

Rapid Detection of Active Tuberculosis Using Coal-Derived Quantum Dots

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Introduction: Tuberculosis (TB) is a zoonotic disease which is spread by the *Mycobacterium tuberculosis* through aerial transmission, it affects both human and cattle. The disease is characterized by classic clinical features which include chronic cough, sputum production, appetite loss, weight loss, fever, night sweats, and hemoptysis. Tuberculosis is a major pulmonary disease of concern, which can potentially be diagnosed via breath analysis. In a recent report, world health organization (WHO) states that almost 9.6 million people worldwide are infected with TB each year, while over 3 million people do not get the basic care they need and over 1.5 million people die of the disease, causing TB to be the leading cause of death worldwide alongside HIV. Rapid Diagnosis of tuberculosis has undergone evolution in the past decade, however, despite efforts in the field, point-of-care (POC) disease diagnostics presents several challenges including cost, detection time, equipment portability, and performance.¹

Current Methods of Detection:

- **Interferon Gamma Release Assay:** This is a diagnostic test tool that measures blood samples *ex-vivo* for cellular interaction of Mtb-specific RD1 antigens [e.g. early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10)]. Two IGRAs are commercially available, the Quanti-FERON-TB Gold In-Tube (QFT) assay (Cellestis Ltd, Carnegie, Australia) and the T-SPOT.TB assay (Oxford Immunotec, Abingdon, UK). However, the detection of active TB infection is limited due to its low sensitivity. Based on this information, IGRA's is not recommended in patients with active tuberculosis or in immunocompromised hosts.²
- **Tuberculin skin test:** Tuberculin is a mixture of antigens obtained from the culture of *M. tuberculosis*. Two different tuberculin preparations are available, Old Tuberculin (OT) and Purified Protein Derivative (PPD). The Mantoux PPD tuberculin skin test involves injecting 0.1 mL of PPD tuberculin standardized to a dose of five units just under the top layer of the skin (intracutaneously). People who have been exposed to tuberculosis will develop an causing a slight redness and swelling at the injection site. However, this finding alone is not diagnostic of active tuberculosis, it is only helpful when it is positive, although false positivity may result in cases where patient is recently inoculated with BCG vaccine.²
- **Smear microscopy:** This is the most common method for diagnosing tuberculosis in low income and middle income countries, most laboratories use smears of unconcentrated sputum (direct smears) with Ziehl-Neelsen staining. Despite being a principal method of diagnoses in latent and active tuberculosis, it has failed to find its efficacy in in expedience.³
- **Xpert MTB/RIF Assay:** The Xpert MTB/RIF assay is an automated nucleic acid amplification test that can simultaneously identify *M. tuberculosis* and detect rifampin resistance. This test performs substantially better than smear microscopy. Among over 900 children in South Africa, the sensitivity of Xpert MTB/RIF was similar for induced sputum and nasopharyngeal aspirate specimens (71 and 65 percent, respectively); specificity was >98 percent.

Proposed solution: Based on clinical research, C-reactive protein (CRP) is found in elevated amounts in the serum of patients with active tuberculosis.⁶ We propose to development of a smart, tunable fluorescent sensor system which can provide a rapid, economic method to detect tuberculosis in a patient's serum through the conjugation of biomarkers (CRP), that can be detected using quantum dots.

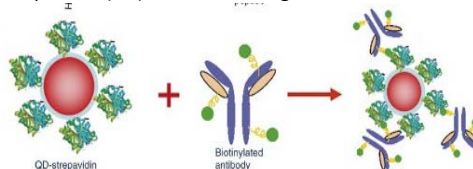
Synthesizing Quantum Dots from Coal

In a typical procedure, 300 mg of coal was suspended in concentrated sulphuric acid (60 ml) and nitric acid (20 ml), and followed by cup sonication (Cole Parmer, model 08849-00) for 2 h. The reaction was then stirred and heated in an oil bath at 100 or 120 °C for 24 h. The solution was cooled to room temperature and poured into a beaker containing 100 ml ice, followed by adding NaOH (3 M) until the pH was 7. The neutral mixture was then filtered through a 0.45-µm polytetrafluoroethylene membrane and the filtrate was dialyzed in 1,000 Da dialysis bag for 5 days. For the larger a-QQDs, the time can be shortened to 1 to 2 h using cross-flow ultrafiltration (Spectrum Labs, KrosFlo Research Ili TFF system with 3 kD cutoff membrane). After purification, the solution was concentrated using rotary evaporation to obtain solid QQDs.⁴

Bioconjugation of Quantum Dots

QD conjugation to biomolecules is carried out by noncovalent biotin-avidin binding Mattoussi and co-workers, through the use of an adaptor or fusion protein for IgG antibody coupling based on electrostatic interactions. The adaptor protein has a positively charged leucine zipper domain for electrostatic binding to QDs and a protein G domain for binding to the antibody Fc region. Using such a "bifunctional" adaptor, the Fc end of the antibody is connected to the QD surface, with the target-specific F(ab')₂ domains facing outward.⁵

Figure 1: Schematic diagram showing bioconjugation of QB-Antibody (QB-Ab) Yun Xing et al., (2007)



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Figure 2: (Bioconjugated quantum dots for multiplexing and immunohistochemistry) Yun Xing et al., (2007)

