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A point-of-need enzyme linked aptamer assay for Mycobacterium tuberculosis detection using a smartphone

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ABSTRACT

Due to the poor sensitivities and specificities of antibody based point of need devices, we have developed a low-cost and reliable latent tuberculosis infection (LTBI) screening system based on enzyme linked aptamers which are cost effective and chemically stable. Specific recognition of mannose-capped lipoarabinomannan on the surface of *Mycobacterium tuberculosis* has been achieved for the development of direct and indirect dot-blot approaches which are designed for simultaneous quantitative analysis of multiple samples and identification of mycobacterial strains, respectively. Compared with traditional acid-fast staining assay used for LTBI screening, our direct dot-blot system has offered a lower limit of quantitation at 10^4 CFU/mL and higher accuracy. In contrast to traditional bacterial culture approach which takes 3–5 weeks, our assay can be completed within 5 h, which is a remarkable increase in assay efficiency. To maximize the portability and fulfill the needs for LTBI screening in developing countries, an Android application has been developed to carry out colorimetric analysis of images captured by a smartphone camera, followed by generating and sharing of diagnostic reports through internet connectivity. Upon promotion, we believe this low-cost and effective LTBI screening system will control the outspread of tuberculosis and save many lives, particularly in developing countries.

1. Introduction

Mycobacterium tuberculosis (*M.tb*) latently infects approximately 23% of the global population (namely 1.7 billion individuals) in 2014 and causes 1.5 million deaths each year [1–3], reminding the public the harsh reality that tuberculosis (TB) is around everyone. "Early diagnosis, early treatment" has been proved to be the effective way to prevent the development of active TB from latent carriers and avoid TB epidemic. However, it is still challenging to identify those people who need medical attention because of the deficiency in rapid and accurate diagnostic technology [4–7].

Currently, active TB diagnosis is primarily based on microscopic examination, culture of body fluids, and chest X-rays radiography [6,7]. Although smear microscopy method is the 'front-line' diagnostic test for pulmonary disease, it suffers from poor sensitivity and disappointed detection rates which range from 20% to 80% of culture-confirmed cases. In addition, smear microscopy method can only obtain qualitative results in a low throughput manner, and not more than 20 samples per day can be processed by one professional personnel according to the national mandatory guidance. Chest radiography is also employed in certain countries, but this method lacks the required specificity for immunocompromised and HIV infected patients. The conventional culture methods remain to be the most sensitive and accurate technique for TB identification, yet it is extremely time-consuming (3–5 weeks). And sophisticated

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laboratory facilities are also required for culture method that limits its accessibility to patients in developing countries.

Obviously, inexpensive, rapid and accurate detection methods implemented using easy-to-use portable devices that does not require laboratory facilities or specialist training, will be crucial for achieving global TB elimination by the year 2050, since more than 95% of deaths occur in low-income countries. Recently, many options are being extensively explored for the precise and cost-effective point-of-care (POC) platforms for TB diagnosis, including enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (PCR) which require an additional instrumental determination and/or specialist training, and immunochromatographic test strips which always give qualitative results [8–10]. In addition, antibody-based POC devices have so far proved to be of little value in TB diagnosis because of the poor sensitivities and specificities of the antibodies [4,9].

Aptamers are attractive alternatives to antibodies due to their cost-effective synthesis and ease of modification at arbitrary positions, high stability, affinity and specificity, and small size [11]. Therefore, in this study, a biotin-labeled aptamer which can specifically recognize mannose-capped lipoarabinomannan (ManLAM) on the cell wall of *M.tb* is employed to develop two dot-blot approaches (direct and indirect) for point-of-need TB diagnosis. The direct dot-blot assay is designed for simultaneous quantitative analysis of multiple samples, while the indirect approach can be used to identify the strains of mycobacterial.

In the meantime, thanks to the integration of high-resolution digital sensors, multi-core processing units, and cloud services that ensure pervasive connectivity, the ubiquitous smartphones have created a golden opportunity to develop easy-to-use "all-in-one" devices *via* the addition of accessories for point-of-need latent TB infection (LTBI) screening. Particularly, colorimetric analysis can be performed with a simple camera-equipped smartphone, thus becoming the handiest and feasible approach for point-of-need analysis [13–21]. In this study, an Android application (App) is developed to analyze the images captured by the rear camera of a smartphone, generate diagnostic reports and share them through internet connectivity, realizing the point-of-need TB diagnosis without the need for any additional instruments and specialist training. The determination of *M.tb* in spiked sputum samples and clinical samples have validated the good sensitivity and specificity of the developed dot-blot assays, which are comparable to traditional TB diagnosis methods such as acid fast stain and microbiological culture. The rapid and cost-effective detection method, and the reliable and user-friendly smartphone-based readout can definitely contribute to develop a comprehensive monitoring and surveillance system that would be attainable by all people, in particular the poor with symptoms suggestive of TB.

