# Mycobacterial Diseases

Commentary

# Immuno-PCR: It's Role in Serodiagnosis of Tuberculosis

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## DESCRIPTION

Tuberculosis (TB) is a serious public health problem, which ranks equal with human immunodeficienc\ virus infection as the top most killer globally among the infectious diseases. Rapid and accurate diagnosis is crucial to control the disease so as to initiate an early antitubercular therapy. He GeneXpert MTB/RIF assay recently endorsed by the world health organization (WHO) has been a major breakthrough in TB diagnostics; however, its wide implementation is restricted in many developing nations due to high cost. Here is an urgent need to devise a rapid, highly sensitive and reproducible pointof-care TB diagnostic test. Polymerase chain reaction (PCR) tests targeting IS6110, mpb64 (Rv1980c), pstS1 (Rv0934), etc. are widely used for the diagnosis of TB patients however PCR can't detect non nucleic acid molecules such as proteins, lipids and carbohydrates, which are abundant in circulation during TB infection. Though enzyme-linked immunosorbent assay (ELISA) is widely used for the detection of proteins, but it fails when there is a low concentration of target proteins in body fluids of TB patients and it also leads to nonspecific binding of body fluids that ultimately leads to reduced specificity. Several commercial antibody detection tests are available with imprecise, inconsistent and invariable results by ELISA, therefore, the WHO has recommended against the use of these tests whereas direct detection of Mycobacterium tuberculosis antigens allow specific diagnosis of active TB independent of the host's immune response. Considering that antigen detection may be translated into a rapid POC TB diagnostic test, technology to improve their detection is urgently needed. Originally discovered by immuno-PCR (I-PCR) combines the versatility and simplicity of ELISA with the enormous DmplificDtion capacity of PCR, which has been used for the ultralow detection of cytokines, protooncogenes and potential biomarkers for an early diagnosis of infectious and non-infectious diseases including TB infection and that showed several-fold lower detection limit than analogous ELISA.

We could detect up to 1femtogram (fg)/mL of mycobacterial recombinant purified protein such as early secreted antigenic target-6 (ESAT-6, Rv3875) and immunodominant antigen 85B (Ag85B, Rv1886c) and up to 10 fg/mL of cord factor (trehalos-6,6'- dimycolate) by I-PCR, which was atleast 105-fold lower than ELISA. We previously developed I-PCR based on streptavidin-biotin system for the detection of cocktail of regions of differences (RD) encoded proteins of M. tuberculosis such as ESAT-6, culture filtrDte protein-10 (CFP-10, Rv3874), CFP-21 (Rv1984c) etc. Furthermore, by paired sample analysis, i.e., the detection of cocktail of RD proteins in sputum and detection of anti-RD antibodies in serum of the same PTB patients by I-PCR exhibited higher sensitivities (91% in smearpositive PTB cases and 72% in smear-negative PTB cases) and specificities (85%). We recently developed I-PCR based on succinimidyl 4-[N-maleimidomethyl]-cyclohexane-1- carboxylate (SMCC) for the detection of Ag85B, ESAT-6, cord factor and the cocktail of these antigens in body fluids of PTB and extra pulmonary TB (EPTB) patients. Strikingly, detection of Ag85B protein in sputum samples of PTB patients by I-PCR exhibited better sensitivity (80-83% in PTB patients and 66.6-68.6% in EPTB patients) and specificity (90.3-92.8% in PTB patients and 90-92% in EPTB patients) than the detection of cocktail of Ag85B, ESAT-6 and cord factor which was also superior to ELISA. I-PCR based on SMCC is quicker to perform due to less incubation/wash steps and lead to reduced background signals. We observed only few false positive results in controls with I-PCR assays based on both antigen and antibody detection thus leading to high specificity, which could be due to proper washing at each step and the use of appropriate blocking solutions. He identification of few false positive results also suggests that I-PCR assay is vulnerable to sense non-specific signals and the sample matrix effect. Overall, I-PCR tests indicate promising results based on Ag85B detection for an early diagnosis of TB. We need to devise a costeffective I-PCR test so that it can be included in the diagnostic panel for a routine use.