Review Article

Tuberculosis – burden and serodiagnosis

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Abstract

Tuberculosis (TB) is one of the leading causes of death worldwide. Clinical features and demonstration of the organism by microscopy/culture are still the mainstay of diagnosis of tuberculosis. The present paper reviews the burden of TB and the role of serology in its diagnosis.

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Introduction

Tuberculosis (TB) is one of the leading causes of death worldwide due to a single infectious agent, *Mycobacterium tuberculosis*. The present review examines the burden of tuberculosis in terms of its prevalence, incidence, and resistance to anti-tubercular drugs and role of serodiagnostic procedures.

Burden of tuberculosis: About one-third of the world's population is latently infected with *M. tuberculosis* [1]. In 2016, an estimated 10.4 million people (10% people living with HIV) fell ill with TB and 1.3 million died among HIV-negative TB people and an additional 374,000 deaths occurred among HIV-positive people. Most of the estimated number of incidence cases in 2016 occurred in the World Health Organization (WHO) South-East Asia Region (45%), the WHO African Region (25%) and the WHO Western Pacific Region (17%); smaller proportions of cases occurred in the WHO Eastern Mediterranean Region (7%), the WHO European Region (3%) and the WHO Region of the Americas (3%). The top five countries with 56% of estimated cases were India, Indonesia, China, Philippines and Pakistan (in descending order). Global efforts to combat TB have saved an estimated 53 million lives since 2000 and reduced the TB mortality rate by 37%. Despite these achievements, the latest picture of TB is grim and TB remains the top infectious killer in 2016. In 2015, an estimated 1 million children became ill with TB and 170,000 children died of TB (excluding children with HIV). It is estimated that there is a large pool of undiagnosed drug resistant *M. tuberculosis* infection in children [1].

Banu et al. reported drug susceptibility pattern of 1,906 *M. tuberculosis* isolates from fourteen sentinel surveillance sites of seven divisions of Bangladesh and showed that 1,481 (77.7%) isolates were susceptible to all first-line anti-tuberculosis drugs. Resistance to streptomycin (SM) was 373 (19.6%), to isoniazid (INH) 145 (7.6%), to rifampicin (RMP) 74 (3.9%) and to ethambutol (EMB) 68 (3.6%). Monoresistance to SM, INH, RMP and EMB was 255 (13.4%), 20 (1.0%), 09 (0.5%) and 7 (0.4%) respectively. The multi-drug resistant-TB (MDR-TB) was 2.3% in new patients and 13.8% in previously treated patients. The overall MDR-TB among the urban population was 3.1% in new and 9.6% in previously treated patients, and among the rural population it was 3.2% in new and 22.9% in previously treated patients [2].

Mohiuddin M and Haq JA conducted a study on drug resistance pattern of isolated *M. tuberculosis* from newly detected (untreated) and previously treated TB cases. Out of the total 192 *M. tuberculosis* isolates, 167 were from newly detected and 25 were from previously treated cases. Among the 167 newly detected cases 46.71% were resistant to any of the four first line

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anti-TB drugs and overall drug resistance pattern was INH 37 (22.15%), rifampicin 16 (9.58%), ethambutol 22 (13.17%), and streptomycin 37 (22.15%). Among the previously treated cases, 100% were resistant to any of the four first line anti-TB drugs and overall drug resistance pattern was INH 13 (52.0%), rifampicin 14 (56.0%), ethambutol 17 (68.0%) and streptomycin 13 (52.0%). The rate of MDR-TB in newly detected cases was 4.2% while it was 36.0% among the previously treated cases [3].

Sinha et al. from India reported the drug resistance pattern of 235 *M. tuberculosis* isolates. Out of 235 isolates, 71.1% was resistant to at least one anti-TB drug, whereas only 28.9% was found to be sensitive to all drugs. The rate of MDR-TB was 52.8%. Interestingly, MDR strain of *M. tuberculosis* was isolated from bone marrow sample of a patient without any treatment history [4]. Sethi et al. in India also reported a high prevalence of MDR-TB in HIV cases. MDR-TB was observed in 17.4% isolates. MDR-TB was found to be associated with 9.9% and 27.6% newly and previously treated cases respectively. There was significantly higher association of MDR-TB (27.3%) with HIV seropositive patients as compared to HIV seronegative patients (15.4%) [5]. Current estimates reported the prevalence of primary and acquired MDR-TB in India as 3.5% and 20.5%, respectively [6].

