Chapter 9
Advances in Diagnosis of Latent TB Infection: What Is the Latest Approach to Diagnose Latent TB Infection to Prevent TB?

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Abstract Both types of tests for the diagnosis of latent tuberculosis infection (LTBI), the tuberculin skin test (TST) and interferon-gamma release assays (IGRAs), rely on detecting evidence of cell-mediated immunity to *M. tuberculosis* antigens. If testing is positive and there are no symptoms, radiographic or microbiologic evidence of TB disease, the patient is typically considered to have LTBI. Advantages of the TST include low cost, ease of administration, no lab requirement, and adjustable interpretation cut-off per individual LTBI risks. Advantages of IGRAs include requiring a single visit, higher specificity compared to the TST, and likely modestly improved sensitivity (particularly with immunocompromised individuals). The TST and IGRA have only poor-to-fair concordance. Both the TST and IGRAs have significant shortcomings. They cannot discriminate asymptomatic infection from past infection, identify individuals at elevated risk of TB disease, or be used to assess response to preventative therapy. A variety of novel technologies are in different phases of investigation, development, or clinical use that may help address these issues.

Keywords Latent TB infection · Tuberculin skin test · Interferon-gamma (IFN-γ) release assay

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1 Introduction

For the purposes of clinical and programmatic management, latent tuberculosis infection (LTBI) is defined as:

- Evidence of cell-mediated immunity to TB antigens (either a positive tuberculin skin test or interferon-gamma [IFN-γ] release assay).
- No clinical signs or symptoms of TB disease.
- No radiographic signs of TB disease.
- If specimens are collected (e.g., sputum), then cultures are negative for *M. tuberculosis*.

There is no test that is capable of directly detecting the presence of *M. tuberculosis* causing LTBI. The commercially available tests rely upon the host immune response to detect evidence of prior exposure to *M. tuberculosis*. A positive test does not distinguish between those who have had a TB exposure with T-cell priming but have cleared their infection, persons with viable TB in a controlled state ("LTBI"), or patients with TB disease. This lack of discrimination contributes to the poor performance of these tests in predicting who will progress to TB.

The tuberculin skin test (TST) was the only widely available test for LTBI diagnosis until 2001, when a commercial interferon-γ release assay (IGRA) became available: the first generation QuantiFERON-TB test (Cellestis Ltd.). After hundreds of studies evaluating the accuracy of IGRAs, it is clear that both the TST and IGRAs are acceptable but less than perfect tests [1]. In the following sections, we discuss the immunology, history, procedures, performance, strengths, and limitations of the TST and IGRAs in the diagnosis of LTBI, as well as new diagnostic approaches under investigation.

2 The Science of LTBI Testing

2.1 TB Exposure and the Immune Response

Exposure to *M. tuberculosis* occurs through the inhalation of droplet nuclei that remain airborne after being expelled by individuals with active pulmonary TB [2, 3]. Those infective droplet nuclei small enough to navigate the bronchi and avoid mucociliary clearance arrive at the terminal alveoli, where they are phagocytized by resident alveolar macrophages [4]. It is thought that some individuals clear the bacilli through innate immune defenses and tests for LTBI (TSTs or IGRAs) will remain negative due to the lack of T-cell activation.

M. tuberculosis has a number of mechanisms for avoiding lysosomal destruction after phagocytosis, allowing for persistence and replication within the macrophage until causing cell death [5, 6]. Infected macrophages release cytokines and chemokines that attract more phagocytic cells, along with neutrophils, to the site of

infection, which in turn leads to more infected cells and expansion and dissemination of the infection [7]. During this process, alveolar dendritic cells take up and transport bacilli or their remnants to regional lymph nodes and present *M. tuberculosis* antigens to naïve $CD4^+$ and $CD8^+$ T-cells, leading to their differentiation into $CD4^+$ helper T-cells type 1 and $CD8^+$ cytotoxic T-cells, respectively [8]. Once activated, these effector T-cells migrate hematogenously to the site of infection. B-cells are also activated and differentiate into specific antibody-secreting cells.

The cell-mediated immune response typically develops within a few weeks after initial infection, in which case TST and IGRAs will normally become positive [9]. The arrival of effector T-cells to the site of infection leads to activation of proximal macrophages, largely mediated by the release of IFN-γ by T-helper cells, causing morphological changes in the macrophages and enhancing their mycobacterial killing [8]. Progressively mononuclear cells aggregate and organize around the infected macrophages, extracellular bacilli, and cellular debris to form nodular structures called granulomas [10]. If cell-mediated immunity is effective, the bacilli are eliminated or contained (i.e., LTBI). If it fails—sometimes months or even years later—the bacteria proliferate and spread, the pro-inflammatory immune response causes worsening collateral tissue destruction, and subclinical disease progresses to symptomatic TB [11, 12].

It is important to note that while patients infected with *M. tuberculosis* are commonly categorized pragmatically as either having LTBI or active TB disease for public health and clinical purposes [13], the natural history of TB involves a spectrum between infection and active TB disease that is likely dynamic over time [14].

2.2 *Measuring the Immune Response to* **M. tuberculosis** *Exposure*

Current methods for diagnosing LTBI rely on detecting evidence of acquired immunity to *M. tuberculosis*, based on the principle that T-cells sensitized to *M. tuberculosis* antigens will predictably release cytokines when re-exposed to these antigens. In the case of the TST, purified protein derivatives from *M. tuberculosis* cultures are injected intradermally. If the person's cellular immune system has previously been exposed to *M. tuberculosis*, specific memory helper T-cells are rapidly activated by local antigen-presenting cells (e.g., dendritic cells), leading to clonal expansion and the release of IFN-γ and other pro-inflammatory cytokines [15]. The subsequent local vasodilation, edema, and infiltration of immune cells into the area near the site of the injection create a visible induration that can be measured to determine if it meets the threshold for a positive result [16].

In contrast to the TST, IGRAs are an ex vivo test to assess for *M. tuberculosis* sensitized T-cells. Depending on the specific test, whole blood or peripheral blood mononuclear cells (PBMCs) from the patient are incubated with *M. tuberculosis* antigens, leading to the release of IFN-γ by sensitized T-cells [17]. The IFN-γ

production is then measured by one of two types of enzyme-linked immunosorbent assays to measure either the total concentration of IFN-γ produced by all cells or the number of cells secreting IFN-γ. Unlike the TST, which uses a nonspecific mixture of mycobacterial proteins and is vulnerable to eliciting false-positive results due to prior BCG vaccination or environmental nontuberculous mycobacteria (NTM) exposure, IGRAs use proteins encoded by genes found in the genome of *M. tuberculosis* and other members of the TB-causing *M. tuberculosis* complex (e.g., *M. africanum*, non-BCG strains of *M. bovis*, *M. canetti*) but not shared by BCG substrains or most NTM species (exceptions include *M. flavescens*, *M. kansasii*, *M. marinum*, and *M. szulgai*) [18].

Although based on similar principles, it has been recognized since the introduction of IGRAs that there is poor-to-fair concordance with the TST. Some of the discrepancies are explained by the superior specificity of IGRAs in high-risk populations due to lack of cross-reactivity with the bacillus Calmette-Guérin (BCG) vaccine and lesser cross-reactivity to antigens from NTM. However, discordance between the tests is observed in individuals without a history of BCG vaccination.

