

FAQs for Health Professionals

QuantiFERON[®]-TB Gold Plus



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Questions and answers

QuantiFERON-TB Gold Plus (QFT-Plus®) is a whole-blood test that measures the cell-mediated immune response of tuberculosis (TB) infected individuals. Approved by the US Food and Drug Administration (FDA), and CE marked, QFT-Plus, like the tuberculin skin test (TST), can be used as an aid in the diagnosis of latent tuberculosis infection and TB disease. This document has been compiled as a result of common questions posed by healthcare professionals on the use of QFT-Plus.

About TB

Tuberculosis (TB) is an airborne disease caused by infection with *Mycobacterium tuberculosis* complex organisms (*M. tuberculosis*, *M. bovis* and *M. africanum*). The transmission of TB occurs through the inhalation of microscopic droplets that are either coughed or sneezed from an individual infected with active TB disease of the lung (active pulmonary TB). Not everyone who becomes infected with TB bacteria develops active TB disease.

What is latent TB? And how is it different from active TB disease?

Latent TB infection (LTBI) is considered a 'carrier state' of *M. tuberculosis* infection where an individual silently carries the TB bacteria in their body. In LTBI, the infection is contained by the host's immune system. Hence, unlike active TB, individuals with LTBI are asymptomatic, not contagious to others and the TB organism is not detectable by any laboratory method. However, in approximately 10% of individuals with this condition, the silent infection may progress or reactivate to active disease weeks to decades later. LTBI is now believed not to be 'latent' at all, but rather, is part of a spectrum of conditions from complete quiescence to subclinical disease, which may be dependent on time from infection, amount of exposure and medical conditions that increase the risk of progression or reactivation. Individuals with LTBI can be offered preventive therapy to prevent active disease from occurring. Systematic testing and treatment of LTBI in at-risk populations is a critical component of WHO's End TB Strategy to eliminate the disease by 2050 (http://www.who.int/tb/post2015_strategy/en/). In 2014, WHO released Guidelines on the management of latent tuberculosis infection (<http://www.who.int/tb/publications/latent-tuberculosis-infection/en/>).

Active TB is a disease state of uncontrolled *M. tuberculosis* growth that occurs when TB bacteria are able to overcome a person's immune system. Active TB can affect any organ of the body, but is most commonly a disease of the lung. A person with active TB will often have symptoms which are not specific for tuberculosis (e.g., a chronic cough, night sweats and weight loss). Direct detection of *M. tuberculosis* bacilli in sputum or specimen culture is the hallmark of disease and is considered the gold standard of TB diagnosis. A person who has active pulmonary TB and is coughing, with the presence of *M. tuberculosis* in their sputum is considered infectious.

QFT-Plus is an assay that indirectly detects TB infection by measuring the interferon- γ (IFN- γ) as a result of cell-mediated immune response to TB-specific antigens. Its intended use is the same as the tuberculin skin test (TST) and can be used as a diagnostic aid for *M. tuberculosis* infection, whether active tuberculosis disease or LTBI. However, when using QFT-Plus in a person suspected of having active TB, it should not replace appropriate microbiological and molecular investigation. QFT-Plus cannot distinguish between active and latent TB infection and hence, a positive result should never be used in isolation to diagnose or exclude active tuberculosis or LTBI. When making a diagnosis of LTBI, active disease must be excluded by radiography and medical evaluation.

What is the meaning of 'remote' or 'recent' TB infection and can QFT distinguish between remote and recent infection?

The term 'remote infection' is an LTBI term that is commonly used in the TB community to distinguish it from 'recent infection', which is defined as a person infected within the past 2 years. Although the lifetime risk of developing TB disease is estimated at 10%, half of that risk is within the first 2 years of infection and therefore progression to disease is highest shortly after infection. It is believed that the farther away from the infection, the lower the likelihood of disease. However, medical conditions including prior healed TB, malnutrition and being elderly increases the likelihood of disease from LTBI, both recent and remote alike. Like the TST, QFT-Plus cannot distinguish between remote and new infection unless serial testing detects recent infection (positive tests) in persons with previously negative results within a 2-year window. Additionally, young children under the age of 5 with positive test results are assumed to have recent TB infection.

Why is latent TB infection important?

