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Interferon-γ release assay

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Abstract

Traditionally, tuberculosis (TB) has been viewed as having two distinct manifestations, known as TB infection (TBI) and TB disease. The spectrum, however, has recently been expanded to include the elimination of TBI with the help of innate and/or adaptive immunity, TBI, incipient and subclinical TB disease, and TB disease. Epidemiologically speaking, identifying individuals with TBI is critical since diagnosis and treatment of TBI are essential in controlling the TB burden. It is important to identify high-risk individuals with TBI who are more likely to progress to active TB disease. There are two diagnostic methods for identifying TBI. These include the conventional tuberculin skin test (TST) and interferon-γ release assay (IGRA). However, these methods are not the 'gold standard.' Furthermore, all of these methods are indirect, relying on the host's adaptive immune response to *Mycobacterium tuberculosis*-derived protein antigens. This review will describe the various tests for TBI, such as TST, IGRAs, newer skin and blood tests, methods for performing IGRAs, interpretation strategies, and limitations.

Key words: *Mycobacterium tuberculosis*, interferon-γ release assay, tuberculosis infection, tuberculin skin test.

Introduction

Tuberculosis (TB) has been known to infect mankind since time immemorial and is caused by *Mycobacterium tuberculosis* (*M.tb*) complex. The World Health Organization (WHO) Global TB Report 2023 stated that the incidence of TB cases in 2022 was 133 [95% uncertainty interval (UI): 124–143] per 100,000 population. In absolute terms, the total number of symptomatic TB cases was 10.6 million. Most cases occurred in Southeast Asia (46%) and Africa (23%) [1]. The total number of deaths was 1.3 million. TB is traditionally described in two forms: TB disease and TB infection (TBI).

After the acquisition of infection by an immunocompetent person, the TB bacilli enter a latent statein 90% of cases. In immunocompetent individuals, the bacilli may undergo reactivation subsequently at a rate of 10% in their lifetime. TBI is defined as a state of persistent immune response to M.tb antigens without evidence of clinically manifested TB disease [2]. Patients with TBI may harbor live *M.tb* bacilli, but presently we do not have the diagnostic modalities to detect the live bacilli. Although, the bacteria are metabolically active and dividing, a potent immune system controls the infection. The immunodiagnostic tests only reflect the persistent adaptive immune response to M.tb and not the true latent infection [3]. The Tuberculosis Network European Trials group consensus statement stated that the TST and IGRA positivity indicates "lasting tuberculosis immune responses" and not necessarily "latent tuberculosis infection," and it is unknown how long the adaptive immune responses towards mycobacterial antigens persist in the absence of live mycobacteria [3]. Approximately 1.7 billion people, or 23% of the world's population were infected with M.tb, and approximately 80% of all TBI cases were from the WHO regions of Southeast Asia, Western-Pacific, and Africa [4,5].

These large pools of infected people are the harbinger of future active TB disease. In a mathematical modeling study, Houben et al. reported that by 2035 and 2050, a pool of 1.7 billion latently infected people will generate 16.5 and 8.3 per 100,000 per year of active TB patients, respectively [5]. In a transmission-dynamic model, TB preventive therapy has a greater impact on overall TB incidence than most other single interventions [6]. A crucial step in TB control is to identify at-risk individuals with TBI who would benefit from TB preventive therapy. Currently, two diagnostic tests are in use, including TST and IGRA for diagnosing TBI.

Tuberculin skin test

Robert Koch, in 1882, discovered the tubercle bacillus. It was named M.tbin 1886 [7]. Clemens von Pirquet, in 1907, developed tuberculin as a diagnostic agent for TB [8]. French physician Charles Mantoux subsequently developed the interpretation strategy for the TST [9]. A purified protein derivative (PPD) from the attenuated M.tb strain was used in the injection. The TST, also known as the Mantoux test, has been used for over a century to diagnose TBI. It involves

injecting 0.1 ml of PPD intradermally into the volar aspect of the forearm. A special buffer, Tween-80, is employed to stabilize the solution. The diameter of the induration is measured and recorded 48 to 72 hours later in millimeters. The test solution was standardized against batch RT-23 produced by the Statens Serum Institute in Denmark. The TST is cost-effective and simple to implement in field settings, allowing for rapid testing of a large number of patients. However, the TST has limitations in terms of performance, sensitivity, and specificity [10]. The test lacks specificity as it may yield positive results in individuals previously vaccinated with Bacillus Calmette-Guérin (BCG) strains. Moreover, there is cross-reactivity with numerous nontuberculous mycobacterium (NTM) species [11]. The sensitivity is also suboptimal in individuals with human immunodeficiency virus (HIV) infection, particularly those with low CD4 counts and not on highly active antiretroviral therapy (HAART), within 10 weeks of TB infection, lymphoma, live virus vaccination (measles, smallpox), immunosuppressive therapy, overwhelming TB (e.g., military TB), extreme age (new-borns, elderly), and other infections. The prevalence of PPD reactivity in HIV-positive patients with CD4 counts below 400 lymphocytes/mm3 is 2.7%, compared to 10.0% in HIV-negative controls ($P<0.001$) [12]. Furthermore, the TST may exhibit a booster effect, characterized by increased skin reactivity one week to one year after the initial negative test, attributed to an immunological recall phenomenon. Boosting is commonly noticed when the time interval between the first and second tests is between one and five weeks. It is less common within 48 hours or longer than sixty days [13]. A false negative test result may occur due to inadequate PPD storage and poor administration technique. Furthermore, the test necessitates two visits, and there is inter-reader and intra-reader variability. The window period between infection and the development of tuberculin positivity is 12 weeks.

Interferon-γ release assay

IGRA is an in vitro diagnostic assay. It uses early-secreted antigenic targets (ESAT-6), culture filtrate proteins (CFP-10), and TB7.7 antigens. Streeton et al. in the late 1990s, developed a novel in vitro blood test that used PPD to stimulate T cells within whole blood before detection of released IFN-γ [14]. They used this test for TBI detection. They measured IFN-γ (IU/mL) responses as % PPD/phytohaemagglutinin response ratios. Using the PPD response ratio of 15%, the sensitivity and specificity of the IFN- γ assay were 90% and 98%, respectively. This assay was later known as QuantiFERON-TB (Cellestis Inc., Victoria, Australia) and subsequently approved by the Food and Drug Administration (FDA), United States of America, in 2001 [15]. Besides PPD, the QuantiFERON-TB assay also used the Mycobacterium avium antigen. Besides these, there are mitogen tubes as positive controls (phytohaemagglutinin) and

nil tubes containing saline as negative controls. The test found positive responses in 83% of those with active TB disease and 59% of the previously treated cases. Other categories that showed positive responses were untreated inactive disease (80%) and exposed but TST-negative group (43%). However, the PPD-based assay lacks specificity as the protein components are shared by Mycobacterium bovis (M.bovis) and non-tubercular mycobacteria (NTM). Advancement in mycobacterial genomics, including the discovery of a region of difference 1 (RD-1) segment of the M. tb genome, has made PPD-based assays irrelevant [16]. The RD-1 is a 9.5-kb DNA fragment that is missing from all BCG sub- strains but found in all virulent laboratory and clinical strains of M.bovis and M.tb. [17].

Two antigenic targets localized to the RD-1 are the 6 kDa ESAT-6 and the 10 kDa CFP-10. Both of these antigenic targets are absent in BCG strains and the majority of NTM species except M.kansasii, M.szulgai, M.mari num, M.gastri, M.leprae, and M.Flavescens [18,19]. The TB7.7 antigen is encoded in RD-11 and is missing from the BCG strains as well as the most common environmental mycobacteria [20]. Following the identification of these RD-1-relared antigens, ESAT-6 and CFP-10 replaced PPD, and the test was known as QuantiFERON-TB Gold (QFT-G) in 2005.

Two more versions of the QuantiFERON-TB assay were developed subsequently: the QuantiFERON Gold In-Tube (QFT-GIT) (Cellestis, Ltd., Victoria, Australia) assay and the QuantiFERON-TB Gold Plus (QFT-Plus) (QIAGEN, Carnegie, Australia) assay. The QFT-GIT assay used three antigens, including EAST-6, CFP-10, and TB7.7. Antigen- coated vacutainer blood collection tubes are used. The QFT-GIT assay also uses a nil tube, a mitogen tube, and an antigen tube. The QFT-GIT assay became commercially available in 2007 [21]. The QFT-Plus assay is a newer version of QFT-GIT and was launched in 2015. The QFT-Plus assay is different from the earlier version because of the presence of two antigen tubes. TB antigen tube 2 (TB2) contains both long and short peptides of ESAT-6 and CFP-10. They are recognized by MHC classes I and II. They stimulate IFN-γ production by both CD4+ and CD8+ T cells [22,23]. The TB antigen tube 1 (TB1) contains long peptides derived from ESAT-6 and CFP-10 and is recognized by MHC class II molecules for presentation to CD4+ T-cells [24]. Therefore, the QFT-Plus assay has better utility for immunosuppressed patients. Professor Lalvani's group in Oxford, United Kingdom, developed the ELISpot assay [25, 26]. The commercially approved test was marketed in 2004 as the T-SPOT.TB test (Oxford Immunotec Limited, Abingdon, United Kingdom). The QFT-GIT and QFT- plus assays are ELISA-based assays that measure the amount of IFN-γ released by lymphocytes in response to specific TB antigens. On the other hand, the T-SPOT.TB is an enzyme-linked immunospot assay that counts the number of IFN-γ-producing cells (spot-forming cells). T-SPOT.TB assay uses peripheral blood mononuclear

cells (PBMCs), which are separated from peripheral blood and are stimulated by ESAT-6 and CFP-10. PBMCs are monocytes and lymphocytes. The T-SPOT.TB stimulates both CD4+ and CD8+ T-cells.

There are a few modifications to the T-SPOT.TB test. T- Cell Select is an automated sample preparation system that includes the automatic isolation of PBMCs [27]. The T-Cell Xtend reagent allows blood samples to be processed up to 36 hours after venepuncture without affecting the accuracy of the test [28]. The Wantai TB-IGRA was launched in 2011 by the Beijing Wantai Biological Pharmacy Enterprise [29]. This test is similar to the QFT-Plus assay. It includes a positive control, a negative control, and an antigen tube. WANTAI TB-IGRA uses a recombinant fusion protein of CFP-10 and ESAT-6, whereas QFT-Plus uses polypeptide antigens of CFP-10 and ESAT-6 [30]. The WHO recommends Beijing Wantai's TB-IGRA and Qiagen QuantiFERON-TB Gold Plus assays [31].

