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Gold nanoparticle-based enhanced lateral flow immunoassay for detection of *Cronobacter sakazakii* in powdered infant formula

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ABSTRACT

Cronobacter sakazakii is an opportunistic foodborne pathogen that can infect newborns through powdered infant formula (PIF). In this study, we developed a novel enhanced lateral flow immunoassay (LFA) with enhanced sensitivity for detection of *C. sakazakii* in PIF by the naked eye. The proposed strategy for signal enhancement of the traditional LFA used concentrated gold nanoparticles (AuNP) as the enhancer to conjugate with capture antibodies, which could increase the immobilized capture antibodies concentration at the detection zone to improve capture efficiency. Besides, the detection signal was further amplified by accumulated AuNP as the *C. sakazakii* labeled with AuNP probes was captured by antibodies conjugated with enhancer at the test line. We also studied the effect of different concentrations of capture antibodies and concentrated AuNP on detection performance, and found that 2.2 mg/mL of capture antibodies and 0.06 n*M* concentrated AuNP were the optimal combination that could avoid a false-positive signal and maximally amplify the detection signal of the enhanced LFA. Using this strategy, the detection sensitivity of the enhanced LFA was 10^3 cfu/mL and improved 100-fold compared with traditional LFA. The strip was highly specific to *C. sakazakii*, and the time for detection of *C. sakazakii* in PIF was shortened by 3 h. In summary, the enhanced LFA developed by the addition of concentrated AuNP as the enhancer can be used as a sensitive, rapid, visual qualitative and point-of-care test method for detecting target analytes.

Key words: enhanced lateral flow immunoassay, gold nanoparticle, *Cronobacter sakazakii*, powdered infant formula

INTRODUCTION

Cronobacter sakazakii, a member of the *Cronobacter* spp. (Joseph et al., 2012), is one of the most frequently reported foodborne pathogens that causes necrotizing enterocolitis, sepsis, and meningitis in infants as well as immuno-compromised populations (Healy et al., 2010). Contaminated powdered infant formula (**PIF**) has been identified as the most likely vehicle of transmission of the infection caused by *C. sakazakii* (Strydom et al., 2012; Li et al., 2014). Traditional microbiological methods take 5 to 7 d to complete analysis of *C. sakazakii*, and thus are time-consuming and laborious (Druggan et al., 2009). Currently, a variety of alternative techniques have been reported to detect *C. sakazakii*, including nucleic acid amplification methods (Zhou et al., 2016), ELISA (Kong et al., 2015), electrochemical biosensor, and immunomagnetic separation assay (Shukla et al., 2016). Although these methods are rapid and sensitive, most of them require laborious procedures, expensive equipment, and professional expertise. Such disadvantages impede these methods from on-site implementation. Thus, it is of great significance to develop a simple, sensitive, and rapid methodology to monitor *C. sakazakii* in PIF and prevent the risk of foodborne diseases caused by *C. sakazakii*.

Lateral flow immunoassay (**LFA**), which uses gold nanoparticles (**AuNP**) as the color marker with unique optical properties, extraordinary chemical stability, and binding capacity for biomolecules (Baptista et al., 2008), is a rapid, simple, and low-cost system for detection of target analytes in food, clinical diagnostics, and the environment with the naked eye (Tao et al., 2016). Nevertheless, the further application of LFA is impeded by poor sensitivity (Chen et al., 2015). To improve the sensitivity, some pioneering researchers replaced the traditional label-AuNP by AuNP nanocomposite and novel nanoparticle, including AuNP-decorated silica nanorods (Xu et al., 2014), quantum dots (Qu et al., 2016), fluorescence-quenching (Shen et al., 2017), and so on, which involved sophisticated preparation procedures and advanced detection tools. Meanwhile, the

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advances based on silver staining (Rodríguez et al., 2016), enzyme labeling (Cho and Irudayaraj, 2013), or $NH_2OH{\cdot}HCl$ enlargement (Li et al., 2013) have been widely used to amplify the detection signals; however, these methods rely on additional procedures to add extra reagents and extend the detection time (Chen et al., 2015). Therefore, it is necessary to significantly improve the sensitivity of traditional LFA by a simple and effective enhancing method.

