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Biotin-exposure-based immunomagnetic separation coupled with nucleic acid lateral flow biosensor for visibly detecting viable *Listeria monocytogenes*

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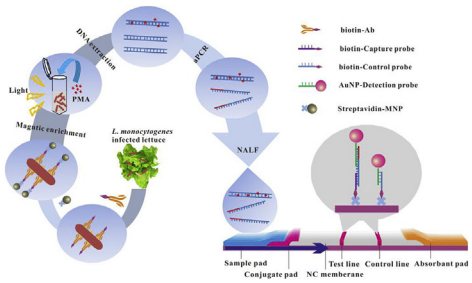
HIGHLIGHTS

- A biotin-exposure-based IMS method for *Listeria monocytogenes* enrichment and isolation was developed.
- The usage of antibody in biotin-exposure-based IMS was reduced 10 times compared with previous study.
- PMA treatment prior to aPCR could selectively detect of viable *Listeria monocytogenes.*

GRAPHICAL ABSTRACT



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ABSTRACT

Infectious diseases caused by *Listeria monocytogenes* pose a great threat to public health worldwide. Therefore, a rapid and efficient method for *L. monocytogenes* detection is needed. In this study, a biotin-exposure-based immunomagnetic separation (IMS) method was developed. That is, biotinylated antibody was first targeted to *L. monocytogenes*. Then, streptavidin-functionalized magnetic nanoparticles were added and anchored onto *L. monocytogenes* cells indirectly through the strong noncovalent interaction between streptavidin and biotin. Biotin-exposure-based IMS exhibited an excellent capability to enrich *L. monocytogenes*. Specifically, more than 90% of *L. monocytogenes* was captured when the bacterial concentration was lower than 10^4 colony-forming units (CFU)/mL. Importantly, the antibody dosage was reduced by 10 times of that in our previous study, which used antibody direct-conjugated magnetic nanoparticles. Propidium monoazide (PMA) treatment prior to PCR amplification could eliminate the false-positive results from dead bacteria and detected viable *L. monocytogenes* sensitively and specifically. For viable *L. monocytogenes* detection, enriched *L. monocytogenes* was treated with PMA prior to asymmetric PCR amplification. The detection limits of the combined IMS with nucleic acid lateral flow (NALF) biosensor for viable *L. monocytogenes* detection were 3.5×10^3 CFU/mL in phosphate buffer solution and 3.5×10^4 CFU/g in lettuce samples. The whole assay process of recognizing viable *L. monocytogenes* was completed within 6 h. The proposed biotin-exposure-mediated IMS combined with a disposable NALF biosensor platform posed no health risk to the end user, and possessed potential applications in the rapid screening and identification of foodborne pathogens.

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1. Introduction

Listeria monocytogenes is among the most significant foodborne pathogens, which can present in various foods, such as milk, meat, eggs, sea food, vegetables, and ready-to-eat foods [1]. *L. monocytogenes* can survive at wide pH range and high salt concentrations; it can also grow despite cold temperatures [2]. Thus, controlling *L. monocytogenes* strictly in food processing is challenging. Consuming foods contaminated with *L. monocytogenes* may cause severe listeriosis [1], meningitis, sepsis, miscarriage, and even death. Therefore, developing a rapid and sensitive method for viable *L. monocytogenes* detection is essential.

The conventional culture-based method is considered as the "gold standard" for *L. monocytogenes* detection. However, this method is usually time consuming and complicated, and labor intensive because of its multiple detection steps, including selective enrichment and differential plating [3]. Nucleic acid amplification based detection methods, such as PCR, which could provide a millionfold amplification of a specific nucleic acid sequence in less than an hour [4], exhibit high specificity and accuracy for *L. monocytogenes* detection. However, for *L. monocytogenes* detection, conventional PCR is usually coupled with agarose gel electrophoresis [5,6], which is labor intensive and instrument dependent. The latter procedure is also harmful to the human body because of the use of a toxic stain.

Recently, the nucleic acid lateral flow (NALF) biosensor has drawn considerable attention due to its rapid and portable features [7–9]. The biosensor has also been widely applied for clinical diagnosis, environmental monitoring, and food safety inspection [10–14]. Meanwhile, asymmetric polymerase chain reaction (aPCR) combined with NALF biosensor can solve the shortcomings of traditional agarose gel electrophoresis. aPCR is a, linear-after-the-exponential, PCR assay. It is performed with unequal concentrations of primers for conventional PCR to amplify single specific nucleic acid sequences. aPCR assay, combined with a NALF biosensor, could detect *L. monocytogenes* within a few minutes by the naked eye through the nucleic acid hybridization reaction and gold nanoparticle colorimetric probes. On the basis of the aPCR assay, Ang et al. [15] developed a NALF biosensor to specifically detect the cholera toxin gene from the diarrhea-causing toxigenic *Vibrio cholerae*. Moreover, Liu et al. [16] combined a paper-based nucleic acid diagnostic system with multiplex aPCR assay to develop a platform to simultaneously recognize multiplex target genes, and the authors also extended the system to *L. monocytogenes* genotyping. Therefore, combined aPCR-based single specific nucleic acid sequence amplification with a NALF biosensor promises the rapid and sensitive detection of *L. monocytogenes*. However, extracting the DNA of *L. monocytogenes* present at low concentrations in complex food matrix or large-volume samples for DNA extraction and PCR assay is not feasible [17,18].

