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Role of interferon gamma release assay in the diagnosis and management of mycobacterium tuberculosis-associated uveitis: A review

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Role of interferon gamma release assay in the diagnosis and management of *Mycobacterium tuberculosis*-associated uveitis: a review

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ABSTRACT

Tuberculosis (TB)-associated uveitis is a common cause of infectious uveitis in the developing world. Diagnosis of TB uveitis remains a challenge. The role of interferon gamma release assays (IGRAs) is uncertain. Herein we summarise the available literature on the utility of IGRAs in the diagnosis and management of TB uveitis. We searched PubMed database from 1 August 2010 to 31 July 2020 using the following keywords alone and in combination: 'interferon-gamma release assay', 'QuantiFERON', 'T-SPOT.TB', 'TB uveitis', 'serpiginous like choroiditis', 'tuberculoma', 'TB vasculitis', 'TB panuveitis' and 'ocular tuberculosis'. Data from 58 relevant studies were collated. The review is focused on currently marketed versions of IGRA tests: QuantiFERON-TB Gold In-Tube assay, QuantiFERON-TB Gold Plus assay (QFT-Plus) and T-SPOT.TB. We found limited evidence regarding the diagnostic utility of IGRA in patients with uveitis. No study was identified evaluating the newer QFT test—the QFT-Plus—in patients with uveitis. Similarly, there is lack of data directly comparing QFT-Plus with T-SPOT.TB specifically for the diagnosis of TB uveitis.

INTRODUCTION

Mycobacterium tuberculosis continues to be a common pathogen infecting an estimated 10 million people in 2018 alone.[1] Ocular tuberculosis (OTB) is an extrapulmonary form of tuberculosis (TB) with a multitude of presentations. The reported incidence varies considerably, but majority of patients present with choroidal involvement.[2] OTB rarely occurs as a primary infection, mostly occurring secondary to haematogenous spread.[3]

Diagnosing OTB is a challenge because a definitive diagnosis of OTB can only be achieved by testing either tissue samples or ocular fluids for *M. tuberculosis*. Obtaining ocular samples is not without risks. Because of the paucibacillary nature of the organism, even the samples collected do not always yield results, even with PCR testing.[4] This problem is compounded by the fact that OTB can present with symptoms similar to other ocular diseases. OTB may be categorised into TB scleritis and TB uveitis (TBU). TBU is further divided into tubercular anterior uveitis, tubercular intermediate uveitis, tubercular posterior uveitis, tubercular panuveitis and tubercular retinal vasculitis. Interestingly, OTB often does not present with other systemic manifestations of TB.[5]

In the absence of a diagnostic gold standard test, in 2015 Gupta *et al*[6] proposed a classification for intraocular TB. It involves labelling cases as confirmed, probable or possible TBU. Microbiological verification from ocular fluid/tissues is required to label a case as *a confirmed TBU*. For a case to be marked as *a probable TBU*, immunological evidence of TB infection or documented exposure to TB is required, along with at least one clinical sign suggestive of OTB and microbiological, radiological or clinical evidence of extraocular TB infection. A more lax criterion is acceptable when diagnosing a patient as *a possible TBU*. Interferon gamma release assay (IGRA) is used in two out of three groups in the classification of TBU.

Historically tuberculin skin test (TST) has been used to provide immunological evidence of infection. However, it has low specificity due to the false-positive results in BCG-vaccinated patients and in patients infected with other non-tuberculous mycobacteria. This has led to its usefulness being called into question.[7] TST also has low sensitivity in immunocompromised patients and is therefore not recommended in these groups based on the current guidelines.[8] IGRA was introduced in 2001. IGRA is an in vitro blood test that measures the interferon gamma (IFN-γ) released by T cells following stimulation by antigens specific to *M. tuberculosis*. These antigens include early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These antigens are encoded by genes in the region of difference 1 (RD1) locus of the *M. tuberculosis* genome.[9] Two types of

commercial IGRA tests are currently available: QuantiFERON-TB assay (Cellestis/Qiagen, Carnegie, Australia) and the T-SPOT.TB assay (Oxford Immunotec, Oxford, UK). Both tests are approved by the US Food and Drug Administration (FDA).

The aim of this review is to summarise the literature about the utility of IGRA test in the diagnosis and management of TBU. Factors that may induce variability in test results and make them less reproducible, such as prevalence of disease, immune status and antituberculous treatment (ATT), and factors not directly related to patients are briefly discussed as well.

Literature search

An electronic literature search was conducted from 1 August 2010 to 31 July 2020 of articles in the PubMed database using the following keywords alone and in combination: 'interferon-gamma release assay', 'QuantiFERON', 'T-SPOT.TB', 'TB uveitis', 'serpiginous like choroiditis', 'tuberculoma', 'TB vasculitis', 'TB panuveitis' and 'ocular tuberculosis'. Manuscripts published in the past 10 years in English were included. References of included articles were also searched for relevant studies.

