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ORIGINAL RESEARCH

Comparative Evaluation of Diagnostic Performance: Standard E TB Feron ELISA vs QuantiFERON-TB Gold Plus for Latent Tuberculosis Infection Detection in Diverse Risk Groups in Bangladesh

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Background: Around one-quarter of the global population has latent tuberculosis infection (LTBI). If left untreated, LTBI has 5–10% lifetime risk of developing into TB. Interferon-gamma release Assays (IGRAs) are more sensitive than the tuberculin skin test for LTBI detection. However, the high cost and complexity of IGRAs are barriers to adoption in resource-constrained settings. This study evaluated the diagnostic performance of a more affordable IGRA, Standard E TB-Feron (TBE), among different risk groups in Bangladesh.

Methods: 532 participants of all age groups were enrolled from the TB Screening and Treatment Centers and Dhaka Hospital of icddr,b between June and September 2023. The participants were categorized into four risk groups: healthy people, healthcare workers/attendants of TB patients, patients with microbiologically confirmed TB, and people with a history of TB. The diagnostic performance of TBE was compared to QuantiFERON-TB Gold Plus (QFT-Plus) for all groups. GeneXpert, culture, and microscopy were used to confirm TB microbiologically.

Results: TBE had an overall agreement of 85.9% (95% CI, 82.5% to 88.7%), positive percent agreement of 86.1% (95% CI, 80.6% to 90.5%), and negative percent agreement of 85.7% (95% CI, 81.3% −89.4%) with QFT-Plus. Among 81 culture-positive patients, TBE and QFT-Plus were positive for 60 (74.1%) and 62 (76.5%) respectively. Among healthy people, TBE and QFT results were positive for 49 (24.5%) and 59 (29.5%) respectively. Among health workers and contacts, TBE and QFT-Plus were positive for 79 (39.5%) and 73 (35.5%) respectively.

Conclusion: We found a substantial agreement (Cohen's kappa of 0.71) between TBE and QFT-Plus in detecting LTBI across different groups, suggesting its potential as a cost-effective diagnostic tool. Implementation of TBE in routine clinical practice could increase accessibility to LTBI diagnosis, facilitating the timely initiation of preventative therapy, and leading to a reduction of active TB incidence.

Keywords: latent tuberculosis infection, interferon-gamma release assay, Standard E TB-Feron ELISA, QuantiFERON-TB Gold Plus

Introduction

Tuberculosis disease (TB) is one of the top infectious killers in the world. In 2022 an estimated 10.6 million people felt ill with TB and leading to 1.3 million deaths.[1] An even greater number of people, with estimates suggesting one-quarter of the global population, is infected with latent tuberculosis infection (LTBI).[2] LTBI occurs when a person is infected with *Mycobacterium tuberculosis* (Mtb) but shows no clinical symptoms. Such individuals cannot transmit the disease

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and may not even know they have been infected unless reactivation occurs. However, if LTBI is left untreated, there is a 5–10% lifetime risk of developing active TB, making it a silent reservoir from which reactivation may occur.[3] To achieve the goals of the current Global End TB strategy, the large reservoir of LTBI needs to be reduced through the identification of LTBI and preventative therapy.[4]

An accurate diagnosis of LTBI is crucial for effectively delivering preventative therapy. However, there is no definitive gold standard test for diagnosing LTBI. The two primary diagnostic tests, tuberculin skin test (TST) and interferon-gamma release assays (IGRA) rely on cell-mediated immunity. TST is prone to the booster effect and can give false positive results due to previous bacillus Calmette-Guérin (BCG) vaccination or non-tuberculous mycobacteria (NTM) infections.[5,6] The false positive results arise from TST's use of purified protein derivative (PPD) from Mtb, which shares antigens with BCG and other NTMs. The TST test also requires two hospital visits and the interpretation of the readings can be subject to inter-/intra-reader variability. As the results of both these tests depend on the host immune response, their positivity rates are lower in immunocompromised populations. TST is more susceptible to this drawback than IGRA.[7,8] On the other hand, IGRA tests offer higher sensitivity and specificity, only require a single visit from the patient, provide a technical readout, have no booster effect due to their in-vitro nature, and are not affected by BCG vaccination since they use antigens absent in BCG.[9,10]

While, Bangladesh, and the Southeast Asia region as a whole, bear a disproportionate burden of TB and LTBI,[1] diagnosis of LTBI remains particularly challenging in these countries as IGRA tests are more expensive and require higher technical capacity and laboratory infrastructure. Cost is a significant factor for resource-limited countries like Bangladesh and is a barrier to replacing the TST with IGRA. The Standard E TB-Feron ELISA (TBE) and Standard F TB-Feron fluorescent immunoassay FIA tests have been developed by SD BIOSENSOR (Republic of Korea) to address some of these issues with current IGRA tests available in the market.