IGRA efficacy

Mori et al. evaluated the two RD-1 region antigens, CFP-10 and ESAT-6, in the detection of TBI in BCG-vaccinated individuals on the QuantiFERON-TB platform [32]. They estimated the sensitivity and specificity in culture-confirmed M.tb and patients with no identified risk of exposure. The reported sensitivity and specificity were 89% and 98.1%, respectively with the cutoff of 0.35. The specificity of the TST test in this study was 35.4%. In a systematic review, the pooled sensitivity of QuantiFERON-TB Gold, QFT-GIT, and T-SPOT.TB as 78% (95% CI, 73% to 82%), 70% (CI, 63% to 78%), and 90% (CI, 86% to 93%), respectively [33]. The pooled specificity of both QuantiFERON tests among non–BCG-vaccinated and BCG-vaccinated participants was 99% (CI, 98% to 100%) and 96% (CI, 94% to 98%), respectively, whereas the pooled specificity of T-SPOT.TB assay was 93% (CI, 86% to 100%). Therefore, IGRA is not affected by prior BCG vaccination status and the T-SPOT.TB test is more sensitive than other IGRA assays. The pooled sensitivity of TST was 77% (CI, 71% to 82%), whereas, the pooled specificity of TST in non-BCG-vaccinated participants was 97% (CI, 95% to 99%). In another systematic review and meta-analysis [34], the pooled specificity IGRA and TST was 98 to 100% and 88.7% (95% CI 84.6–92.0), respectively for the diagnosis of TBI. The pooled negative predictive value (NPV) for QFT-GIT and T-SPOT.TB was 99.8% (95% CI 99.4–100) and 97.8% (95% CI 94.5–99.4%), respectively. The positive predictive value (PPV) value for developing active TB among subjects who had tested positive for TBI and refused preventive treatment was 2.3 to 3.3% for TST, 2.8 to 14.3% for QFT-GIT, and 3.3 to 10% for T- SPOT.TB. Therefore, IGRA showed higher specificity and a higher PPV compared with TST. The high NPV indicates that TST and IGRAs will reliably exclude progression to TB. The low PPV of IGRA and TST suggests that the number needed to be treated (NNT) to prevent a single case of TB is 25 to 33,

with the caveat that there is 100% adherence to and effectiveness of the preventive therapy [35]. Two systematic reviews and meta-analyses had shown a higher sensitivity of QFT-plus compared to that of QFT-GIT for detecting TBI [36,37]. The increased sensitivity might be due to the CD8+ T-cell immune responses in the TB2 tube. CD8+ T lymphocytes indicate exposure to M.tb pathogens in situations where there are few CD4+ T lymphocytes, such as HIV infection. Xu et al. compared QFT-Plus and QFT-GIT in immune-compromised individuals and reported a good agreement between the two assays (Cohen's κ of 0.859) [38]. Furthermore, QFT-plus detected more cases in immune-compromised patients. The predictors of indeterminate results were lower lymphocyte counts, lower CD4+ T cell and CD8+ T cell absolute counts, and a lower CD4/CD8 ratio. They also reported the following thresholds that will reduce indeterminate results: absolute lymphocyte counts of >1.15 × 109 cells, and absolute CD4+ T cell counts of >467.7 × 106 to 478.5 × 106 cells. Therefore, both QFT- Plus and QFT-GIT assays showed good agreement even in immune-compromised individuals. A meta-analysis showed higher IFN-γ production in the TB2 tube than TB1 tube [39]. Venkatappa et al. compared and evaluated the agreement of test results for QFT-Plus with those of QFT-GIT, T-SPOT.TB, and the TST for the diagnosis of TBI [40]. They reported 94% agreement between QFT-Plus and QFT-GIT, 77% agreement between QFT-Plus and QFT-GIT with TST, 92% agreement between QFT- Plus and T-SPOT.TB, and 91% agreement between QFT-GIT and T-SPOT.TB, respectively. Gupta et al. found a modest increase in PPV with a higher quantitative IGRA assay [41]. The PPV was 3.0% for QFT-GIT 0.35 IU/ml and 3.6% for 4.00 IU/ml, whereas with T-SPOT.TB, it was 3.4% for 5 spots and 5.0% for 50 spots. The WHO consensus statement suggested the features of an optimal target product profile (TPP) of a test that should predict progression from LTBI to active disease within two years after infection with a sensitivity and specificity of 90% or at least 75%, a high PPV, and a low NNT [42]. Therefore, the available diagnostic assays for TBI are not ideal.

IGRA an immunocompromised patients

In HIV-infected individuals, IGRAs show better performance than TST in identifying TBI as its sensitivity remains relatively unimpaired in moderately advanced HIV infection, unlike TST [43,44]. T-SPOT.TB may have an advantage over TST and QFT- GIT in patients with low CD4 lymphocyte counts, as the T-SPOT.TB test functions independently of CD4 lymphocyte counts [45]. Cattamanchi et al., in a systematic review and meta-analysis, assessed the role of IGRA in TBI among HIV-infected individuals [46]. They included 37 studies with a total of 5736 HIV-infected individuals. The pooled sensitivities for T-SPOT.TB and QFT-GIT were 72% (95% CI 62–81%) and 61% (95% CI 41–75%), respectively. Therefore, in immunocompromised individuals, T-SPOT.TB is more sensitive than IGRA. T-SPOT.TB retains sensitivity in

immunosuppression as this test requires an adequate number of PBMCs in each test well. Table 1 shows the difference between IGRA and TST.

Methods of performing IGRA

Both TST and IGRA depend on the detection of cell-mediated immune responses to M.tb antigens. IGRA assays are based on the principle that patients infected with M.tb have primed T-cells. When whole blood is incubated with M.tb antigens, exposure of primed T-cells to M.tbantigens occurs, which leads to the production of IFN-γ. The released IFN-γ is detected by enzyme-linked immunosorbent assay (ELISA) or enzyme-linked immunospot (ELISpot) method. The QFT-plus assay uses the ELISA platform. The QFT-Plus assay uses four tubes: a nil tube, a mitogen tube, a TB1 antigen tube, and a TB2 antigen tube. The nil tube and mitogen tube are used as negative and positive control, respectively. The nil tube measures the background or circulating IFN-γ level in the absence of antigens. The mitogen tube uses phytohaemaglutinin as mitogen which is a universal T-cell stimulus. It assesses the capacity of T cells to produce IFN-γ upon stimulation with a mitogen. Both TB1 and TB2 tubes contain peptide antigens consisting of ESAT-6 and CFP-10. In the TB1 tube, the antigens stimulate CD4+T-helper lymphocytes. The TB2 tube contains an additional set of shorter peptides that also stimulate CD8+ cytotoxic T lymphocytes [47]. First, 4 ml of whole blood is collected by venepuncture into specialized blood collection tubes, namely the nil tube, the mitogen tube, and the two antigen tubes. Another option is to collect the blood into a lithium or heparinized vacutainer and subsequently transfer it into the four specialized tubes. Approximately 1mL of blood was added to each of the nil, TB antigen, and mitogen tubes. Now the tube should be shaken for proper mixing of blood with the antigens dried on the wall. Samples should be sent to the referral laboratory within 16 hours. The samples in the reference laboratory are now incubated for 16 to 24 hours at 37 °C [48]. After incubation, tubes should be centrifuged at 2000–3000 relative centrifugal force (RCF) for 15 minutes, and the plasma should be separated. The released IFN-γ concentrations from lymphocytes are measured using an ELISA test. The levels of IFN-γ are measured in international units (IU) [49]. Plasma samples can be stored for up to 28 days at 2°C to 8°C or below -20°C for extended periods. The negative control or the nil tube must have an IFN-γ value of 8 IU/ml, otherwise the test will be considered invalid. A high value in nil tube indicates high levels of circulating heterophile antibody or non-specific, circulating IFN-γ. For example, in patients with rheumatoid arthritis and systemic lupus erythematosus, the circulating IFN-γ levels are often elevated [19]. The positive control or the mitogen tube must have an IFN-γ value of 0.5 IU/ml higher than the nil tube. Low response in mitogen tube indicates insufficient IFN-γ production. The mitogen tube

indicates patients' immune status and whether the blood handing and incubation was correctly done [49]. Results are expressed after subtracting the nil tube value from the values of the TB1 and TB2 tube. The result is considered positive when the IFN-γ levels of TB1 or TB2 tube minus the nil tube is 0.35 IU/ml. The result must be interpreted along with nil and mitogen tubes values. A negative test result means IFN-γ levels of less than 0.35 IU/mL in both TB antigen tubes. Indeterminate results include a nil tube value of more than > 8 IU/ml with any mitogen level and antigen tube values or a mitogen tube value of <0.5 IU/ml with any nil and antigen tubes values. Table 2 shows the interpretation strategy of QFT-Plus assay [47].

Method of performing the T-SPOT.TB assay

T-SPOT.TB is an in vitro diagnostic test performed on the ELISpot platform. It measures the IFN-γ producing T cells in response to stimulation with ESAT-6 and CFP10. Both the QFT-plus assay and the T-SPOT.TB test will be positive, not only for M.tb but also for other members of the complex including *M.b ovis*, *M.africanum*, *M.microti*, *M. canetti* [50] After a venepuncture into a heparinized tube, PBMCs are isolated, washed, counted, and normalized to create a standard cell suspension. A standard number of PBMCs (250,000 PBMCs per well) are added to a specially designed 96 well plastic microtiter plate. A total of four wells are required for each patient. Two quality control wells, a negative and a positive control, are run with each individual sample. The remaining two wells are reserved for the TB antigens. These include panel A (ESAT-6) and panel B (CFP10) antigens. If primed T cells are present, they will bind to TB-specific antigens and produce IFN-γ. High-affinity antibodies have already been pre-coated on the plates, allowing them to capture the released IFN-γ [50]. A 16 to 20 hours incubation in a humidified incubator at 37°C with 5% carbon dioxide, is followed by addition of a secondary enzyme-labeled antibody is added. [50] This will bind to the IFN-γ secreted by the T cells and the captured anti- IFN-γ antibodies. The number of IFN-γ producing cells is quantified as a spot number. Blood samples to be processed with the T-SPOT.TB test must be used within 8 hours post venepuncture. Samples can be used up to 32 hours post venepuncture with the addition of the T-Cell Xtend reagent prior to running the T-SPOT.TB test.