While IGRAs require only a single visit for testing, they are more expensive to perform than the TST, require a laboratory, have a single approved cut-off for a positive result regardless of LTBI risk, and may have lesser specificity in low-risk populations [19–21]. IGRAs and the TST share similar limitations including dependence on an individual's immune system for accurate test performance, inability to differentiate between points on the TB spectrum of disease, and poor positive predictive value.

3 Tuberculin Skin Test

3.1 TST History

Robert Koch, in 1890 presented his findings on "tuberculin," a discovery that he believed would prevent and cure TB [22]. Subsequently called "Koch's Old Tuberculin," it was prepared by taking a liquid broth in which *M. tuberculosis* had been cultured, sterilized with heat, and reduced in volume through evaporation [23]. Although not of therapeutic value, in the early part of the twentieth century Clemens von Pirquet discovered that when children with TB were inoculated with Koch's Old Tuberculin, a papule would transiently appear at the site of inoculation. Mantoux's description of the intradermal injection of 0.1 ml of tuberculin, published in 1910, describes a test that is used to this day [23]. In 1934, Florence Seibert developed a process for preparing tuberculin based on steaming cultures of *M. tuberculosis* and purifying the proteins through precipitation with ammonium sulfate resulting in purified protein derivative or PPD [24]. PPD-S, for "standard," was developed for use in the USA in 1944, and the current standard US preparation is PPD-S2. Two commercially available forms of PPS-S2 are Aplisol (JPH Pharmaceuticals, Inc.) and Tubersol (Sanofi Pasteur Ltd.), differing in the methods

of protein precipitation. PPD formulations used outside of the USA include PPD RT23 produced by the Statens Serum Institute, the Japanese product PPD-s, and PPD RT23 Mexico, used in Latin America [24]. Some studies have demonstrated differences in potency between different formulations of PPD [24] and even differences between different forms of PPD-S2 [25].

3.2 Performing TST

Variability in TST results may be introduced by differences in tuberculin placement technique and should be performed by medical personnel with adequate training and experience. The preferred method for TST application is the Mantoux method, in which an intradermal injection of tuberculin is applied away from skin lesions and veins. In the USA, 0.1 ml of 5 tuberculin units of PPD-S2 are injected into the volar surface of the forearm. When performed correctly, a 6–10 mm diameter wheal (raised area of the skin) will occur. When there are concerns about TST administration, a second TST can be performed at a site at least several centimeters from the first or on the alternate forearm [26].

TSTs should be read between 48 and 72 h after placement, which is when induration is maximum. It is important to note that the size of induration (swelling), not erythema, determines a person's response. If a test is read after 72 h and meets the size criteria for a positive test, then the patient may be diagnosed with latent TB infection. However, if a test is read after 72 h and is interpreted as negative, a repeat test should be performed. The tuberculin response should be read by a trained examiner. Induration should be determined by palpation with the fingertip, marking of the borders, and measured across the forearm (transverse diameter) with the measured diameter recorded in millimeters. The tuberculin formulation and lot number, forearm used, dates of placement and reading, and size in millimeters should be recorded.

When TSTs are placed in both arms of a single person, the variation in the size of induration averages 15% [27]. Variability in interpreting tuberculin responses by a single trained reader is also about 15%, while variability between different trained readers is greater, on average 2.5 mm [28]. Patients should not be permitted to interpret their own tuberculin responses.

Multiple puncture tests (e.g., Tine and Heaf tests) were frequently used in the past due to ease of administration. However, it is difficult to control the dose of tuberculin with these tests and they are no longer recommended for use in the USA [29]. In the 1990s, the US CDC recommended the use of anergy testing in conjunction with TST in people living with HIV to assess cellular immune function. As most people have been exposed to common fungi and vaccine antigens, preparations (e.g., tetanus toxoid, mumps, or Candida antigens) were injected intradermally to assess for an intact delayed hypersensitivity response. However, this is no longer recommended as a positive delayed-type hypersensitivity response to antigens other than PPD may remain despite loss of tuberculin reactivity [26].

3.3 Safety

Local reactions at the site of tuberculin placement, including blistering and local necrosis, may occur in 1–2% of positive skin tests [30, 31]. Rarely (<0.1/1,000,000 tests), serious hypersensitivity reactions including anaphylaxis may occur due to the TST [32]. A positive TST reaction may cause long-lasting skin discoloration [8]. Patients with local or hypersensitivity reactions should be instructed to never again receive tuberculin and should instead undergo an IGRA if indicated.

3.4 TST Interpretation

TST interpretation is risk-stratified using three different sizes of induration to determine a positive response. These thresholds for positivity are based on TST accuracy in different populations, LTBI prevalence in different groups, and risk for progression to active TB [13] (see Fig. 9.1).

For persons who are subject to repeat TST testing, for example, as a workplace requirement, a TST conversion is defined as a newly positive test (based on the cut-off categories in Fig. 9.1) that is at least 10 mm larger than the previous value [29]. There is no role for repeating TSTs in patients who have been treated for LTBI in order to assess for a treatment response [33].

3.5 Accuracy

Due to the lack of tests to directly identify *M. tuberculosis* in latently infected persons, most studies of LTBI test accuracy use surrogate outcomes, usually estimating sensitivity in patients with current or prior culture-confirmed TB and specificity in persons who are at low risk for TB exposures.

The sensitivity of the TST, based on responses in healthy persons with previously treated TB is 95–98% [13]. Several factors are associated with false-negative TST results. During the period immediately following infection with *M. tuberculosis*, tuberculin responses may be negative as T-cell reactivity can take 6–10 weeks to fully develop. False-negative reactions are also more common in infants and young children, in the presence of severe illness (including extensive TB), after recent viral and bacterial infections, in immunosuppressed persons, and in persons receiving immunosuppressive drugs including corticosteroids and TNF-alpha blockers [13]. Prednisone doses of >10–15 mg/day have been associated with a depressed tuberculin response [34, 35]. One study of patients with TB and positive TST were administered 40 mg of prednisone daily and found that the mean time to TST reversion occurred on day 14 of corticosteroid treatment and that return of TST positivity occurred an average of 6 days after discontinuation of prednisone [36]. Methotrexate has not been associated with an altered tuberculin response [37].