TBE utilizes whole recombinant proteins of ESAT-6, CFP-10, and TB7.7 degraded into diverse peptides that present multiple epitopes to the T cells.[11,12] This is expected to give TBE an advantage over other IGRA assays using TB-specific synthetic peptides since presenting multiple epitopes stimulates T cells more effectively, leading to higher sensitivity. QFT-Plus utilizes two antigen tubes containing TB-specific synthetic long peptides of ESAT-6 and CFP-10, while the second tube of the QFT-Plus test also contains short peptides of these antigens.[13] While widely accepted, QFT-Plus availability in Bangladesh is limited to private healthcare providers and research settings due to the high cost per test. TBE kits cost significantly less per test (half the price of QFT per test during the study period) and require 3 mL blood vs 4 mL for QFT-plus. The introduction of newer tests that are affordable and accurate will be a major advance in reducing the LTBI reservoir in resource limited settings.

The diagnostic performance of TBE has only been evaluated in South Korea,[11,14,15] an intermediate-TB burden country. We aim to assess the diagnostic performances of TBE in Bangladesh a high-burden, and resource-constrained country by comparing it with QFT-Plus among diverse risk groups in our population.

Methods

Study Design and Enrolment

In this prospective study, we targeted enrolling 110 individuals with TB and 420 without TB were enrolled from the TB Screening and Treatment Centre (TBSTC) and Dhaka Hospital of icddr,b between June and September 2023.

The study enrolled four distinct groups of individuals. Group 1 was comprised of participants who had no prior history of tuberculosis, no symptoms of the disease, and no known contact with anyone who had an active case of TB. Group 2 consisted of healthcare workers and individuals who had close contact with patients infected with TB. Group 3 included people newly diagnosed with TB during or within a month before the study enrolment. Finally, Group 4 included participants who had previously been treated for TB and had completed their treatment before the time of enrolment. Each group was also divided into different subgroups based on the enrolment of patients with diabetes, smoking history, and children (Table 1). Individuals deemed unfit to provide 7 mL of blood or those without consent or assent were excluded from the study.

Table 1 Enrollment Targets of Clinical Characteristics and Risk Factors Associated with the Study Participants

Sub-group	Group 1	Group 2	Group 3	Group 4	Total
Diabetes	5	5	5	5	20
Smoker	4	3	10	3	20
Pediatrics	7	8	7	8	30
Others	184	184	88	4	460
Total	200	200	110	20	530

The study complies with the Declaration of Helsinki, and the protocol and consent forms were approved by the Research Review Committee and Ethical Review Committee of the Institutional Review Board of icddr,b (Research protocol # PR-23012). All participants aged 18 and over provided written informed consent. For participants 11–17 years of age, written parental/guardian consent or assent was obtained. Additionally, children aged 0–10 provided parental/guardian written assent. Laboratory staff was blinded to the status of the patient, the enrolled group, and the result of other tests until all tests were completed for the patient. Indeterminate results in IGRA tests were repeated once, but discrepant results were not.

Collection and Incubation of Blood

7 mL of blood was collected from the participants, 3 mL of which were evenly distributed into three TBE tubes, and 4 mL were evenly distributed into a set of QFT-Plus tubes. The order of blood insertion was Nil tube, followed by TB-antigen tube(s), and finally, the Mitogen tube. After 16–24 of incubation at 37°C, the tubes were then subjected to centrifugation for 15 minutes at 2500×g. The tests were performed immediately or stored at 4°C, and performed within three days of collection.

Standard E TB-Feron ELISA (TBE) and QuantiFERON TB Gold-Plus (QFT-Plus) Assay

Both TBE and QFT-Plus are ELISA-based and were conducted according to the instructions provided by the manufacturers.[13,16] The procedures for both tests were similar, except for an additional TB2 antigen tube in QFT-Plus. Additionally, TBE required 1 hour of incubation at 37°C, whereas QFT-Plus required 2 hours at room temperature.

