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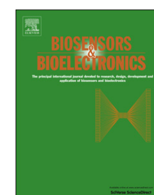

Short communication

Advantages of fluorescent microspheres compared with colloidal gold as a label in immunochromatographic lateral flow assays

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ABSTRACT

Label selection is of vital importance for immunochromatographic assays. In this study, the fluorescent microsphere test strip and colloidal gold immunochromatographic test strip (FM-ICTS and CG-ICTS) were developed for the detection of *Escherichia coli* O157:H7 on the basis of the sandwich format. Two types of labels, namely, colloidal gold particles (CG) and carboxyl-modified fluorescent microspheres (FMs), were compared while coupling with anti-*E. coli* O157:H7 monoclonal antibody (mAb). The FM-ICTS and CG-ICTS were also compared. Results show that the coupling rate between FMs and mAb was higher than that between CG and mAb. Under optimum conditions, the sensitivity of FM-ICTS was eight times higher than that of CG-ICTS. Approximately 0.1 μg of mAb was used in every FM-ICTS, whereas 0.4 μg of mAb was used in every CG-ICTS. The coefficient of variation of FM-ICTS and CG-ICTS was 4.8% and 16.7%, respectively. The FM-ICTS and CG-ICTS can be stored at room temperature for 12 months and specific to five *E. coli* O157:H7 strains. Milk sample inoculated with *E. coli* O157:H7 were tested by the FM-ICTS and CG-ICTS. The FM-ICTS sensitivity was 10^4 CFU/ml while the CG-ICTS sensitivity was 10^5 CFU/ml. The sensitivity, consumption of antibodies, and coefficient of variation of FM-ICTS were better than those of CG-ICTS for the detection of *E. coli* O157:H7.

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1. Introduction

Colloidal gold immunochromatographic test strip (CG-ICTS) has recently seen widespread applications in the fields of medical diagnosis (Chou, 2013; Tripathi et al., 2012), food safety (Chalinan et al., 2013; Chen et al., 2012; Lai et al., 2007; Le et al., 2013), animal health (Kilic et al., 2012; Sheng et al., 2012), agriculture (Hua et al., 2012; Lee et al., 2013; Wang et al., 2013a), and the environment (Liu et al., 2012; Na et al., 2012). This technique is based on an immunochromatographic procedure that utilizes antigen–antibody properties and provides rapid detection of analytes. Colloidal gold (CG) was introduced into immunochromatographic test strip to increase stability. Colloidal gold is essentially inert and forms almost perfectly spherical particles when properly manufactured. Antibodies bind to the surfaces of these gold particles with high strength when correctly coupled, thus providing a high degree of long-term stability in both liquid and solid forms (Chandler et al., 2000). CG-ICTS possesses four benefits: user-friendly format, a short period of time to acquire test results, long-term stability over a wide range of climates, and relatively inexpensive production. These characteristics render CG-ICTS ideally suitable for on-site testing by untrained personnel.

However, CG-ICTS shows serious limitations when high sensitivity is needed. Lateral flow immunoassays have been developed with the advancement of labels, and more sensitive assays using fluorescent microspheres (FMs) instead of CG have appeared in recent years (Song et al., 2013; Xu et al., 2013). However, studies comparing the new labels with CG focused only on sensitivity (Etvi et al., 2012). To our knowledge, direct comparative evaluations of FMs versus CG on the basis of their coupling rate, properties of relative test strip from sensitivity, consumption of antibodies, and coefficient of variation (CV) have not been published.

Escherichia coli O157:H7 was used as a model target analyte. The CG and carboxyl-modified FMs were conjugated with anti-*E. coli* O157:H7 monoclonal antibody (mAb). The coupling rate and amount of antibody conjugating to the surface of the two labels were studied. Label-antibody complex was sprayed on the conjugate and prepared immunochromatographic test strips (ICTS). Two types of ICTS detecting *E. coli* O157:H7 (FM-ICTS and CG-ICTS) were compared for the sensitivity and CV by different readers.

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2. Materials and methods

2.1. Bacterial strains and growth condition

E. coli O157:H7 strain ATCC 43888 was used in this study. The strain was cultured in Luria–Bertani medium (LB, Oxoid, Basingstoke, UK) at 37 °C for 20 h before use. To determine the number of viable cells, serial dilutions of cultures in phosphate-buffered saline (PBS, Sigma Chemical Company, St. Louis, MO, 0.01 M, pH 7.4) were made and plated onto trypticase soy agar (TSA; Becton, Dickinson and Company, Sparks, MD). The plates were then incubated at 37 °C for 24 h.

2.2. Reagents

Fluorescein isothiocyanate fluorescent microspheres (diameter = 175 nm; excitation wavelength = 470 nm; emission wavelength = 525 nm; COOH = 443 μeq/g) were obtained from Merck company (Darmstadt, Germany). The CG particles (40 nm) were prepared in our laboratory (Lai et al., 2009). Nitrocellulose membrane, absorbent pad, sample pad, and conjugate pad were purchased from Millipore (Bendford, MA, USA). The antibody pairs, namely, murine anti-*E. coli* O157:H7 mAb and goat anti-*E. coli* O157:H7 polyclonal antibody, and Donkey anti-mouse IgG were purchased from Meridian Life Science, Inc (Memphis, TN, USA). Bovine serum albumin (BSA), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), and *N*-hydroxy-sulfosuccinmide (sulfo-NHS) were from Sigma (St. Louis, MO, USA). All solvents and other chemicals were analytical reagent grade.

