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Novel immunochromatographic assay based on Eu (III)-doped polystyrene nanoparticle-linker-monoclonal antibody for sensitive detection of *Escherichia coli* O157:H7

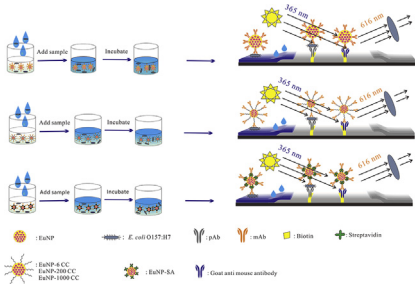
Ke-Yu Xing [a], Juan Peng [a], Dao-Feng Liu [b], Li-Ming Hu [a], Chun Wang [a], Guo-Qiang Li [a], Gang-Gang Zhang [a], Zhen Huang [a], Song Cheng [a], Fang-Fei Zhu [a], Na-Mei Liu [a], Wei-Hua Lai [a, *]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China
[b] Jiangxi Province Center for Disease Control and Prevention, Nanchang 330047, China

HIGHLIGHTS

- Novel ICA based on EuNP-linker-mAb was used to sensitively detect *E. coli* O157:H7.
- SA not only conjugated more Bio-mAb but also provided more active sites of the mAb.
- EuNP-SA-ICA had advantages over other four ICAs in sensitivity and linear range.
- EuNP-SA-ICA offers promising application in the detection of milk sample.

GRAPHICAL ABSTRACT



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ABSTRACT

Colloidal gold immunochromatographic assay (ICA) has poor sensitivity when used for *Escherichia coli* O157:H7 (*E. coli* O157:H7) detection. Eu (III)-doped polystyrene nanoparticle (EuNP) has a large range of stokes shift, long decay time, and wide excitation spectrum and narrow emission spectra. EuNP has been used as novel probe in ICA to improve sensitivity. In this study, carboxyl-modified EuNPs were prepared with different linkers. ICA based on EuNP, EuNP-6 carbon chain (CC) complex, EuNP-200CC complex, EuNP-1000CC complex, and EuNP-streptavidin (EuNP-SA) complex were systematically compared for the detection of *E. coli* O157:H7. Under optimized working conditions, the limits of detection (LOD) of EuNP-ICA, EuNP-6CC-ICA, EuNP-200CC-ICA, EuNP-1000CC-ICA, and EuNP-SA-ICA were 9.54×10^2, 1.59×10^2, 3.18×10^2, 2.98×10^2, and 1.08×10^2 colony-forming units (CFU) mL^{-1}, respectively. The linear ranges of EuNP-ICA, EuNP-6CC-ICA, EuNP-200CC-ICA, EuNP-1000CC-ICA, and EuNP-SA-ICA were $6.36 \times 10^2 - 1.59 \times 10^5$, $3.18 \times 10^2 - 1.59 \times 10^5$, $6.36 \times 10^2 - 1.59 \times 10^5$, $6.36 \times 10^2 - 1.59 \times 10^5$, and $8.0 \times 10^1 - 1.59 \times 10^5$ CFU mL^{-1}, respectively. EuNP-SA-ICA exhibited the highest sensitivity and the widest linear range with good specificity, accuracy, and precision. It could be a promising analytical method for

Abbreviations: ICA, Immunochromatographic assay; *E. coli* O157:H7, *Escherichia coli* O157:H7; EuNP, Eu (III)-doped polystyrene nanoparticle; CC, Carbon chain; SA, Streptavidin; LOD, Limits of detection; CFU, Colony-forming units; ELISA, Enzyme-linked immunosorbent assay; SA-Bio, Streptavidin-biotin; mAb, Monoclonal antibody; pAb, Polyclonal antibody; NC, Nitrocellulose; EDC·HCl, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodimide hydrochloride; BSA, Bovine serum albumin; BB, Borate buffer; PBS, Phosphate-buffered saline; TMB, 3,3′,5,5′-Tetramethylbenzidine; HRP, Horseradish peroxidase; ICTS, Immunochromatographic test strip; Bio-mAb, Biotin-*anti-E. coli* O157:H7 monoclonal antibody; TEM, Transmission electron microscope; DLS, Dynamic light scattering; PDI, Polydispersity index; CV, Coefficient of variation.

* Corresponding author. Tel.: +86 13879178802; fax: +86 791 88333708.
E-mail address: talktolaiwh@163.com (W.-H. Lai).

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detecting *E. coli* O157:H7 in food samples. EuNP-SA-ICA may be a good model for detecting low concentrations of other food-borne pathogens.

1. Introduction

Enterohemorrhagic *Escherichia coli* (*E. coli*) is a group of *E. coli* that causes human hemorrhagic diarrhea and enteritis. A particularly dangerous type from this group is referred to as enterohemorrhagic *E. coli* O157:H7, which is associated with foodborne outbreaks traced to undercooked meats, apple juice or cider, salad, salami, and milk [1]. *E. coli* O157:H7 was first recognized as a pathogen during a severe hemorrhagic diarrhea in 1982. The US Federal Center for Disease Control and Prevention reported a case on 6 October 2016, which led to the infection of 199 people. The infective strain was *E. coli* O157:H7.

Some methods for detection of pathogens include pure culture [2,3], polymerase chain reaction [4–7], electrochemical strategies [8,9], enzyme-linked immunosorbent assay (ELISA) [10–13], surface plasmon resonance [14,15], gene chip [16], free-flow magnetophoresis [17], and surface enhanced Raman spectroscopy [18,19]. However, these methods are time-consuming, laborious, and not suitable for on-site detection.