Interpretation

Results are reported as the number of IFN-γ producing T cells, calculated by subtracting spot-forming cells in nil well from that of the panel A and B. The positive control or mitogen tube typically has a spot count 20 spots. If the spot count in the positive control is less than 20 spots, it indicates that a subset of patients may have T cells with poor response to PHA. The test result is positive if (panel A-nil) and/or (panel B-nil) result shows 8 spots. Nil control value

is 10 and mitogen spot count can be any [50]. A negative result is reported if (panel A-nil) and (panel B-nil) result shows 4 spots. Nil control value is 10 and mitogen spot count is 20 spots. A nil control spot count >10 spots should be reported as 'Invalid' or 'Indeterminate' and the test should be repeated. If the mitogen well shows <20 spots and both (panel A- nil) and (panel B-nil) shows 4 spots, the test should be considered invalid [50]. A borderline (equivocal) result means the highest of either 'panel A-nil' or 'panel B-nil' counts is 5, 6 or 7 spots. The test should be repeated. Table 3 shows the interpretative strategy of T-SPOT.TB test [51].

Limitations

The IGRA test has a few limitations too. The assays require sophisticated laboratories with trained manpower and costly logistics. Furthermore, there are issues related to sample transport and storage. This is particularly relevant in developing nations. Both TST and IGRA cannot differentiate between TBI and TB disease. IGRA should not be used for active TB diagnosis in high burden countries such as India [52]. Otherwise, there will be a significant over diagnosis of TB due to the high prevalence of TBI in India [53]. A positive IGRA result may indicate active TB disease or TBI. A negative IGRA result cannot be used as a rule-out test [54].

False+ve IGRA

IGRA can show false-positive in NTM infections such as M.kansasii, M.szulgai, M.marinum, and M.flavescens, as they also possess the RD-1 genes encoding CFP- 10 and ESAR-10 [18,19,55]. IGRA may show false positive in M bovis infection but not in the case of the BCG strain as the RD-1 was eliminated early in the process of attenuating the pathogenic strain to a nearly avirulent BCG strain [56]. Pre-analytical processing error may also cause false positive IGRA.

False-negative IGRA

False-negative results have been identified in 8-19% of cases [57]. Elderly, underweight, HIV co-infection, extra pulmonary TB, and an increased number of human leukocyte antigen DRB1*0701 alleles were associated with false-negative IGRA results in earlier studies [57-60]. However, the limitations of these studies included small sample sizes, observational design, and performed at a single center [57]. The Tuberculosis Network European Trials Group (TBNET) identified advanced age as the only risk factor for false-negative IGRA results in patients with active TB [57]. Yamasue et al. [61], in a systematic review, reported the following factors as contributing to false negative IGRA assay results: advanced age and low peripheral lymphocyte counts (pooled odds ratio 2.06; 95% CI, 1.68–2.52 and 2.68; 95% CI, 2.00–3.61,

respectively). Wang et al. reported on multivariate analysis that younger age (9 years; OR = 4.782; 95% CI: 1.689, 13.539), weight for age (z-score > 0.37; OR = 4.256; 95% CI: 1.458, 12.428), and hypoproteinemia (total protein 68.4 g/L; OR = 7.131; 95% CI: 1.864, 27.271) were risk factors for false-negative T- SPOT.TB results in childhood TB [62]. Advanced age alters the TB antigens-induced IFN-γ production, whereas, low peripheral blood lymphocyte count determines the IFN-γ production in QFT but not T-SPOT. This is because the T-SPOT assay needs a certain minimum amount of PBMCs. Compartmentalization of T cells within the site of pathology may explain the false-negative result. Technical-operational variability may also cause a false-negative assay. False-negative QFT test results are more common in HIV-infected individuals with lower CD4 counts [63].

Indeterminate results

Indeterminate results indicate either high IFN-γ levels (> 8 IU/ml) in the nil tube or low IFN-γ levels (<0.5 IU/ml) in the mitogen tube. The indeterminate result with T- SPOT.TB is defined by a nil control spot count >10 spots. Zhou et al. in a systematic review and meta-analysis, reported a pooled indeterminate rate of 3.9% (95% CI 3.5%–4.2%) for IGRAs in the detection of TBI [64]. They also found a similar indeterminate rate for QuantiFERON®-TB and T-Spot.TB; however, QuantiFERON- Plus had a lower indeterminate rate than T-Spot.TB. Diel et al. [65], reported the pooled rate of indeterminate results of the T-Spot.TB as 3.8% (95% CI, 0.035-0.042) and 6.1% (95% CI, 0.052-0.071), among immunocompetent and immunosuppressed patients, respectively. Important risk factors for indeterminate IGRA results are immunosuppression and children, especially those younger than 5 years. The indeterminate rate in the immunocompromised population was significantly higher than that in healthy controls [odds ratio (OR) 3.51, 95% CI 2.11–5.82]. A lower CD4+ cell count in HIV-positive patients further increased the rate of indeterminate values. Compared to adults, children's pooled indeterminate rates (OR = 2.56, 95% CI 1.79–3.57) were significantly higher. These rates increased as the children's age dropped.[64] The majority of indeterminate results are due to failed positive controls (94.6%, 95% CI 89.6%–98.0%) [65]. This indicates dysfunctional T lymphocytes or technical errors. Failed nil tubes may be due to the presence of heterophilic antibodies (e.g., human anti-mouse) or spontaneous IFN-γ secretion during an infection or following vaccination [66]. Sun et al. [67], in a multivariate analysis, reported that male (OR = 1.882, 95% CI: 1.222–2.899), Behcet's disease (OR = 7.764, 95% CI: 1.714–35.167), heavy use of corticosteroids within a month (OR = 0.357, 95% CI: 0.138–0.921, for > 1000 mg group), and hypoalbuminemia (OR = 0.385, 95% CI: 0.241–0.615) are significantly associated with high nil responses. Another cause of indeterminate QuantiFERON-Plus result

is severely-ill COVID-19 patients. Ward et al. found a 6-fold reduction of IFN-γ levels compared to control patients in severely ill COVID-19 patients [68]. More than 60% of these severely ill COVID-19 patients showed poor mitogen responses. Low mitogen levels are associated with age < 10 years [adjusted OR (aOR) 3.7, 95%CI 2.4-5.9], females (aOR 1.4, 95%CI 1.1-1.8), Asians (aOR 2.1, 95%CI 1.3-3.4), and the US-born (aOR 1.9, 95%CI 1.4-2.6). A high nil results are more frequent among Hispanics (aOR1.7, 95%CI 1.0-2.8) [69]. The indeterminate IGRA result has been reported in 27.1% of the HIV-infected patients (95% CI: 17.8-38.1%), and the risk increased in patients with advanced immunosuppression (CD4+ T-cell count < 200 cells/mm3) [70]. The T-SPOT.TB is associated with significantly less frequent indeterminate results than QuantiFERON- TB Gold (3% versus 11%, p<0.0001) [71]. Patients on immunosuppressive therapy and peripheral blood lymphocytopaenia has the highest rate of indeterminate results. Therefore, T-SPOT.TB is not only more sensitive in immunocompromised persons, it also produces significantly less indeterminate results compared to QuantiFERON-TB Gold assay. Therefore, in an HIV-infected patient with a lower CD4+ lymphocyte counts, T-SPOT.TB would be a reasonable choice [72]. IGRA tests is also preferred in subjects vaccinated with BCG and in children >5 years [73]. The increased sensitivity of the T-SPOT.TB test should be considered with the caveat that T-SPOT.TB test may detect a transient immune response to TBI [74]. Table 4 shows the difference between QuantiFERON-TB plus assay T-SPOT.TB (T-SPOT) assay.

Variability of IGRA results

Another issue with IGRA assay is the poor reproducibility on serial testing, particularly among healthcare workers (HCWs). On serial testing, IGRA show a higher rate of reversions and conversions. Conversions occur in the apparent absence of TB exposure, whereas, reversions occur in the absence of treatment [75]. Joshi et al. retrospectively evaluated the long-term reproducibility, conversions, reversions, and predictive value data of QFT-GIT among 2303 HCWs in the Unites States [76]. They found reversions and conversions in 45% and 3.2%, respectively. They performed a third QFT-GIT among 41 of 71 convertors and 90% of them had reversions. This indicates poor reproducibility of this test. Therefore, QFT-GIT is not suitable for serial testing. Pai et al. considered true conversions for individuals with fluctuating QFT-GIT results close to the cutoff value of 0.35 IU/mL [77]. These conversions and reversions tend to occur more frequently when the initial result is close to the cut-off (0.35 IU/ml). Thanassi et al. conducted serial QuantiFERON-GIT test in US healthcare workers, a low-risk population [78]. They reported reversions in 52.2% of initial positive cases and occurred most likely when the TB antigens –nil was 1.11 IU/ml. Dorman et al. in the same low-risk cohort reported reversions of QFT-GIT and T- SPOT.TB in 76.4% and 77.1%, respectively when

retested after 6 months [79]. This indicates false positive IGRA in low-risk population which can result in unnecessary chest x-rays, unwarranted anxiety and worry, and often unnecessary treatment and sometimes side effects [80]. van Zyl-Smit et al. found within-subject variability of IGRA of 16 to 80% [81]. IGRA variability can occur due to manufacturing sources, pre-analytical sources, analytical sources, and immunological sources [82]. The pre- analytical factors that may explain IGRA variability include blood volume, sample agitation, temperature during transport, delay in incubation, duration of incubation, plasma separation delay, and plasma/PBMC storage [83]. The variability also includes intra-laboratory and inter-laboratory variability [84]. One important pre- analytical delay is the incubation delay. Although the manufacturer of the QuantiFERON assay recommends a 0 to 16-h range of delay before incubation, Doberne et al. showed that 6-and 12-hour delays caused reversion rates of 19% and 22%, respectively when compared to immediate incubation [85]. Incubation delays may also cause a rise in indeterminate result [82]. Other pre-analytical factors include methods of shaking, variation in blood volume, and incubation duration [82]. Tagmouti et al. in a systematic review found that under ideal conditions (i.e. repeat testing of an aliquot of the same sample) the QuantiFERON IFN- γ result could vary by ± 0.47 IU/ml (coefficient of variation, 13%) and ± 0.26 IU/ml (30%) for individuals with an initial IFN- γ response in the borderline range (0.25–0.80 IU/ml) [86]. They identified blood volume inoculated into QuantiFERON tubes and pre-analytic delay as key sources of variability. Immunological source of variability includes immune boosting phenomenon as reported by van Zyl-Smit et al. [81]. They showed a TST induced "boosting" of IGRA responses which occurred after 3 days and may persist for several months. IGRA should be performed within 72 hours of PPD placement [87]. The Canadian guideline on IGRA for LTBI stated that if both TST and IGRA test are performed, blood sample for IGRA should be obtained on the same day as the TST [88]. If a TST was administered, the Wisconsin TB Program recommends IGRA testing at least 90 days after a TST to avoid potential boosting [89].