It is estimated that up to 10% of people infected with *M. tuberculosis* will develop active TB in their lifetime. With an estimated 2 billion people (or one-third of the world's population) infected, the large global reservoir of LTBI represents a huge pool of future contagious disease.

Diagnosing LTBI and preventive treatment is important because it, can significantly reduce the risk of disease, and prevent outbreaks from recent transmission. On a global level, achieving a significant reduction in the burden of TB cases cannot be achieved without also including the detection and treatment of LTBI (1). Multiple modeling studies like the one below have shown accelerated decline in TB rates when including LTBI detection and treatment. These data are the basis for inclusion of LTBI and prevention in the WHO End TB strategy.

How should screening for TB and LTBI be prioritized?

Prioritized or targeted TB screening focuses on screening individuals and populations at highest risk of being infected, progressing or reactivating TB disease, or having both or multiple risks present. The purpose of TB screening is to find cases at an early asymptomatic phase that is less contagious and easily curable, and find LTBI among individuals who may benefit from preventive treatment. Targeted testing can be applied as follows:

1. Contact investigation: Identifying newly infected contacts tops the priority list as the risk of infection is high and new infection carries a much higher risk of disease progression compared to old or chronic infection (2). Contact screening and prevention of transmission is a WHO recommendation (3).
2. Congregate settings: Congregate settings are places where transmission of communicable diseases is a real risk. Focused screening for disease and LTBI prior to entry into congregate settings reduces TB transmission through early identification of TB. Preventive treatment among those with LTBI prevents high transmission TB outbreaks from reactivation disease.

Congregate settings may include:

- Hospitals/healthcare institutions
- Residential facilities
- Prisons/correctional facilities
- Renal dialysis units
- Homeless shelters
- Higher educational facilities and dormitories*
- Military barracks*
- Certain employment settings, e.g., the mining industry*
- Refugee camps

* Relevant in medium- and high-burden countries only.

3. Populations with high prevalence of TB infection: Targeted screening of individuals that are at high risk of being infected, such as individuals from TB-endemic countries entering low-burden countries or known populations with higher TB prevalence such as impoverished, homeless persons can make a significant individual and public health impact, especially when TB prevention is focused on those with LTBI that have clinical conditions that increase the risk of TB disease progression or reactivation.
4. Clinical conditions that increase the risk of developing TB disease: Prevention of disease in these individuals with LTBI prevents the need for long and more toxic multidrug treatment regimens and protects against developing lung and organ destruction, long term disability, death, economic loss and transmission of disease to family and those close to the individual (see Table, next page).

Medical risks of reactivation

Relative risk reactivation of TB in various clinical settings	Times risk
AIDS	110–170
HIV infection	50–110
Solid organ transplant	20–74
Silicosis	30
Recent TB infection (<2 years)	15
Chronic renal failure	10–25
Carcinoma of head and neck	16
Abnormal chest radiograph with upper lobe fibro nodular disease typical of healed TB infection	6–19
TNF- α inhibitor therapy	1.7–9
Glucocorticoid therapy	4.9
Children less than 4 years old	2.2–5
Diabetes mellitus	2–3.6
Underweight (BMI <20)	2–3
Smoker (1 pack/day)	2–3
Normal healthy individual	1

Table adapted from Lobue et al (2)

Individuals with LTBI and medical co-morbidities should be targeted for LTBI treatment after active tuberculosis has been excluded by thorough medical evaluation and radiography. This also applies to individuals newly infected from recent exposure to TB, such as contacts of known active TB cases, especially child contacts under 5 years of age.

Similarly, patients should be tested before initiation of immunosuppressive therapy since immunosuppression can negatively impact TB test results (IGRAs and TST).

Is latent TB contagious?

No, TB in its latent form cannot spread as the bacterial burden is very low and undetectable. However, it can become active pulmonary TB, which is contagious, often before the individual is aware that they have it. Hence, excluding active TB with a chest x-ray and medical evaluation is essential before LTBI treatment is initiated.

Doesn't everybody in high-incidence countries have latent TB?

No, this is a common misconception that is used to avoid the need for testing. One in three people worldwide is thought to be infected with LTBI, (4) although there is significant variance in high-incidence countries based on the demographics of the population being studied.