We included studies that met the following criteria: investigated the use of currently marketed versions of both tests (QuantiFERON-TB Gold In-Tube assay (QFT-GIT), QuantiFERON-TB Gold Plus assay (QFT-Plus), T-SPOT.TB or the premarket ELISpot version of T-SPOT.TB). Studies were excluded if authors had failed to specify which version of the QFT was being used and if the assays were being used on a bodily fluid other than blood. For data extraction, one reviewer abstracted relevant data from the eligible studies, which was double-checked by a second reviewer. Details have been provided in figure 1.

Types of tests

QuantiFERON-TB assay (QFT)

The first version of the QuantiFERON-TB test (QIFN) was approved by the FDA as a diagnostic tool for latent TB infection in 2001.[10] It is an ELISA-based whole blood test that uses peptides from the *M. tuberculosis*-specific antigens. The result is reported as quantification of IFN-γ in international units (IU) per millilitre. The patient is considered positive for latent TB infection if the reported value is above the cut-off specified by the manufacturer. The QFT assay has four versions: QIFN, QFT-Gold,

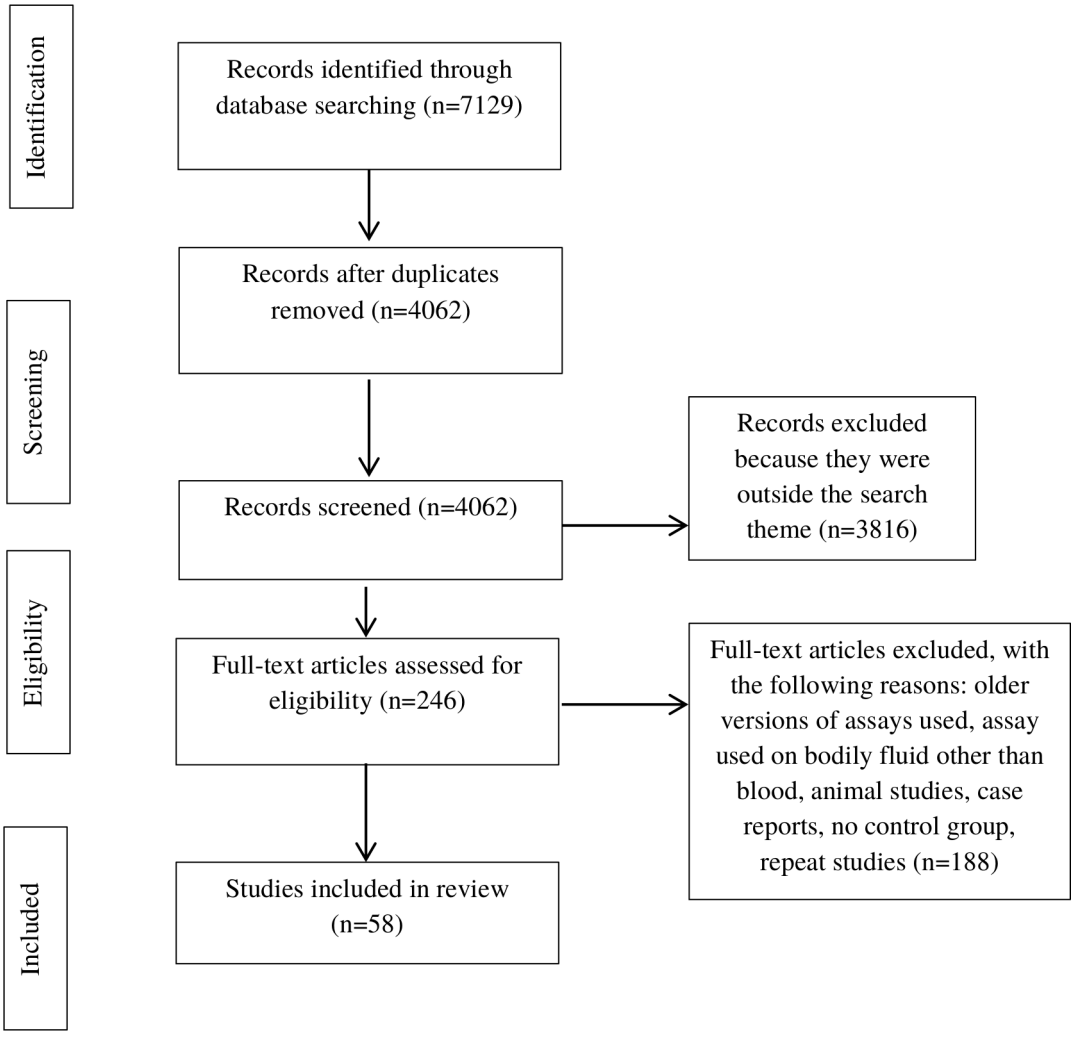

Figure 1 Flowchart of study selection process.