Another issue is the discordance between IGRA and TST results, especially in children. The most common discordant pattern in the low TB endemic setting is TST- positive and IGRA-negative. It may be due to false-negative IGRA or false-positive TST. IGRA can be false negative during the window period. Usually, it takes 4 to 6 weeks after exposure for IGRA conversion. However, sometimes, 14 to 22 weeks may elapse after exposure for IGRA conversion [90]. Pregnancy is an immunosuppressive state. Weinberg et al. found that from entry to delivery, 24% of 284 QFT-GIT-positive women reverted to negative or indeterminate state [91]. A decreased IFN-γ production in response to TB antigen and/or mitogen during pregnancy may explain the loss of QFT-positivity during pregnancy. Unlike TST, IGRAs are not affected by

prior BCG vaccination. Therefore, in countries where adult BCG vaccination has been adopted, IGRA should be done instead of TST. Intravesicular BCG administration for bladder cancer also affects the result of TST but not IGRA. Silverman et al. showed that intravesicular BCG administration predicted a positive TST ($p<0.001$) response but not a positive IGRA ($p=0.35$) [92].

Novel skin tests

Newer Mtb antigen-based skin tests (TBST) using the Mtb specific antigens (ESAT6 and CFP10) seems very promising for the diagnosis of TB infection. The WHO assessed the outcomes of these three TBST: Cy-Tb (Serum Institute of India, India); C-TST (formerly known as ESAT6-CFP10 test, Anhui Zhifei Longcom, China); and Diaskintest (Generium, Russian Federation). All the three TBST use intradermal injection of antigen and are read after 48–72 hours as induration similar to the method suggested for Mantoux test. The sensitivity of the new TBST were similar to TST and IGRA, specificity similar to that of IGRA and better than that of TST, particularly in populations with prior BCG vaccination history [93]. These tests combine the operational advantages of the TST and the specificity of IGRAs for the diagnosis of TBI [94]. Similar to TST, all these three new skin tests require two clinic visits.

Cy-Tb

It was formerly known as C-Tb test. It consists of a 1:1 mixture of two RD-1 antigens as recombinant dimer (rdESAT-6 and CFP-10). These antigens are produced by recombinant method in Lactococcus lactis by the Statens Serum Institut (SSI), Denmark [95]. The dose is applied intradermally (0.1 mL) and contains 0.05 µg of rdESAT-6 and 0.05 µg of rCFP-10 [93]. The cutoff of induration is 5 mm. The storage is at 2-80c. [96] The Serum Institute of India in a partnership with SSI manufactures and distributes this test. Ruhwald et al. [94], in a double-blind, randomised, controlled phase 3 trial evaluated the efficacy and safety of the C-Tb skin test in the diagnosis of TBI in Spain. They also compared the result with QFT-GIT and TST tests. Unlike TST, there was no impact of BCG vaccination on C-Tb result. The Cy-Tb positivity was 43% in close contacts. Furthermore, there was strong concordant between Cy- Tb and QFT-GIT results (94%). They reported a similar safety profile of C-Tb with that of the TST. The specificity of C-Tb test was 99.3% (95% CI 96–100%) [95]. The sensitivity using a cut-point of 5 mm was 73.9 (95% CI 67.8-79.3) [96,97].

Diaskintest

Diaskintest is produced and marketed by the Generium Pharmaceutical, Russia. The recombinant protein is produced by genetically modified Escherichia coli BL21 and

subsequently diluted with sterile isotonic phosphate buffer solution along with a preservative (phenol) [93]. The dose is applied intradermally (0.1 mL) and contains 0.2 µg of ESAT-6 and CFP10 recombinant protein and auxiliary ingredients. The result is read after 48 to 72 hours. Presence of induration or papule of any size is considered positive reaction. The storage is at 2-80c [96]. Starshinova et al. [98], in a meta-analysis included results of 3,777,083 patients tested with Diaskintest (83.0%). They reported the overall diagnostic sensitivity and specificity of 86.0% and 98.0%, respectively with an accuracy of 95.1% in the total studied population.

C-TST

Formerly called the ESAT6-CFP10 test (Anhui Zhifei Longcom, China), the C-TST test uses the recombinant fusion protein ESAT6-CFP10 (EC) produced in genetically modified E. coli. The test dose is applied intradermally (0.1 mL) and contains 5 units (U) of recombinant Mtb fusion protein, phosphate-buffered saline (PBS, 1.0 mmol/liter), phenol (3%) and Tween 80 (0.0005%) [99]. An average diameter of induration or erythema is measured by summing the transverse and longitudinal diameters and the whole divided by 2. Zhang et al. reported that an induration or erythema diameter not less than 5 mm was useful in the diagnosis of TBI [99]. The manifestations that are considered as strong positive reactions include blister, skin necrosis, and lymphadenopathy. In a phase 2b trial, Xu et al. found sensitivity and specificity of 87.5% (95% CI, 77.8–97.2) and 98.9% (95% CI, 96.0–99.9) which were similar to that of T-SPOT.TB test 86.5% (95% CI, 79.5–93.4) and 96.1% (95% CI, 93.5–97.8), respectively [100].

Newer IGRA tests

The LIAISON QFT-plus assay is a chemiluminescence immunoassay (CLIM) analyser which measures IFN-γ using the fully automated LIAISON® XL analyzer [101]. The manufacturer is the DiaSorin S.p.A., Italy. This test is called QFT-plus CLIA or LIAISON QFT-plus assay. Fernández-Huerta et al. compared the QFT-plus assay on Liaison XL and ELISA platform in Barcelona, Spain [102]. They observed substantial agreement (κ, 0.872) and correlation between (r, >0.950) between the two assays. The QFT-plus assay on Liaison XL platform generated significantly higher levels of IFN-γ IU/ml than the ELISA ($P = 0.004$ for the TB1 tube and $P = 0.010$ for the TB2 tube). The higher IFN-γ levels also allowed interpretable results which were indeterminate on ELISA platform. Therefore, LIAISON QFT-plus assay is more sensitive compared to ELISA based QFT-plus assay. It measures IFN-γ produced by both CD4+ and CD8+ lymphocytes. Bisognin et al. similarly reported substantial agreement between the two (75.4%) [103]. The Italian Clinical Microbiologist Association defined borderline results

as TB1 and/or TB2 values within the range of 0.20 to 0.70 IFN-γ IU/ml and recommended retesting. The chemiluminescence-based assay significantly increased the IFN-γ levels from 0.29 to 0.59 IU/ml in TB1 and 0.32 to 0.60 IU/ml in TB2, respectively, although, it remained in the borderline range. The Receiver operating characteristic (ROC) analysis suggested the optimal cut-off value of 0.45 IU/ml for TB1 and 0.46 IU/ml for TB2. Being automatic in nature, it has reduced assay time. The incubation period is 16 to 24 hours with assay time of 45 minutes. It can perform up to 25 tests per hour [104]. Altawallbeh et al. reported excellent agreement of 97.8% agreement between the LIAISON and QFT-plus assay among 185 potential TB sample with Cohen's kappa value of 0.88 [105]. Buron et al. found higher IFN-γ measurements across the range of values with the CLIA than with the ELISA [106]. CLIA was positive in 42.7% of borderline-negative result with ELISA. Therefore, there is risk of false-positive result with the Liaison CLIA for QFT- Plus in low-incidence settings. Confirming borderline positives with ELISA is a viable strategy to mitigate false-positive CLIA results.

QIAreach® QuantiFERON-TB (QIAreach QFT)

The QIAreach QuantiFERON-TB (QIAreach) (QIAGEN, Carnegie, Australia) is a semi-automated assay that incorporates digital fluorescence lateral flow nanoparticle technology to capture the IFN-γ level released by both CD4 and CD8 T-cells in plasma [107]. It uses only the TB2 tube of QTF plus assay and does not use mitogen or nil tubes. It measures IFN-γ in plasma via its unique digital detection cartridge known as eStick attached to the power source (8-port eHub portable platform). The assay has the capability to perform 24 tests per eHub per hour. The test result is available on eHub display in 3–20 minute. Stieber et al. [108], in a feasibility study, reported overall agreement between QIAreach-QFT and QFT-plus assay of 95.6% (two-sided 95% CI 91.8–98), with a positive percentage agreement (i.e., sensitivity) of 100% (95% CI 94.7–100) and a negative percentage agreement (i.e., specificity) of 95.6% (95% CI 90.6–98.4). Main advantage of QIAreach-QFT is requirement of less laboratory infrastructure and resources. It is also portable and requires only 1 ml of blood [109]. Furthermore, it has rapid turnaround time. In one study, sensitivity of QIAreach QFT for diagnosing TBI was 93.7% (two-sided 95% CI 82.2–98.7%) and 95.1% (two-sided 95% CI 88–98.7%), respectively for the untreated and treated groups, whereas the specificity was 97.7% (two-sided 95% CI 94.2–99.4%). The overall percentage agreement with QFT-plus was 95.7% (two-sided 95% CI 92.8.3–97.7%) with a Cohen's κ of 0.96 [107]. In a systematic review and meta- analysis, the pooled sensitivity, specificity, positive predictive value, and negative predictive value of QIAreach QFT for diagnosing TBI were 99% (95% CI 95–100%), 94% (95% CI 85–97%), 88% (95% CI 70–98%), and 100% (95% CI 99–100%), respectively [110].

VIDAS TB-IGRA (bioMérieux, France)

VIDAS® TB-IGRA is a fully automated in vitro diagnostic for TBI. It reduces the chance of human error as it fully motivated. It requires incubation at 370c for 16 hours with turnaround time is 17 hours [111]. It uses three tubes: nil, antigen, and mitogen tubes. The positive result and negative results are defined by antigen-Nil IFN-γ concentrations 0.35 IU/mL and 0.35 IU/mL, respectively. Indeterminate result is defined by mitogen-Nil IFN-γ concentrations of <1.1 IU/mL or by a Nil IFN-γ concentrations of 6.4 IU/mL. In one multicentric study, VIDAS® TB-IGRA produced a smaller number of indeterminate results compared to QFT-Plus (1/107 vs. 23/107) in patient with TB disease [111].

