

Interferon-gamma release assays in tuberculous uveitis: a comprehensive review

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Abstract

• Tuberculous uveitis (TBU) comprises a broad clinical spectrum of ocular manifestations, making its diagnosis challenging. Ophthalmologists usually require evidence from investigations to confirm or support a clinical diagnosis of TBU. Since direct isolation of the causative organism from ocular specimens has limitations owing to the small volume of the ocular specimens, resultant test positivities are low in yield. Immunodiagnostic tests, including the tuberculin skin test and interferon-gamma release assays (IGRAs), can help support a clinical diagnosis of TBU. Unlike the tuberculin skin test, IGRAs are *in vitro* tests that require a single visit and are not affected by prior Bacillus Calmette-Guerin vaccination. Currently, available IGRAs consist of different techniques and interpretation methods. Moreover, newer generations have been developed to improve the sensitivity and ability to detect active tuberculosis. This narrative review collates salient practice points as a reference for general ophthalmologists, such as evidence for the utilization of IGRAs in patients with suspected TBU, and summarizes basic knowledge and details of clinical applications of these tests in a clinical setting.

• **KEYWORDS:** ocular tuberculosis; tuberculous uveitis; interferon-gamma release assays; review

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INTRODUCTION

Tuberculosis (TB) is an infection caused by *Mycobacterium tuberculosis* (*Mtb*). The global TB report 2020 revealed that a quarter of the world's population was infected with *Mtb*, and the incidence rate for developing the disease was 30 000 people per day^[1]. It was reportedly one of the top ten causes of death, responsible for 1.4 million fatalities worldwide in 2019^[1]. Currently, active TB and latent tuberculosis infection (LTBI) are believed to constitute a comprehensive spectrum rather than being considered as separate stages of the infection. World Health Organization guidelines define LTBI as a state of persistent immune response stimulated by *Mtb* antigens without evidence of clinical manifestations of active TB^[2]. LTBI has several stages that affect individuals who could be asymptomatic; they may experience a controlled infection with nonreplicating but viable organisms. LTBI is therefore considered an important reservoir of TB infection that can subsequently develop into an active disease^[3].

Besides lungs, TB can affect multiple organs throughout the body, including the eyes. Ocular TB is an extrapulmonary infection that may involve any part of the ocular tissue and may occur without a history of pulmonary TB. Tuberculous uveitis (TBU) is one of the most common clinical presentations of ocular TB. The prevalence of TBU varies according to geography. While endemic areas, including India and Saudi Arabia reported a high prevalence of 5.6%-26.2%, non-endemic countries, such as the United States, Europe, and Japan, showed a lower prevalence of 0.2%-7%^[4-5].

Considering the immune-related pathogenesis of TBU, immunological tests can be useful for its diagnosis. The tuberculin skin test (TST) and interferon-gamma release assays (IGRAs) are well-known immunological tests. Poor specificity of the TST for diagnosing TB in those who had been vaccinated with Bacillus Calmette-Guerin (BCG) and in residents of endemic regions, led to the subsequent development of IGRAs that enabled immunodiagnosis of LTBI^[6]. These assays performed along with or without the TST, are now increasingly used to diagnose TBU.

In this narrative review, we provide a brief description of the clinical characteristics of TBU. We further comparatively

review the principles of IGRAs, including diagnostic accuracy, advantages and disadvantages, interpretation, and clinical application in adults with suspected TBU.

Clinical Characteristics of Tuberculous Uveitis TBU has a wide spectrum of clinical presentations that may vary from anterior uveitis to intermediate uveitis, posterior uveitis, and panuveitis. It may present as unilateral or bilateral, granulomatous or non-granulomatous inflammation involving the eye. The clinical findings of TBU include iris nodules, ciliary body tuberculoma, organizing exudates in the pars plana/peripheral uvea, choroidal tubercle/tuberculoma, subretinal abscess, serpiginoid choroiditis, retinitis, retinal vasculitis, neuroretinitis, optic neuropathy, endophthalmitis, and panophthalmitis^[7].