Table 1 Mechanisms of actions of IGRAs

Test	Mechanism of action
QFT	ELISA-based whole blood assay. Blood is collected in heparinised tubes containing antigens, phytohaemagglutinin as a positive control and saline as a negative control. IFN-γ is quantified from supernatant plasma using ELISA. The antigens used for each version are different (see below).
QIFN	Antigens used: human, avian and bovine type PPDs.
QFT-G	Antigens used: ESAT-6 and CFP-10.
QFT-GIT	Antigens used: ESAT-6, CFP-10 and TB7.7.
QFT-Plus	Two antigen tubes used: TB1 and TB2. TB1 contains ESAT-6 and CFP-10. TB2 contains an additional set of peptides targeted to induce response from CD8+ cells.
T-SPOT.TB	ELISPot assay in which peripheral blood mononuclear cells are processed. The cells are washed and counted, and a standard number of cells are added into plates and stimulated with TB-specific antigens, ESAT-6 and CFP-10. Cells responding to these antigens release IFN-γ, which is captured by IFN-γ antibodies. A secondary enzyme-labelled antibody is added and binds to the captured IFN-γ. A detection reagent is added which reacts with the enzyme-labelled antibody. This reaction produces spots, showing where the IFN-γ was released. Spots are then counted.

CFP-10, culture filtrate protein 10; ESAT-6, early secreted antigenic target 6; IFN-γ, interferon gamma; IGRA, interferon gamma release assay; PPDs, purified protein derivatives; QFT, QuantiFERON-TB assay; QFT-G, QuantiFERON-TB Gold; QFT-GIT, QuantiFERON-TB Gold In-Tube assay; QFT-Plus, QuantiFERON-TB Gold Plus assay; QIFN, QuantiFERON-TB test; TB, tuberculosis.

QFT-GIT and QFT-Gold Plus. The mechanisms of action of tests are summarised in table 1.

QuantiFERON-TB assay (QIFN)

This version used the whole purified protein derivative (PPD) as the antigen: human, avian and bovine types. Whole heparinised blood is incubated with these PPDs, phytohaemagglutinin as a positive control and saline as a negative control. The plasma is then collected, and the IFN-γ is quantified using an ELISA kit.[10] This product is no longer marketed.

QuantiFERON-TB Gold assay

The second version received approval in 2005. Fresh heparinised whole blood is incubated with synthetic peptides representing ESAT-6 and CFP-10, rather than with PPDs. IFN-γ concentration is measured using ELISA.[11] This product is no longer marketed.

QuantiFERON-TB Gold In-Tube assay

The QFT-GIT assay was approved by the FDA in 2007.[12] In QFT-GIT, whole blood is collected in three heparinised tubes, one containing stimulating antigens ESAT-6, CFP-10 and TB7.7, a mitogen (positive control) tube, and a nil (antigen-free) tube. After incubation, IFN-γ concentration is measured using an ELISA kit.[13]

Diagnostic accuracy

The sensitivity of QFT-GIT reported in meta-analyses ranged between 69% and 89% and the specificity between 76% and 99% for active TB. Similarly, for latent TB infection, sensitivity was reported between 58% and 84% and specificity between 71% and 89%.[14–25] For TBU, sensitivity was 64% (95% CI 60% to 69%) and specificity was 99.5% (95% CI 98.8% to 99.9%) in a study conducted in Singapore.[17]

QuantiFERON-TB Gold Plus assay

The fourth generation of QFT uses two antigen tubes: TB1 and TB2. Both tubes contain the antigens ESAT-6 and CFP-10 (TB7.7 is not used in this assay). TB2 contains an additional set of peptides targeted to induce a response from CD8+ cells.[26 27] It was approved by the FDA in June 2017.[28]

Diagnostic accuracy

The sensitivity of QFT-Plus reported in meta-analyses for active TB was 94% and the specificity was 96%.[20 21] For latent TB infection, sensitivity was 91% and specificity was 95%.[29 30] No study reported the diagnostic accuracy of QFT-Plus among patients with TBU.

T-SPOT.TB assay

The T-SPOT.TB is an enzyme-linked immunosorbent spot (ELISpot) assay in which peripheral blood mononuclear cells are processed.[31] It was licensed in the European Union in July 2004 and received FDA premarket approval in July 2008.[32] These cells react with synthetic peptides representing ESAT-6 and CFP-10, and IFN-γ antibodies are used to bind to the IFN-γ as the cells release it. A secondary antibody, which is enzyme-labelled, is added which attaches to the bound IFN-γ. A detection reagent is introduced which reacts with the enzyme-labelled antibody. This reaction produces IFN-γ producing spots, which are then counted. The patient is considered to be positive for TB infection if the number of spots in the TB antigen well exceeds the provided threshold value.[33]

Diagnostic accuracy

The sensitivity of T-SPOT.TB reported in meta-analyses ranged between 74% and 89% and the specificity between 59% and 96.8% for active TB. Similarly, for latent TB infection, sensitivity ranged between 55% and 93% and

specificity ranged between 76% and 93%.[14 17 20 23 32 34–37] Three primary studies reported the diagnostic accuracy of T-SPOT.TB assay for uveitis.[17 34 36]

Direct comparison of QFT-GIT and T-SPOT.TB for diagnosis of TBU

In a 2-year prospective study conducted in Singapore, QFT-GIT and T-SPOT.TB were performed in patients with newly diagnosed uveitis.[17] The authors stated that regardless of the prevalence of TBU, QFT-GIT was a superior choice and should be used as a first-line test. Regarding the usefulness of performing both tests in combination, if both tests give a positive result, then the likelihood of a tuberculous cause rises to 100%. However, even if both tests are negative, the possibility of TB still remains. For discordant results, QFT-GIT provided more accurate readings than T-SPOT.TB and both were more accurate than the TST. The authors concluded that QFT-GIT was the optimal choice in tests to diagnose patients with TBU. We were unable to find any study comparing QFT-Plus and T-SPOT.TB tests. All reported diagnostic accuracies of IGRAs in TBU are provided in table 2.