2.3. Instruments

The FM-ICTS reader was from Shanghai Huguo Science Instrument Co, Ltd (excitation wavelength = 470 nm; emission wavelength = 520 nm), and the SkanFlexi BioAssay Reader Systems for detecting CG-ICTS was from Skannex Biotech Co., Ltd (Oslo, Norway).

2.4. Preparation of FM probe

The EDC-mediating method was used to cross-link carboxyl-modified FMs with amine-containing mAb (Wang et al., 2013b). Approximately 10 ml of carboxylated FMs was mixed with sulfo-NHS and EDC in MES-buffered saline (pH 4.7) at room temperature for 120 min to form an amine-reactive sulfo-NHS ester. After adding 1 ml of anti-*E. coli* O157:H7 mAb at concentrations of 25, 50, 100, 200, and 300 μg/ml, the solutions were incubated at room temperature for 2 h. Afterward, 10% BSA was added into the mixture for blocking unreacted active sites for 30 min. The reaction mixture was centrifuged at 8000*g* for 15 min. After centrifugation, the pellet was suspended in 500 μl of neutral PBS containing 1% BSA, 5% sucrose, 3% trehalose, and 0.4% Tween 20. The FM-labeled mAb compound was dried on an enzyme-linked immunosorbent assay (ELISA) plate for 2 h at 30 °C.

2.5. Preparation of colloidal gold probe

About 1 ml of the same anti-*E. coli* O157:H7 mAb at concentrations of 25, 50, 100, 200, and 300 μg/ml were added into 10 ml of pH-adjusted CG solution and was agitated for 30 min. Afterward, 2 ml of 1% (w/v) BSA solution was added and was agitated for 15 min. The mixture was centrifuged at 9000*g* for 30 min. After centrifugation, the gold pellets were dissolved in 50 mM of Tris/HCl buffer. The CG-labeled mAb compound was dried on an ELISA plate for 2 h at 30 °C.

2.6. Evaluation of coupling rate of FM probe and CG probe by ELISA

A total of 100 μl of *E. coli* O157:H7 (10^8 CFU/ml) solution was added to the bottom of each well and incubated for 2 h at 37 °C. After washing, 100 μl of anti-*E. coli* O157:H7 mAb (0, 0.01, 0.02, 0.04, 0.08, 0.16, and 0.32 mg/ml) was added to separate duplicate wells and incubated for 2 h at 37 °C. After washing, 100 μl of substrate/chromogen solution was added and incubated for 15 min at room temperature in the absence of light. The reaction was stopped by adding 100 μl of 2 M H_2SO_4. The absorbance was measured at 450 nm by a Labsystem microplate reader (Helsinki, Finland). The calibration curve was made according to the experimental data. The concentration of residual mAb in supernatant was detected and could be read from the calibration curve. The coupling rate of FM-mAb and CG–mAb could be determined by using ELISA.

2.7. Preparation of test strips

The sample pad, which was treated with 50 mM of borate buffer, pH 7.4, containing 1% BSA, 0.5% Tween-20, and 0.05% sodium azide, was dried at 60 °C for 2 h. The goat anti-*E. coli* O157:H7 polyclonal antibody (1.5 mg/ml) and rabbit anti-mouse antibody (1.0 mg/ml) were applied to the test and control lines on the nitrocellulose membrane, respectively, and dried at 35 °C. The NC membrane was blocked with 5% BSA in pH 7.2 PBS (0.02 M) for avoiding the background in the strip. Absorption pad and glass-fiber membrane were used without treatment. The nitrocellulose membrane, absorption pad, glass fiber membrane, and pretreated sample pad were assembled as the strip.

2.8. Sensitivity comparison between FM-ICTS and CG-ICTS

The *E. coli* O157:H7 culture was diluted from 2.0×10^2 to 1.6×10^6 CFU/ml by sterile PBS. About 100 μl aliquot of sample was pipetted into the ELISA well, which was added with FM-labeled mAb compound and CG-labeled mAb compound. The test was allowed 10 min for the antibody–antigen complex to form. Thereafter, the samples were transmitted into sample pads of FM-ICTS and CG-ICTS. After 10 min, the FM-ICTS was detected by the FM-ICTS reader, and the CG-ICTS was detected by the SkanFlexi BioAssay Reader. The properties of FM-ICTS were compared with that of CG-ICTS. All experiments were performed in triplicate.

2.9. Stability comparison between FM-ICTS and CG-ICTS

The FM-ICTS and CG-ICTS were subjected to accelerated stability study. They were kept at 37 °C and taken out at different time intervals and tested using *E. coli* O157:H7 (ATCC 43888, approx. 10^5 CFU/ml).

2.10. Specificity comparison between FM-ICTS and CG-ICTS

Five *E. coli* O157:H7 strains and 23 non-*E. coli* O157:H7 strains were tested by the FM-ICTS and CG-ICTS for evaluating the specificity of two methods.

2.11. Detection of food sample between FM-ICTS and CG-ICTS

Ultra high temperature sterilized milk samples were purchased from a local supermarket in Nanchang. Each milk sample (25 g) transferred to a stomacher bag was inoculated with 0.1 ml of *E. coli* O157:H7 innocula (10^6, 10^5, 10^4, and 10^3 CFU per ml of milk). The samples were tested by the FM-ICTS and CG-ICTS, respectively.

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