Gupta *et al*^[8] suggested that the presence of cellular infiltrate either in the anterior chamber or in the vitreous along with broad posterior synechiae, retinal perivasculitis with or without discrete choroiditis/scars, multifocal serpiginoid choroiditis, choroidal granuloma (single or multifocal), optic disc granuloma, and optic neuropathy were ophthalmic signs consistent with a diagnosis of ocular TB. Affected patients in endemic areas often present with broad-based posterior synechiae, retinal vasculitis, choroiditis, and serpiginoid choroiditis findings that are highly specific to TBU^[9]. Studies from nonendemic areas demonstrated occlusive retinal vasculitis and serpiginoid choroiditis as more common clinical presentations of TBU^[10]. Figure 1 illustrates clinical characteristics of TBU.

Current Immunodiagnostic Tests for Tuberculous Uveitis Generally, *Mtb* may not be isolated from the ocular tissue in all TBU patients. Ocular TB may be classified as such, based on an affected patient's clinical findings meeting diagnostic criteria or a clinical definition^[4,8]. Figueira *et al*^[11] proposed that investigations for TB should be performed in any of the following situations: 1) Uveitis of unknown etiology, either recurrent or unresponsive to conventional therapy; 2) Ocular findings highly suggestive of ocular TB; 3) Before initiating immunosuppressive therapy, particularly that with biologic agents.

It should be noted that most patients with ocular TB have no history of pulmonary or other systemic TB infections. An absence of pulmonary TB; therefore, does not exclude the possibility of ocular TB. Up to 60% of patients with extrapulmonary TB do not have evidence of pulmonary disease, and chest X-rays are normal in those with LTBI^[4,12].

A definitive diagnosis of TBU requires isolation of *Mtb* from intraocular tissue specimens obtained through invasive procedures such as aqueous paracentesis, vitreous aspiration, or retinal biopsy^[11]. *Mtb* culture remains the gold standard for a definitive diagnosis of ocular TB. Other methods

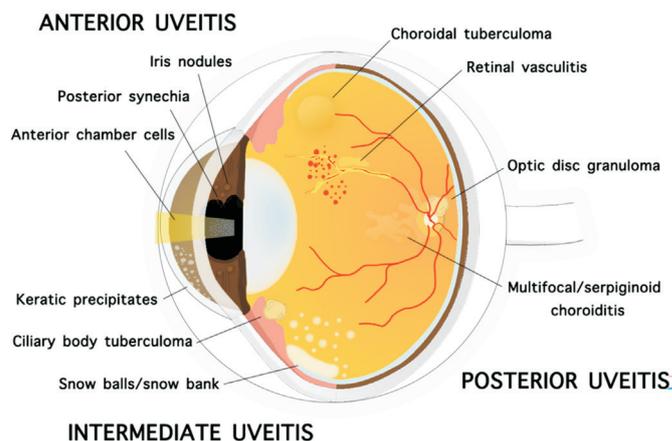


Figure 1 Clinical findings of tuberculous uveitis include keratic precipitates, anterior chamber cells, posterior synechia, iris nodules, ciliary body tuberculoma, snowballs/snow bank, retinal vasculitis, multifocal/serpiginoid choroiditis, choroidal tuberculoma (single or multifocal), optic disc granuloma.

include detection of acid-fast bacilli on smear examination, amplification of *Mtb* nucleic acids, and histopathological examination of ocular tissues^[6,13]. However, there are limitations in identifying the organism in the eye. Usually, only a limited amount of ocular tissue sample can be extracted, and associated procedural complications may potentially damage vision. The Collaborative Ocular Tuberculosis Study showed that patients with presumed TBU demonstrated low positive yield of *Mtb* from intraocular fluid samples on polymerase chain reaction^[14]. Given the limitations of ocular sampling and associated low positivity yield, TBU was rarely diagnosed using this method. This may contribute to delays in diagnosis and treatment, resulting in poor visual outcomes. Consequently, a finding of uveitis with intraocular features characteristic of TBU in conjunction with positive results of indirect tests, is considered sufficient evidence for a diagnosis. Two indirect investigations, including the TST and IGRAs, evaluate the intensity of the host immunological reaction to TB antigens, which may manifest as a T lymphocyte-mediated immune response or as a delayed hypersensitivity reaction. The TST is an *in vivo* test performed using a purified protein derivative consisting of >200 protein precipitates derived from a heat-inactivated *Mtb*. The diagnostic feature of a positive TST is the development of skin induration, interpreted within 48-72h after an intradermal injection of the purified protein derivative. The American Thoracic Society and Centers for Disease Control and Prevention have provided guidelines for the interpretation of positive TST findings, as shown in Table 1^[15]. Notably, a technique that helped identify different mycobacterial antigens, including early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10), was developed into a test for blood-based *in vitro* evaluation of the immune response to TB. In the absence of cross-reactivity due to prior BCG