Active TB Diagnosis

For group 3 participants, who tested positive for *Mycobacterium tuberculosis* complex (MTBC) in the GeneXpert MTB/RIF assay at the TBSTC, the remaining sputum sample was transferred to the Mycobacteriology laboratory within 24 hours for further testing. Acid-fast bacillus (AFB) microscopy and culture on Löwenstein–Jensen (LJ) slants were performed after sputum decontamination using N-Acetyl-L-Cysteine-Sodium Hydroxide (NALC-NaOH) method.[17]

Statistical Analysis

The qualitative concordance between tests was determined by calculating positive percent agreement (PPA), negative percent agreement (NPA), and Cohen's kappa using a 2-by-2 crosstab analysis. Fisher's exact test was used to calculate the statistical significance of the effect of Diabetes or Smoking on IGRA results. The chi-square test of independence was used to determine if there was a significant difference between TBE and QFT-plus across the groups. Indeterminate results were excluded from statistical calculations. A p-value less than 0.05 was considered statistically significant. Indeterminate results were excluded from statistical calculations. Statistical analysis was performed using R Studio (R Studio Team, 2023) and R program (version 4.3.2).

Results

Demographic and Clinical Characteristics

A total of 532 participants were enrolled. 200 healthy participants were enrolled in Group 1, 200 in contact with TB were enrolled in Group 2, 110 TB patients were enrolled in Group 3, and 22 with a history of TB were enrolled in Group 3

(Table 2). Among all participants, 280 (52.6%) were male, 49 (9.2%) were diabetic, 75 (14.1%) were smokers, and 9 (1.7%) were 5 years old or younger. These characteristics are shown in Table 2.

QFT-Plus, and TBE results Among the Groups

Table 3 presents the distribution of TBE and QFT-Plus test results across the groups. While some differences in results are observed between the tests, they were statistically insignificant ($p > 0.05$) across all groups. TBE had a slightly higher overall positive rate (n = 226, 42.5%) than QFT-Plus (n= 212, 39.8%). Among TB patients in group 3, QFT-Plus had a slightly higher positive rate (n= 77, 70.0%) than TBE (n= 74, 67.3%). Conversely, QFT-Plus had a lower positive rate (n= 49, 24.5%) than TBE (n=59, 29.5%) in group 1. In groups 2 and 4, the positive rates for TBE were 39.5% (n= 79) and 63.6% (n= 14), respectively, while those for QFT-Plus were 36.5% (n=32) and 59.1% (n= 13). QFT-Plus had 4 indeterminate results (1 per group) and TBE had 12 (5 in group 1 and 7 in group).

Comparison of Results Between TBE and QFT-Plus

Indeterminate results were not considered when calculating the percentage agreements in Table 4. All indeterminate results were repeated for both tests, and the final result has been shown and used for the agreement and Cohen's kappa

Table 2 Demographic and Clinical Characteristics of Enrolled Participants

Characteristic	Group 1, N = 200[A]	Group 2, N = 200[A]	Group 3, N = 110[A]	Group 4, N = 22[A]	Overall, N = 532[A]
Sex					
Male	93 (46.5%)	99 (49.5%)	75 (68.2%)	13 (59.1%)	280 (52.6%)
Female	107 (53.5%)	101 (50.5%)	35 (31.8%)	9 (40.9%)	252 (47.4%)
Age Category in years					
0–5	1 (0.5%)	3 (1.5%)	1 (0.9%)	4 (18.2%)	9 (1.7%)
5–17	16 (8.0%)	7 (3.5%)	11 (10.0%)	5 (22.7%)	39 (7.3%)
18–25	58 (29.0%)	43 (21.5%)	28 (25.5%)	2 (9.1%)	131 (24.6%)
25–35	70 (35.0%)	72 (36.0%)	27 (24.5%)	4 (18.2%)	173 (32.5%)
36–50	36 (18.0%)	54 (27.0%)	24 (21.8%)	4 (18.2%)	118 (22.2%)
>50	19 (9.5%)	21 (10.5%)	19 (17.3%)	3 (13.6%)	62 (11.7%)
Diabetic					
Diabetic	14 (7.0%)	11 (5.5%)	21 (19.1%)	3 (13.6%)	49 (9.2%)
Non-diabetic	186 (93.0%)	189 (94.5%)	89 (80.9%)	19 (86.4%)	483 (90.8%)
Smoker					
Smoker	21 (10.5%)	23 (11.5%)	27 (24.5%)	4 (18.2%)	75 (14.1%)
Non-smoker	179 (89.5%)	177 (88.5%)	83 (75.5%)	18 (81.8%)	457 (85.9%)

Note: [A]n (%).

