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Rapid quantitative detection of *Yersinia pestis* by lateral-flow immunoassay and up-converting phosphor technology-based biosensor

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Abstract

Up-converting phosphor technology (UPT)-based lateral-flow immunoassay has been developed for quantitative detection of *Yersinia pestis* rapidly and specifically. In this assay, 400 nm up-converting phosphor particles were used as the reporter. A sandwich immumoassay was employed by using a polyclonal antibody against F1 antigen of *Y. pestis* immobilized on the nitrocellulose membrane and the same antibody conjugated to the UPT particles. The signal detection of the strips was performed by the UPT-based biosensor that could provide a 980 nm IR laser to excite the phosphor particles, then collect the visible luminescence emitted by the UPT particles and finally convert it to the voltage as a signal. V_T and V_C stand for the multiplied voltage units for the test and the control line, respectively, and the ratio V_T/V_C is directly proportional to the number of *Y. pestis* in a sample. We observed a good linearity between the ratio and log CFU/ml of *Y. pestis* above the detection limit, which was approximately 10^4 CFU/ml. The precision of the intra- and inter-assay was below 15% (coefficient of variation, CV). Cross-reactivity with related Gram-negative enteric bacteria was not found. The UPT-LF immunoassay system presented here takes less than 30 min to perform from the sample treatment to the data analysis. The current paper includes only preliminary data concerning the biomedical aspects of the assay, but is more concentrated on the technical details of establishing a rapid manual assay using a state-of-the-art label chemistry.

Keywords: Up-converting phosphor technology; Lateral-flow immunoassay; Sandwich immunoassay; *Yersinia pestis*

1. Introduction

Yersinia pestis, a small Gram-negative rod belonging to the family *Enterobacteriaceae*, is the etiological agent of plague. This organism is one of the three human pathogenic species, including *Y. pestis*, *Yersinia pseudotuberculosis*, and *Yersinia enterocolitica*, in the genus *Yersinia* [1]. Plague occurs predominantly in three different forms, that is, bubonic, pneumonic, and septicaemic, depending on the route of exposure to the pathogen. Bubonic plague usually involves one group of lymph nodes, contracted following the bite by a flea that has previously fed on an infected rodent and developed a blocked proventriculus, and the infection can spread to the lungs (pneumonic plague) when the suitable treatment is not administered in time. Septicaemic plague is the result of haematogenous dissemination of the bacteria from a bubo. Pneumonic plague can also be transmitted by airborne droplets. The infection takes place within a few hours and causes bronchopneumonia. What is more, the *Y. pestis* is the most dangerous bacterial agent that could potentially be used for biological warfare or bioterrorism [2].

Plague is a typical zoonosis distributed in Asia, Africa and America and it was redefined by the World Health Organization (WHO) as a reemerging infectious disease due to the increased

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outbreak of plague around the world [3]. The rapid detection of *Y. pestis* is critical for controlling the spread of plague. The laboratory detection of *Y. pestis* is based on bacteriological, serological and nucleic acid-based methods [1]. The bacterial culture is not well suitable for rapid detection of *Y. pestis* due to the time delay. The passive haemagglutination assay (PHA), which is used to detect *Y. pestis*-specific antibodies, has repeatedly shown to lack sensitivity and specificity [4]. Therefore, rapid and sensitive assays are desirable for the detection of *Y. pestis*, its specific antigens or nucleic acid sequences. At present, sensitive PCR (polymerase chain reaction)-based detection methods have been described to identify *Y. pestis* [5–7]. Especially, real-time PCR is highly specific and less prone to cross-contamination [8]. Although offering reasonable sensitivity, these assays are time consuming (include both DNA/RNA preparation and the longer detection procedure), and not readily available for on-site detection. Detection of F1 capsular antigen (F1 CA) of *Y. pestis* by immunoassay methods such as immunogold chromatographic dipstick assay and ELISA (enzyme-linked immunosorbent assay) has been developed [9,10].

Immunochromatographic assay, also called lateral-flow (LF) immunoassay, with benefits of low-cost, easy-to-use, rapid and sensitive detection of various analytes, has been developed for many years and mainly been used to detect drugs of abuse and for pregnancy testing at the early stage [11]. Now, it has been surging in infectious disease diagnostics [12,13]. Jung et al. developed a colloidal gold particles-based LF strip for detecting *Escherichia coli* O157 at a minimum of 1.8×10^5 CFU/ml [22]. LF procedure utilizes the specific interaction between antigens and antibodies and provides rapid detection of various analytes. In the field of biomedical diagnostics, the search for the increased detection sensitivity and the possibility of quantitative detection using simple inexpensive assays is an ongoing challenge. In this respect, LF assays have become the popular diagnostic tools in a variety of settings because they are sensitive, simple to perform, inexpensive to manufacture, and perhaps most important, well suitable for rapid on-site detection and can be carried out nearly anywhere by non-technical personnel [14].