Table 1 Positive tuberculin skin testing interpretation according to risk groups

Risk groups	Induration
HIV infection	≥5 mm
History of recent contact with an active TB patient	
Fibrotic changes in chest X-ray suggestive of TB	
Transplanted and immunocompromised patients (including patients under treatment at least 1mo with ≥15 mg/d of prednisone or equivalent) ^a	
Immigrants coming from high-endemic areas (migration in the last 5y)	≥10 mm
Intravenous drugs users	
Residents and employees of prisons, hospitals and so on ^b	
Microbiology laboratories staff	
Individuals with pathological risk conditions (Silicosis, diabetes, chronic renal failure, hematological disorders and neoplasms, malnutrition, gastrectomy or jejunioileal bypass)	
Children under 4 years old and/or young adults in contact with adults at risk	
Individuals without TB risk factors	≥15 mm

HIV: Human immunodeficiency virus; TB: Tuberculosis. ^aThe risk of tuberculosis in patients treated with corticosteroids increases with higher doses and longer durations; ^bFor persons who are otherwise at low risk and are tested at the start of employment, a reaction of ≥15 mm induration is considered positive. Adapted from the Center for Disease Control and Prevention. Screening for tuberculosis and tuberculosis infection in high-risk populations recommended by the Advisory Council for the Elimination of Tuberculosis.

vaccination, measurement of interferon-gamma (IFN- γ) produced by T lymphocytes in response to these antigens, forms the basis of IGRAs performed for the diagnosis of TB^[16].

Principles of Interferon-Gamma Release Assays and Interpretation IGRAs are *in vitro* immunodiagnostic tests that use antigens to stimulate specific T lymphocyte responses to *Mtb*. Once specific T-cells are stimulated, they activate an immune response by releasing cytokines, including IFN- γ . IFN- γ is a crucial cytokine of the CD4 T helper 1 (Th1) subset and is responsible for the delayed hypersensitivity immune response. *In vitro* T-cell responses can be evaluated by estimating either the number of IFN- γ producing T-cells or by measuring IFN- γ production using an enzyme-linked immune absorbent spot assay (ELISpot) and an enzyme-linked immunosorbent assay (ELISA), respectively^[16]. The United States Food and Drug Administration has approved commercial IGRAs as indirect and adjunct tests for identifying TB, to be performed in conjunction with risk assessment, radiography and other medical and diagnostic evaluations.

Enzyme-linked immune absorbent spot assay technique The ELISpot technique was used for the T-SPOT. TB test (Oxford Immunotec, Abingdon, United Kingdom) facilitates the enumeration of peripheral blood mononuclear cells that produce IFN- γ after stimulation with *Mtb*-specific antigens^[17]. Peripheral blood mononuclear cells are added into 4 separated 2-microtiter wells coated with monoclonal antibodies to IFN- γ . The first two wells are each filled with either of the two different antigens, ESAT-6 and CFP-10, which are peptide antigens located in the region of difference-1 (RD-1) of the *Mtb* genome but are not found in the BCG vaccine strains and most strains of non-tuberculous mycobacteria, except

for *M. kansasii*, *M. marinum*, *M. szulgai*, and *M. flavescens*. Likewise, there is a report of cross-reactivity between antigens of *M. tuberculosis* and *M. leprae*^[18].

Both ESAT-6 and CFP-10 have multiple epitopes for T lymphocytes and can, therefore, generate an intense reaction. The two remaining wells act as controls for internal test validation. The positive control well contains phytohemagglutinin (mitogen) for stimulating nonspecific T-cell response, whereas no antigen is added in the negative control (nil) well. The production of IFN- γ is then detected *via* a sandwich capture technique by conjugation with secondary antibodies revealing a “spot”. These spots, considered “footprints” of effector T-cells producing IFN- γ , are then enumerated^[3,19].