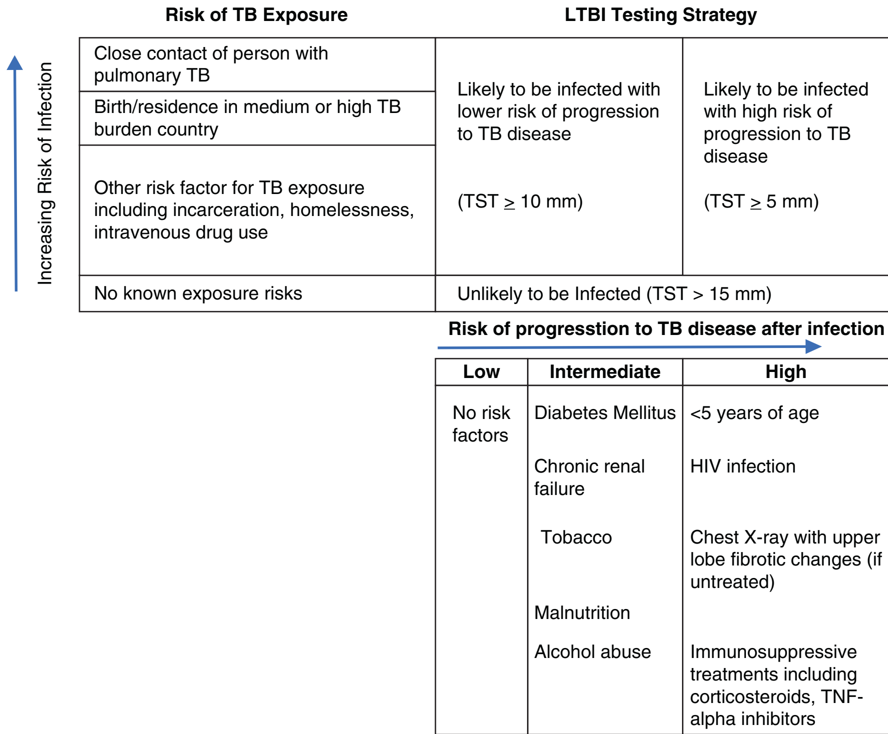

Fig. 9.1 Recommended tuberculin skin test cut-offs for diagnosing latent TB infection and rationale based on risk of infection and risk of progression to TB disease if a person is infected with *M. tuberculosis*. Adapted from Lewinsohn DM et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. Clinical Infectious Diseases 2017;64(2):111–5

It has long been dogma that recent vaccination with live-attenuated viruses is associated with lower tuberculin responses [26]. In fact, studies have found mixed effects on TST responses after live-attenuated vaccines. Additionally, impacts on TST responses after vaccination with killed virus vaccines have also been noted. The effect of mRNA-based vaccines, such as those against SARS-CoV-2, are unknown. When possible, tuberculin testing should be performed on the same day as a vaccination or at least 4 weeks following vaccination [26].

The specificity of the TST varies by patient group, depending on the frequency of BCG vaccination and exposures to environmental NTM. TST specificity among non-US-born persons (most of whom were BCG vaccinated) has been estimated at 70%, compared to 92% for US-born persons (where BCG vaccination has never been universally recommended) [38]. The likelihood that prior BCG vaccination will cause a false-positive TST is much greater if the individual was vaccinated after age 1 year or within 8 years prior to tuberculin testing [39]. TST may be more specific than IGRAs when testing low-risk populations [19, 20], likely related to the use of risk-based cut-points to define TST positivity.

Following exposure to *M. tuberculosis* or certain other mycobacteria, the resultant hypersensitivity is typically lifelong and reflected in all future TSTs. However, it can wane over time to the point where the reaction disappears. This is more common in older adults and people whose hypersensitivity was due to BCG vaccination or NTM exposure [40]. In these cases, an initial TST may be negative, but the antigen exposure from the TST stimulates the cell-mediated immune system such that if a second TST is done within weeks, it will become positive [28]. This is called the booster phenomenon. The boosting is most often seen when the interval between TSTs is about 1–4 weeks, but the effect can last months. Because boosting can be falsely interpreted as a new *M. tuberculosis* infection, for people such as healthcare providers who will undergo TSTs regularly (as well as for some at-risk groups like residents of long-term care facilities), repeat testing in 1–4 weeks is suggested for all individuals who initially test negative in order to assess for boosted reactions and confirm the person's baseline tuberculin response [13]. Such two-step TSTs are done only once to document for future reference. Note, TSTs alone (without any prior exposure to mycobacteria) do not sensitize individuals and will not cause false-positive tests [26].

3.6 Summary

The TST has a number of advantages including >100 years of experience, no need for phlebotomy or a laboratory, low cost, differential interpretation cut-offs based on LTBI risk and risk of progression, and serial testing criteria for conversion. This is balanced against limitations that include errors in performing the TST placement, variability in assessing induration size, lower specificity due to BCG vaccination and NTM exposures, requirements for two healthcare encounters, frequent patient and/or clinician resistance to a positive finding, and poor positive predictive value.

4 Interferon-Gamma Release Assays

4.1 History of IGRAs, Discussion of QFT, T-SPOT

An assay to diagnose bovine TB, based on the detection of IFN-γ in response to a specific antigen, was developed in 1990 [41]. In 1998, a report on the performance of the QuantiFERON-TB assay, an IGRA for the detection of LTBI in humans, was published that showed high specificity and sensitivity in comparison to the TST [42]. The second generation assay, QFT-Gold (QFT-GIT), was approved in Japan and the USA in 2005. QuantiFERON-TB Gold Plus assay (QFT-Plus; Qiagen), the currently available fourth-generation QFT assay, received European approval in 2016, US approval in 2017, and Japanese approval in 2018. T-SPOT.TB, developed

in the late 1990s by Lalvani et al. [43], is manufactured by Oxford Immunotec (Abingdon, UK). T-SPOT.TB has been available in Europe since 2004, in the USA since 2008, and in Japan since 2012.

At the time of this writing, there are two commercially available IGRAs: QuantiFERON-TB Gold Plus assay and T-SPOT.TB. Like the TST, IGRAs rely on host T-cell responses to *M. tuberculosis* antigens, with prior exposure eliciting IFN-γ production. The two IGRAs differ in that T-SPOT is an enzyme-linked immunospot (ELISPOT) assay whereas QFT is enzyme-linked immunosorbent assay (ELISA) based. The result from each test is based on the spot-forming cells (SFC) or quantification of IFN-γ in international units (IU) per milliliter for T-SPOT and QFT, respectively.

4.2 Procedure and Results

4.2.1 QFT-Plus Procedure

QFT-Plus differs from prior generations of the assay in that an additional antigen tube (TB2) containing peptides is used to elicit both $CD8^+$ and $CD4^+$ T-lymphocyte responses. Like its predecessors, QFT-Plus includes an antigen tube (TB1) designed to assess IFN-γ responses from $CD4^+$ helper T-lymphocytes. QFT-Plus no longer includes the antigen TB-7.7, present in prior generations of this assay, but retains the two other antigens, ESAT-6 and CFP-10. The theoretical advantages of assessing a $CD8^+$ T-cell response include improved performance in immunocompromised states that affect $CD4^+$ T-cell responses (e.g., HIV), discrimination between LTBI and active TB, and increased activity in patients with recently acquired infection [44–46]. However, to date studies have not supported these theoretical advantages.

To perform the QFT assay, whole blood is collected directly into four collection tubes, 1 ml of blood per tube, and shaken ten times [47]. Alternatively, at least 5 ml of whole blood may be collected into a lithium-heparinized tube, inverted several times, and maintained at room temperature for up to 12 h or refrigerated for up to 48 h until transfer into the QFT-Plus collection tubes [47]. After incubation for 16–24 h at 37 °C, plasma is harvested from each tube and the concentration of IFN-γ is determined for each by ELISA. The QFT-Plus assay is considered positive if the difference between TB antigen tube and the Nil tube is ≥0.35 IU/ml. To control for high background IFN-γ levels in the Nil tube, the IFN-γ response to antigen must be 25% greater than the IFN-γ concentration in the Nil control. The plasma sample from the Mitogen tube serves as an IFN-γ positive control, demonstrating T-cell activity after stimulation (Fig. 9.2).