LIOFeron®TB/LTBLIGRA (LIONEX Diagnostics & Therapeutics, Germany)

LIOFeron®TB/LTBI IGRA assay includes two components: the human blood stimulation tubes and IFN-γ ELISA. This test is a chemiluminescent immunoassay (CLIA). There are four blood tubes: positive control, negative control, TB A, and TB B [112]. ESAT-6, CFP-10, and TB7.7 antigens are present in TB A tube, whereas alanine dehydrogenase (Ala-DH) is present in TB B tube. [49] This test measures IFN-γ produced by both CD4+ and CD8+ lymphocytes. Ala-DH is the proprietary antigen of LIONEX. It has several advantages. It is absent in BCG [113]. It contains the MHC class I- restricted T CD8+ lymphocytes epitopes [114]. This antigen is also involved in adaptation to the anaerobic dormant stage of latent TB infection [115]. There is less manual handling as the test measures IFN-γ concentrations automatically [104]. The incubation time is 16-24 hours at 37^0c with assay time of 2.5 hours [104,112]. The positive result is defined by antigen-Nil IFN-γ concentrations 0.35 IU/mL and 25% of the negative control. The negative result is defined by antigen-Nil IFN-γ concentrations <0.35 IU/mL or <25% of the negative control and the positive control is 0.5 IU/ml. Indeterminate result includes negative control IFN-γ concentrations >8 IU/mL or the antigen tube-Nil IFN-γ concentrations of <0.35 IU/ml or <25% of the negative control with a mitogen control of <0.5 IU/mL. Bella et al. reported sensitivity and specificity of LIOFeron®TB/LTBI IGRA assay for TBI diagnosis as 90% and 98%, respectively, whereas, that of the QFT-plus assay were 85% and 94%, respectively [116]. Therefore, it has higher sensitivity than QFT-plus assay.

Ichroma™ IGRA-TB

The Ichroma™ IGRA-TB is produced and marketed by the Boditech Med Inc., Chuncheon, Republic of Korea. It is a fully automated point-of-care device with three tubes namely nil, mitogen, and TB antigen tubes. It uses a fluorescent lateral flow immunoassay to measure IFN-

γ concentration [117]. The antigens used are ESAT-6 and CFP-10. The incubation time is 16 to 24 hours and results are obtained in 15 minutes [117]. The ichroma™ IGRA- TB showed an acceptable performance compared to the QFT-plus assay. However, there was a higher frequency of indeterminate results (2.2%) compared to QFT-plus assay [118]. The total agreement rate and kappa value between the ichroma™ IGRA- TB and QFT-plus were 95.2% and 0.70 (strong agreement), respectively [118].

STANDARD F TB-Feron FIA

The STANDARD F TB-Feron FIA (SD Biosensor, Republic of Korea) is a fluorescent lateral flow immunoassay [119]. It contains three tubes with the TB antigen tube containing ESAT-6, CFP-10, and TB7.7 similar to QFT-GIT [120]. However, there are certain differences between the STANDARD F TB-Feron FIA and QFT-GIT. The TB antigens in the former contain recombinant whole proteins of ESAT-6, while the latter contains TB-specific synthetic peptide antigens. [104] Whole proteins break down into several small peptides with various epitopes and the advantage of using them is their enhanced sensitivity. Therefore, it can stimulate T cells vigorously and has thereby increased IFN-γ levels [121]. The incubation time of the STANDARD F TB-Feron FIA is 16 to 24 hours at 37°C and the result is available in 15 minutes [49]. Yoo et al. reported an agreement rate between STANDARD F TB-Feron FIA and QFT-plus of 92.0% with a Cohen's kappa value of 0.77 (95% CI, 0.68–0.87) [122].

Erythra TB-KIT (Erythra Inc., Stanford, CA, USA)

This test uses PPS as antigen stimulation for whole blood. The technology is based on lateral flow chromatography assay. It does not require any incubation. The assay time is 20 minutes with throughput of one sample per run [96].

AdvanSure 13

It is a chemiluminescence-based assay manufactured by LG Chem, Seoul, Korea. It measures IFN-γ response to ESAT-6, CFP-10, and TB7.7 antigens quantitatively and automatically. [96] It uses small assay volumes of 50 microliters of incubated plasma from each of the three tubes for analysis [123]. The incubation time is 16 to 24 hours and the analysis time post-incubation is 15 minutes [96,124]. Magnetic- coated anti-human IFN-γ antibodies was used for the detection. The resultant anti- human IFN-γ antibodies- IFN-γ complex produces a chemiluminescent signal which was measured as relative light unit (RLU). It correlates with the amount of IFN-γ [123]. Kim et al. evaluated the performance of AdvanSure I3 and QFT-GIT assay using 341 blood samples from healthcare workers and patients and reported 99.1%

overall agreement (kappa coefficient=0.98) between the two assays [123].

IP-10 IGRA

Serum levels of cytokine such as interferon-γ, induced protein 10 (IP-10) in patients with TB [96]. IP-10 is secreted by antigen-presenting cells upon stimulation by IFN-γ and has a higher expression compared to IFN-γ. The IP-10 IGRA is a quantitative lateral flow assay developed by R-Biopharm (Pfungstadt, Germany). The incubation time is 16 to 24 hours and the analysis time post-incubation is 20 minutes. Ortiz-Brizuela et al. [125], in a systematic review and meta-analysis, evaluated the diagnostic performance of new commercial IGRA for TBI. They included a total of 87 studies (QFT-plusd 44, 10 for Standard E TB-Feron, 4 for QFT-Plus CLIA, 3 for QIAreach, 26 for TB-IGRA, and 1 T-SPOT.TB). Furthermore, as compared to the QFT-GIT, the sensitivity of QFT- plus was 0.1 percentage points lower (95% CI, −2.8 to 2.6; certainty of evidence (CoE): moderate), and its specificity was 0.9 percentage points lower (95% CI, −1.0 to −.9; CoE: moderate). However, as compared to QFT-GIT, the sensitivity and specificity of TB-IGRA were 3.0 percentage points higher (95% CI, −.2 to 6.2; CoE: very low) and 2.6 percentage points lower (95% CI, −4.2 to −1.0; CoE: low), respectively. They also found an excellent agreement between the QFT-Plus CLIA, QIAreach, and QFT-Plus.

Conclusions

Diagnosis of TB infection and TB preventive therapy is an important step in achieving the End TB strategy. There is no gold-standard for the diagnosis of TB infection. Interferon-γ-based assays are a reliable alternative to the old tuberculin skin test for diagnosing tuberculous infection. Both tuberculin and IGRA measure the adaptive immune response to Mycobacterium Tuberculosis antigens and both the tests do not differentiate between TB infection and TB disease.

Currently available IGRA assays includes the QuantiFERON-TB Gold in tube, QuantiFERON-plus, and the ELISPOT assay. The IGRA assay is more specific compared to tuberculin test as this assay utilizes the RD-1 antigens such as ESAT-6 and CFP-10. These antigens are not shared by BCG strain and the majority of non-tubercular mycobacterial infection. However, both TST and IGRA have low positive predictive value of progression into TB disease. The ELISPOT assay is more sensitive in immunocompromised population. The QFT- Plus assay has comparable sensitivity and specificity with the QuantiFERON-TB Gold in tube test. IGRA should be advised in BCG vaccinated individuals. IGRA testing is not ideal for serial testing, particularly in low-risk settings. There are many newer skin and IGRA-based tests. An ideal test should fulfil WHO criteria of Target product profile (TPP) for a test in order to predict

progression from tuberculosis infection to active disease. The newer Mtb antigen-based skin tests (TBST) have the advantage of good specificity as they use the Mtb specific antigens such as ESAT6 and CFP10. In addition, these tests can be used at the peripheral level unlike the IGRA tests. The newer skin-based test is also cost-effective compared to the conventional IGRA test which needs sophisticated laboratory infrastructure.

Moreover, in a resource-poor countries, shifting of IGRA sample is often difficult. This is particularly advantageous for low and middle-income countries. The Cy-TB test has already been rolled down in the National TB elimination program by the Government of India. There is heterogeneity in screening approach to patients harbouring TB infection which depends on various factors such as cost-effectiveness, availability, requirement of specialised training, and last but not the least requirement of sophisticated laboratory infrastructures. The implementation of TB preventive therapy faces several gaps worldwide. Mitigation of these gaps including gap in testing will be a crucial step in achieving the End TB target.

In future, we need newer assays for diagnosing TB infection which will not only meet WHO criteria of target product profile (TPP), but also possess capability to differentiate TB infection from TB disease. It should be suitable for point-of-care applicability. It should possess high sensitivity, specificity, positive predictive value, cost-effective, stability in different climates, and not requiring stringent storage facility.

References

1. WHO. Global tuberculosis report 2023. Available from: https://www.who.int/publications/i/item/9789240083851.
2. Kiazyk S, Ball TB. Latent tuberculosis infection: an overview. Can Commun Dis Rep 2017;43:62-6.
3. Mack U, Migliori GB, Sester M, et al. LTBI: latent tuberculosis infection or lasting immune responses to M. tuberculosis? A TBNET consensus statement. Eur Respir J 2009;33:956-73.
4. WHO. WHO Global tuberculosis report 2018. Available from: https://www.who.int/publications-detail-redirect/9789240013131.
5. Houben RMGJ, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS Med 2016;13:e1002152.
6. Menzies NA, Cohen T, Hill AN, et al. Prospects for tuberculosis elimination in the United States: results of a transmission dynamic model. Am J Epidemiol 2018;187:2011-20.
7. Grange JM. Koch's Tubercle Bacillus - a centenary reappraisal. Zentralbl Bakteriol Mikrobiol Hyg A Med Mikrobiol Infekt Parasitol 1982;251:297-307.
8. Daniel TM. The history of tuberculosis. Respir Med 2006;100:1862-70.