Sources of variability

Prevalence of disease (endemic versus non-endemic)

Spectrum bias or effect refers to the phenomenon in which there is a variance in the performance of a test in different clinical settings. This may arise due to a variation in prevalence of the disease in different studies. Because of this effect, tests may not be as accurate in populations different from the ones they were developed in.[38] Leeflang *et al*[39] analysed data from 23 meta-analyses and found there to be a statistically significant ($p<0.05$) negative correlation between specificity and prevalence. They theorised that this association was caused by clinical variability (range in patient spectrum, different referral pathways and reader expectations) and artefactual differences (distorted inclusion of patients, verification bias and imperfect reference standard). WHO recommends that IGRAs should not replace microbiological methods of diagnosis of pulmonary and extrapulmonary TB in low-income and middle-income countries (which may have a higher incidence than high-income countries).[40]

Babu *et al*[41] stated that in countries where TB is endemic immune-based tests might not be very useful as they do not directly detect the organism but show that an immune response has taken place. In a study of 687 contacts of patients with culture-confirmed systemic TB in France, QFT-GIT's positive predictive value for progression to TB was 1.96%, while the negative predictive value was 99.8%. This study supports the utility of QFT-GIT in a low prevalence setting.[42] Kang *et al*[43] conducted a multicentre study to investigate the clinical usefulness of the T-SPOT.TB in diagnosing active TB in China, a high burden country. They found that there was a high number of false positives when using this test (43.6%, 95% CI 40.9% to 46.3%) and did not recommend its use in a high burden country.

Immune suppression

Immune suppression secondary to corticosteroids reduces the release of IFN-γ and therefore leads to the test being less accurate in patients under treatment. Edwards *et al*[44] demonstrated this in an ex vivo model study. They also stated that in these patients, inducible protein 10 (IP-10) might serve as a more reliable biomarker. Glucocorticoids are noted to have a significant influence on IGRAs in children if treated for more than a week.[45] Bao *et al* found glucocorticoids to affect the mitogen-stimulated response and therefore negatively affect the IGRA results in both adults and children.[46 47] QFT-Plus sensitivity was noted to be lower among people with HIV/AIDS with severe immunosuppression.[48]

An older study evaluating the clinical usefulness of T-SPOT.TB in immunocompromised patients (53 with malignancy, 29 patients with diabetes mellitus, 23 taking immunosuppressive drugs and 14 with end-stage renal disease) found T-SPOT.TB cannot be used alone to rule in TB due to its poor specificity in these groups. They found the usefulness to be particularly less in the group taking immunosuppressive drugs.[49] T-SPOT.TB test was found not to be affected by CD4+ T lymphocyte count ($p=0.289$), and it was stated that in HIV-infected patients with low CD4+ T lymphocyte counts, T-SPOT.TB test may be considered for latent TB infection diagnosis.[50]

Antituberculous treatment

In a study by Bao *et al*,[46] 44.4% of patients with active (pulmonary or extrapulmonary) TB converted to a negative reading with the QFT-GIT after less than 3 months of ATT. This is thought to be due to the bactericidal effect of ATT on M. Tuberculosis, which reduces antigen production and hence reduces antigen-stimulated IFN-γ production.

However, another study reported that the effects of therapeutic concentrations of ATT (isoniazid, rifampicin and ciprofloxacin) on IFN-γ and other cytokines were comparatively small, but found that dexamethasone caused a marked change in IFN-γ concentrations. The authors hypothesised that large changes in IFN-γ were due to effects of the treatment, rather than the immunomodulatory effects of these drugs.[51]

Factors unrelated to patients

Sources of variability unrelated to patients (eg, due to manufacturing or analytical sources) can also reduce the reliability of IGRA tests. The presence of variability means that the changes in the results may not have an immunological basis. A dichotomous cut-off value leads to greater ambiguity in the interpretation of results. Pai *et al*[9] categorised the known sources of variability into manufacturing sources, preanalytical sources, analytical sources (random errors causing fluctuations in measurements) and immunological sources (boosting by PPD and modulation by Pathogen Associated Molecular patterns (PAMP)). Preanalytical sources represent the main