An interpretation of T-SPOT. TB test is based on the enumeration of spot-forming units. A test result is considered positive if a spot count induced by either antigen ≥6 spots after subtracting the spots from the negative control well. A negative result is indicated if ≤5 spots are induced by both antigens after subtracting the spots from the negative control well. An “indeterminate” result refers to >10 spots in the negative control well, or when the positive control well shows <20 spots unless either of the antigen well shows a positive result. This suggests that another sample should be collected and repeatedly tested. If the spot counts from the antigen wells minus the negative control well are equal to or near the cut-off value (5, 6, or 7 spots), these results may be considered as “borderline” or “equivocal”, leading to less reliable results and warranting a repeat test with another sample. Therefore, in the United States, different cut-off values for positive and negative results are indicated by ≥8 spot counts and ≤4 spot counts, respectively^[3,20].

Table 2 Interpretation of results of interferon-gamma release assays

Negative control	Positive control minus negative control	First antigen tube minus negative control	Second antigen tube minus negative control	IU/mL
≤8.00	Not relevant	≥0.35 and ≥25% of negative control value	Not relevant	
≤8.00	Not relevant	Not relevant	≥0.35 and ≥25% of negative control value	
≤8.00	≥0.50	<0.35	<0.35	
≤8.00	≥0.50	≥0.35 and <25% of negative control value	≥0.35 and <25% of negative control value	
≤8.00	≥0.50	<0.35	≥0.35 and <25% of negative control value	
≤8.00	≥0.50	≥0.35 and <25% of negative control value	<0.35	
≤8.00	<0.50	<0.35	<0.35	
≤8.00	<0.50	≥0.35 and <25% of negative control value	≥0.35 and <25% of negative control value	
≤8.00	<0.50	<0.35	≥0.35 and <25% of negative control value	
>8.00	Not relevant	Not relevant	Not relevant	

Adapted from Della Bella *et al*^[22].

Enzyme-linked immunosorbent assay technique The ELISA technique has been commercially developed through many generations, which include QuantiFERON (QFT), QuantiFERON-TB Gold (QFT-G), QuantiFERON-TB Gold in-tube (QFT-GIT), and QuantiFERON-TB Gold Plus (QFT-Plus). Initially, purified protein derivative was used as the antigen in the QFT test. Then, TB-specific antigens, ESAT-6 and CFP-10, were representatively used in subsequent versions named QFT-G. The third-generation test, QFT-GIT, includes three tubes, the TB antigen-coated tube, the positive control (mitogen) tube, and the negative (nil) control tube. TB antigen-coated tube contains ESAT-6, CFP-10, and an additional peptide antigen TB7.7^[3,19].

CD4 T lymphocytes were believed to play a significant role in the immune response to TB until recent studies provided evidence to support the additive role played by CD8 T lymphocytes^[21]. RD-1-specific CD8 T lymphocytes are also more frequently detected in recent or active TB infections than in LTBI. These findings led to the development of the fourth generation of IGRAs, QFT-Plus. This test package contains two tubes with TB-specific antigens, TB1 and TB2. The TB1 tube containing long peptides derived from ESAT-6 and CFP-10, was designed to induce a specific CD4 T-cell response. The TB2 tube also contains the same long peptides as TB1 along with shorter peptides that can stimulate the CD8 T-cell response. The ability to elicit both CD4 and CD8 T-cell responses is believed to improve the sensitivity of this generation of IGRAs^[19].

With application of ELISA, IFN- γ levels from plasma are reported as international units per milliliter (IU/mL). Similar to the technique used in the T-SPOT.TB test, a quantitative result is calculated by subtracting the estimated IFN- γ level in the negative (nil) control tube from that in the antigen-specific tubes. The interpretation of the results is summarized in Table 2. An estimated value of ≥ 0.35 IU/mL and that $\geq 25\%$ of the negative control value found in either one or both TB

antigen-coated tubes is considered positive. While values falling outside these prescribed limits are considered negative, a mitogen control level < 0.5 IU/mL or a negative (nil) control value > 8.0 IU/mL, is interpreted as an indeterminate report^[22]. Affected patients may show poor response to the mitogen (positive control tube) due to two possible reasons. First, the test may not have been performed correctly, due to errors in specimen collection, delayed specimen processing, incubator malfunction, or technical issues. Second, anergy can lead to a persistently diminished response to mitogens. The reproducibility and reportability of an indeterminate result may provide clinically useful information^[15]. Figure 2 illustrates the common commercial IGRAs, including T-SPOT.TB test, QFT-GIT, and QFT-Plus.