9. Yang H, Kruh-Garcia NA, Dobos KM. Purified protein derivatives of tuberculin--past, present, and future. FEMS Immunol Med Microbiol 2012;66:273-80.
10. Pollock L, Basu Roy R, Kampmann B. How to use: interferon γ release assays for tuberculosis. Arch Dis Child Educ Pract Ed 2013;98:99-105.
11. Huebner RE, Schein MF, Bass JB Jr. The tuberculin skin test. Clin Infect Dis 1993;17:968-75.
12. Markowitz N, Hansen NI, Wilcosky TC, et al. Tuberculin and anergy testing in HIV-seropositive and HIV-seronegative persons. Pulmonary Complications of HIV Infection Study Group. Ann Intern Med 1993;119:185-93.
13. Menzies D. Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. Am J Respir Crit Care Med 1999;159:15-21.
14. Streeton JA, Desem N, Jones SL. Sensitivity and specificity of a gamma interferon blood test for tuberculosis infection. Int J Tuberc Lung Dis 1998;2:443-50.
15. Food and Drug Administration, Center for Devices and Radiological Health. QuantiFERON®-TB - P010033 [Letter]. Rockville, MD: Food and Drug Administration, 2002. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?ID=P010033.
16. Lalvani A, Richeldi L, Kunst H. Interferon gamma assays for tuberculosis. Lancet Infect Dis 2005;5:322-4; author reply 325-7
17. Mahairas GG, Sabo PJ, Hickey MJ, et al. Molecular analysis of genetic differences between Mycobacterium bovis BCG and virulent M. bovis. J Bacteriol 1996;178:1274-82.
18. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet 2000;356:1099-104.
19. Prevention Committee of the Japanese Society for Tuberculosis. Guidelines for using QuantiFERON® TB Gold In-Tube August 2011. Kekkaku 2013;88:33-7.
20. Brock I, Weldingh K, Leyten EM, et al. Specific T-cell epitopes for immunoassay-based diagnosis of Mycobacterium tuberculosis infection. J Clin Microbiol 2004;42:2379-87.
21. Whitworth HS, Scott M, Connell DW, et al. IGRAs--the gateway to T cell based TB diagnosis. Methods 2013;61:52-62.
22. QIAGEN GmbH. QuantiFERON-TB Gold Plus, ELISA Package Insert. Available from: https://www.quantiferon.com/wp-content/uploads/2017/04/English_QFTPlus_ELISA_R04_022016.pdf.
23. Haas MK, Belknap RW. Diagnostic tests for latent TB infection. Clin Chest Med 2019;40:829-37.
24. Manuel O, Kumar D. QuantiFERON-TB Gold assay for the diagnosis of latent tuberculosis infection. Expert Rev Mol Diagn 2008;8:247-56.

25. Lalvani A, Pathan AA, McShane H, et al. Rapid detection of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. Am J Respir Crit Care Med 2001;163:824-8.
26. Lalvani A, Pathan AA, Durkan H, et al. Enhanced contact tracing and spatial tracking of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. Lancet 2001;357:2017-21.
27. Revvity. T-SPOT technology (ELISPOT). Available from: https://www.oxfordimmunotec.com/international/products-services/t-cell-select/.
28. Li J, Liu Y, Ma L, et al. The performance of T-cell Xtend reagent in increasing blood storage times for interferon gamma release assays. J Clin Lab Anal 2018;32:e22253.
29. WHO. WHO operational handbook on tuberculosis. Module 3: diagnosis. Tests for TB infection. 2022. Available from: https://iris.who.int/bitstream/handle/10665/363237/9789240058361-eng.pdf.
30. WHO. ELISA-based WANTAI TB-IGRA. Available from: https://tbksp.org/en/node/2427.
31. WHO. Use of alternative interferon-gamma release assays for the diagnosis of TB infection. WHO policy statement. Available from: https://iris.who.int/bitstream/handle/10665/351180/9789240042360-eng.pdf.
32. Mori T, Sakatani M, Yamagishi F, et al. Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. Am J Respir Crit Care Med 2004;170:59-64.
33. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. Ann Intern Med 2008;149:177-84.
34. Diel R, Goletti D, Ferrara G, et al. Interferon-γ release assays for the diagnosis of latent Mycobacterium tuberculosis infection: a systematic review and meta-analysis. Eur Respir J 2011;37:88-99.
35. Lalvani A, Berrocal-Almanza LC, Halliday A. Predicting progression to active tuberculosis: A rate-limiting step on the path to elimination. PLoS Med 2019;16:e1002814.
36. Sotgiu G, Saderi L, Petruccioli E, et al. QuantiFERON TB Gold Plus for the diagnosis of tuberculosis: a systematic review and meta-analysis. J Infect 2019;79:444-53.
37. Pourakbari B, Mamishi S, Benvari S, Mahmoudi S. Comparison of the QuantiFERON-TB Gold Plus and QuantiFERON-TB Gold In-Tube interferon-γ release assays: a systematic review and meta-analysis. Adv Med Sci 2019;64:437-43.
38. Xu Y, Yang Q, Zhou J, et al. Comparison of QuantiFERON-TB gold in-tube and QuantiFERON-TB gold-plus in the diagnosis of Mycobacterium tuberculosis infections in immunocompromised patients: a real-world study. Microbiol Spectr 2022;10:e0187021.

39. Darmawan G, Liman LMS, Hamijoyo L, et al. Comparison of interferon-gamma production between TB1 and TB2 tubes of QuantiFERON-TB Gold Plus: a meta-analysis. Clin Chem Lab Med 2023;61:2067-75.
40. Venkatappa TK, Punnoose R, Katz DJ, et al. Comparing QuantiFERON-TB gold plus with other tests to diagnose Mycobacterium tuberculosis Infection. J Clin Microbiol 2019;57:e00985-19.
41. Gupta RK, Lipman M, Jackson C, et al. Quantitative IFN-γ release assay and tuberculin skin test results to predict incident tuberculosis. a prospective cohort study. Am J Respir Crit Care Med 2020;201:984-91
42. WHO. Consensus meeting report: development of a target product profile (TPP) and a framework for evaluation for a test for predicting progression from tuberculosis infection to active disease. Available from: http://www.who.int/iris/handle/10665/259176.
43. Rangaka MX, Wilkinson KA, Seldon R, et al. Effect of HIV-1 infection on T-Cell-based and skin test detection of tuberculosis infection. Am J Respir Crit Care Med 2007;175:514.20.
44. Stephan C, Wolf T, Goetsch U, et al. Comparing QuantiFERON tuberculosis gold, T-SPOT tuberculosis and tuberculin skin test in HIV-infected individuals from a low prevalence tuberculosis country. AIDS 2008;22:2471-9.
45. Leidl L, Mayanja-Kizza H, Sotgiu G, et al. Relationship of immunodiagnostic assays for tuberculosis and numbers of circulating CD4+ T-cells in HIV infection. Eur Respir J 2010;35:619-26
46. Cattamanchi A, Smith R, Steingart KR, et al. Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in HIV-infected individuals: a systematic review and meta-analysis. J Acquir Immune Defic Syndr 2011;56:230-8.
47. Laboratory Procedure Manual. Tuberculosis (TB) QUANTIFERON gold plus (Interferon-gamma). Available from: https://www.quantiferon.com/wp-content/uploads/2017/04/English_QFTPlus_ELISA_R04_022016.pdf.
48. Belknap R, Daley CL. Interferon-gamma release assays. Clin Lab Med 2014;34:337-49.
49. Pagaduan JV, Altawallbeh G. Advances in TB testing. Adv Clin Chem 2023;115:33-62.
50. Revvity. T-SPOT technology (ELISPOT). Available from: https://www.oxfordimmunotec.com/international/wp-content/uploads/sites/3/Final-File-PI-TB-US-V6.pdf.
51. Testing for TB infection video: tuberculin skin test & IGRA blood test Available from: https://www.michigan.gov/-/media/Project/Websites/mdhhs/Folder2/Folder90/Folder1/Folder190/6_Participant_Guide_Testing_for_TB_Infection_Video_-_2017.pdf?rev=65b6c1708ee14792ab6467636d22e4f1.

52. WHO. South-East Asia immunizes a record-high number of children in 2024. Available from: http://www.searo.who.int/india/mediacentre/events/2014/stci_book.pdf.
53. Pai M, Rodrigues C. Management of latent tuberculosis infection: An evidence-based approach. Lung India 2015;32:205-7.
54. Sester M, Sotgiu G, Lange C, et al. Interferon-γ release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. Eur Respir J 2011;37:100-11.
55. van Ingen J, de Zwaan R, Dekhuijzen R, et al. Region of difference 1 in nontuberculous Mycobacterium species adds a phylogenetic and taxonomical character. J Bacteriol 2009;191:5865-7.
56. Behr MA, Wilson MA, Gill WP, et al. Comparative genomics of BCG vaccines by whole-genome DNA microarray. Science 1999;284:1520-3.
57. de Visser V, Sotgiu G, Lange C, et al. False-negative interferon-γ release assay results in active tuberculosis: a TBNET study. Eur Respir J 2015;45:279-83.
58. Kobashi Y, Shimizu H, Ohue Y, et al. False negative results of QuantiFERON TB-2G test in patients with active tuberculosis. Jpn J Infect Dis 2009;62:300-2.
59. Kobashi Y, Mouri K, Yagi S, et al. Clinical utility of the QuantiFERON TB-2G test for elderly patients with active tuberculosis. Chest 2008;133:1196-1202.
60. Hang NT, Lien LT, Kobayashi N, et al. Analysis of factors lowering sensitivity of interferon-γ release assay for tuberculosis. PLoS One 2011;6:e23806.
61. Yamasue M, Komiya K, Usagawa Y, et al. Factors associated with false negative interferon-γ release assay results in patients with tuberculosis: a systematic review with meta-analysis. Sci Rep 2020;10:1607.
62. Wang MS, Liu XJ. Risk factors for false-negative interferon-γ release assay results in culture-confirmed childhood TB. Am J Trop Med Hyg 2019;101:1303-7.
63. Overton K, Varma R, Post JJ. Comparison of interferon-γ release assays and the tuberculin skin test for diagnosis of tuberculosis in human immunodeficiency virus: a systematic review. Tuberc Respir Dis 2018;81:59-72.
64. Zhou G, Luo Q, Luo S, et al. Indeterminate results of interferon gamma release assays in the screening of latent tuberculosis infection: a systematic review and meta-analysis. Front Immunol 2023;14:1170579.
65. Diel R, Loddenkemper R, Nienhaus A. Evidence-based comparison of commercial interferon-gamma release assays for detecting active TB: a metaanalysis. Chest 2010;137:952-68.