About QFT-Plus

What is QFT-Plus?

QuantiFERON-TB Gold Plus (QFT-Plus) is blood-based laboratory test that measures responses to TB-specific peptide antigens in whole blood. Like the tuberculin skin test (TST), it is an indirect test for *M. tuberculosis* infection, but is more specific than TST and is unaffected by prior Bacille Calmette-Guerin (BCG) vaccination and most environmental mycobacterial infections. A modern replacement to the TST, QFT-Plus provides clinicians with an accurate, reliable and efficient tool for aiding the diagnosis of TB infection.

Compared to its predecessor QFT-Gold In-Tube, which contains peptide antigens optimized to stimulate CD4 T cells, QFT-Plus contains new proprietary antigens optimized for both CD4 and CD8 T cell stimulation. This provides a broader picture of the immune response. CD8 T cells play an important role in TB immunology and may be an important biomarker of intracellular TB burden. CD8 T cell responses are more frequent in active TB, new infection and function well in young children and individuals with HIV. To date research indicates the new formulation of QFT-Plus has shown good correlation and performance compared to its predecessor (5) with publications on sensitivity, specificity, performance in HIV-infected persons (6), immigrant students, healthcare workers and utility in monitoring treatment (7) Like its predecessors, a positive QFT result correlates better to TB risk and is a better predictor of true infection from *M. tuberculosis* compared to TST (8). However, until there is adequate evidence, QFT-Plus cannot be used to distinguish between active tuberculosis disease and LTBI, and interpretation of results should always include a risk assessment, radiography and other medical and diagnostic evaluations.

What is the intended use of QFT-Plus?

The QuantiFERON-TB Gold Plus (QFT-Plus) assay is an in vitro diagnostic test using a peptide cocktail simulating ESAT-6 and CFP-10 proteins to stimulate cells in heparinized whole blood. Detection of interferon- γ (IFN- γ) by enzyme-linked immunosorbent assay (ELISA) is used to identify in vitro responses to these peptide antigens that are associated with *Mycobacterium tuberculosis* infection.

QFT-Plus is an indirect test for *M. tuberculosis* infection (including disease) and is intended for use in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations.

How does QFT-Plus differ from QFT

The principal differences from the previous QFT-Gold In-Tube (QFT) version are in the new, proprietary formulation, adding CD8 stimulating antigens and new options for blood collection and processing. Compared to QFT, which contains antigens that are designed to primarily stimulate CD4 T cells, QFT Plus adds an additional antigen tube containing a proprietary formulation designed for optimal CD4 and CD8 T cell stimulation and thereby provides a broader picture of the immune response. CD8 T cells play an important role in TB immunology and may be an important biomarker of intracellular TB burden. CD8 T cell responses reported in the literature (9) show more frequent activity in active TB, new infection and function well in young children and individuals with HIV. To date, research indicates that the new formulation of QFT-Plus has shown good correlation and performance compared to its predecessor (10) with publications on sensitivity (5, 10, 11), specificity (7, 11), performance in HIV-infected persons (6), healthcare workers (7) and utility in monitoring treatment (12). Like its predecessors, a positive QFT result correlates better to TB risk and is a better predictor of true infection from *M. tuberculosis* compared to TST (11). However, until there is adequate evidence, QFT-Plus should not be used to distinguish between active tuberculosis disease and LTBI, and interpretation of results should always include a risk assessment, radiography and other medical and diagnostic evaluations.

QFT-Plus has a four-tube format with an additional antigen tube (TB2) containing both CD4 and CD8 stimulating antigens. The Nil and Mitogen tubes remain the same, while the TB Antigen Tube 1 (TB1) contains CD4 T cell stimulating ESAT-6 and CFP-10 antigens as before, without TB7.7. The second TB Antigen Tube 2 (TB2) contains the same CD4 antigens of TB1 and proprietary CD8 antigens.

Having the two separate TB Antigen tubes in QFT-Plus has the potential to provide additional clinical information that was not available in QFT. Active research focused on the performance and CD8 antigen response of QFT-Plus in active TB, contacts, disease progression, pediatrics, persons living with HIV, healthcare workers in high burden settings and pregnancy are ongoing all over the world.