WHO estimated that there were 600,000 new cases with resistance to rifampicin of which 490,000 were MDR-TB. Almost half (47%) of these cases were in India, China and Russian Federation [1]. Recently, the emergence and dissemination of extensively drug-resistant TB (XDR-TB) worldwide is of great threat to public health and tuberculosis control, raising concerns of a future epidemic of virtually untreatable tuberculosis [4]. XDR-TB is defined as MDR-TB with additional resistance to any fluoroquinolone and to at least one of the three injectable anti-tubercular drugs like capreomycin, kenamycin and amikacin [7]. In fact, the emergence of drug resistant *M. tuberculosis* has unfavorably affected the efforts of TB control being made by different countries with limited access to second-line anti-TB drugs [8]. A number of outbreaks of MDR-TB require the continuous surveillance of drug resistance for effective treatment of TB patients and also for initiating adequate public health assessment. The latest anti-TB drug resistance surveillance data (WHO MDR-TB update 2017) showed that 4.1% of new and 19% of previously treated TB cases in the world were estimated to have rifampicin or multidrug-resistant tuberculosis (RR/MDR-TB) and about 6.2% of MDR-TB cases in 2016 were XDR-TB. It was also reported that in 2016 an estimated 600,000 new cases of RR/MDR-TB emerged globally of which 240,000 died. Most of the cases and deaths occurred in Asia. In 2016, 8,000 cases of XDR-TB were reported worldwide. To date, 121 countries have reported at least one XDR-TB case [1]. A summary of TB, MDR-TB and RR-TB cases in different WHO regions for 2016 is shown in Table-1.

Table-1: *TB, MDR-TB and RR-TB cases in different WHO regions for 2016 [1]*

Region	Total notified TB cases	MDR-TB and RR-TB (%)
Western Pacific	1,400,638	1.5
South East Asia Region	2,898,482	1.6
Europe	260,464	18.9
Eastern Mediterranean	527,693	0.9
Americas	233,793	1.6
Africa	1,303,483	2.1
Global total	6,624,523	2.3

Note: MDR-TB = multidrug-resistant tuberculosis; RR-TB = rifampicin-resistant tuberculosis

Tuberculosis may involve any organ or system in the body and is classified as pulmonary (PTB) and extra pulmonary tuberculosis (EPTB). Common sites of EPTB include lymph nodes, pleura, abdominal organs and osteo-articular areas [9]. Lymph node involvement is the commonest form of EPTB. In developing countries where the incidence of TB is high, tubercular lymphadenitis (TBL) is one of the most frequent causes (30-52%) of lymphadenopathy [9,10]. In Bangladesh, lymph node tuberculosis was found to be common

(36.2%) among the EPTB [11]. Therefore, rapid and accurate diagnosis of TBL is of prime importance because delayed chemotherapeutic intervention is associated with poor prognosis [12,13]. Despite T and B cell mediated immunity against *M. tuberculosis*, approximately 90-95% infected individuals develop latent tuberculosis infection (LTBI) following primary infection. If LTBI is left untreated, there is a 10% life time risk of developing active tuberculosis, usually localized in the lungs [14]. In HIV infected patients, there is an even greater risk, ~10% per year, with a higher incidence of disseminated infection [15].

Diagnosis of TB: Diagnosis of tuberculosis (TB) mainly depends on sputum smear microscopy, chest radiography and tuberculin skin test (TST). Microscopic examination of sputum and other specimens by Ziehl-Neelsen staining is the only rapid, relatively simple and inexpensive test for diagnosis of active pulmonary TB and EPTB. But, the reported sensitivity of Ziehl-Neelsen staining of unprocessed sputum smears from adults is only 40 to 70% because 5×10^3 to 5×10^4 organism/ml specimen is needed for the detection of bacilli [16]. Culture is also done for isolation and identification of *M. tuberculosis* but it is time consuming, bio-hazardous and needs bio-safety facilities. It needs an average time of 23.6 days in Lowenstein-Jensen media [17]. Sensitivity and specificity of this method are 48.9% and 100% respectively [18]. In newer liquid culture method like Microscopic Observation of Drug Susceptibility (MODS) assay, about nine days are required for culture and drug susceptibility and its sensitivity is 92% and specificity 94.4% [19,20]. But in this method, chance of contamination is more and skilled laboratory personnel are required and it is bio-hazardous also. The average turnaround time for other liquid based culture methods like mycobacterial growth indicator tube (MGIT) and automated systems like BACTEC is around 6.5 to 9 days with specificity between 80-00% [21]. Improved diagnostic tests like nucleic acid amplification tests are often too expensive and complex to be used as routine method in low-income settings. The GeneXpert MTB/RIF assay, being claimed as a major advance in TB diagnostics and endorsed by the WHO, provides simultaneous detection of *M. tuberculosis* and rifampicin resistance. However, high cost is a barrier for scaling-up this new technology in many resource poor areas where the need is most severe [22].