66. Zrinski Topic R, Zoric ic -Letoja I, Pavic I, Dodig S. Indeterminate results of QuantiFERON-TB gold in-tube assay in nonimmunosuppressed children. Arch Med Res 2011;42:138-43.

67. Sun X, Wan S, Zhang L, et al. Prevalence and influencing factors of the high nil-control spot count in T-SPOT.TB: A matched case-control study. Clin Chim Acta 2018;487:96-100.

68. Ward JD, Cornaby C, Schmitz JL. Indeterminate QuantiFERON gold plus results reveal deficient interferon gamma responses in severely ill COVID-19 patients. J Clin Microbiol 2021;59:e0081121.

69. Banach DB, Harris TG. Indeterminate QuantiFERON®-TB Gold results in a public health clinic setting. Int J Tuberc Lung Dis 2011;15:1623-30.

70. Sharninghausen JC, Shapiro AE, Koelle DM, Kim HN. Risk factors for indeterminate outcome on interferon gamma release assay in non-US-born persons screened for latent tuberculosis infection. Open Forum Infect Dis 2018;5:ofy184.

71. Kobashi Y, Mouri K, Obase Y, et al. Clinical evaluation of QuantiFERON TB-2G test for immunocompromised patients. Eur Respir J 2007;30:945-50.

72. Redelman-Sidi G, Sepkowitz KA. IFN-γ release assays in the diagnosis of latent tuberculosis infection among immunocompromised adults. Am J Respir Crit Care Med 2013;188:422-31.

73. Gualano G, Mencarini P, Lauria FN, et al. Tuberculin skin test - Outdated or still useful for Latent TB infection screening? Int J Infect Dis 2019;80S:S20-2.

74. Ewer K, Deeks J, Alvearez L, et al. Longitudinal tracking of M. tuberculosis-specific T cells ex vivo in latently infected, tuberculin skin test-negative tuberculosis contacts. In: Tuberculosis: integrating host and pathogen biology. Keystone Symposia 2003 Abstract Book; Abstract 317. Silverthorne, CO: Keystone Symposia

75. Herrera V, Perry S, Parsonnet J, Banaei N. Clinical application and limitations of interferon-gamma release assays for the diagnosis of latent tuberculosis infection. Clin Infect Dis 2011;52:1031-7.

76. Joshi M, Monson TP, Joshi A, Woods GL. IFN-γ release assay conversions and reversions. Challenges with serial testing in U.S. health care workers. Ann Am Thorac Soc 2014;11:296-302.

77. Pai M, Joshi R, Dogra S, et al. Serial testing of health care workers for tuberculosis using interferon-gamma assay. Am J Respir Crit Care Med 2006;174:349-55.

78. Thanassi W, Noda A, Hernandez B, et al. Delineating a Retesting Zone Using Receiver Operating Characteristic Analysis on Serial QuantiFERON Tuberculosis Test Results in US Healthcare Workers. Pulm Med 2012;2012:291294.

79. Dorman SE, Belknap R, Graviss EA, et al. Interferon-γ release assays and tuberculin skin testing for diagnosis of latent tuberculosis infection in healthcare workers in the United States. Am J Respir Crit Care Med 2014;189:77-87.
80. Pollock NR, McAdam AJ, Pai M, et al. Interferon γ-release assays for diagnosis of latent tuberculosis in healthcare workers in low-incidence settings: pros and cons. Clin Chem 2014;60:714-8.
81. van Zyl-Smit RN, Zwerling A, Dheda K, Pai M. Within-subject variability of interferon-g assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review. PLoS One 2009;4:e857.
82. Pai M, Denkinger CM, Kik SV, et al. Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. Clin Microbiol Rev 2014;27:3-20.
83. Banaei N, Gaur RL, Pai M. Interferon gamma release assays for latent tuberculosis: what are the sources of variability? J Clin Microbiol 2016;54:845-50.
84. Menzies D. Use of interferon-gamma release assays for diagnosis of tuberculosis infection (tuberculosis screening) in adults. Available from: https://www.uptodate.com/contents/use-of-interferon-gamma-release-assays-for-diagnosis-of-tuberculosis-infection-tuberculosis-screening-in-adults.
85. Doberne D, Gaur RL, Banaei N. Preanalytical delay reduces sensitivity of QuantiFERON-TB gold in-tube assay for detection of latent tuberculosis infection. J Clin Microbiol 2011;49:3061-4.
86. Tagmouti S, Slater M, Benedetti A, et al. Reproducibility of interferon gamma (IFN-γ) release Assays. A systematic review. Ann Am Thorac Soc 2014;11:1267-76.
87. Pai M, Banaei N. Occupational screening of health care workers for tuberculosis infection: tuberculin skin testing or interferon-γ release assays? Occup Med 2013;63:458-60.
88. Canadian Tuberculosis Committee (CTC). Updated recommendations on interferon gamma release assays for latent tuberculosis infection. An Advisory Committee Statement (ACS). Can Commun Dis Rep 2008;34:1-13.
89. Tuberculosis blood test: interferon gamma release assay (IGRA). Available from: https://www.dhs.wisconsin.gov/publications/p01182.pdf.
90. Lee SW, Oh DK, Lee SH, et al. Time interval to conversion of interferon-gamma release assay after exposure to tuberculosis. Eur Respir J 2011;37:1447-52.
91. Weinberg A, Aaron L, Montepiedra G, et al. Effects of pregnancy and isoniazid preventive therapy on mycobacterium tuberculosis interferon gamma response assays in women with HIV. Clin Infect Dis 2021;73:e3555-62.

92. Silverman MS, Reynolds D, Kavsak PA, et al. Use of an interferon-gamma based assay to assess bladder cancer patients treated with intravesical BCG and exposed to tuberculosis. Clin Biochem 2007;40:913-5.
93. WHO. Rapid communication: TB antigen-based skin tests for the diagnosis of TB infection. Available from: https://www.who.int/publications/i/item/WHO-UCN-TB-2022.1.
94. Ruhwald M, Aggerbeck H, Gallardo RV, et al. Safety and efficacy of the C-Tb skin test to diagnose Mycobacterium tuberculosis infection, compared with an interferon γ release assay and the tuberculin skin test: a phase 3, double-blind, randomised, controlled trial. Lancet Respir Med 2017;5:259-68.
95. Aggerbeck H, Giemza R, Joshi P, et al. Randomised clinical trial investigating the specificity of a novel skin test (C-Tb) for diagnosis of M. tuberculosis infection. PLoS One 2013;8:e64215.
96. Hamada Y, Cirillo DM, Matteelli A, et al. Tests for tuberculosis infection: landscape analysis. Eur Respir J 2021;58:2100167.
97. Hoff ST, Peter JG, Theron G, et al. Sensitivity of C-Tb: a novel RD-1-specific skin test for the diagnosis of tuberculosis infection. Eur Respir J 2016;47:919-28.
98. Starshinova A, Dovgalyk I, Malkova A, et al. Recombinant tuberculosis allergen (Diaskintest®) in tuberculosis diagnostic in Russia (meta-analysis). Int J Mycobacteriol 2020;9:335-46.
99. Zhang H, Wang L, Li F, et al. Induration or erythema diameter not less than 5 mm as results of recombinant fusion protein ESAT6-CFP10 skin test for detecting M. tuberculosis infection. BMC Infect Dis 2020;20:685.
100. Xu M, Lu W, Li T, et al. Sensitivity, specificity, and safety of a novel ESAT6-CFP10 skin test for tuberculosis infection in China: 2 randomized, self-controlled, parallel-group phase 2b trials. Clin Infect Dis 2022;74:668-77.
101. DiaSorin. LIAISON® QuantiFERON®-TB Gold Plus ([REF] 311020). Avaialable from: https://www.accessdata.fda.gov/cdrh_docs/pdf18/P180047D.pdf.
102. Fernández-Huerta M, Moreto C, Vila-Olmo N, et al. Evaluation of the fully automated chemiluminescence analyzer liaison XL for the performance of the QuantiFERON-TB gold plus assay in an area with a low incidence of tuberculosis. J Clin Microbiol 2021;59:e0060321
103. Bisognin F, Lombardi G, Re MC, Dal Monte P. QuantiFERON-TB gold plus with chemiluminescence immunoassay: do we need a higher cutoff? J Clin Microbiol 2020;58:e00780-20.
104. Kobashi Y. Current status and future landscape of diagnosing tuberculosis infection. Respir Investig 2023;61:563-78.

105. Altawallbeh G, Gabrielson D, Peters JM, Killeen AA. Performance of an advanced interferon-gamma release assay for Mycobacterium tuberculosis detection. J Appl Lab Med 2021;6:1287-92.
106. Buron V, Banaei N. Inflated gamma interferon response with QuantiFERON-TB gold plus using the automated liaison XL analyzer: a testing algorithm to mitigate false-positive results in low-incidence settings. J Clin Microbiol 2023;61:e0029523.
107. Saluzzo F, Mantegani P, Poletti de Chaurand V, Cirillo DM. QIAreach QuantiFERON-TB for the diagnosis of Mycobacterium tuberculosis infection. Eur Respir J 2022;59:2102563.
108. Stieber F, Howard J, Manissero D, et al. Evaluation of a lateral-flow nanoparticle fluorescence assay for TB infection diagnosis. Int J Tuberc Lung Dis 2021;25:917-22.
109. Aziz ZA, Noordin NM, Wan Mohd WM, Kasim MA. First evaluation of the performance of portable IGRA, QIAreach® QuantiFERON®-TB in intermediate TB incidence setting. PLoS One 2023;18:e0279882.
110. Mahmoudi S, Nourazar S. Evaluating the diagnostic accuracy of QIAreach QuantiFERON-TB compared to QuantiFERON-TB Gold Plus for tuberculosis: a systematic review and meta-analysis. Sci Rep 2024;14:14455.
111. Diagbouga S, Djibougou AD, Pease C, et al. Preliminary diagnostic performance of the VIDAS® TB-IGRA for the detection of Mycobacterium tuberculosis infection and disease. medRxiv 2022.04.01.22271763.
112. Lionex. LIOFeron®TB/LTBI IGRA. Avaialble from: https://lionex.de/product/lioferon/.
113. Jungblut PR, Schaible UE, Mollenkopf HJ, et al. Comparative proteome analysis of Mycobacterium tuberculosis and Mycobacterium bovis BCG strains: towards functional genomics of microbial pathogens. Mol Microbiol 1999;33:1103-17.
114. Dong Y, Demaria S, Sun X, et al. HLA-A2-restricted CD8+-cytotoxic-T-cell responses to novel epitopes in Mycobacterium tuberculosis superoxide dismutase, alanine dehydrogenase, and glutamine synthetase. Infect Immun 2004;72:2412-5.
115. Tripathi SM, Ramachandran R. Crystal structures of the Mycobacterium tuberculosis secretory antigen alanine dehydrogenase (Rv2780) in apo and ternary complex forms captures "open" and "closed" enzyme conformations. Proteins 2008;72:1089-95.
116. Della Bella C, Spinicci M, Alnwaisri HFM, et al. LIOFeron®TB/LTBI: a novel and reliable test for LTBI and tuberculosis. Int J Infect Dis 2020;91:177-81.
117. Ichroma™ IGRA-TB 25. Available from: https://labindustrias.com/wp-content/uploads/2023/10/INS-TBE-EN-ichroma-IGRA-TB-25_Rev.00_2002111.pdf.
118. Kweon OJ, Lim YK, Kim HR, et al. Performance evaluation of newly developed fluorescence immunoassay-based interferon-gamma release assay for the diagnosis of latent tuberculosis infection in healthcare workers. J Microbiol Immunol Infect 2022;55:328-31.