Table 2 Diagnostic accuracies of IGRAs for uveitis

Assay	Study (reference)	Year	N	Country incidence	Population	Target condition	Setting	Sensitivity (95% CI)	Specificity (95% CI)	Gold standard
QFT-GIT	Prospective head-to-head study comparing 2 commercial interferon gamma release assays for the diagnosis of tuberculous uveitis[17]	2014	120	Singapore (mid TB incidence)	Patients with uveitis	TB uveitis	Eye hospital	0.64 (0.60 to 0.69)	0.995 (0.988 to 0.999)	Not specified.
T-SPOT.TB	Interferon-gamma release assay as a diagnostic test for tuberculosis-associated uveitis[34]	2012	138	Singapore (mid TB incidence)	Patients with uveitis	TB uveitis	Eye hospital	0.36	0.75	Not specified.
	Interferon γ release assay for the diagnosis of uveitis associated with tuberculosis: a Bayesian evaluation in the absence of a gold standard[67]	2013	191	Singapore (mid TB incidence)	Patients with uveitis	TB uveitis	Eye hospital	0.50 (0.33 to 0.67)	0.91 (0.88 to 0.93)	None, a Bayesian latent class model used instead.
	Prospective head-to-head study comparing 2 commercial interferon gamma release assays for the diagnosis of tuberculous uveitis[17]	2014	120	Singapore (mid TB incidence)	Patients with uveitis	TB uveitis	Eye hospital	0.67 (0.60 to 0.74)	0.91 (0.88 to 0.93)	Not specified.
	Evaluation of the accuracy of T-SPOT.TB for the diagnosis of ocular tuberculosis in a BCG-vaccinated, non-endemic population[36]	2017	40	Chile (low TB incidence)	Patients with uveitis	TB uveitis	Tertiary referral centre	0.80 (0.53 to 0.94)	0.85 (0.62 to 0.96)	Positive TST with intraocular inflammation and positive response to ATT.

ATT, antituberculous treatment; IGRAs, interferon gamma release assays; QFT-GIT, QuantiFERON-TB Gold In-Tube assay; TB, tuberculosis; TST, tuberculin skin test.

component of causes of variability and are the ones most extensively researched. Gaur *et al*[52] found that blood volume variability affected the results, and standardising the blood volume to 0.8 mL may increase assay sensitivity. They also found that shaking the nil and TB antigen tubes separately at different strengths contributed to variability in results. Multiple authors have reported that removing the dichotomous cut-off value and instead introducing a range would increase the reliability and validity of the QFT-GIT.[53 54] Jonsson *et al*[53] found that retesting of results that fell in either the borderline negative or positive ranges resulted in reversions or conversions that would otherwise have been classified as false positives or negatives, leading to possible undertreatment or overtreatment. The T-SPOT.TB uses a borderline category to indicate that results in that range should be retested. Rego and colleagues[55] found that this range, rather than a cut-off value, resulted in less false positives, as most of those in the borderline range that were retested turned out to be negative.

Utility of IGRA

Utility of IGRA in the diagnosis of TBU

Diagnostic test results should be interpreted carefully based on the patient's ocular signs and symptoms, age, and comorbidities. Gupta *et al*[56] defined this as the pretest likelihood of a patient having OTB. If the pretest likelihood is determined to be low, a positive test may not determine that the patient has TB. Ang *et al*[57] evaluated the usefulness of IGRA in the diagnosis of TBU. They reported low sensitivity to exclude OTB. A Bayesian latent class study by Agrawal *et al*[58] also concluded that QFT-GIT alone could not separate TBU from non-TBU. Gineys *et al*[59] state that the cut-off value for QFT, that is, 0.35 IU/mL, is too low for the diagnosis for uveitis, and that raising this value might prevent unnecessary anti-TB treatment. In another study testing 50 patients suspected with TBU with QFT-Gold in India, Sudharshan *et al*[60] found that the percentage of positive results was higher in patients with conditions affecting the posterior uveitis, especially serpiginous-like choroiditis. Testing in 181 patients in a hospital in Korea led to similar results, with posterior uveitis being a finding in 44.6% of patients with a positive IGRA result.[61] In a retrospective cohort study conducted in Singapore, a mid-level burden country, including cases from August 2006 to February 2007, Ang and colleagues[62] found that QFT-GIT was not superior to TST in sensitivity and was only slightly more specific. They suggested that since QFT-GIT was only slightly superior to TST in the diagnosis of TBU, a combination of both tests should be used.

Utility of IGRA in screening for latent TB before TNF treatment

Antitumour necrosis factor (TNF) agents are used to treat immune-mediated inflammatory disorders, including uveitis. The use of these therapies leads to immune suppression and may precipitate infections. One concern is the conversion of a latent TB infection to an active one. This concern has led many countries to make latent TB screening mandatory before the commencement of anti-TNF treatment. In contrast to what has been reported about the effects of corticosteroid use on QFT-Gold, Sargın *et al*[63] found QFT-Gold and T-SPOT. TB to be good alternatives to TST in rhuematoid arthritis (RA) patients for latent TB detection as they were not significantly affected by prior vaccinations or by corticosteroid use. Other studies have also found QFT-GIT to be appropriate for screening latent TB.[64 65] Due to poor agreement between TST and QFT, researchers in Spain recommended using both tests.[66]

CONCLUSION

This review highlights the lack of high-quality diagnostic accuracy studies evaluating IGRA tests for the diagnosis of TBU. As the newer versions of QFT tests are introduced, further studies are needed to determine their usefulness in clinical settings. No study was identified evaluating the latest QFT assay—QFT-Plus—for the diagnosis of TBU. Similarly, there is lack of data directly comparing QFT-Plus with T-SPOT.TB specifically for the diagnosis of TBU.