QFT-Plus has three potential outcomes: positive, negative, or indeterminate. A low response to Mitogen (<0.5 IU/ml) indicates an indeterminate result when a blood sample also has a negative response to the TB antigens. An indeterminate response may also result from a Nil tube that has a very high background IFN-γ level (>8 IU/ml).

Interpretation criteria for the QuantiFERON-TB Gold In-Tube Test (QFT-GIT)

Interpretation	Nil*	TB Response†	Mitogen Response§
Positive¶	≤8.0	≥0.35 IU/ml and ≥25% of Nil	Any
Negative***	≤8.0	<0.35 IU/ml or <25% of Nil	≥0.5
Indeterminate††	≤8.0	<0.35 IU/ml or <25% of Nil	<0.5
	>8.0	Any	Any

Source: Based on Cellestis Limited. QuantiFERON-TB Gold In-Tube [Package insert]. Available at http://www.cellestis.com/IRM/content/pdf/QuanitFeron%20US%20VerG-Jan2010%20NO%20TRIMS.pdf.

*The interferon gamma (IFN-γ) concentration in plasma from blood incbate without antigen.

†The IFN-γ concentration in plasma from blood stimulated with a single cocktail of peptides representing early secretory antigenic target-6 (ESAT-6) culture filtrate protein-10 (CFP-10), and part of TB 7.7 minus Nil.

§The IFN-γ concentration in plasma from blood stimulated with mitogen minus Nil.

¶Interpretation indicating that *Mycobacterium tuberculosis* infection is likely.

**Interpretation indicating that *M. tuberclosis* infection is not likely.

††Interpretation indicating an uncertatin likelihood of *M. tuberculosis* infection.

Interpretation criteria for the T-SPOT.TB Test (T-Spot)

Interpretation	Nil*	TB Response†	Mitogen§
Positive¶	≤10 spots	>8 spots	Any
Broderline**	≤10 spots	5, 6, or 7 spots	Any
Negative††	≤10 spots	<4 spots	
Indeterminate**	>10 spots	Any	Any
	≤10 spots	<5 spots	<20 spots

Source: Based on Oxford Immunotec Limited T-Spot. TB [Package insert]. Available at http://www.oxfordimmunotec.com/USpageInsert.

*The number of spots resulting from incubation of PBMCs in culture media without antigens.

†The greater number of spots resulting from stimulation of peripheral blood mononuclear cells (PBMCs) with two separate cocktails of peptides representing early secretory antigenic target-6 (ESAT-6) or culture filtrate proten-10 (CFP-10) minus Nil.

§The number of spot resulting from stimulation of PBMCs with mitogen without adjustment for the number of spots resulting from incubation of PBMCs without antigens.

¶Interpretation indicating that *Mycobacterium tuberculosis* infection is likely.

**Interpretation indicating an uncertain likelihood of *M. tuberclosis* infection.

††Interpretation indicating that *M. tuberculosis* infection is not likely.

Fig. 9.2 Interpretation of results for QuantiFERON-TB Gold In-Tube Test (QFT-GIT) and T-SPOT.TB Test (T-Spot). Reproduced from Centers for Disease Control and Prevention. Updated Guidelines for Using Interferon Gamma Release Assays to Detect Mycobacterium tuberculosis Infection—United States, 2010. MMWR 2010;59(No. RR-5): p. 16

4.2.2 T-SPOT.TB Procedure

For the T-SPOT assay, whole blood (minimum 2 ml) is drawn into either lithium heparin or Vacutainer CPT tube, inverted 8–10 times, and stored at room temperature [48]. Processing must occur within 8 h unless T-cell Xtend additive is used, in which case the tubes should be kept between 10 °C and 25 °C and processed within 32 h. After centrifugation, PBMCs are extracted and washed, enumerated, and added to microtiter wells at 2.5×10^5 PBMCs per well. Although the T-SPOT step of WBC separation is technically more complex to process than QFT, it ensures a fixed number of WBCs in the assay, which may be important for immunosuppressed patients [13].

The T-SPOT assay uses four wells per patient: the negative-control (Nil) well that measures background IFN-γ-producing T-cells (spot-forming cells [SFC]), the two antigen wells (Panels A and B) separately containing ESAT-6 and CFP-10 that measure *M. tuberculosis*-specific SFC, and the positive control (Mitogen) well that measures nonspecific SFC. PBMCs and panels/controls are added to each well and placed in an incubator for 16–20 h. Wells are washed away and IFN-γ is detected via a sandwich capture technique by conjugation with secondary antibodies revealing "spots," which are counted to determine the resulting interpretation.

T-SPOT results are interpreted by subtracting the spot count in the Nil well from that in Panels A and B. T-SPOT.TB testing can result in positive, negative, or invalid (equivalent to an indeterminate QFT result) (Fig. 9.2). However, different from the rest of the world, in the USA there is a fourth category, termed "borderline." Outside of the USA, the T-SPOT is positive if Panel A-Nil and/or Panel B-Nil ≥6 spots and negative if both Panel A-Nil and Panel B-Nil ≤5 spots [48]. A Nil spot count in excess of 10 spots is considered invalid. When the mitogen well spot count is <20 spots, the result should be considered invalid unless either Panel A or Panel B are positive or borderline in which case the result is valid. In the USA, T-SPOT is considered positive when Panel A-Nil and/or Panel B-Nil ≥8 spots and negative if both Panel A-Nil and Panel B-Nil ≤4 spots; if the higher of Panel A-Nil or Panel B-Nil is 5, 6, or 7 spots, then the result is "borderline." Borderline results should be interpreted in conjunction with the patient's pretest probability of infection with *M. tuberculosis* [49].

4.2.3 Indeterminate/Invalid Results

Optimally, an indeterminate result indicates that knowledge of *M. tuberculosis* infection cannot be obtained from the IGRA due to either a low lymphocyte count or low lymphocyte response to mitogen. A study from New York City reviewed the frequency of QFT indeterminate results from public health clinics and found that 2% were indeterminate, approximately equally divided between high Nil and low Mitogen results [50]. Indeterminate frequencies of 4% have been reported in QFT results from outpatients [51] and QFT and T-SPOT results in children [52]. Very high frequencies of indeterminate results have been reported from the testing of

inpatients [53, 54]. An increased frequency of IGRA indeterminate/invalid results has been associated with young age [50, 55], Asian ethnicity [50, 51], HIV infection [55, 56], non-HIV-related immunocompromise [52], and anemia [51]. Delays in incubating the tubes are associated with indeterminate results [21]. Limited data suggests that indeterminate/invalid results are more common for QFT than T-SPOT when the CD4 count is <200 cells/μl [13].

In the case of indeterminate/invalid results, IGRA manufacturers recommend recollection of blood and retesting with the same assay; approximately two-third of repeat tests will give an actionable result (i.e., positive or negative) [50].