Currently, other products of IGRAs launched in the market include STANDARD E TB-Feron ELISA/STANDARD and F TB-Feron FIA (IFN-gamma; both SD Biosensor), LIOFERON TB/LTBI (LIONEX Diagnostics & Therapeutics GmbH), and Advansure TB IGRA and Advansure i3 TB-IGRA (both LG Chem). Additionally, products of IGRAs that are continuously developing include T-Track(R) TB (Lophius Biosciences GmbH, Germany), VIDAS TB-IGRA (bioMérieux, France), Access QuantiFERON[®]-TB (QIAGEN, USA), ichroma[™] IGRA-TB (Boditech Med Inc., Republic of Korea) and IP-10 IGRA elisa/lateral flow (rBioPharm, Germany)^[11].

Another adding antigen other than ESAT-6, CFP-10, and TB 7.7 used for LIOFeron TB/LTBI is alanine dehydrogenase, which can induce CD8 T-cell response. This antigen is not found in BCG and is reportedly involved in the adaptation of *Mtb* to an anaerobic dormant stage observed in LTBI. Like the QFT-Plus, LIOFeron TB/LTBI test includes four tubes (positive control, negative control, and two antigen-coated tubes). The first antigen tube, TB-A, contains long peptides of ESAT-6, CFP-10, and TB7.7. However, the second antigen tube called TB-B is coated only with highly purified recombinant alanine dehydrogenase, a different antigen from that used in QFT-Plus.

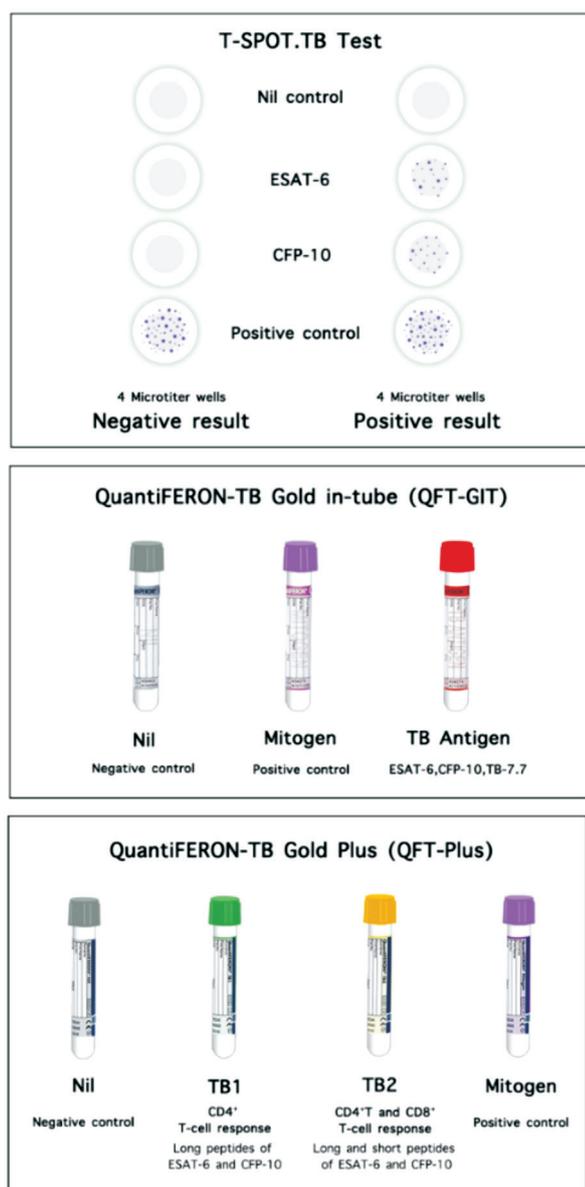


Figure 2 Current interferon-gamma release assays approved by the Food and Drug Administration for commercial use.