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REFERENCES

1 Global tuberculosis report, 2019. Available: https://www.who.int/tb/publications/global_report/en/
2 Cutrufello NJ, Karakousis PC, Fishler J, *et al*. Intraocular tuberculosis. *Ocul Immunol Inflamm* 2010;18:281–91.
3 Albert DM, Raven ML. Ocular tuberculosis. *Microbiol Spectr* 2016;4.
4 Teixeira-Lopes F, Alfarroba S, Dinis A, *et al*. Ocular tuberculosis - A closer look to an increasing reality. *Pulmonology* 2018;24:289–93.
5 Figueira L, Fonseca S, Ladeira I, *et al*. Ocular tuberculosis: position paper on diagnosis and treatment management. *Rev Port Pneumol* 2017;23:31–8.
6 Gupta A, Sharma A, Bansal R, *et al*. Classification of intraocular tuberculosis. *Ocul Immunol Inflamm* 2015;23:7–13.
7 Huebner RE, Schein MF, Bass JB. The tuberculin skin test. *Clin Infect Dis* 1993;17:968–75.
8 Diagnostic standards and classification of tuberculosis in adults and children. this official statement of the American thoracic Society and the centers for disease control and prevention was adopted by the ats board of directors, July 1999. this statement was endorsed by the Council of the infectious disease Society of America, September 1999. *Am J Respir Crit Care Med* 2000;161:1376–95.