4.2.4 Sources of Variation/Error

Like any laboratory test, IGRAs are subject to variability at every step of the process. These sources have been categorized as preanalytical, analytical, manufacturing, and immunological [57]. Although most evaluations have been with QFT, these sources of variation likely apply to T-SPOT.

Significant preanalytical causes of variability include inadequate blood volume, failure to invert tubes after collecting blood, overly vigorous shaking of tubes, and delays in the processing of tubes. A systematic review identified blood volume inoculated into IGRA tubes and delay in processing as key sources of variability [58].

Analytical sources include variation in laboratory techniques and imprecision in measurements. One study that retested the same blood samples with QFT-GIT found that the within-subject variability in IFN-γ response on retesting was 0.60 IU/ml for all persons, and 0.24 IU/ml in individuals whose initial TB response was near the QFT cut-off (0.25–0.80 IU/ml) [59]. This "normal" variation resulted in conversion and reversion rates of 9% and 7%, respectively. A systematic review on the reproducibility of IGRAs, found that the estimated range of variability of IFN-γ response in QuantiFERON under identical conditions was ±0.47 IU/ml (coefficient of variation, 13%) overall, and ±0.26 IU/ml (30%) for individuals with an initial IFN-γ response 0.25–0.80 IU/ml, near the QFT cut-point [60]. Due to this variation in QFT result, a change in LTBI diagnosis was not uncommon: 26% of samples converted to positive if the baseline result ranged between 0.25 and 0.34 IU/ml, and 18% of samples reverted to negative if baseline IFN-γ results were between 0.35 and 0.8 IU/ml.

Immunological sources of variation include immune boosting in the setting of recent TST. Boosting of an IGRA result has been reported in the setting of recent TST administration. Dorman et al performed repeat IGRA testing (both QFT-GIT and T-SPOT) 7–21 days after IGRA and TST testing in persons with IGRAs that were negative at baseline [20]. A boosted response (i.e., conversion to a positive IGRA) was observed in 9.1% of participants by QFT-GIT and 11.3% by T-SPOT. This phenomenon was more common if the baseline testing result was TST+/IGRA−, in agreement with prior studies [61]. IGRA boosting due to TST administration appears to start 3 or more days post-TST placement and may wane by 6 months

after the TST. ATS/IDSA/CDC guidelines recommend that when dual testing (i.e., TST and IGRA) is considered that the IGRA be collected either concurrently with or prior to TST placement [13].

4.3 Accuracy

As previously noted, the lack of direct tests to identify *M. tuberculosis* infection has led to most studies of LTBI test accuracy using surrogate outcomes: estimating sensitivity in patients with current or prior TB and specificity in persons at low risk for *M. tuberculosis* infection.

Across many studies, IGRA sensitivity was equal to (QFT 81–86%) or superior to (T-SPOT 90–95%) the sensitivity of the TST (71–82%) when the outcome was microbiologically confirmed or clinical TB [13]. False-negative IGRA results are associated with advanced age, HIV infection, non-HIV-related immunocompromise, low lymphocyte count, and extrapulmonary forms of TB including CNS, pleural, and bone and joint TB [60].

Among persons who have not received vaccination with BCG, the specificity of IGRAs and TST is similar (>95%) [13]. In BCG vaccinated individuals, IGRAs offer improved specificity over the TST: >95 vs. 60% [13, 21]. Despite findings of similar or superior specificity with IGRAs compared to the TST, IGRAs have proved less specific in populations at low risk for infection with *M. tuberculosis*, including US health care workers [20, 62] and US-born individuals [19].

Using data from the National Health and Nutrition Examination (NHANES), a sample representative of the US population, Ghassemieh et al investigated QFT and TST agreement in more than 6000 individuals [19]. Using a TST response of 10 mm of induration and the manufacturer's recommended QFT-GIT cut-point, test agreement in US-born participants was 97.0% although the kappa statistic was only fair at 0.27 (95% CI, 0.18–0.36). Among US-born participants, 0.6% were positive by both tests, 0.8% were TST-positive only, and 2.2% were IGRA-positive only. Among non-US-born participants, test agreement was 81.6% with kappa statistic 0.38 (95% CI, 0.33–0.44). Test results in non-US-born participants were: 9.1% positive by both tests, 11.2% TST-positive only, and 7.2% IGRA-positive only.

Dorman and colleagues performed a longitudinal study of QFT-GIT, T-SPOT.TB, and TST in over 2400 US healthcare workers [20]. All participants were considered at low risk for infection with *M. tuberculosis* and follow-up visits occurred at 6, 12, and 18 months after enrollment. A positive test at study enrollment was present in 1.8% by TST, 3.8% by QFT-GIT, and 5.0% by T-SPOT. Among participants with negative test results at enrollment, conversion to a positive test result (likely a false-positive result) occurred in 0.9% by TST, 6.1% by QFT-GIT, and 8.3% by T-SPOT. Test conversions were six to nine times more frequent with IGRAs than TST. For both IGRAs, the likelihood of a conversion or reversion increased if the baseline quantitative value was closer to the test cut-point. Among participants

who were positive at enrollment, reversions occurred in 57% with QFT and 64% with T-SPOT; reversions were very common for baseline QFT values <0.7 IU/ml and T-SPOT values ≤10 SFCs. The Ghassemieh and Dorman studies point to compromised IGRA specificity in populations at low risk for LTBI.

A study from a moderate TB-burden setting, rural China, where LTBI risk is high, enrolled over 21,000 participants to undergo testing with the TST and QFT [63]. Age- and sex-standardized rates of TST positivity (≥10 mm) ranged from 15 to 42%, and QFT positivity rates ranged from 13 to 20%. TST-only positive results were associated with the presence of a BCG scar. Out of this cohort, 7505 participants with a positive TST and/or QFT who were not treated for LTBI were followed for 2 years [64]. The TB incidence rate was 0.87 per 100 person-years among participants who tested positive with QFT, 0.50 per 100 person-years for those who tested positive with TST, and 0.82 per 100 person-years for those who tested positive with both tests.

Several points are worth emphasizing based on the above studies. These results support the preferential use of IGRAs in BCG vaccinated individuals. However, QFT and T-SPOT specificity seem to be lower than the TST in low-risk populations, related in part to the use of a single cut-point for IGRAs. Second, people at low risk for LTBI should not be tested. For example, using an IGRA with sensitivity of 86% and specificity of 95% to test 1000 US-born individuals (LTBI prevalence of 2.7%) [19] would result in 23 true-positive persons and 49 false positives: 68% of the positive test results would be false positive. Whereas using the same test in foreign-born residents of the USA (15.9% LTBI prevalence) would identify 137 true-positives and 42 false-positive results: 23% of the positive results would be false positive. Third, IGRA values close to the cut-point (0.35 IU/ml for QFT and 6 SFCs for T-SPOT) may be read as positive due to laboratory variability. It is important to examine the magnitude of the test result and the pretest probability of LTBI in interpreting an IGRA result.