Role of serodiagnostic procedures for diagnosis of TB: Detection of antibodies or antigens, as serological marker, is being used in regular practice for the diagnosis of many viral and bacterial infections. Many *M. tuberculosis* cell wall components have antigenic properties. Following its infection different antibodies like IgG, IgM, IgA are reported to be produced against different cell wall antigens. Many serological tests have been used to detect *M. tuberculosis* antigens and antibodies. In comparison to microscopy, serological TB tests have the advantages of rapid diagnosis, technological simplicity, and modest training requirements. In addition, these tests could be performed at peripheral health facilities.

M. tuberculosis infection can be categorized into three main stages: latent, reactivating, and active TB. Each stage represents differences in *M. tuberculosis* gene expression and hence antibody response to *M. tuberculosis* infection varies in different stages of *M. tuberculosis* infection due to stage specific antigens [23]. Antibody response to *M. tuberculosis* infection may also vary due to heterogeneity of the geographical background [24]. Hsp16.3 is secreted during the latent phase of mycobacterial growth and is an important component that facilitates the survival of *M. tuberculosis* during latent human infection [25]. Immune responses to *M. tuberculosis* antigens, ESAT6 (early secretory antigen target), CFP10 (culture filtrate protein) and Ag85B have been shown to be significantly higher in active TB than in latent TB [26]. Thus, it is rational to evaluate the *M. tuberculosis*-secreted antigens in serodiagnosis of active TB or latent TB infection.

The proteins of *M. tuberculosis* induce a variable degree of humoral immune responses in infected person. The most studied secreted proteins of *M. tuberculosis* are ESAT-6, CFP-10, 38kDa, 16kDa and Ag85 complex. The ability of these proteins to elicit serological response has in fact made them to be utilized as the candidates for serodiagnosis. The other proteins eliciting humoral immune response are cell wall fraction (CWF) and lipoarabinomannan (LAM). Serological methods

have been regarded as attractive tools for rapid diagnosis of tuberculosis due to their simplicity, rapidity and low cost. Serodiagnosis also does not require safety measures associated with handling of live bacilli as in culture and offers the possibility of detecting cases often missed by routine sputum smear microscopy.

Many investigators assayed humoral immune response to tubercular antigens and evaluated different antigens as candidate for serodiagnostic test to detect active and latent tubercular infection. The success is so far variable.

Previously, we determined antibody response to four mycobacterial antigens namely Ag85 complex, culture filtrate protein (CFP), cell wall fraction (CWF) and lipoarabinomannan (LAM) in the sera of 30 confirmed cases of tuberculosis and 30 healthy subjects. The sensitivity and specificity of anti-Ag85 complexes and anti-CFP IgM and IgG antibody ranged from 60% to over 95%. It appeared that IgM and IgG antibody response to Ag85 complex was better compared to that of CFP. Therefore, determination of IgM and IgG against Ag85 complex could be used as a serological marker for diagnosis of active tuberculosis in cases where other tests do not give conclusive information [27]. It is particularly applicable in children where they are unable to provide sputum samples for either staining or culture.

Many authors investigated antibody response against Ag85 complex, CFP and LAM and found sensitivity and specificity similar to our findings [28-34]. Ag85 complex also showed immunodominant positivity in the studies conducted by Imaz et al. [35] and Sanchez-Rodriquez et al. [36]. However, Suraiya et al. found poor positivity to Ag85 complex [37]. This might be due to difference in stages of infection and heterogeneity of the geographical background [24]. Suraiya et al. conducted a study on 60 confirmed pulmonary tuberculosis patients to test the presence of IgG and IgA against *M. tuberculosis* proteins like ESAT6, SCWP (soluble cell wall protein), LAM (lipoarabinomannan), Ag85 and observed that the sensitivity of IgA ELISA was 81.7%, 83.3%, 11.7%, 53% and specificity was 96.6%, 93.3%, 100.0%, 96.6% respectively. The sensitivity of IgG ELISA was 71.0%, 71.0%, 71.0%, 21.7% and specificity was 93.3%, 96.6%, 96.6%, 100.0% respectively [37].