9 Pai M, Denkinger CM, Kik SV, *et al*. Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. *Clin Microbiol Rev* 2014;27:3–20.
10 Guidelines for using the QuantiFERON®-TB test for diagnosing latent Mycobacterium tuberculosis infection. Available: https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5202a2.htm
11 Guidelines for using the QuantiFERON®-TB gold test for detecting Mycobacterium tuberculosis infection, United States. Available: https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5415a4.htm
12 Center for Devices and Radiological Health. Center for devices and radiological health, 2007. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/PMA.cfm?ID=4827
13 Lee C, Agrawal R, Pavesio C. Ocular Tuberculosis--A Clinical Conundrum. *Ocul Immunol Inflamm* 2016;24:237–42.
14 Tavast E, Salo E, Seppälä I, *et al*. IGRA tests perform similarly to TST but cause no adverse reactions: pediatric experience in Finland. *BMC Res Notes* 2009;2:9.
15 Lee JE, Kim H-J, Lee SW. The clinical utility of tuberculin skin test and interferon-γ release assay in the diagnosis of active tuberculosis among young adults: a prospective observational study. *BMC Infect Dis* 2011;11:96.
16 Vallada MG, Okay TS, Del Negro GMB, *et al*. Accuracy of the QuantiFERON-TB gold in tube for diagnosing tuberculosis in a young pediatric population previously vaccinated with Bacille Calmette-Guerin. *Revista Paulista de Pediatria* 2014;32:04–10.
17 Ang M, Wong WL, Kiew SY, *et al*. Prospective head-to-head study comparing 2 commercial interferon gamma release assays for the diagnosis of tuberculous uveitis. *Am J Ophthalmol* 2014;157:1306–14.
18 Kussen GMB, Dalla-Costa LM, Rossoni A, *et al*. Interferon-Gamma release assay versus tuberculin skin test for latent tuberculosis infection among HIV patients in Brazil. *Braz J Infect Dis* 2016;20:69–75.
19 Petrucci R, Lombardi G, Corsini I, *et al*. Quantiferon-Tb gold in-tube improves tuberculosis diagnosis in children. *Pediatr Infect Dis J* 2017;36:44–9.
20 Takasaki J, Manabe T, Morino E, *et al*. Sensitivity and specificity of QuantiFERON-TB gold plus compared with QuantiFERON-TB gold in-tube and T-SPOT.TB on active tuberculosis in Japan. *J Infect Chemother* 2018;24:188–92.
21 Horne DJ, Jones BE, Kamada A, *et al*. Multicenter study of QuantiFERON®-TB Gold Plus in patients with active tuberculosis. *Int J Tuberc Lung Dis* 2018;22:617–21.
22 Chien J-Y, Chiang H-T, Lu M-C, *et al*. Quantiferon-Tb gold plus is a more sensitive screening tool than QuantiFERON-TB gold in-tube for latent tuberculosis infection among older adults in long-term care facilities. *J Clin Microbiol* 2018;56. doi:10.1128/JCM.00427-18. [Epub ahead of print: 26 Jul 2018].
23 Whitworth HS, Badhan A, Boakye AA, *et al*. Clinical utility of existing and second-generation interferon-γ release assays for diagnostic evaluation of tuberculosis: an observational cohort study. *Lancet Infect Dis* 2019;19:193–202.
24 Stevens JP, Ballengee CR, Chandradevan R, *et al*. Performance of interferon-gamma release assays for tuberculosis screening in pediatric inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2019;69:e111–6.
25 Hong JY, Park SY, Kim A, *et al*. Comparison of QFT-Plus and QFT-GIT tests for diagnosis of *M. tuberculosis* infection in immunocompetent Korean subjects. *J Thorac Dis* 2019;11:5210–7.
26 Knierer J, Gallegos Morales EN, Schablon A, *et al*. QFT-Plus: a plus in variability? - Evaluation of new generation IGRA in serial testing of students with a migration background in Germany. *J Occup Med Toxicol* 2017;12:1
27 Kamada A, Amishima M. QuantiFERON-TB® Gold Plus as a potential tuberculosis treatment monitoring tool. *Eur Respir J* 2017;49. doi:10.1183/13993003.01976-2016. [Epub ahead of print: 22 Mar 2017].
28 2017-06-08-QFT-Plus-FDA-Approval. Available: http://corporate.qiagen.com/newsroom/press-releases/2017/2017-06-08-qft-plus-fda-approval [Accessed 7 Jul 2020].
29 Pourakbari B, Mamishi S, Benvari S, *et al*. Comparison of the QuantiFERON-TB gold plus and QuantiFERON-TB gold in-tube interferon-γ release assays: a systematic review and meta-analysis. *Adv Med Sci* 2019;64:437–43.
30 Sotgiu G, Saderi L, Petruccioli E, *et al*. Quantiferon TB gold plus for the diagnosis of tuberculosis: a systematic review and meta-analysis. *J Infect* 2019;79:444–53.
31 T-SPOT®.TB. Available: http://www.oxfordimmunotec.com/international/products-services/t-spot-tb-test/#:~:text=The%20product%20was%20licensed%20in,skin%20test%20gives%20poor%20results
32 Cattamanchi A, Ssewenyana I, Davis JL, *et al*. Role of interferon-gamma release assays in the diagnosis of pulmonary tuberculosis in patients with advanced HIV infection. *BMC Infect Dis* 2010;10:75.
33 Fact Sheets | Testing & Diagnosis | Fact Sheet - Recommendations for Human Immunodeficiency. Clinics | TB | CDC, 2019. Available: https://www.cdc.gov/tb/publications/factsheets/testing/igra.htm [Accessed 25 Mar 2020].
34 Ang M, Wong W, Ngan CCL, *et al*. Interferon-Gamma release assay as a diagnostic test for tuberculosis-associated uveitis. *Eye* 2012;26:658–65.
35 Sun L, Tian J-ling, Yin Q-qin, *et al*. Performance of the interferon gamma release assays in tuberculosis disease in children five years old or less. *PLoS One* 2015;10:e0143820.
36 Urzua CA, Liberman P, Abuauad S, *et al*. Evaluation of the accuracy of T-SPOT.TB for the diagnosis of ocular tuberculosis in a BCG-vaccinated, non-endemic population. *Ocul Immunol Inflamm* 2017;25:455–9.
37 Zhang L, Shi X, Zhang Y, *et al*. Analysis of factors influencing diagnostic accuracy of T-SPOT.TB for active tuberculosis in clinical practice. *Sci Rep* 2017;7:7764.
38 Usher-Smith JA, Sharp SJ, Griffin SJ. The spectrum effect in tests for risk prediction, screening, and diagnosis. *BMJ* 2016;353:i3139.
39 Leeflang MMG, Rutjes AWS, Reitsma JB, *et al*. Variation of a test's sensitivity and specificity with disease prevalence. *Can Med Assoc J* 2013;185:E537–44.
40 Use of interferon-γ release assays (IGRAs) in tuberculosis control in low and middle-income settings expert group meeting report. Available: https://www.who.int/tb/publications/tb-igraexpertmeeting-report/en/
41 Babu K, Satish V, Satish S, *et al*. Utility of quantiferon TB gold test in a South Indian patient population of ocular inflammation. *Indian J Ophthalmol* 2009;57:427–30.
42 Bergot E, Haustraete E, Malbruny B, *et al*. Observational study of QuantiFERON®-TB gold in-tube assay in tuberculosis contacts in a low incidence area. *PLoS One* 2012;7:e43520.
43 Kang W-L, Wang G-R, Wu M-Y, *et al*. Interferon-Gamma release assay is not appropriate for the diagnosis of active tuberculosis in high-burden tuberculosis settings: a retrospective multicenter investigation. *Chin Med J* 2018;131:268–75.
44 Edwards A, Gao Y, Allan RN, *et al*. Corticosteroids and infliximab impair the performance of interferon-γ release assays used for diagnosis of latent tuberculosis. *Thorax* 2017;72:946–9.
45 Li T, Bao L, Diao N, *et al*. Influencial factors of the performance of interferon-γ release assays in the diagnosis of childhood tuberculosis. *Clin Exp Med* 2015;15:303–9.
46 Bao L, Li T, Diao N, *et al*. Fluctuating behavior and influential factors in the performance of the QuantiFERON-TB gold in-tube assay in the diagnosis of tuberculosis. *PLoS One* 2015;10:e0103763.
47 Petruccioli E, Chiacchio T, Vanini V, *et al*. Effect of therapy on Quantiferon-Plus response in patients with active and latent tuberculosis infection. *Sci Rep* 2018;8:15626
48 Telisinghe L, Amofa-Sekyi M, Maluzi K, *et al*. The sensitivity of the QuantiFERON®-TB Gold Plus assay in Zambian adults with active tuberculosis. *Int J Tuberc Lung Dis* 2017;21:690–6.
49 Jung JY, Lim JE, Lee H-J, *et al*. Questionable role of interferon-γ assays for smear-negative pulmonary TB in immunocompromised patients. *J Infect* 2012;64:188–96.
50 Binay UD, Fincancı M, Fersan E, *et al*. [Comparison of Tuberculin Skin Test (TST) and T-SPOT.TB Tests for Diagnosis of Latent Tuberculosis Infection (LTBI) in HIV-infected Patients]. *Mikrobiyol Bul* 2019;53:388–400.
51 Clifford V, Zufferey C, Germano S, *et al*. The impact of anti-tuberculous antibiotics and corticosteroids on cytokine production in QuantiFERON-TB gold in tube assays. *Tuberculosis* 2015;95:343–9.
52 Gaur RL, Pai M, Banaei N. Impact of blood volume, tube shaking, and incubation time on reproducibility of QuantiFERON-TB gold in-tube assay. *J Clin Microbiol* 2013;51:3521–6.
53 Jonsson J, Westman A, Bruchfeld J, *et al*. A borderline range for quantiferon gold in-tube results. *PLoS One* 2017;12:e0187313.
54 Whitworth WC, Goodwin DJ, Racster L, *et al*. Variability of the QuantiFERON®-TB gold In-Tube test using automated and manual methods. *PLoS One* 2014;9:e86721.
55 Rego K, Pereira K, MacDougall J, *et al*. Utility of the T-SPOT®.TB test's borderline category to increase test resolution for results around the cut-off point. *Tuberculosis* 2018;108:178–85.
56 Gupta V, Shoughy SS, Mahajan S, *et al*. Clinics of ocular tuberculosis. *Ocul Immunol Inflamm* 2015;23:14–24.
57 Ang M, Wong WL, Li X, *et al*. Interferon γ release assay for the diagnosis of uveitis associated with tuberculosis: a Bayesian evaluation in the absence of a gold standard. *Br J Ophthalmol* 2013;97:1062-7.