119. SD Biosensor STANDARD F TB-Feron FIA. Available from: https://www.sdbiosensor.com/product/product_view? product_no=167.
120. Migliori GB, Ong CWM, Petrone L, et al. The definition of tuberculosis infection based on the spectrum of tuberculosis disease. Breathe 2021;17:210079.
121. Kweon OJ, Lim YK, Kim HR, et al. Evaluation of standard E TB-feron enzyme-linked immunosorbent assay for diagnosis of latent tuberculosis infection in health care workers. J Clin Microbiol 2019;57:e01347-19.
122. Yoo IY, Lee J, Choi AR, et al. Comparative evaluation of standard E TB-Feron ELISA and QuantiFERON-TB gold plus assays in patients with tuberculosis and healthcare workers. Diagnostics 2021;11:1659.
123. Kim JJ, Park Y, Choi D, Kim HS. performance evaluation of a new automated chemiluminescent immunoanalyzer-based interferon-gamma releasing assay AdvanSure I3 in comparison with the QuantiFERON-TB gold in-tube assay. Ann Lab Med 2020;40:33-39.
124. TB diagnostics pipeline: needs & solutions by Samuel Schumacher, Deputy Head TB. FIND & NDWG joint symposium, 50th Union World Conference, 30 October 2019. https://www.stoptb.org/sites/default/files/imported/article/oldweb/wg/new_diagnostics/assets/documents/TB_diagnostics_pipeline_needs_and_solutions.pdf.
125. Ortiz-Brizuela E, Apriani L, Mukherjee T, et al. Assessing the diagnostic performance of new commercial interferon-γ release assays for mycobacterium tuberculosis infection: a systematic review and meta-analysis. Clin Infect Dis 2023;76:1989-99.

Table 1. Differences between IGRA and TST.

	Interferon Gamma Release Assay (IGRA)	Tuberculin test (TST)
Setting of the test	In vitro	In vivo
Basic principle	It measures adaptive immune response to mycobacterial tuberculosis specific antigens	Delayed-type hypersensitivity response to the intradermal inoculation of purified protein derivative (PPD)
Antigens	ESAT-6, CFP-10, and TB7.7	PPD which contains more than 200 antigens
Antigenic cross-reactivity and false-positivity	*Mycobacterium bovis* and NTM such as *M.kansasii, M.szulgai, M.marinum, M.gastri, M.leprae*, and *M. flavescens*.	*Mycobacterium bovis*, bacilli Calmette-Guérin (BCG) and many NTM
Instrumentation and laboratory infrastructure	ELISA or ELIspot based test so instrumentation and sophisticated laboratory infrastructure required	Do not need sophisticated instrument and can be done3 in field condition
Interpretation	Laboratory based	Operator based
Ease of use	Can be used in specialized laboratory	Can be applied in field condition
Outcome	Measures the levels of IFN-γ	Measures induration
Number of visit	Single patient visit	Two patient visits
Boosting	TST induced "boosting" of IGRA responses occurs after 3 days and may persist for several months [81]	Boosting occurs after repeated testing
Inter-reader and intra-reader variability.	No	Yes
TB infection versus TB disease	Don't distinguish	Don't distinguish
Negative test	A negative test does not exclude LTBI or active TB disease	A negative test does not exclude LTBI or active TB disease
Positive predictive value[34]	QFT-GIT: 2.8 to 14.3% T-SPOT.TB: 3.3 to 10%	2.3 to 3.3%
Negative predictive value	QFT-GIT 98.0% T-SPOT.TB 98.3%	TST (10mm) 100%
Sensitivity		
Immunosuppressive state	IGRA is better than TST	Low sensitivity in immunosuppressed patients
serial testing	Potential boosting	Conversions and spontaneous reversions
Window period		12 weeks
Specificity [33]	QuantiFERON assay among non–BCG-vaccinated: 99% (CI, 98% to 100%) BCG-vaccinated: 96% (CI, 94% to 98%) T-SPOT.TB : 93% (CI, 86% to 100%).	88.7% (95% CI 84.6–92.0)[34]
Sensitivity[33]	QuantiFERON-TB Gold: 78% (95% CI, 73% to 82%) QFT-GIT 70% (CI, 63% to 78%) T-SPOT.TB 90% (CI, 86% to 93%)	77% (CI, 71% to 82%)

Table 2. Interpretation strategy of QFT-Plus assay [47].

NIL (IU/ml)	TB1-NIL (IU/ml)		TB2-NIL (IU/ml)	Mitogen-NIL (IU/ml)	Results	Interpretation
8 IU/mL	IFN-γ levels 0.35 and 25% of the NIL	and/or	IFN-γ levels 0.35 and 25% of the NIL	Any	Positive	*M.tb*infection likely
	IFN-γ levels < 0.35 or 0.35 and <25% of the NIL		IFN-γ levels < 0.35 or 0.35 and <25% of the NIL	0.50	Negative	M.tb infection NOT likely
	IFN-γ levels < 0.35 or 0.35 and <25% of the NIL		IFN-γ levels < 0.35 or 0.35 and <25% of the NIL	< 0.50	Indeterminate	Likelihood of M.tb infection cannot be ruled out. Repeat testing is indicated
>8 IU/mL	Any		Any	Any		

Table 3. Interpretative strategy of T-SPOT.TB test [51].

Interpretation	Spot counts				
	Nil	*M.tuberculosis* antigen panel			Positive control/Mitogen
		Panel A - Nil		Panel B -Nil	
Positive	10	8	and/or	8	Any
Negative	10	4	and/or	4	20
Borderline/equivocal	10	5, 6 or 7 spots			Any
Indeterminate/invalid	>10	Any		Any	Any
	10	4	and/or	4	<20

Table 4. Difference between QuantiFERON-TB plus assay T-SPOT.TB (T-SPOT) assay.

	QuantiFERON-TB assay	T-SPOT.TB (T-SPOT) assay
Setting of the test	In vitro diagnostic Indirect test to assess adaptive immunity for TB	In vitro diagnostic Indirect test to assess adaptive immunity for TB
Basic feature	Enzyme-linked immunosorbent assay (ELISA)-based assay	Enzyme-linked immunospot (ELISpot) assay
	It uses whole-blood	It uses peripheral blood mononuclear cells
	Used Nil, positive control, and one antigen tube in QFT-GIT QFT-plus assay uses both long and short peptides of ESAT-6 and CFP10 in TB1 &TB2 tube	Used Nil, positive control, and 2 antigen tubes (TB1 and TB2)
Antigens used	QuantiFERON-TB: PPD QuantiFERON-TB Gold: ESAT-6 and CFP10 QFT-GIT: ESAT-6, CFP10, and TB7.7 QFT-plus: ESAT-6 and CFP10	ESAT-6 and CFP10
Principle of the test	Detect IFN-γ produced by Mycobacterium tuberculosis primed T lymphocytes following exposure to TB antigens	Detect IFN-γ produced by Mycobacterium tuberculosis primed T lymphocytes following exposure to TB antigens
Infection and disease	Unable to differentiate infection from active TB disease	Unable to differentiate infection from active TB disease
Effect on T lymphocytes	QFT-plus stimulates both CD4+ and CD8+ T-lymphocytes	Stimulates both CD4+ and CD8+ T-lymphocytes
Boosting effect on repeat test	No	No
Need for return visit	No	No
Output	Measures serum concentration of IFN-γ produced by T cells on contact with ESAT-6 and CFP-10	Measures spots produced by IFN-γ secreted by T cells on contact with ESAT-6 and CFP- 10
Number of venipuncture	Single blood draw	Single blood draw
Incubation time	16–24 h[48]	16–20 h [48]
Interpretation	Positive (0.35 IU/ml) Negative (< 0.35 IU/ml) Indeterminate: low mitogen and/or high Nil	Positive (8 Spots) Negative (4 spots) Indeterminate/Invalid: low mitogen and/or high Nil Borderline (5 to 7 spots)
Sensitivity	QuantiFERON-TB Gold: 78% (95% CI, 73% to 82%) QFT-GIT: 70% (CI, 63% to 78%)[33] QFT-Plus; 0.91 (95% CI = 0.84–0.96)[36]	90% (CI, 86% to 93%)[33]
HIV-infected individuals[49]	Sensitivity: QFT-GIT 61% (95% CI 41-75%)	Sensitivity: 72% (95% CI 62-81%)
Specificity	Non–BCG-vaccinated: 99% (CI, 98% to 100%) BCG-vaccinated: 96% (CI, 94% to 98%)[33] 0.95 (95% CI = 0.93–0.97)[36]	93% (CI, 86% to 100%)[33]
Effect of BCG	No effect	No effect
PPV[34]	QFT-GIT 2.8 to 14.3%	3.3 to 10%
NPV[34]	QFT-GIT 99.8% (95% CI 99.4–100)	97.8% (95% CI 94.5-99.4%)
Indeterminate results	QFT-G: 2%[69] Immunosuppressed: 11%[71]	Immunocompetent: 3.8% (95% CI, 0.035-0.042)[65] Immunosuppressed: 6.1% (95% CI, 0.052-0.071)[65]