No other antigens or peptides are included in the TB-B tube^[22]. Besides IFN- γ , several markers such as interleukin (IL)-2, IFN- γ -inducible protein of 10 kDa (IP-10), IL-5, and IL-10 have been investigated to improve diagnostic performance and discrimination of TB status. These biomarkers are beyond our scope; thus, they are not included in this review^[23-25].

Sensitivity, Specificity, and Accuracy of Interferon-Gamma Release Assays The quality of evidence supporting the use of both IGRAs and the TST for diagnosing active TB infection is low^[17]. IGRAs are insufficiently accurate diagnostic tests for active TB and show limited specificity in distinguishing an active infection from an immune response to LTBI^[6]. Considering the lack of a gold-standard diagnostic test for LTBI, sensitivity and specificity of IGRAs are typically estimated using representative reference standards. Sensitivity was assessed in patients with microbial culture-confirmed

Table 3 Sensitivity and specificity of interferon-gamma release assays collated from systematic review and Meta-analysis

Tests	Pooled sensitivity (95%CI)	Pooled specificity (95%CI)
Sester <i>et al</i> ^[6] , 2011		
TST	0.65 (0.61-0.68)	0.75 (0.72-0.78)
T-SPOT.TB	0.81 (0.78-0.84)	0.59 (0.56-0.62)
QFT-GIT	0.80 (0.75-0.84)	0.79 (0.75-0.82)
Metcalf <i>et al</i> ^[26] , 2011		
T-SPOT.TB	0.88 (0.81-0.95)	0.61 (0.40-0.79)
T-SPOT.TB ^a	0.76 (0.45-0.92)	0.52 (0.40-0.63)
QFT-GIT	0.84 (0.78-0.91)	0.52 (0.41-0.62)
QFT-GIT ^a	0.60 (0.34-0.82)	0.50 (0.35-0.65)
Sotgiu <i>et al</i> ^[19] , 2019		
QFT-Plus	0.94 (0.90-0.98)	0.95 (0.93-0.97)
QFT-Plus ^b	0.91 (0.84-0.96)	
TB1 tube	0.91 (0.79-0.98)	
TB2 tube	0.95 (0.88-0.95)	

TB: Tuberculosis; TST: Tuberculin skin test; QFT-GIT: QuantiFERON-TB Gold in-tube; QFT-Plus: QuantiFERON-TB Gold Plus. ^aAmong HIV-infected individuals; ^bAmong latent tuberculosis infections.

TB, while specificity was evaluated in low-risk individuals from low-incidence areas without any known history of exposure to the disease^[18]. Some studies compared sensitivity and specificity of IGRAs with that of the TST or between generations of the immunoassay itself. Table 3 summarizes the pooled sensitivity and specificity of IGRAs as reported in systematic reviews and Meta-analyses^[6,19,26]. A systematic review and Meta-analysis reported a high agreement and no significant difference in sensitivity and specificity between QFT-GIT and QFT-Plus^[27]. IGRA performance also varies between regions with high and those with low TB incidence, with lower sensitivity observed in the former endemic areas^[17]. The new generation of IGRAs is under development to improve test performance. Della Bella *et al*^[22] compared test parameters of LIOFeron TB/LTBI test with QFT-Plus in application for diagnosis of active TB and LTBI. While the accuracy of both tests was comparable, LIOFeron TB/LTBI assay was more sensitive than the QFT-Plus for detecting LTBI. However, the former test was unable to distinguish active TB from LTBI.

IGRAs performed in patients with human immunodeficiency virus (HIV) infection demonstrated lower sensitivity than those performed in immunocompetent individuals. A Meta-analysis showed lower sensitivity for T.SPOT.TB and QFT-GIT, particularly in affected patients with low CD4 counts of <100 cells/mL^[26,28]. In low- and middle-income countries, IGRAs may have a role in identifying TB infection in HIV-infected patients due to the decreased utilization of TST in

Table 4 Comparison between tuberculin skin testing and interferon-gamma release assays.

