



# The Place Importance of Serologic Techniques in Tuberculosis Diagnosis



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## Abstract

Diagnosing active TB accurately and rapidly is a key challenge for eradicating the TB epidemic. Conventional culture methods are slow and staining methods not sufficiently sensitive. Nucleic acid amplification techniques (NAATs) tend to be costly and in some cases lack sensitivity. Antibody detection tests (serological tests) have a long history and have been used successfully for the rapid diagnosis of many infectious diseases (e.g., HIV, syphilis, and viral hepatitis). TB serological tests almost exclusively rely on antibody recognition of antigens of *Mycobacterium tuberculosis* by the humoral immune response, as opposed to antigen recognition by the cellular immune response (e.g. interferon-gamma release assays). These tests use various modifications of enzyme-linked immunosorbent assay (ELISA) or immunochromatographic methods to detect different antibody classes. Cellular immunodiagnosics, including tuberculin skin test (TST) and Interferon-Gamma Release Assays (IGRAs) have been used to diagnose latent tuberculosis infection (LTBI). The TST is the only universally accepted test for the diagnosis of LTBI.

**Keywords:** Tuberculosis; Diagnosis; Serological tests

## Introduction

Tuberculosis (TB) is an important global public health problem all over the world. Diagnosing active TB accurately and rapidly is a key challenge for eradicating the TB epidemic [1-3]. Traditional culture methods are slow and staining methods not sufficiently sensitive. Nucleic acid amplification techniques (NAATs) tend to be costly and in some cases lack sensitivity [4-10]. Antibody detection tests (serological tests) have a long history and have been used successfully for the rapid diagnosis of many infectious diseases (e.g., HIV, syphilis, and viral hepatitis) [11-14]. These test are based on the patients' immune response to TB antigens. Previous or current infections may result in positive response depending on the patients' immune status. TB serological tests almost exclusively rely on antibody recognition of antigens of *Mycobacterium tuberculosis* by the humoral immune response, as opposed to antigen recognition by the cellular immune response (e.g. interferon-gamma release assays) [15,16]. Different mycobacterial proteins have been used for diagnosis of of TB. These tests use various modifications of enzyme-linked immunosorbent assay (ELISA) or immunochromatographic methods to detect different antibody classes (mostly IgG) [17-20]. Compared to convantional methods (microscopy, culture); antibody detection methods may enable rapid TB diagnosis, as these tests have the advantages of being quick and technologically easy, requiring minimal training. In

addition, these tests could be adapted to point-of-care formats that can be implemented at lower levels of health services in low- and middle-income countries [15,16].

Although detection of antibodies to *Mycobacterium tuberculosis* complex in the blood is a relatively simple and cost-effective, the existing serological tests for TB are inaccurate to diagnosis pulmonary TB as well as extrapulmonary TB. Thus, the WHO issued a policy statement against the use of serological tests for the diagnosis of active TB [17]. No serological TB test is recommended by international guidelines for clinical use nor approved by the US Food and Drug Administration, dozens of distinct commercial serological tests are marketed in many parts of the world, especially in developing countries with weak regulatory systems. Cellular immunodiagnosics, including tuberculin skin test (TST) and Interferon-Gamma Release Assays (IGRAs) have been used to diagnose latent tuberculosis infection (LTBI). The TST is the only universally accepted test for the diagnosis of LTBI. However, sensitivity of TST can be compromised in individuals with immunodeficiency; and specificity can be affected by previous BCG vaccination and exposure to non-tuberculosis mycobacterium. Furthermore, subjectivity of its interpretation, the need for a return visit, and the fact that TST does not distinguish latent and active TB limits the usefulness of TST Compared to TST, IGRAs have several advantages including superior specificity and sensitivity,

requirement of a single visit, and it does not cause bias to read the test result [18-20].