One of the principal reasons for poor sensitivity of LFA is that the reaction is transient, caused by capillary action, which leads to low binding rate between the probe-labeled targets and the capture antibodies immobilized at the nitrocellulose (**NC**) membranes (Moghadam et al., 2015; Ren et al., 2016). Increasing the immobilized amount of capture antibodies can enhance the binding efficiency, whereas this methodology is ultimately limited by the protein binding capacity of NC membranes (Moghadam et al., 2015). Thus, the application of a novel strategy to overcome the limitation of protein binding capacity of NC membranes may improve the sensitivity of LFA.

Considering all aspects described above, the aim of present study was to increase the immobilized amount of capture antibodies to significantly improve the sensitivity of traditional LFA by a simple and effective method. Herein we proposed the enhanced LFA with concentrated AuNP as the enhancer to increase the immobilized amount of capture antibodies and amplify the signal for ultrasensitive detection of *C. sakazakii* in PIF.

MATERIALS AND METHODS

Chemicals and Equipment

Gold (III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$), sodium citrate ($C_6H_5Na_3O_7{\cdot}2H_2O$), and BSA were obtained from Sigma (St. Louis, MO). The pair of anti-*C. sakazakii* monoclonal antibodies (2H1 as the detection antibody, 5C4 as the capture antibody) and goat anti-mouse IgG was purchased from Hangzhou Goodhere Biotechnology Co. Ltd. (Hangzhou, China). The sample pad, NC membrane, conjugate pad, absorption pad, and polyvinyl chloride pad used to fabricate the LFA were obtained from Hangzhou Goodhere Biotechnology Co. Ltd. Ponceau S was acquired from Beyotime Institute of Biotechnology (Shanghai, China). Other reagents used were of analytical purity.

The XYZ 3050 strip dispenser from BioDot (Irvine, CA) was used to spray the test (**T**) and control (**C**) line. A HGS201 cutter purchased from AUTOKUN (Hangzhou, China) was used to cut the strips. A JH250Windows AuNP-based strip reader supplied by Weifang Bainuo Di Biotechnology Co. Ltd. (Weifang, China) was used for measuring the signal of the T line. A SU-8010 high-resolution scanning electron microscope (Hitachi, Japan) was applied to observe the AuNP at the T line of the strip.

Bacterial Strains

Four *C. sakazakii* strains (ATCC 29544, ATCC 12868, ATCC 29004, ATCC BAA-894), 7 other *Cronobacter* spp., and 11 non-*Cronobacter* strains were used. All strains were cultured in nutrient broth for 12 to 16 h at 37°C shaking at 160 rpm.

Synthesis of Detection Antibody Conjugated with AuNP

The AuNP (average diameter 35 nm) were synthesized according to previously reported methods (Frens, 1973). The synthesis procedures of anti-*C. sakazakii* monoclonal detection antibodies (2H1) conjugated with AuNP based on the following procedures constructed by our team (Zhao et al., 2017) were performed by adding 10 μg of detection antibodies to pH-adjusted 1-mL AuNP solutions with 4 μL of the 0.2 *M* K_2CO_3 followed by incubation for 1 h at room temperature. Subsequently, 100 μL of blocking solution (10% BSA) was added and then incubated for another 1 h at room temperature to block the residual surface of AuNP. Then, the mixtures were centrifuged at 7,000 × *g* for 20 min at 4°C to discard unbound antibodies, and the precipitate was re-dispersed in 100 μL of 10 m*M* Tris-HCl buffer containing 1% BSA, 2% trehalose, and 2 μL of 10% BSA to increase flow closure. The obtained AuNP probes were stored at 4°C for further uses.

Preparation of the Test-Line Solution for the Enhanced LFA

Then, 35 nm AuNP was centrifuged at 7,000 × *g* for 20 min at 4°C followed by removing the supernatant. The pellet was suspended in 10 m*M* Tris-HCl buffer as concentrated AuNP solution, which was used as an enhancer conjugated with anti-*C. sakazakii* monoclonal capture antibodies (5C4) followed by incubation at 4°C for 1 h. After the incubation, the entire solution was used as the test-line solution to spot onto the T lines of enhanced LFA. To evaluate the effect of concentration of AuNP and capture antibodies on detection performance, the concentrated AuNP and capture antibodies at different concentrations were optimized to construct the enhanced LFA and this experiment was performed in triplicate.