UPT particles are submicrometer-sized, lanthanide-doped ceramic particles that possess anti-Stokes shift emission by up-converting infrared excitation light (980 nm) to emit visible light. Up-converting phosphors have also been used to realize quantitative assays and increase sensitivity. UPT reporters are 10- to 100-fold more sensitive than assays using conventional reporter systems such as colloidal gold or colored latex beads [15]. They have attracted considerable attention as the relatively novel luminescent labels, especially in LF assays. UPT has already been demonstrated for the sensitive detection of nucleic acids in microarray [16], single-stranded nucleic acids in a sandwich-hybridization assay [17] and *Streptococcus pneumonia* in an amplification-free hybridization-based DNA [18]; cell and tissue surface antigens by immunocytochemistry [19]; human chorionic gonadotropin [15], drugs of abuse, pathogenic *E. coli* by lateral-flow immunoassay [20]. In this study, up-converting phosphor is used as a reporter in rapid LF immunoassay for quantitative detection of *Y. pestis*.

2. Experimental

2.1. Materials

Affinity-purified polyclonal antibody from rabbit against F1 antigen of *Y. pestis* and affinity-purified antibody from goat against rabbit IgG were prepared in our laboratory. Materials used in preparation of reagents, including HNO_3 (concentrated), tetraethylorthosilicate (TEOS), $NH_4{\cdot}OH$, isopropanol, triethoxyaminopropylsilane (APES), $CHCl_3$, ninhydrin, glycine, glutaraldehyde, sodium cyanoborohydride ($NaCNBH_3$), polyoxyethylene bis-amino (NH_2-PEG-NH_2, with an average molecule weight of 3350), Na_2CO_3, $NaHCO_3$, NaCl, Na_2HPO_4, NaH_2PO_4, NaN_3, albumin bovine V from bovine serum (BSA), *p*-nitrophenyl phosphate (pNPP), Tween-20, NP-40, sucrose, etc., were all obtained from Sigma Chemical Co. (St. Louis, MO). UPT particles ($NaYF_4$:Yb^{3+}, Er^{3+}) with 400 nm in diameter, which emit visible green light of 541 nm when excited by infrared light of 980 nm, were obtained from Kerune Phosphor Technology Co. (KPT, Shanghai).

Strains of *Y. pestis* EV76, *Y. enterocolitica* (LAM 00980, LAM 1310 and LAM 1311), *Y. pseudotuberculosis* (LAM00942, LAM00943 and LAM00644) and other Gram-negative enteric bacteria [*E. coli* (LAM00001 and LAM00003) and *Salmonella choleraesuis* (LAM00993)] were collected by our laboratory [Laboratory of Analytical Microbiology (LAM)]. All bacteria were cultured in Luria–Bertani (LB) broth at 37 °C with shaking at 200 rpm.

2.2. Preparation of UPT-labelled antibody conjugates

UPT particles (400 nm in diameter) were used to conjugate with the antibody against F1 antigen of *Y. pestis*. The biological molecules cannot be directly coupled to the untreated UPT particles. Therefore, a series of surface modifications and activations of UPT particles were required to realize covalent coupling of antibodies to them [14]. Firstly, UPT particles were coated with a thin layer of silica by using TEOS. Then the silica-coated surface of UPT particles can be functionalized with amino-, aldehyde-functional groups using APES, polyoxyethylene bis-amino and glutaraldehyde, respectively. Eventually these aldehyde-functionalized UPT particles can be conjugated to the antibody directly in a 4 °C-prechilled Na_2CO_3–$NaHCO_3$ buffer (50 mM, pH 9.5) under stirring. Uncoupled antibodies to the UPT particles were separated from the conjugated ones by a series of washing steps involving centrifugation and resuspension of pelleted UPT particles in a phosphate buffer of 30 mM pH 7.2. After several washes, UPT conjugates (1 mg/ml) were stored at 4 °C in 30 mM phosphate buffer [pH 7.2, containing 0.1% (w/v) BSA, 0.05% (v/v) Tween-20, 0.02% (w/v) NaN_3].

2.3. Preparation of UPT-based lateral-flow test strips

The shape of the test strip is rectangle with dimension of 74 mm × 4 mm. The components of the strip were the same as those used in immunogold chromatographic dipstick assay (Fig. 1). To prepare LF strips, the sample pad (15 mm,