5 Comparisons of TST, QFT, and T-SPOT.TB

The majority of studies on LTBI test accuracy have used surrogate outcomes. However, at least one large study from a low TB-burden setting has evaluated the performance of the three commercially available tests in predicting progression to active TB [65]. This is significant as the goal of LTBI screening and treatment is to prevent TB disease. UK PREDICT TB was an English study that enrolled persons at high risk for LTBI (i.e., close contacts to a patient with infectious TB, recent immigrants from high TB-burden countries) to undergo QFT-GIT, T-SPOT, and TST and followed enrollees until study completion (median 2.9 years) [65]. Several thresholds were evaluated for determining a positive TST including 5 mm, 10 mm, and a novel strategy of 5 mm for BCG-naïve and 15 mm for BCG vaccinated participants (TST-15). Among 6380 participants, 83% of whom were born outside the UK, 1.2% developed TB. In these patients, QFT was positive in 61%, T-SPOT positive in 68%, TST (5 mm) positive in 83%, and TST-15 positive in 68%. The positive

predictive values for the tests were: T-SPOT 4.2%, QFT-GIT 3.3%, and TST-15 3.5%. A positive T-SPOT result was a significantly better predictor of progression to TB than the other tests. Negative predictive values were similar across tests and TST thresholds ranging from 99.4 to 99.6%.

5.1 Alternative Cut-Points

Unlike the TST, for which risk-based thresholds are applied in determining a positive result, IGRAs were approved with a single cut-point value (excepting T-SPOT in the USA which includes a borderline category). As discussed in *Sources of Variation* and demonstrated by the Dorman study [20], patients with IGRA results close to the cut-point may experience conversions and/or reversions during serial testing; most of these patients will not have infection with *M. tuberculosis*. Studies have evaluated whether alternative IGRA cut-points could improve test accuracy in diagnosing *M. tuberculosis* infection.

Higher quantitative IGRA results are associated with an increased risk of progression to active TB [66–69]. A systematic review and meta-regression analysis evaluated whether antigen-nil IFN-γ levels correlate with risk of progression to TB [70]. Based on 34 included studies, the investigators found that higher levels of IFN-γ were associated with increased risk of progression to TB in a dose–response relationship. Whether these findings are reflective of a biologic mechanism or increasing specificity with higher IFN-γ levels is not known.

A study from South Africa, a very high-burden setting, found that QFT values >0.7 IU/ml were strongly associated with progression to TB whereas "positive" QFT results below this range (i.e., 0.35–0.7 IU/ml) did not have an increased risk of TB compared to QFT-negative individuals [71]. However, given the small number of participants who progressed to TB, this study may have been underpowered and should not be applied to low-burden settings.

In 2010, Sweden introduced a borderline range for QFT results between 0.2 and 0.99, with a recommendation to repeat the test if initial values fall within this range [72]. Swedish investigators evaluated over 40,000 QFT-GIT test results, from which 9% were within the borderline range. On retesting of these borderline results, 54% were <0.35 IU/ml (i.e., negative), 27% remained borderline positive (0.35–0.99 IU/ml), and 17% had a value >0.99 IU/ml. No patients with an initial borderline result developed TB within 3–24 months. Similar findings have been reported from South Korea [73].

However, other studies from low-burden settings have called into question the use of a borderline category. Gupta and colleagues evaluated data from a UK cohort of 9610 TB contacts and recent immigrants to identify QFT, T-SPOT, and TST cut-points that would improve on test specificity while maintaining adequate sensitivity [67]. Although TB incidence increased with the magnitude of test responses, loss of sensitivity with higher thresholds supported keeping the current QFT and T-SPOT cut-points. A study of Portuguese healthcare workers suggested restricting the use of borderline categories to low TB risk populations only [74].

It remains unclear whether a different threshold for IGRA positivity would be of use in clinical practice to improve specificity without significantly decreasing sensitivity. The authors follow the US guidelines to test for LTBI only when risk factors are present [13]. When patients with a low pretest probability for *M. tuberculosis* infection are tested, we recommend evaluating the magnitude of the test response. For low-risk patients, if the first test result is weakly positive, we perform a second test and offer treatment only if both tests are positive.

6 Test Application in Special Situations and Populations

6.1 Marker of Treatment Response

LTBI treatment has no consistent effect on IGRA values [75–79]. This means that measuring IFN-γ levels pre- and post-treatment is not useful as a measure of treatment response.

6.2 Pediatrics

The 2017, American Thoracic Society/Infectious Diseases Society of America/CDC guidelines on TB diagnosis recommend IGRAs as preferable or equivalent to the TST in children aged 5 years and older [13]. However, for children <5 years of age, the guidelines recommend the TST over IGRAs. This was based on limited direct evidence at the time of publication that suggested that the TST has greater sensitivity but lower specificity than IGRAs in young children. Because of the high risk of progression to active TB in children <5 years, the guidelines prioritized sensitivity over specificity in this population. Differing slightly from these recommendations, the American Academy of Pediatrics preferentially recommends the TST in children <2 years of age [80], based on greater experience and understanding of its performance in this young population compared to IGRAs. In addition, phlebotomy is more difficult in young children and insufficient blood volumes may be an added reason for performing TST in this population.

Using TBESC data, Ahmed and colleagues evaluated the performance of QFT-GIT, T-SPOT, and TST in more than 3500 children <15 years of age [81]. Four children developed active TB. The negative predictive values for TST, QFT-GIT, and T-SPOT were 99.9 (95% CI: 99.7–100), 100 (95% CI: 99.8–100), and 99.9 (95% CI: 99.8–100), respectively. Of 533 children with TST-positive/IGRA-negative results who were not treated for LTBI (including 54 children <2 years old), none developed active TB.

These findings suggest that IGRAs are likely accurate in testing for LTBI in children of all ages. The TST remains an acceptable alternative, although its lower specificity will result in a higher frequency of positive results.

6.3 Pregnancy

A systematic review from 2016 identified three studies of LTBI performance in pregnant women from low-burden settings, with concordance between TST and IGRAs of 77, 88, and 91%. The authors concluded that in low-burden settings, test performance was not impacted by pregnancy [82]. Differing from low-burden settings, this systematic review identified one study from a high-burden setting (India) in which all participants were HIV-negative and found that QFT was positive twice as often as the TST. In addition, they observed that the positivity rate for both QFT and TST increased in the postpartum period compared to antepartum [83]. Among pregnant women with HIV in high TB-burden settings, IGRAs were found to have about twice the positivity rate of the TST, and the positivity rate was higher 3 months postpartum compared to antepartum [84, 85]. Collectively these studies suggest that LTBI testing should be delayed during pregnancy unless there is a strong indication (e.g., recent contact to a person with infectious TB, or newly diagnosed HIV). In addition, IGRAs may be more sensitive tests than the TST in peripartum women.

6.4 Serial Testing

As discussed above, serial testing in low-risk populations (e.g., health care workers) is discouraged by the US CDC [86]. When testing is performed, IGRAs may result in a much higher proportion of conversions than the TST (6–8 times higher [20]). New IGRA conversions should be carefully evaluated for the magnitude of the result (IU/ml or SFCs) and repeat testing should be considered when the conversion value is near the cut-point of the test.

