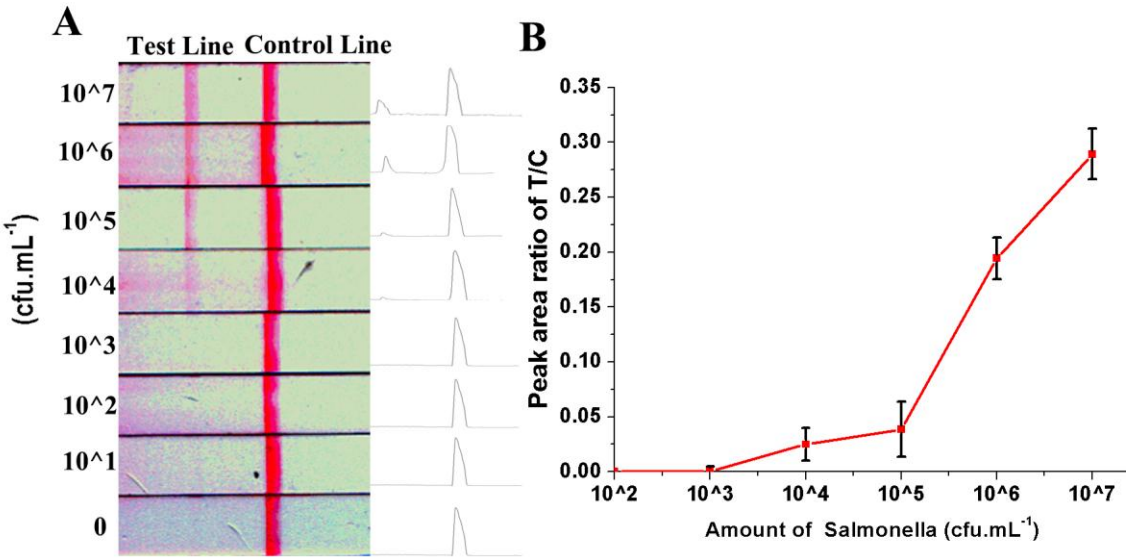


Figure 5



Highlights

1. The assay is visualized and the signal is amplified by enzyme-free and isothermal reaction --hybridization chain reaction(HCR)
2. With HCR, the LOD of synthetic DNA is 1.76 pM, which is decreased by about 2 orders of magnitude comparable to the assay without HCR. And the detection limit of *Salmonella* is 3×10^3 cfu mL-1.
3. The biosensor is suitable for non-specialist personnel and point-of-care (POC) diagnosis in low-resource settings