### Immunocromatographic tests (ICT)

The ICT Tuberculosis test is a rapid, card-based immunochromatographic test for detection of antibodies directed against a various purified *M. tuberculosis* antigens immobilized in lanes on a test strip and generally uses an anti-human IgG labeled with colloidal gold [21-23]. In 2005, the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) performed an evaluation of 19 commercially available rapid diagnostic TB tests. The evaluation reported that, in comparison with culture plus clinical follow-up, commercial tests provided sensitivity and specificity values of 1% to 60% and 53% to 99%, respectively [24].

### Antibody- based tests for detection TB

There are numerous commercial antibody based serological tests for TB diagnostics. Different mycobacterial proteins have been used for serological diagnosis of TB. Initially complex antigens like culture filtrate and later on single purified antigens like Ag85 are being used for this purpose [15,21,22]. Advancement in molecular biology techniques have identified novel antigens of *M. tuberculosis*, some of them have been used to detect specific antibodies raised against them in TB patients. In order to develop a sensitive and a specific serodiagnostic test for TB, purified *M. tuberculosis* secreted proteins were tested to check their potential for use in the serodiagnosis. The antigens used were antigen 85a (ag85a), antigen 85b (ag85b), antigen 85c (ag85c), culture filtrate protein-10 kiloDalton (CFP-10), early secretory antigenic target-6 kiloDalton (ESAT-6) and heat shock proteins (HSP), MPT64. WHO has reviewed 67 studies for pulmonary TB and 25 studies for extrapulmonary TB in which serological tests have been evaluated. The sensitivity and specificity of serological tests have varied from 0% to 100% and from 31% to 100%, respectively. These tests are not recommended for use in TB diagnostics [25-33].

### Interferon-gamma release assays (IGRA Test)

IGRAs are in vitro immune tests based on the detection of antigen-specific T cell immune responses. The methods have been introduced as an alternative to TST for the diagnosis of latent tuberculosis infection (LTBI) [34,35]. Two commercial IGRA tests are available. QuantiFERON®-TB Gold In-Tube (QFT-IT) (Qiagen, Germany) quantitates released IFN- $\gamma$  from the supernatant after whole-blood antigen stimulation using an enzyme-linked immunosorbent assay (ELISA). T-SPOT®.TB (Oxford Immunotec Ltd, Abingdon, UK) measures the frequencies of antigen-specific IFN- $\gamma$ -producing cells from a purified lymphocyte fraction with an enzyme-linked immune spot assay (ELISPOT) (35). Both assays use the following *M. tuberculosis*-specific antigens, the early secretory antigenic target-6 (ESAT-6), the culture filtrate protein-10 (CFP-10), and TB7.7 (p4) (only in QFT-IT), which all are absent in most of the NTMs and BCG strains. This feature is advantageous over PPD, especially in populations with a high NTM exposure and general BCG vaccination. Neither of the IGRAs can distinguish between

active TB and LTBI [17-20]. The TB Network European Trials Group and ECDC have carried out systematic reviews and meta-analyses to assess the accuracy of IGRAs in the diagnosis of active TB and LTBI in different populations. As concluded in the ECDC guidance, no added value of IGRAs combined with standard methods for active TB diagnostics has been found [20]. However, in certain clinical situations, including patients with EPTB or negative AFB staining or culture, children, or the differential diagnosis of infection with NTM, IGRAs can be used to supplement the diagnostic work-up. IGRAs should not be used as a rule-out test of active TB. For the diagnosis of LTBI in low-incidence countries, clear advantages over TST have been shown, and IGRAs can be used in contact-tracing algorithms and in risk assessment to identify individuals for preventative treatment. A negative IGRA result does not rule out LTBI. Only subjects who have an increased risk of developing active TB from LTBI, and would benefit from preventative therapy, should be tested by IGRA. The estimated sensitivity of IGRAs for the detection of LTBI is 80–90%, when culture-confirmed TB patients have been tested. In low TB settings, the specificity of over 95% has been identified. The reproducibility of the IGRAs is limited and highly susceptible to numerous factors related to manufacturer, sample processing, analytical testing and immunological variability [36-40].

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