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Latex bead and colloidal gold applied in a multiplex immunochromatographic assay for high-throughput detection of three classes of antibiotic residues in milk

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ABSTRACT

Two rapid and sensitive high-throughput immunochromatographic assays were successfully developed for simultaneous determination of 12 sulfonamide, 18 quinolone and six tetracycline residues in milk. Under optimized conditions, residues from these three classes of antibiotics can be qualitatively and quantitatively determined on a single strip within 10 min. The detection limits were much lower than the maximum residue limits established by the European Union. Comparative evaluations of sensitivity, antibody consumption, coefficient of variation and user experience between these two immunochromatographic assays showed that latex beads have advantages over colloidal gold when employed as a label in the assay. Therefore, the latex bead-based high-throughput immunochromatographic assay has the best potential for on-site testing of a large number of samples as part of food safety applications.

1. Introduction

Antibiotic residues in animal-derived food are caused by improper use in animal disease treatment or overuse as food additive for growth promotor (Livingston, 1984; Netting, 1993). This issue has caught increasing attention due to the threat to the public health and ecosystem (Boxall et al., 2004, pp. 1–91; Cháfer-Pericás, Maquieira, & Puchades, 2010; García-Galán, Díaz-Cruz, & Barceló, 2009). Sulfonamides (SAs), quinolones (QNs) and tetracyclines (TCs) are widely used in the food animal production because of their broad-spectrum antibacterial properties. When used without following the withdrawal time guidelines, these antibiotics can accumulate in the animal-derived food products. Reported incidences of these antibiotic residues in the food were very high: 20%, 19% and 8% for SAs, QNs and TCs, respectively (Cháfer-Pericás et al., 2010). Moreover, with their widespread use on dairy farms, residues from these three classes of antibiotics usually coexist in the milk (Bilandžić et al., 2011; Dorival-García, Junza, Zafra-Gómez, Barrón, & Navalón, 2016; Fonseca et al., 2009). The presence of these residues in the milk may cause adverse reactions in humans, including allergic reactions and intoxication (Conzuelo et al., 2013; Marshall & Levy, 2011). Furthermore, the alarming emergence of pathogens resistant to multiple antibiotics is associated with the excessive use of these antibiotics. For these reasons, maximum residue limits of these antibiotics in the milk have been established by the European Union in 2010 (Commission, 2010).

To enhance the food quality and safety, the development of multiplex and high-throughput detection methods are urgent and necessary. A number of high-throughput instrumental methods have already been developed for the detection of multiple antibiotics in the animal-derived food samples such as liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Han et al., 2015; O Mahony, Clarke, Whelan, O Kennedy, Lehotay & Danaher, 2013). Despite its high accuracy and sensitivity, instrumental analysis requires expensive instruments, complicated sample pretreatment processes, professional expertise and relative long time to process. Immunoassays, including the enzyme-linked immunosorbent assay (ELISA), immunochromatographic assay (ICA), immunochip and other immunosensors as simple and

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sensitive methods, are more suitable for rapid and high-throughput analysis of multiple compounds. (Jawaid et al., 2015; Jiang et al., 2013; Li et al., 2011; 2013; Li & Shen et al., 2015, Li & Li et al., 2015; Li & Wen et al., 2015, Song et al., 2014; Song et al., 2015). Among these assays, the immunochromatographic assay is the easiest to use, time-saving and cost-effective.

In order to improve the detection efficiency and achieve high-throughput detection, lots of efforts have been made on ICA. First, developing antibodies or other recognition materials that are class-specific to contaminants, can then be used to develop class detection methods (Song et al., 2015; Wang, Liang, Wen, Zhang, Li & Shen, 2015). Second, the design of several test lines or dots on an immunochromatographic strip can be altered to improve the spatial resolution for detecting multiple analytes (Li & Shen et al., 2015, Li & Li et al., 2015; Li & Wen et al., 2015; Song et al., 2015; Taranova, Berlina, Zherdev, & Dzantiev, 2015). Third, detection of multiple analytes can be achieved when several strips share one sample pad (Li et al., 2013). Fourth, by taking advantage of the unique property of nano-material like quantum dots, multiple analysis can be achieved in one test line (Wang, Hou, & Ma, 2015). However, the last two strategies need complicated production processes or could not quantify the compounds, which significantly reduce their practical use. Therefore, majority research of multiplex ICAs seeks to combine the first two strategies using colloidal gold (CG), quantum dots or fluorescence microspheres as labels. A shortcoming of fluorescence nanoparticles is that they require use of an external device for excitation, so colored nanoparticles that can be visualized by the naked eye have been investigated more extensively (Li et al., 2013; Li & Shen et al., 2015, Li & Li et al., 2015; Li & Wen et al., 2015; Song et al., 2015; Xing et al., 2015). CG is the most widely adopted nanoparticle among optical labels, and has the advantages of easy to manipulate, biocompatible and highly visible, with its intense red color. However, it exhibits the same color in two or more test lines, which can be confusing in on-site test. Latex beads (LBs), impregnated with diversified vibrant dyes, have shown more advantages such as easy synthesized, multicolor, are desirable to develop robust ICAs.