Table 3 Distribution of QFT-Plus, and TBE Results Among Enrolled Participants

		Group 1, N = 200[A]	Group 2, N = 200[A]	Group 3, N = 110[A]	Group 4, N = 22[A]	Overall, N = 532[A]
QFT-Plus	Positive	49 (24.5%)	73 (36.5%)	77 (70.0%)	13 (59.1%)	212 (39.8%)
	Negative	150 (75.0%)	126 (63.0%)	32 (29.1%)	8 (36.4%)	316 (59.4%)
	Indeterminate	1 (0.5%)	1 (0.5%)	1 (0.9%)	1 (4.5%)	4 (0.8%)
TBE	Positive	59 (29.5%)	79 (39.5%)	74 (67.3%)	14 (63.6%)	226 (42.5%)
	Negative	136 (68.0%)	121 (60.5%)	29 (26.4%)	8 (36.4%)	294 (55.3%)
	Indeterminate	5 (2.5%)	0 (0.0%)	7 (6.4%)	0 (0.0%)	12 (2.3%)
p-value[#]		0.210	0.56	0.847	0.907	0.277

Notes: [A]n (%); [#] Pearson's Chi-squared test.
Abbreviations: QFT-Plus, QuantiFERON-TB Gold Plus; TBE, Standard E TB-Feron ELISA.

Table 4 Comparison of TBE with QFT-Plus Among the Enrolled Participants

		QuantiFERON-TB Gold Plus			PPA (%)[B]	NPA (%)[B]	Overall Agreement[B]	Kappa Value[B]
		Positive, N = 212[A]	Negative, N = 316[A]	Indeterminate N = 4[A]				
TBE	Positive, N= 226 Negative, N= 294 Indeterminate, N= 12	179 (84.4%) 29 (13.7%) 4 (1.9%)	44 (13.9%) 264(83.5%) 8 (2.5%)	3 (75.0%) 1 (25.0%) 0 (0.0%)	86.1% (80.6%–90.5%)	85.7% (81.3%–89.4%)	85.9% (82.5%–88.7%)	0.71 (0.65–0.77)

Note: [A]n (%) [B](95% CI).
Abbreviations: PPA, positive percentage agreement; NPA, negative percentage agreement; TBE, Standard E TB-Feron ELISA.

Table 5 Culture Result, Diabetes, and Smoking Status Among Active TB Individuals

		Culture Result			Culture Positive N = 81[A]			
		Positive, N = 81[A]	Negative, N = 15[A]	Unavailable, N = 14[A]	Smoker, N = 23 [A]	Non-smoker, N = 58[1]	Diabetic, N = 17 [A]	Non-diabetic, N = 64 [A]
QFT-Plus	Positive Negative Indeterminate	62 (76.5%) 18 (22.2%) 1 (1.2%)	6 (40.0%) 9 (60.0%) 0 (0.0%)	9 (64.3%) 5 (35.7%) 0 (0.0%)	15 (65.2%) 7 (30.4%) 1 (4.3%)	47 (81.0%) 11 (19.0%) 0 (0.0%)	14 (82.4%) 2 (11.8%) 1 (5.9%)	48 (75.0%) 16 (25.0%) 0 (0.0%)
TBE	Positive Negative Indeterminate	60 (74.1%) 17 (21.0%) 4 (4.9%)	6 (40.0%) 8 (53.3%) 1 (6.7%)	8 (57.1%) 4 (28.6%) 2 (14.3%)	15 (65.2%) 8 (34.8%) 0 (0.0%)	45 (77.6%) 9 (15.5%) 4 (6.9%)	15 (88.2%) 2 (11.8%) 0 (0.0%)	45 (70.3%) 15 (23.4%) 4 (6.3%)

Note: [A] n (%).

calculations in Table 4. The PPA and NPA of TBE with QFT-Plus were 86.1% (95% CI, 80.6%–90.5%) and 85.7% (95% CI, 81.3% − 89.4%), respectively. The overall agreement of TBE with QFT-Plus was 85.9% (95% CI, 82.5% to 88.7%). If all indeterminate results are grouped with negative results the PPA, NPA, and overall agreement of TBE with QFT-Plus becomes 84.43% (95% CI, 78.84% to 89.04%), 86.08% (95% CI, 81.76% to 89.70%) and 85.42% (95% CI, 82.11% to 88.32%) respectively. When all indeterminate results are grouped with positive results the PPA, NPA, and overall agreement of TBE with QFT-Plus becomes 86.32% (95% CI, 80.95% to 90.64%), 83.54% (95% CI, 78.99% to 87.46%) and 84.66% (95% CI, 81.30% to 87.63%) respectively.

Among 110 individuals in group 3, culture results were available for 96 (Table 5) samples, of which 81 were culture-positive and 15 were culture-negative. Among the 81 culture-positive individuals, 62 (76.5%), and 60 (74.1%), tested positive in QFT-Plus, and TBE respectively. Among culture-positive individuals, smokers had a lower positive rate in all three IGRA tests compared to non-smokers, and diabetics had a higher positive rate compared to non-diabetics. However, these differences were not statistically significant in Fisher's exact tests and thus have not been reported.