Colloidal gold immunochromatographic assay (ICA) is convenient and fast but has low sensitivity [20–24]. Eu (III)-doped polystyrene nanoparticle (EuNP) was developed in ICA [25–28]. The nanoparticle has a large stokes shift (>150 nm) and a long fluorescence lifetime and can effectively eliminate the interference of various non-specific fluorescence, thereby leading to high sensitivity.

Steric hindrance of label-monoclonal antibody (mAb) is presumed to limit the immunoreaction between target and antibody. Thus, some label complexes conjugating with label, linkers, and antibodies are used in ICA to reduce steric hindrance [27–29].

In this study, novel EuNP was conjugated with antibody via four different linkers and used in ICA for the sensitive detection of *E. coli* O157:H7 in milk. Linkers enhanced the immunoreaction between target and antibody. Among these linkers, streptavidin-biotin (SA-Bio) system is expected to be more sensitive for an immunoreaction, because the high binding of biotin and streptavidin is a strong combination of multi-level amplification [30–33]. The complex label was first introduced in ICA.

2. Materials and methods

2.1. Materials and reagents

EuNP (excitation wavelength = 365 nm, emission wavelength = 615 nm), EuNP-6 carbon chain (CC), EuNP-200CC, and EuNP-1000CC were purchased from Microdetection Bio-tech Co., Ltd (Nanjing, China). Streptavidin was purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). Biotin was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Anti-*E. coli* O157:H7 monoclonal antibody (mAb) and rabbit anti-*E. coli* O157:H7 polyclonal antibody (pAb) were purchased from Meridian Life Science, Inc. (Memphis, TN, USA). Nitrocellulose (NC) membrane, sample pad, conjugate pad, and absorbent pad were purchased from Millipore (Bedford, MA, USA). Polystyrene plates with 96 wells were purchased from Hangzhou Sheng-you Biotech Co., Ltd. (Hangzhou, China). *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodimide hydrochloride (EDC·HCl), bovine serum albumin (BSA), borate buffer (BB; 0.05 M, pH 6.0), phosphate-buffered saline (PBS; 0.01 M, pH 7.4), and Tween 20 were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 3,3′,5,5′-Tetramethylbenzidine (TMB) and H_2O_2 were obtained from Beijing Ke-wei (Beijing, China). Horseradish peroxidase (HRP)–conjugated goat anti-mouse IgG was purchased from ZSGB-BIO (Beijing, China). All other chemicals and reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

The ELISA reader was purchased from Beijing Pulang New Technology Co., Ltd. (Beijing, China). The portable strip reader for the EuNP-immunochromatographic test strip (ICTS) (excitation wavelength = 365 nm, emission wavelength = 615 nm) was obtained from Fenghang Scientific Instrument Co., Ltd. (Zhejiang, China).

2.2. Bacterial strains culturing

E. coli O157:H7 (ATCC 43888) and 14 non-*E. coli* strains, namely, *Staphylococcus aureus* (CMCC 26003), *Enterobacter sakazakii* (CMCC 45407), *Shigella flexneri* (CMCC 2457), *Shigella sonnei* (CMCC 51592), *Candida albicans* (ATCC 10231), *Bacillus subtilis* (BD 168), *Listeria welshimeri* (ATCC 35897), *Proteusbacillus vulgaris* (CMCC 49027), *Pseudomonas aeruginosa* (CMCC 11997), *Micrococcus luteus* (CMCC 28001), *Salmonella typhimurium* (ATCC 13311), *Salmonella choleraesuis* (ATCC 10708), *Salmonella enteritidis* (ATCC 13076), and *Salmonella paratyphi A* (ATCC 9150) were cultured in Luria–Bertani medium (LB, Oxoid, Basing-stoke, UK) at 37 °C for 20 h. All these strains were conserved in our laboratory.

2.3. Preparation of biotin-anti-E. coli O157:H7 monoclonal antibody (Bio-mAb)

Biotin (10 mM) at 13.3 μL was added to 2 mL of 0.5 mg mL^{-1} anti-*E. coli* O157:H7 mAb and mixed in a DH-II Rotary Mixer from Ningbo Xinzhi Biotechnology Co., Ltd. (Zhejiang, China) at 30 rpm for 45 min at room temperature. Bio-mAb was purified by dialyzing at 4 °C for 3 days.

2.4. Preparation of EuNP-SA

EuNP was added to 0.5 mL of 0.05 M BB (pH 6.0) containing 1.75 μg of EDC to activate the carboxyl of surface of EuNP for 15 min. Then, SA was added to form EuNP-SA complexes. The remaining active sites of the complexes were blocked with 50 μL of 0.05 M BB containing 10% (w/v) BSA. Finally, the mixtures were centrifuged at 12,000 rpm at 4 °C for 20 min. The pellet was suspended in 1 mL of 0.05 M BB (pH 7.0) containing 0.2% (w/v) BSA and 0.5% (v/v) Tween 20. The residual SA in supernatant was analyzed in a subsequent experiment.

2.5. Detection of coupling rate of EuNP-SA with ELISA

The SA solution (100 μL; 1.25 μg mL^{-1}) was coated in the ELISA plate and incubated at 4 °C for 10 h. After washing thrice with 250 μL of 0.01 M PBS containing 1‰ (v/v) Tween 20, 300 μL of 3% (w/v) BSA solution was added into each well to block active sites