Test	Advantages	Disadvantages
TST	Low cost for reagents	Requires two visits
	Easy to perform in field setting	Subjective results with inter-reader and intrareader variability in measurement
	Ability to test a large number of people quickly	Low specificity in BCG-vaccinated people
	Minimal test-retest variability in low-risk populations	
IGRAs	Single-visit	Higher cost for reagents
	Positive and negative controls built into the test	Requires transporting samples to a laboratory
	Objective results	More complicated settings (laboratory registration, drawing blood, labelling, and transporting tubes to a laboratory)
	Electronic laboratory reporting	
Both tests	More specific in BCG-vaccinated people	
	Limited sensitivity for active disease and inability to distinguish active TB from LTBI	
	Low ability to predict short-term progression to active TB	
	Reduced sensitivity in immunosuppressed populations	
	Inability to differentiate a resolved infection from a new or ongoing infection	

BCG: Bacillus Calmette-Guerin; IGRAs: Interferon-gamma release assays; LTBI: Latent tuberculosis infection; TB: Tuberculosis; TST: Tuberculin skin test. Adapted from Haas and Belknap^[3].

immunosuppressed individuals^[17]. However, a study performed in a population with a high prevalence of HIV reported three times the number of ocular TB patients with a positive QFT tested positive on a TST. Therefore, the investigators concluded that QFT should not replace TST in a limited-resource setting^[29].

Two studies reported the application of IGRAs in the diagnosis of TBU. A study from Korea evaluated the usefulness of QFT-G in diagnosing presumed TBU in 181 patients. The sensitivity and specificity were reported as 100% and 72%, respectively, with a high positive predictive value in younger patients (≤ 40 y) presenting with posterior uveitis and retinal vasculitis^[30]. Another prospective head-to-head study by Ang *et al*^[31] reported that the QFT-GIT test was statistically more accurate in diagnosing true-positive TBU patients than the T-SPOT.TB test (98% vs 76%, respectively). Due to the greater positive predictive value of QFT-GIT compared to that of the T-SPOT.TB test, they suggested application of the former as a first-line diagnostic test rather than the latter or a TST.

Advantages and Disadvantages of Interferon-Gamma Release Assays Features of IGRAs and the TST are compared in Table 4. The most important advantage of the former test over the latter is the elimination of false-positive results associated with prior BCG vaccination or with previous exposure to environmental mycobacteria. The time-frame of a patient's exposure to the BCG vaccine may impact the TST result. If the patient had only been inoculated at birth or during the infantile period without having received subsequent booster shots, the impact on TST specificity was minimal. False-positive TST interpretation usually decreases over time, although, it can persist in some individuals and reduce diagnostic accuracy of the test for LTBI^[3].

TST also has limitations in its sensitivity. Approximately 75%-90% of patients with active TB show a positive TST. TST performed in patients with immunosuppressive conditions, including HIV infection and disseminated TB, is associated with a high rate of false-negative results (>50%) and, therefore, requires careful interpretation. In such patients, especially in the immunocompromised and in the pediatric age-group, IGRAs demonstrate higher sensitivity than the TST. In addition, IGRAs become more valuable because of the higher pretest probability for identification of TBU, particularly in uveitis patients from endemic areas^[16].

Furthermore, the technique of IGRAs is more convenient for patients than that of the TST, as it requires only a single visit for blood sampling. Unlike TST, IGRAs are performed *in vitro* and can be repeated without risk of sensitization or a boosting effect. These characteristics make IGRAs practical tests for application in TB screening programs, particularly those conducted in an occupational setting^[18].

A systematic review reported high variation in reversion rates of IGRAs in either active or latent TB among studies, which makes these tests unreliable for monitoring response to anti-tuberculous therapy (ATT)^[32]. However, an advantage of QFT-Plus in monitoring the response to ATT was recently reported^[33-34]. A decrease in IFN- γ response following QFT-Plus-TB2 stimulation was observed after subjects with active TB had received ATT. However, the study was conducted in a region with low endemicity. Further studies are indicated to confirm the utility of QFT-Plus in monitoring treatment response. Similar to the TST, IGRAs perform an indirect assessment of immune response to *Mtb*. False-negative and indeterminate IGRA results have been reported in infants and young children <5 years of age, advance age, early infection phase (<6-8wk),

low peripheral lymphocyte counts, in individuals with a recent history of viral vaccination, in immunosuppressed patients (e.g., HIV infection with ≤ 200 cells/ μ L CD4 T-cell count, disseminated TB), in those with recent viral and bacterial infections, and in those on immunosuppressant therapy (e.g., high-dose corticosteroids, TNF inhibitors)^[15,19,35-38]. In addition, IGRAs demonstrate cross-reactivity and may give positive results due to presence of some mycobacterial antigens within the RD-1 locus, as mentioned before^[19].