Currently, the antigens including 38kD, 16kD, ESAT-6, MPT63, 19kD, MPT64, MPT32, Rv1009, MTB48, MTB81, MTC28, Ag85B and KatG have been evaluated for their serodiagnostic potential. The use of any single *M. tuberculosis* antigen as a serodiagnostic marker generated false positive rate of 30-40%, but a combined use of multiple antigens improves the positive diagnostic rate. Some researchers reported that the detection of antibodies directed against multiple antigens could provide an improvement in sensitivity compared to single antigen in *M. tuberculosis* infection. Zhang et al. focused on the analysis and comparison of the four potential *M. tuberculosis* secreted proteins - ESAT6, CFP10, Ag85B, Hsp16.3 and the fusion protein Ag85B-Hsp16.3 as new markers in the serodiagnosis between active TB and LTBI. The result showed that in active TB the specificity for detecting *M. tuberculosis* antibody responses to antigens Ag85B-Hsp16.3, Ag85B, Hsp16.3, ESAT6 and CFP10 was 95.65%, 80.43%, 88.04%, 95.65% and 80.43% respectively and sensitivity was 61.67%, 63.33%, 63.33%, 96.67%, and 80.00% respectively. In case of LTBI, the serological responses to Ag85B-Hsp16.3, Ag85B, Hsp16.3, ESAT6 and CFP10 showed that the specificity was 73.91%, 97.83%, 88.04%, 84.78% and 69.57% respectively and the sensitivity was 60.00%, 53.33%, 53.33%, 60.00% and 73.33% respectively [38]. Burbelo et al. used luciferase immunoprecipitation system (LIPS) to screen antibody responses against seven potential *M. tuberculosis* antigens (PstS1, Rv0831c, FbpA, EspB, BfrB, HspX, and Ssb) for the diagnosis of pulmonary TB. LIPS mixture format of seven antigens showed 74-90% sensitivity and 96-100 % specificity [39]. A summary of the different studies regarding antibody detection tests for serodiagnosis of active tuberculosis is given in Table-2.

Dai et al. detected *M. tuberculosis* antigens (ESAT-6, CFP-10, 38kD) by multi-target antibodies as capture antibodies and showed that the diagnostic performance was significant with sensitivity of 68% (95% CI – 53.3, 80.48) and specificity of 97.5% (95% CI – 86.84, 99.94) [42]. Attallah et al.

Table-2: *Evaluation of antibody detection tests for serodiagnosis of active tuberculosis*

Antigen used	Antibody detected	Sensitivity (%)	Specificity (%)	Study authors, year [reference]
16kDa+r38kDa	IgG	52.5	93.3	Senol et al., 2007 [40]
Ag85 complex	IgG	82-84.1	85.2-86	Kumar et al., 2010 [29] Kashyap et al., 2007 [30]
ESAT-6	IgG	64.9	88.9	Kumar et al., 2010 [29]
CFP-10	IgG	66	85.2	Kumar et al., 2010 [29]
LAM	IgG	80-93	72-100	Brown et al., 2003 [31] Chan et al., 2000 [32] Sada et al., 1992 [33] Boechme et al., 2005 [34]
CFP-10/ESAT-6	IgG	60.4	73.8	Wu et al., 2010 [35]
38kDa	IgG	59-73.6	85.4	Wu et al., 2010 [35]
MTB48	IgG	73.2	77.7	Wu et al., 2010 [35]
PPD	IgG	94.7	NA	Agarwal et al., 1989 [41]
Intact cell	IgG	8.7	NA	Agarwal et al., 1989 [41]

Note: ESAT = early secretory antigen target; CFP = culture filtrate protein; LAM = lipoarabinomannan; MTB = Mycobacterium tuberculosis; PPD = protein derivative; NA = not available

detected 55kDa *M. tuberculosis* antigen in serum samples of pulmonary TB patients by dot-ELISA format with sensitivity of 87% and specificity of 93% [43]. Liu et al. conducted a study for detection of *M. tuberculosis* antigen peptides of CFP-10 and ESAT-6 by antibody labeled and energy focusing porous discoloidal silicon nanoparticles, NanoDisc-MS method and detected target peptides in 92.6% TB cases with 100% sensitivity in smear positive cases and 91% sensitivity in smear negative cases and no target peptides were detected in healthy controls [44].