2. Materials and methods

2.1. Materials

The aptamer design was based on a previous report [22] and synthesized by Sangon Biotech (Shanghai, China). The aptamer sequence for direct dot-blot assay is 5′-***GGCGCCATAGCGACGGGGCCATTCCAAGAA***-biotin-3′, and the sequence of aptamer for the indirect assay is 5′-GCGGAATTCTAATACGACTCACTATAGGGA ACAGTCCGAGCC-***GGCGCCATAGCGACGGGGCCATTCCAAGAA***-GGGTCAATGCGTCATA-3′. Nitrocellulose (NC) membrane (0.2 μm), streptavidin-labeled horseradish peroxidase (HRP), biotin-labeled Concanavalin A (Con A), and 3,3′,5,5′-tetramethylbenzidine (TMB) chromogen were purchased from Beyotime Biotechnology (Shanghai, China). Polyester cloth was purchased from Japanese Standards Association (JSA). Tween-20 was obtained from Sangon Biotech (Shanghai, China). Tris base, *N*-acetyl-L-cysteine (NALC) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (USA). Ziehl-Neelsen kits which contain carbolfuchsin, 3% hydrochloric acid in 95% ethanol and methylene blue were purchased from Baso Biotechnology (Zhuhai, China) and used for acid-fast staining. Chloroform, methanol, sodium chloride, hydrochloride, sodium hydroxide and all other chemicals were of analytical grade and purchased from Guangzhou Chemical Reagents (Guangzhou, China).

2.2. Bacteria culture and pretreatment

Attenuated strain of *M.tb*, H37Ra, was purchased from Microbiologics Inc (MBL). The standard strains were inoculated in the Löwenstein-Jensen medium (Baso Biotechnology, Zhuhai, China). After three weeks, the H37Ra cells were inoculated into the tube containing glass beads by ring scraper. The colonies were broken up by oscillation and washed by Tris-buffered Saline (TBS, containing 10 mM Tris-HCl and 153 mM NaCl) three times with centrifugation at 5000 rpm. The precipitant bacteria were diluted by TBS to 10^8 colony-forming unit per milliliter (CFU/ml), 10^7 CFU/mL and 10^6 CFU/mL, each of which was at least 3 mL. The three samples were inactivated in 90 °C water bath for 30 min. In order to remove mycolic acid on the cell wall of H37Ra, 200 μL of the delipidation solution (Chloroform: methanol = 1:1) was added into the TBS to wash the bacteria, followed by centrifugation at 13,000 rpm twice [23]. The precipitant were dried at 50 °C and then re-dissolved in TBS.

2.3. Direct dot-blot assay

2.0 or 5.0 μL of the samples were dotted on the NC membrane. After air dried for 30–60 min, the membrane was incubated at 37 °C for 30 min and then blocked in the blocking buffer (1% BSA, 10 mM Tris-HCl, 153 mM NaCl, pH 7.5) for 1 h. The biotin-labeled aptamer was diluted into the blocking buffer and incubated with the membrane for 0.5, 1.0, 2.0, 18.0 h. The resultant membrane was washed by TBST buffer (10 mM Tris-HCl, 153 mM NaCl, 0.05% Tween 20, pH 7.5) three times, each of which for 5 min. The streptavidin-labeled HRP was 1:1000 diluted into the blocking buffer and then incubated with the membrane for 1 h. The membrane was washed by TBST buffer three times followed by washing with TBS (10 mM Tris-HCl, 153 mM NaCl, pH 7.5) for 10 min. Finally, color development was produced using H_2O_2 and TMB at room temperature. The obtained results could be observed with naked eyes, recorded by a scanner (HP LaserJet M1213nf, 900 dpi resolution) or a smartphone with imaging function for data sorting, storage, and transmission.

2.4. Indirect dot-blot assay

Polyester cloth was rinsed by 95% of the ethanol, washed with purified water three times under the vacuum filter, and then air dried. Label-free aptamer was dissolved in high salt buffer containing 30% ethanol (100 mM Tris-HCl, 153 mM NaCl and 10 mM $MgCl_2$). 1 μL of the aptamer solution was spotted on the polyester cloth, and the spotting process was repeated for five times for each dot, followed by dried at 37 °C for 3 h and cross-linked under UV irradiation (254 nm) [25]. The polyester cloth was washed by TBST buffer (10 mM Tris-HCl, 153 mM NaCl 0.05%, Tween 20, pH 7.5) three times under the vacuum filter and blocked in the blocking buffer (1% BSA, 2% Tween 20, 10 mM Tris-HCl, 153 mM NaCl, pH 7.5) for 1 h. The polyester cloth was then washed by 3 mL of the TBST buffer for three times and used for the indirect dot-blot assay. The pretreated sample was re-dissolved in blocking buffer and incubated with the dotted polyester cloth followed by three