Nevertheless, interpretation of IGRAs is still complicated by the immunologic recall of preexisting hypersensitivity to TB (e.g., a booster BCG vaccine shot), conversions due to new infection, reversions from positive to negative results, lack of consensus on a cut-off value, and inconsistent test reproducibility^[18]. Eventually, higher costs and the technically advanced lab facilities required to perform IGRAs become obstacles to their application in some settings. Therefore, the TST still remains a comparably cost-effective and technically easier alternative diagnostic test for TB^[3,16].

Role of Interferon-Gamma Release Assays in Tuberculous Uveitis Some experts suggest performing TST or IGRAs for all patients with idiopathic uveitis, whereas others recommend testing patients with either suspected signs of TBU or with additional risks for TB, including birth outside the US, history of living in an endemic area, history of incarceration or homelessness, history of intravenous drug abuse, HIV positive status, failure to respond to oral corticosteroid or immunosuppressive therapy, or presence of granulomatous inflammation on presentation^[16].

Several studies have provided evidence for the use of IGRAs in TBU. Most studies that were performed using the previous generation of IGRAs showed supportive evidence for TBU diagnosis. Groen-Hakan *et al*^[39] described the QFT-G test as a helpful diagnostic tool for uveitis patients in non-endemic countries. They suggested that a positive IGRA result in uveitis of unexplained cause may indicate a possible benefit of initiating ATT, particularly in affected patients with severe and sight-threatening inflammation. Pathanapitoon *et al*^[40] found that most patients with positive QFT-G tests experienced uveitis of unknown etiology and occlusive retinal vasculitis. A more significant percentage of positive IGRA results was reported in patients with posterior uveitis^[29,41]. Using QFT-G, Gineys *et al*^[42] considered that the standard laboratory cut-off value of ≥ 0.35 IU/mL may lead to over-treatment. They proposed that a cut-off value of 2.00 IU/mL was recommended for the diagnosis of TBU.

Ang *et al*^[43-44] demonstrated the usefulness of QFT-GIT. They found that while the sensitivity of QFT-GIT was not superior to that of the TST, the former was slightly more specific than the latter. Similarly, the T-SPOT.TB test was found to be more

specific but less sensitive than the TST. They recommended using IGRAs together with the TST during screening for TBU, as the overall diagnostic accuracy increased when both tests were performed together. Additionally, utilizing both TST and IGRAs for patients at presentation was found to be cost-effective for the diagnosis of TBU. Nevertheless, negative IGRA and TST results in patients with clinical signs suggestive of TBU should be interpreted cautiously, as these do not exclude a diagnosis of the disease^[41].

The utility of IGRAs was also proposed for the management of TBU in the Collaborative Ocular Tuberculosis Study consensus guidelines. Initiation of ATT is considered based on several supportive factors, including endemic/non-endemic geography, clinical findings suggestive of TBU, immunologic test (IGRAs or TST) results, and radiographic evidence of healed or active pulmonary TB^[45-46].

To our knowledge, several studies have been conducted on the usefulness of monitoring the response to ATT in active or latent TB; however, the exact role of IGRAs in monitoring treatment response in TBU remains undefined. Additionally, no study has reported the accuracy and clinical application of QFT-Plus in TBU^[47]. The evidence of using new generations of IGRAs in TBU is still lacking.

CONCLUSION

TBU comprises a broad spectrum of clinical presentations, and its diagnosis remains challenging. IGRAs are considered useful immunodiagnostic tests that may supplement TST findings to enable diagnosis of TBU in affected patients. Understanding the principles and careful interpretation of IGRAs is helpful for ophthalmologists in clinical settings. However, studies on the new versions of IGRAs in TBU are still lacking, and further studies are needed to determine the role of IGRAs in monitoring the response to ATT in TBU.

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