In this study, multi-color LBs and CG were used to develop multiplex ICAs for simultaneous detection of SAs, QNs and TCs in individual milk sample. Both the latex bead-based immunochromatographic assay (LBIA) and colloidal gold-based immunochromatographic assay (CGIA) combine the advantages of the ICA and multiplex analysis strategies, and result in a significant advancement in high-throughput detection research. To investigate the performance of these two methods, comparative evaluations of the sensitivity, antibody consumption, and coefficient of variation (CV) for the LBIA and CGIA were conducted. To our knowledge, this is the first report on multi-color LB-based ICA.

2. Materials and methods

2.1. Chemicals and equipment

Enrofloxacin (ENR) and other QNs, sulfadimethoxine (SDM) and other SAs, tetracycline (TC) and other TCs, bovine serum albumin (BSA) and goat anti-mouse immunoglobulin were purchased from Sigma (St. Louis, MO, USA). Carboxyl-functionalized latex beads (solid content 5%) were purchased from Bangs Laboratories, Inc. (Indiana, USA). Anti-QNs monoclonal antibody (mAb), anti-SAs mAb, anti-TCs mAb, QNs-BSA, SAs-BSA and TCs-BSA were prepared in our laboratory (Jiang et al., 2013; Wang et al., 2014; Wang, Zhang, Nesterenko, Eremin, & Shen, 2007). The nitrocellulose (NC) membranes were purchased from Millipore Corporation (Bedford, MA, USA). The sample pad (CFKJ-0328) and the absorbent pad (CH37K) were supplied by Shanghai Liangxin Co. Ltd. (Shanghai, China). The microtiter plates were supplied by Guangzhou JET BIOFIL Co. (Guangzhou, China). Other chemical substances were purchased from Beijing Chemical Reagent Company (Beijing, China). The ZX1000 Dispensing Platform and the CM4000 Guillotine Cutting Module were supplied by BioDot Inc. (Irvine,CA, USA). The ESE Quant LFR reader was purchased from QIAGEN (Dusseldorf, Germany). Water was purified using a Milli-Q system from Millipore Corporation (Bedford, MA, USA).

2.2. Bioconjugating LBs with antibodies

Three kinds of detection probes were prepared by conjugating antibodies against three classes of antibiotics to different colored LBs. Firstly, 15 μL of freshly prepared N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) (0.5 mg/mL) solution and 18 μL of N-hydroxysuccinimide (NHS) (0.5 mg/mL) solution were sequentially added to the Basic Black LBs (dissolved in 0.05 M MES, pH 6.0) solution for 15 min with gentle stirring. Secondly, 30 μL of 0.20 mg/mL anti-SAs mAb was added and incubated for another 20 min. Thirdly, 20 μL of 20% BSA (w/v) was added to block unreacted active groups for 10 min. Finally, the mixture was centrifuged at 11,337 g for 10 min at 4 °C, and the precipitate was ultrasonically resuspended with 200 μL phosphate buffer (containing 0.5% tween-20, 0.5% BSA, 5% trehalose, 0.2% Polyvinyl Pyrrolidone and 0.03% procline-300). Another two conjugations (QNs mAb-Crimson Red LBs probes and TCs mAb-Slate Blue LBs probes) were produced using similar steps. These three LBs-mAb probes were mixed at a ratio of 1:1:1 and stored at 4 °C until use.

2.3. Bioconjugating CG with antibodies

The three kinds of CG-mAb probes were produced using the same mAbs following the procedure of Li et al., with some modifications (Li & Shen et al., 2015, Li & Li et al., 2015; Li & Wen et al., 2015). Briefly, CG nanoparticles with a mean diameter of 40 nm were prepared using the sodium citrate reduction method. The pH of 1 mL CG solution was adjusted with 30 μL 1% potassium carbonate. Fifty microliters of 0.20 mg/mL anti-SAs mAb was added, with stirring for 3 min at room temperature. Twenty microliters of BSA solution (20%, w/v) was added to block free CG for 5 min. After centrifugation (4293 g, 4 °C) for 8 min, the pellet was resuspended with 20 μL phosphate buffer. The probes of QNs and TCs were produced using similar steps, and these three probes were mixed for later use.

2.4. Preparation of immunochromatographic strip

The immunochromatographic strip consists of four parts: sample pad, NC membrane, absorbent pad and adhesive backing card. The capture reagents QN-BSA (0.84 mg/mL), SA-BSA (0.82 mg/mL), TC-BSA (1.30 mg/mL) and goat anti-mouse immunoglobulin (0.21 mg/mL) were sprayed onto the NC membrane as the test lines and control line. The dispense rate was 0.8 μL/cm and the interval between two lines was 3.8 mm. The prepared NC membrane was then dried overnight at 37 °C. Finally, the NC membrane, sample pad and absorbent pad were laminated and pasted to the adhesive backing card as the test strip (Fig. 1).

2.5. Qualitative and quantitative immunoassay procedure

The principle of the immunoassay procedure is based on the competitive binding of analytes in a sample and coating antigen conjugate to the probes (Fig. 1). In the LBIA, 6 μL of mixed detection probes and 200 μL of milk sample were added into the microwell first. After incubating for 3 min, an immunochromatographic strip