Immunomagnetic separation (IMS) method, which enables the enrichment and separation of *L. monocytogenes* selectively and efficiently, is an ideal pretreatment platform. Importantly, IMS could eliminate the matrix effect of the food samples and help easily detect *L. monocytogenes*. However, immobilizing the antibody onto the magnetic nanoparticle surface may cause the inevitable loss of antibody activity due to the antibody's random orientation [19]. This occurrence leads to a low capture efficiency for *L. monocytogenes* [20]. To improve the capture capability, we developed an IMS method based on the streptavidin–biotin system in our previous study [5,20]. In the study, biotinylated antibody was immobilized on streptavidin-modified magnetic nanoparticles (MNP-SA) to enhance the orientation and conformation of the antibodies bound to the nanoparticles. However, to obtain a high capture efficiency, the method required high antibody amounts.

In the present study, we developed a IMS method for *L. monocytogenes* enrichment that required less antibody, based on biotin exposure strategy. For *L. monocytogenes* separation, biotinylated antibody first targeted *L. monocytogenes* cells with specific recognition between antibody and antigen. Then, MNP-SA were added. Given the strong noncovalent interaction between streptavidin and biotin, MNP-SA was anchored onto *L. monocytogenes* cells efficiently. Thereafter, magnetically-enriched *L. monocytogenes* was subjected to propidium monoazide (PMA) treatment to eliminate the false-positive results of dead cells. Then, the genomic DNA of *L. monocytogenes* was extracted and aPCR was performed to generate single-stranded DNA (ssDNA) amplicons. Finally, through the NALF biosensor, the ssDNA amplicons were detected by the naked eye. Biotin-exposure-based IMS combined with a disposable NALF biosensor poses no health risk to the end user. As such, the proposed approach is a potential application for rapidly screening and identifying *L. monocytogenes*.

2. Experimental

2.1. Regents and apparatus

Magnetic nanoparticles (MNPs) with diameter of 180 nm were bought from Allrunnano Technology Co., Ltd. (Shanghai, China). Ultrafiltration centrifuge tube (Amicon® Ultra-0.5, MWCO 30 KDa) was bought from Millipore (Temecula, CA). Anti-*Listeria* antibody was provided by Wuxi Zodolabs Biotech (Wuxi, China). PMA was bought from Biotium, Inc. (Hayward, CA). Streptavidin was obtained from Shanghai Hualan Chemical Technology Co., Ltd. (Shanghai, China). Sulfo-NHS-LC-biotin was bought from Thermo Fisher Scientific Inc. (Waltham, MA). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC·HCl) and n-hydroxysuccinimide sodium salt (NHSS) were bought from Sigma-Aldrich Chemical Co. (St. Louis, MO). Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and Tris-acetate were bought from Yuan Ye Sheng-wu (Shanghai, China). Nitrocellulose (NC) membrane, sample pad, conjugate pad, and absorbent pad were obtained from Millipore (Bedford, MA, USA). PALCAM was bought from Land Bridge Technology Co. Ltd. (Beijing, China). All oligonucleotides used in this work were synthesized and purified at Sangon Biotech (Shanghai) Co.,Ltd. (Shanghai, China), and their sequences were listed in Table 1.

A CG-ICTS reader from Skannex Biotech (Oslo, Norway) was used. The BioDot XYZ Platform combining motion control with BioJet Quanti3000k dispenser and AirJet Quanti3000k dispenser were from BioDot (Irvine, CA). UV/vis was from Purkinje General Instrument Co. (Beijing, China).

2.2. Bacterial strains and culture conditions

L. monocytogenes (ATCC 13932) was grown in Luria–Bertani (LB) medium at 37 °C with continuous shaking for 18 h. Other common pathogenic bacterial strains used in this study for verifying the

Table 1
Oligonucleotide sequences used in this study.

Oligonucleotide	Sequences (5′-3′)
hly-207-F	CCGTAAGTGGGAAATCTGTCTC
hly-207-R	AGTTTGTTGTATAGGCAATGGG
Capture probe	biotin-TTTTTTTTTTAGTTTGTTGTATAGGCAATGGG
Control probe	AAAAATTCTTCCTTCAAAGCCGTAATTTTTTTTTT-biotin
Detection probe	TTACGGCTTTGAAGGAAGAATTTTTTTTTTTTTT-SH

F = forward primer, R = reverse primer.

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