Discussion

Our study found that the overall agreement of TBE with QFT-Plus was 85.9% (95% CI, 82.5%–88.7%). If an individual tested indeterminate in QFT-Plus or TBE they were excluded from agreement calculations as categorizing the individuals as negatives would falsely increase specificity and lower sensitivity. With indeterminate results excluded, Cohen's kappa values for agreement between TBE and QFT-Plus was 0.71 (95% CI 0.65–0.77)[15] which shows strong agreement between them. TBE had a PPA of 86.1% (95% CI, 80.6%–90.5%) with QFT-Plus. Two studies conducted among health workers in South Korea found higher agreements between TBE and QFT-Plus/ QFT-GIT (QFT-Gold in Tube, a previous version QFT assay). Young et al reported an agreement of 92.0% (Cohen's kappa value of 0.77) and Kweon et al reported an agreement of 95.3% (Cohen's kappa value of 0.78).[11,15] The higher agreements between TBE and QFT-Plus/ QFT-GIT in these studies compared to the current study might be due to the population variation.

Surrogate gold standards have previously been used to measure the sensitivity and specificity of LTBI diagnostic tests due to the absence of a definite gold standard. The specificity of a test is estimated from the performance among healthy individuals in a population with low TB prevalence. However, using such a standard might not be helpful for the population of Bangladesh due to the high TB burden. Even when a patient reports no known contact with a TB patient, LTBI cannot be ruled out. Among the group with healthy individuals with no reported contact with TB, QFT-Plus, and TBE, had 26.1%, and 29.1% positive rates, respectively. The result from TBE is closest to the estimations of a modelling study by Houben et al, who reported a TB infection prevalence of 30.8% (95% CI, 28.3%–34.8%) for the Southeast Asia region.[2] Similarly, culture positivity has been used as a surrogate standard to estimate the sensitivity of LTBI tests. Out of 81 individuals who tested positive in culture, the QFT-Plus and TBE tests had similar PPA of 76.5% and 74.1%, respectively.

Eight individuals tested negative in both IGRA tests despite testing positive in culture. Among them, 7 suffered from weight loss, 5 were smokers, and none of them had diabetes. The overall culture results presented in Table 5 also indicate that positivity rate of the IGRA tests were higher among people with diabetes, and lower among smokers and individuals who reported weight loss as a symptom during screening. However, the associations between the two tests and these factors were statistically insignificant ($p > 0.05$). Existing research on the relationship between diabetes, smoking, and IGRA results also remains inconclusive, with conflicting or statistically insignificant reports.[18–22]

In group 2, which consisted of healthcare workers and people in regular contact with TB patients, the positive rates of QFT-Plus, and TBE were 38%, and 41.6% respectively. A recent study in Bangladesh by Islam et al reported that 40% of healthcare workers tested positive for TST, and 48% had tested positive for QuantiFERON-TB Gold in-Tube results.[23] The study sites were chest disease hospitals with large PTB patient intakes, while healthcare workers in our study sites had lower levels of involvement with PTB patients for brief periods.

In group 4, which consisted of individuals with a history of TB, the positive rates of QFT-Plus, and TBE were 59.1% and, 63.6% respectively. IGRA tests are not recommended for treatment monitoring, with previous studies showing increases, decreases, and no changes in levels of interferon-gamma in IGRA tests after treatment completion.[23–26]

Our study had a few limitations. Firstly, we did not include TST as a comparator. The performance of TBE compared to TST could have provided further insight into the cost-effectiveness of adopting IGRA assays nationally. Secondly, gender differences in tuberculosis patients have been reported in previous studies, but not to the extent found in our study.[27,28] The overall female-to-male ratio of our study participants was 0.83. However, among active TB patients, the ratio was 0.47. Our differences likely arise because a higher percentage of male patients agreed to participate in our study, which is unlikely to reflect the gender difference among TB patients accurately.

As high-burden countries work towards eradicating active TB cases, reducing the large reservoir of LTBI will be needed to prevent disease resurgence and implementation of affordable, accurate LTBI testing will become increasingly important. TBE has shown comparable performance to the more expensive QFT-Plus, making it a promising candidate for implementation in resource-constrained settings. Future studies could validate the performance of TBE in different regions of the globe, conduct longitudinal studies to assess the impact on TB prevention and conduct cost-effectiveness analyses to inform policy.

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Disclosure

The authors report no conflicts of interest in this work.

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