6.5 People with HIV

The sensitivity of IGRAs and the TST are decreased in people with HIV. After reviewing the literature, the US guidelines found that the sensitivity of both IGRAs for detecting LTBI in people living with HIV is between 65 and 100%, compared to the estimated sensitivity of the TST at 43% [13]. Although this data suggests that IGRAs are at least as sensitive as TST in people living with HIV, the guideline committee decided that there was insufficient data to recommend IGRAs over TST.

A TBESC study used Bayesian latent class analysis, a statistical technique that provides an understanding of test characteristics when no gold standard is available, to estimate the accuracy of the three commercially available tests for the diagnosis of LTBI in US-born people living with HIV [87]. The investigators found that T-SPOT had a significantly higher positive predictive value (90.0%) than QFT (50.7%) and TST (45.4%) and similar negative predictive values across the three tests. The estimated sensitivity was higher for QFT (72.2%) than T-SPOT (51.9%) or TST (54.2%) [87].

Differing from these results, a recent systematic review of TB risk after a positive LTBI test included 9 cohorts of people living with HIV from low TB-burden settings [88]. The incidence of TB was 16.9 per 1000 person-years with a positive IGRA result and 27.1 per 1000 person-years with a positive TST result of ≥5 mm. Based on conflicting findings and a small evidence base, it seems appropriate that the US guidelines do not endorse one test over the other in the setting of HIV infection.

6.6 Other Immunocompromise

The performance of LTBI tests likely varies between immunocompromising conditions give differences in etiologies and immune dysfunction. For example, in one study 41% of pre-liver transplant patients had indeterminate QuantiFERON results compared to 12% of non-liver transplant patients [89]. A recent systematic review of LTBI diagnosis in transplant candidates found that IGRAs were more sensitive and specific than the TST with regard to the diagnosis of LTBI in transplant candidates, although all tests had sub-optimal performance: sensitivity was 46%, 58%, and 55% for the TST, QFT, and T-SPOT, respectively [90]. Specificity of TST, QFT, and T-SPOT were 86%, 89%, and 92%, respectively. Likewise, among patients dependent upon hemodialysis, IGRAs had superior accuracy to the TST [91].

There is no consensus among the various transplant societies as to the preferred LTBI diagnostic strategy for transplant candidates [16]. Among the different transplant organizations, recommended strategies include preferential use of an IGRA, the TST, 2-step TSTs (to take advantage of boosting), either TST or IGRA, and both tests (if the first is negative). The transplant program at the authors' institution preferentially uses an IGRA for LTBI testing [92]. In the setting of LTBI risk factors (e.g., birth in a moderate- or high-burden country), a second test may be performed if the first is negative and treatment offered if the second test is positive. As most solid organ transplant recipients who develop TB will have had a negative TST and/

or IGRAs on pretransplant testing [93], when there is a high pretest probability for LTBI or concern over false-negative test results, some experts offer LTBI treatment regardless of TST and IGRA results [92].

6.7 Society Recommendations

The US ATS/IDSA/CDC guidelines, published in 2017, make the following recommendations (Fig. 9.3):

- A strong recommendation to perform an IGRA rather than a TST in individuals ≥5 years of age who are likely to be infected with *M. tuberculosis*, have a low or intermediate risk of disease progression, and either have a history of BCG vaccination or are unlikely to return to have their TST read.
- A conditional recommendation is to perform an IGRA rather than a TST in all other individuals ≥5 years of age who are likely to be infected with *M. tuberculosis* and who have a low or intermediate risk of disease progression.
- Based on a lack of data, no test preference recommendation for individuals ≥5 years of age who are likely to be infected with *M. tuberculosis* and who have a high risk of progression to disease.
- A recommendation to NOT test persons at low risk for *M. tuberculosis* infection and disease progression. If diagnostic testing for LTBI is performed in individuals who are unlikely to be infected with *M. tuberculosis,* an IGRA instead of a TST is recommended for persons ≥5 years. If this test is positive, then a second test is recommended. The confirmatory test may be either an IGRA or a TST and the patient is considered infected only if both tests are positive.
- TST rather than an IGRA is recommended for healthy children <5 years of age.

Guidance published by the European Centre for Disease Prevention and Control (ECDC) in 2018 recommends the preferential use of IGRAs in people with a history of BCG vaccination, migrant populations, and hard-to-reach populations (the latter two groups based on the need for only a single visit) [94]. The TST is recommended for children <5 years and a combination of TST and IGRA in immunocompromised patients to maximize sensitivity. For other tested populations, no general recommendation is made as to which test is preferred but rather it should be based on country-specific circumstances, operational issues, and patient considerations.

Group	Testing Strategy	Considerations
Likely to be Infected **High** Risk of Progression (TST ≥ 5mM)	**Adults** **Acceptable:** IGRA OR TST Consider dual testing where a positive result from either result would be considered **positive** **Children ≤ 5years of age** **Preferred:** TST **Acceptable:** IGRA OR TST Conside dual testing where a positive result from either would be considered **positive**[1]	Prevalence of BCG vaccination Expertise of staff and/or laboratory Test availability Patient perceptions Staff perceptions Programmatic concerns
Likely to be Infected **Low** to **Intermediate** Risk of Progression (TST ≥ 10mM)	**Preferred:** IGRA where available **Acceptable:** IGRA or TST	
Unlikely to be Infected (TST ≥ 15mM)	**Testing for LTBI is not recommended** **If necessary:** **Preferred:** IGRA where available. **Acceptable:** Either IDRA OR TST **For serial testing:** **Acceptable:** Either IGRA OR TST Consider repeat or dual testing where a negative result from either would be considered **negative**[2]	

Fig. 9.3 Summary of recommendations for testing for latent tuberculosis infection (LTBI). (1) Performing a second diagnostic test when the initial test is negative is a strategy to increase sensitivity. This may reduce specificity, but the panel decided that this is an acceptable trade-off in situations in which the consequences of missing LTBI (i.e., not treating individuals who may benefit from therapy) exceed the consequences of inappropriate therapy (i.e., hepatotoxicity). (2) Performing a confirmatory test following an initial positive result is based upon both the evidence that false-positive results are common among individuals who are unlikely to be infected with *Mycobacterium tuberculosis* and the committee's presumption that performing a second test on those patients whose initial test was positive will help identify initial false-positive results. Abbreviations: *IGRA* interferon-γ release assay, *LTBI* latent tuberculosis infection, *TST* tuberculin skin test. Reproduced with permission [13]

7 Future Directions of LTBI Testing

There is an urgent need for new LTBI diagnostics that address the limitations of the current testing platforms. Performance characteristics of critical importance for future LTBI diagnostics include the capability to:

- Discriminate between subclinical disease, LTBI, and past infection.
- Identify those at elevated risk of progression to TB disease.
- Assess response to therapy.

Other needed characteristics include ease of use, affordability, and ability to provide rapid, easily interpretable results at the point of care.

While no new diagnostic platform is currently poised to replace the current TST and IGRA tests for the diagnosis of LTBI, a wide variety of technologies are at different phases of investigation, development, or clinical use. Many aim to improve on current immunodiagnostics while others use entirely new diagnostic strategies.