Three systematic reviews were commissioned by the WHO Special Program for Research and Training in Tropical Diseases. Two reviews evaluated the performance of commercial serological tests for diagnosis of PTB and EPTB and one review evaluated the performance of non-commercial (in-house) serological tests for PTB. The reference standards were culture and/or smear microscopy and in addition, for EPTB, histopathological examination. The reviews of commercial serological tests for the diagnosis of PTB and EPTB found highly variable sensitivity and specificity. For the review of non-commercial (in house) tests for PTB, only purified antigens were included and purified protein derivative (PPD), culture filtrates or sonicated antigens were excluded. The review yielded 254 test evaluations (including 51 distinct single antigens and 30 distinct multiple antigens combinations) and found potential candidate antigens for inclusion in a serological test in both HIV uninfected and infected individuals. Multiple antigens provided higher sensitivity than single antigen. However, no antigen achieved sufficient sensitivity to replace smear microscopy [45]. The sensitivity and specificity of antigen detecting serological tests for the diagnosis of PTB and EPTB are summarized in Table-3 and 4.

In order to develop policy guidance concerning commercial serological TB tests, WHO commissioned an updated systematic review. The review included 67 studies (5,147 participants) in PTB group and 25 studies (1,809 participants) in EPTB group. The results demonstrated that serological tests for both PTB and EPTB provided inconsistent and imprecise sensitivity and specificity. Anda-TB IgG (Anda Biologicals, Strasbourg, France) yielded pooled sensitivities of

Table-3: *Evaluation of antigen detection tests for serodiagnosis of pulmonary tuberculosis*

Antigen(s) detected	No. of patients with/without TB	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Study authors, year, country [reference]
45/47kDa	64/23	28 (18, 41)	96 (78, 100)	Chanteau et al., 2000, Madagascar [48]
20kDa	175/65	91 (86, 95)	89 (79, 96)	El-Marsy et al., 2008, Egypt [49]
Ag85 complex	24/49	96 (79, 100)	80 (66, 90)	Kashyap et al., 2007, India [30]
M. tuberculosis antigens (unspecified)	41/30	90 (77, 97)	90 (73, 98)	Khomenko et al., 1996, Russia [50]
M. tuberculosis antigens (unspecified)	18/26	94 (73, 100)	77 (56, 91)	Mamun et al., 1990, Bangladesh [51]
M. tuberculosis antigens (unspecified)	19/26	47 (24, 71)	77 (56, 91)	Mamun et al., 1990, Bangladesh [51]
65kDa	24/74	100 (86, 100)	82 (72, 90)	Rajan et al., 2007, India [52]
LAM	50/63	88 (76, 95)	100 (94, 100)	Sada et al., 1992, Mexico [33]
LAM	21/63	57 (34, 78)	100 (94, 100)	Sada et al., 1992, Mexico [33]
PPD	30/37	73 (54, 88)	84 (68, 94)	Sood et al., 1991, India [53]
M. tuberculosis antigens (unspecified)	42/201	79 (63, 90)	75 (68, 80)	Stavri et al., 1990, Italy [54]
38kDa	44/120	48 (32, 63)	97 (92, 99)	Verbon et al., 1993, Netherlands [55]

Note: LAM = lipoarabinomannan; PPD = purified protein derivative; CI = confidence interval

Table-4: *Evaluation of antigen detection tests for diagnosis of extra pulmonary tuberculosis*

Antigen(s) detected	No. of patients with/without TB	Form of tuberculosis	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Study authors, year, country [reference]
ES-31	22/15	Lymph node	73 (50, 89)	87 (60, 98)	Shende et al., 2007, India [56]
ES-20	30/35	Lymph node	82 (60, 95)	87 (60, 98)	Shende et al., 2007, India [56]
ES-20	30/35	Lymph node	90 (73, 98)	89 (73, 97)	Shende et al., 2008, India [57]
ES-31, ES-43, EST-6	32/75	Multiple	47 (29, 65)	91 (82, 96)	Upadhye et al., 2007, India [58]