58 Agrawal R, Grant R, Gupta B, *et al*. What does IGRA testing add to the diagnosis of ocular tuberculosis? A Bayesian latent class analysis. *BMC Ophthalmol* 2017;17:245.
59 Gineys R, Bodaghi B, Carcelain G, *et al*. Quantiferon-Tb gold cut-off value: implications for the management of tuberculosis-related ocular inflammation. *Am J Ophthalmol* 2011;152:433–40.
60 Sudharshan S, Ganesh SK, Balu G, *et al*. Utility of QuantiFERON®-TB gold test in diagnosis and management of suspected tubercular uveitis in India. *Int Ophthalmol* 2012;32:217–23.
61 Ahn SJ, Kim KE, Woo SJ, *et al*. The usefulness of interferon-gamma release assay for diagnosis of tuberculosis-related uveitis in Korea. *Korean J Ophthalmol* 2014;28:226–33.
62 Ang M, Htoon HM, Chee S-P. Diagnosis of tuberculous uveitis: clinical application of an interferon-gamma release assay. *Ophthalmology* 2009;116:1391–6.
63 Sargın G, Şentürk T, Ceylan E, *et al*. TST, QuantiFERON-TB gold test and T-SPOT.TB test for detecting latent tuberculosis infection in patients with rheumatic disease prior to anti-TNF therapy. *Tuberk Toraks* 2018;66:136–43.
64 Vassilopoulos D, Tsikrika S, Hatzara C, *et al*. Comparison of two gamma interferon release assays and tuberculin skin testing for tuberculosis screening in a cohort of patients with rheumatic diseases starting anti-tumor necrosis factor therapy. *Clin Vaccine Immunol* 2011;18:2102–8.
65 Kim H-C, Jo K-W, Jung YJ, *et al*. Diagnosis of latent tuberculosis infection before initiation of anti-tumor necrosis factor therapy using both tuberculin skin test and QuantiFERON-TB gold in tube assay. *Scand J Infect Dis* 2014;46:763–9.
66 Casas S, Andreu A, Juanola X, *et al*. Diagnosis of tuberculosis infection by tuberculin skin test and a whole-blood interferon-γ release assay in patients considered for anti-tumor necrosis factor-α therapy. *Diagn Microbiol Infect Dis* 2011;71:57–65.
67 Ang M, Wong WL, Li X, *et al*. Interferon γ release assay for the diagnosis of uveitis associated with tuberculosis: a Bayesian evaluation in the absence of a gold standard. *Br J Ophthalmol* 2013;97:1062–7.