7.1 Novel Skin-Based Tests

A number of novel skin-based tests for LTBI diagnosis have been designed to elicit a more *M. tuberculosis*-specific immune response than the conventional TST while remaining low cost and low technology. The C-Tb (Serum Institute of India, Pune, India), Diaskintest (Generium, Moscow, Russia), and C-TST (Anhui Zhifei Longcom, Hefei, China) all use the same recombinant ESAT-6 and CFP-10 proteins used in IGRAs [95–97], whereas the DPPD test (Host Directed Therapeutics Bio Corp, Seattle, WA, USA) uses a different recombinant protein specific to *M. tuberculosis* [98].

A recent systematic review and meta-analysis evaluated the evidence for the diagnostic performance of these novel skin-based tests compared to the standard TST or IGRAs [99]. All four tests were found to have similar performance to the TST and IGRAs. While test specificity can be as high as IGRAs [95, 100], overall, variations between the performance of the novel skin-based tests and TST or IGRA were small. All four novel tests were judged to likely have similar value as the conventional tests in identifying people most at risk of developing active TB.

7.2 Novel Cytokine-Based Assays

Strategies to improve the performance of current IGRAs have primarily focused on the use of different immunogenic antigens or the detection of alterative, non-IFN-γ cytokines.

7.2.1 Use of New Antigens

One novel IGRA test that uses a combination of conventional and novel antigens is the LIOFeron TB/LTBI (Lionex GmbH, Braunschweig, Germany), which was introduced in 2019 [101]. The assay is similar to older versions of the QFT assay, where the first antigen tube has ESAT-6 and CFP-10, as well as TB-7.7. However, the test differs in that it has a second antigen tube that contains a recombinant version of *M. tuberculosis* alanine dehydrogenase (Ala-DH). The Ala-DH is not found in BCG (similar to the other antigens) and is known to have a number of epitopes for $CD8^+$ T-cells [102]. While one peer-reviewed study suggested that the LIOFeron test may have a similar performance to QFT [101], more controlled studies are needed.

Many other novel antigens are in the preclinical stages of evaluation [103–106]. To address the need for an IGRA that does not contain ESAT-6, a fundamental component of many experimental TB vaccine candidates which render conventional IGRAs nonspecific following vaccination, Ruwald and colleagues developed an ESAT-6 free IGRA that uses the antigens EspC, EspF, and Rv2348c in combination with CFP-10 [107]. On initial assessment, the ESAT-6-free IGRA had a similar diagnostic performance to QFT. In another study, combining the same novel antigens with a standard QFT assay yielded higher sensitivity, particularly among patients with impaired immune systems, without loss of specificity [108].

7.2.2 Use of Alternative Cytokines

A wide range of cytokine responses beyond IFN-γ has been investigated for their potential to improve the diagnosis of LTBI. A recent systematic review of studies of ESAT-6/CFP-10 cytokine responses for the differentiation of LTBI from active TB identified 100 different cytokines under investigation, with the most frequently studied being IL-2, TNF-α, IP-10, IL-10, and IL-13, in addition to IFN-γ [109]. One of the best studied non-IFN-γ cytokines is IP-10, an IFN-γ-induced protein that is expressed at 100-times higher levels than IFN-γ [110]. Multiple studies have suggested that IP-10 release assays perform as well as IGRAs [110–112]. Moreover, IP-10 release assays have some advantages, including robust performance even when used on dry blood spots, which are convenient for collection, transport, and storage [113, 114].

In recent years, investigations into the use of alternative cytokine detection for diagnosing LTBI have begun to move away from profiling individual cytokine responses and toward identifying complex, multi-cytokine signatures [115]. In one recent study, researchers isolated PBMCs from the blood of 92 subjects including those with active TB, LTBI, and healthy controls, and cytokine production in response to PPD stimulation was measured by a multiplex immunoassay system [116]. Analyzing the results with a machine learning algorithm, the researchers were able to identify a two-cytokine combination that distinguished LTBI from active TB better than any individual cytokine (84% sensitivity, 89% specificity). In another similar study, researchers isolated PBMCs from 65 subjects with various

known TB risk factors including LTBI and assessed cytokine response to a variety of *M. tuberculosis*-associated antigens using a 13-target multiplex immunoassay [117]. Using a machine learning algorithm, the investigators identified multi-cytokine signatures that could predict LTBI diagnosis and relative risk designation with >80% accuracy.

7.3 Serology

Serologic tests based on antibodies against *M. tuberculosis* have the advantages of being simple, inexpensive, and amenable to point-of-care diagnostics. Historically, though, such assays have not been oriented toward LTBI diagnosis and their poor sensitivity and specificity have limited their utility [118]. However, a growing understanding of the association between specific immunogenic antigens and different phases of infection and disease [119], including LTBI [120–122], suggests that antibody-based testing may play a role in future diagnostics for LTBI.

7.4 Transcriptomics

RNA transcriptomics, the study of host gene expression by whole blood RNA sequencing, has shown substantial potential for advancing the diagnosis of LTBI. Numerous studies have suggested that transcriptomic signatures may help identify individuals at elevated risk of active TB and may help differentiate between different phases of infection and disease [123–128]. However, it is an imperfect predictor of risk—as underscored by the results of the large CORTIS trial in 2021, which sought to assess the use of a transcriptomic signature (RISK11) to identify individuals at high risk of TB and prevent disease through targeted use of TB preventative therapy [129]. RISK11 had only very modest predictive performance, and the targeted TB preventative therapy did not reduce progression to active TB over the follow-up period. While transcriptomic signatures may play a role in at least short-term prediction of TB risk [130], it is still unclear how to best interpret positive tests, how much absolute risk they predict, and whether they should be combined with other predictive factors [131].

7.5 Other Novel Diagnostic Strategies

While beyond the scope of this chapter, there are many other potential approaches to improving the diagnosis of LTBI that are under investigation including proteomic and metabolomic profiling, the characterization of $CD4^+$ T-cell activation markers, and the use of combinatorial algorithms that bring together clinical, immunologic, and other factors.

8 Conclusion

The current approach to the diagnosis of LTBI to prevent TB relies on detecting evidence of cell-mediated immunity to *M. tuberculosis* antigens. If testing is positive and there are no clinical signs or symptoms of TB disease, the patient is typically considered to have LTBI. There is no current test capable of directly determining the presence of infection with *M. tuberculosis*.

The two types of LTBI diagnostics widely available are the TST and IGRAs. The advantages of the TST include lower cost, ease of administration, no lab requirement, and adjustable interpretation cut-off per individual LTBI risks. The advantages of IGRAs include requiring a single visit, no cross-reactivity with BCG vaccination or most NTM, and likely modestly improved sensitivity (particularly with immunocompromised individuals). Of note, the TST and IGRA have an only poor-to-fair concordance, like due in part to greater cross-reactivity issues with the TST.

The shortcomings of both the TST and IGRAs are significant. These include the fact that they cannot discriminate asymptomatic infection from past infection, identify individuals at elevated risk of TB disease, or be used to assess response to preventative therapy. For these reasons, a variety of novel technologies are in different phases of investigation, development, or clinical use that may help address these issues. These include novel skin-based tests, enhanced cytokine-release assays, and testing for RNA transcriptomic signatures.

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