Note: ES = excretory-secretory; CI = confidence interval

76% (95% CI – 63, 87) in studies of smear-positive and 59% (95% CI – 10, 96) in studies of smear negative patients; corresponding pooled specificities were 92% (95% CI – 74, 98) and 91% (95% CI – 79, 96) respectively. The key finding in the analysis regarding the popularity of serological tests was

that – it met the perceived need among the private providers and the patients, though it showed the absence of an accurate, validated point of care test for TB [46]. In 2011, World Health Organization has issued policy statement that commercial serological tests for the diagnosis of MTB provides inconsistent and variable results for sensitivity and specificity, do not improve patient-important outcomes and adversely affect the patient safety [45,47]. In view of this, India and Cambodia imposed ban on import and sale of TB serological tests.

The gamma interferon (IFN-γ) release assay (IGRA) is an in vitro test based on release of IFN-γ by foreign epitope-stimulated T cells. The promising antigens for use in such assays are the ESAT-6, CFP-10 and the TB7.7, which are absent from BCG strains and from most non-tuberculous mycobacteria. ESAT-6 and CFP-10 have been shown to elicit strong IFN-γ responses from the T cells of persons infected with *M. tuberculosis* but not from the T cells of those vaccinated with BCG or at low risk of infection. Tsiouris et al. evaluated the sensitivity of an "in-tube" gamma interferon release assay using TB-specific antigens in comparison to the tuberculin skin test (TST) and the sputum smear for acid fast bacilli (AFB) in TB cases in South Africa. Among 154 patients with a positive culture for *M. tuberculosis*, the sensitivity of the IGRA for the diagnosis of TB varied by clinical subgroup from 64% to 82%, that of the TST varied from 85% to 94%, and that of two sputum smears for AFB varied from 35% to 53%. The sensitivity of the IGRA in HIV-infected TB cases was 81%. HIV-infected TB patients were significantly more likely to have indeterminate IGRA results and produced quantitatively less gamma interferon in response to TB-specific antigens than HIV-negative TB patients. The combined sensitivities of the TST plus IGRA and TST plus a single sputum smear were 96% and 93%, respectively. The overall sensitivity of the IGRA was 75% in all the patients with pulmonary TB, which increased to 82% in new cases of pulmonary TB. A single sputum smear combined with the IGRA resulted in a sensitivity of 86% (95% CI- 79, 91) for culture-proven pulmonary TB. A single sputum smear combined with the TST resulted in a sensitivity of 93% (95% CI- 87, 96) for culture-positive pulmonary TB. The sensitivity of the IGRA for TB was considered a surrogate of sensitivity in LTBI [59].

Doan et al. performed the meta-analysis to evaluate the performance of TST and IGRA for LTBI diagnosis in various patient populations using Bayesian latent class modeling. A total of 157 studies were included in the analysis. In immunocompetent adults, the sensitivity of TST and QuantiFERON-TB Gold In-Tube (QFT-GIT) test were estimated to be 84% (95% credible interval [CrI] 82–85%) and 52% (50–53%), respectively. The specificity of QFT-GIT was 97% (96–97%) in non-BCG-vaccinated and 93% (92–94%) in BCG-vaccinated immunocompetent adults. The estimated figures for TST were 100% (99–100%) and 79% (76–82%), respectively. T-SPOT.TB had comparable specificity (97% for both tests) and better sensitivity (68% versus 52%) than QFT-GIT in immunocompetent adults. In immunocompromised adults, both TST and QFT-GIT displayed low sensitivity but high specificity. QFT-GIT and TST were equally specific (98% for both tests) in non-BCG-vaccinated children; however, QFT-GIT was more specific than TST (98% versus 82%) in BCG-vaccinated group. TST was more sensitive than QFT-GIT (82% versus 73%) in children [60].

In summary, the serological tests for diagnosis of PTB and EPTB demonstrate inconsistent and imprecise sensitivity and specificity. However, it may be useful in LTBI where specimens for diagnosis are not available. Serological tests in association with smear microscopy would provide better result. Determination of antibodies directed against multiple antigens might provide improved result compared to single antigen. Similarly, detection of multiple *M. tuberculosis* antigens rather than single antigen could increase the positive diagnostic rate.

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