Additionally, there has been global harmonization of laboratory standards and workflow. As an option, blood can be drawn into a single lithium-heparin tube (non-EDTA) and transferred into the QFT-Blood Collection Tubes at a later stage.

Is QFT-Plus replacing QFT?

Yes. Due to certain regulatory requirements, QFT-Plus will replace QFT, with a transition period of approximately one year. For customers currently running QFT, QIAGEN will assist in the transition from QFT to QFT-Plus to ensure an easy, successful implementation. Technical assistance from QIAGEN is available upon request. Sufficient time will be granted to laboratories switching from QFT to QFT-Plus to ensure a successful implementation and thorough validation.

What is the benefit of detecting immune responses from CD8 T cells

In the natural history of *M. tuberculosis* infection, CD4 and CD8 T cells play a critical role in immunological control of the bacillus. Evidence now supports a role for CD8 T cells participating in the host defense to *M. tuberculosis* by producing IFN- γ and other soluble factors, which activate macrophages to suppress growth of *M. tuberculosis*, kill infected cells or directly lyse intracellular *M. tuberculosis*. *M. tuberculosis*-specific CD8 cells have been detected in subjects with LTBI and with active TB disease. However, CD8 T cell responses are described as being more frequently detected in patients with higher bacterial burden such as active TB disease versus LTBI, and may be associated with a recent *M. tuberculosis* exposure. In addition, research indicates *M. tuberculosis*-specific CD8 T cells producing IFN- γ have also been detected in active TB subjects with HIV co-infection and in young children with TB disease.

Why not have only CD8 T cell response in TB2 instead of CD4 and CD8?

The new formulation of QFT-Plus, using a proprietary combination of peptides designed for both CD4 and CD8 T cell stimulation had synergistic effects and was better able to detect CD8 T cell activity. This synergy was important to deliver the highest possible clinical performance. Using CD8 T cell response in isolation often did not result in any detectable response and the performance benefit was only seen when the CD8 and CD4 responses are acting synergistically.

Has TB7.7 been removed? Why?

The new formulation of QFT-Plus, using a proprietary combination of peptide antigens from ESAT 6 and CFP-10 had excellent performance compared to QFT containing TB7.7.

Why the fourth tube?

The 'fourth tube' in the QFT-Plus assay is essentially the TB2 tube. The Nil and Mitogen tubes serve the same purpose as with QFT – a negative and positive control, respectively. The TB1 tube is very similar to the TB Antigen tube in QFT; the TB1 tube contains peptides from ESAT-6 and CFP-10 that are optimized to elicit cell-mediated immune (CMI) responses primarily from CD4 T cells. The new TB2 tube contains an additional set of peptides optimized for the induction of CMI responses from both CD4 and CD8 T cells. In addition to providing information on the reproducibility of the response, the interpretation of the test is set to optimize for sensitivity by considering a positive result from either tube alone or both. In the Package Insert data, one culture confirmed active TB patient person was positive by TB1 only and 9 by TB2 only. Based on available literature on the role CD8 T cells play in host defense to *M. tuberculosis* infection, the four-tube format provides additional information, but the application of this information in different clinical settings needs to be studied further.

In what clinical situations can QFT-Plus be used?

QFT can be used for those being evaluated for possible *M. tuberculosis* infection, whether active disease or LTBI. For LTBI detection, WHO states that interferon gamma release assays (IGRAs; such as QFT-Plus) are interchangeable with TST in their 2014 guidelines on the management of latent tuberculosis (targeting countries that have a case rate less than 100 per 100 thousand population). In the USA, where QFT use is well established, the 2016 guidelines have expanded preference of interferon gamma release assays (IGRAs inclusive of QFT) over the TST beyond BCG-vaccinated populations and patients unlikely to return for skin test reading. The preference for IGRAs now includes all testing required for low-to-intermediate risk adults. Gamma Release Assays for Detecting *M. tuberculosis* infection, United States 2010 and the Official American Thoracic Society/Infectious Disease Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children (2016) are summarized below:

- IGRAs can be used interchangeably with the TST in all situations in which the CDC recommends TST as an aid in diagnosing *M. tuberculosis* infection.
- IGRAs are recommended, rather than the TST for testing persons from groups that historically have poor rates of return for TST reading (strong recommendation; moderate-quality evidence).
- IGRAs are recommended, rather than the TST for testing persons who have received BCG (strong recommendation; moderate-quality evidence).
- IGRAs are suggested, rather than the TST for testing persons with low or intermediate risks for TB infection (conditional recommendation; moderate-quality evidence).
- Either an IGRA or a TST may be used (without preference) to test recent contacts of persons with infectious tuberculosis with special considerations for follow-up testing. In contact investigations, negative results obtained prior to 8 weeks typically should be confirmed by repeat testing 8–10 weeks after the end of exposure.
- Either an IGRA or a TST may be used (without preference) for periodic screening that addresses occupational exposure to TB (e.g., surveillance programs for healthcare workers (HCW) with special considerations regarding conversions and reversions (see full CDC guideline (4)). Two-step testing is not required because IGRA testing does not boost subsequent test results.
- TST is preferred for testing children younger than 5 years old, due to insufficient evidence on the performance of IGRAs in young children. However, use of an IGRA in conjunction with TST may increase diagnostic sensitivity in this age group (conditional recommendation; very-low-quality evidence).
- **Disclaimer:** The performance of the USA format of the QFT-Plus test has not been extensively evaluated with specimens from individuals younger than age 17 years.

Can QFT-Plus distinguish between active TB and LTBI?

Current evidence may suggest higher CD8 activity in active TB however there is insufficient evidence to state that QFT-Plus can distinguish between active TB and LTBI. Therefore, QFT-Plus should never be used in isolation to diagnose active TB or LTBI. Anyone testing positive should be assessed for active TB with a medical evaluation, chest radiograph and other tests indicated by the clinical symptoms and medical evaluation.

How does it work?

QFT-Plus measures cell-mediated immune (CMI) response in TB-infected individuals. T cells of these individuals are sensitized to TB and thereby respond by secreting a cytokine called interferon-gamma (IFN- γ) when stimulated with specific TB peptides.

QFT-Plus uses peptides from specific proteins made almost exclusively by *M. tuberculosis* complex organisms. Those proteins are absent from all BCG vaccine preparations and from most non-tuberculous mycobacteria (NTM), with the exceptions of *M. kansasii*, *M. marinum*, and *M. szulgai* (13).

Special blood collection tubes coated with the stimulants are used for blood collection and incubation of the patient's blood. IFN- γ is released when the blood from infected individuals is stimulated by the TB-specific antigens (16–24 hours at 37°C). An enzyme-linked immunosorbent assay (ELISA) laboratory test is used to detect and quantify the amount of IFN- γ that has been released.

Why measure interferon-gamma?

M. tuberculosis is an intracellular pathogen primarily residing within macrophages. During the latent phase of the TB infection, specialized effector T cells are generated, which will circulate in the individual's bloodstream. IFN- γ is a good biomarker for cell mediated immunity and is rarely found in the circulation of healthy individuals.

When blood is taken from an infected individual and stimulated with *M. tuberculosis*-specific antigens, effector T cells release the cytokine IFN- γ . The production and subsequent measurement of IFN- γ by a rapid ELISA forms the basis of QFT.

How does QFT-Plus differ from the TST?

The tuberculin purified protein derivative (PPD) used in the TST is an ill-defined mix of proteins and protein fragments, derived from *M. tuberculosis* complex bacilli. However, the vast majority have homologs that are shared with environmental mycobacteria and BCG vaccine strains. It is largely for this reason that the TST test has poor specificity, especially in BCG-vaccinated individuals. False-positive rates can exceed 40% in BCG-vaccinated persons with a positive TST result (14).

QFT-Plus uses peptides from specific proteins made almost exclusively by *M. tuberculosis* complex organisms. Those proteins are absent from all BCG vaccine preparations and from most non-tuberculous mycobacteria (NTM) (with the exceptions of *M. kansasii*, *M. marinum*, and

M. szulgai) (13). Therefore, false-positive reactions from QFT-Plus due to BCG vaccination and most environmental bacteria are uncommon.

The TST assesses delayed-type hypersensitivity in the skin by measuring induration with a ruler, whereas QFT-Plus measures IFN- γ in the blood. The TST measures response to PPD, a polyvalent antigenic mixture, whereas QFT-Plus measures responses to a well-defined mix of synthetic peptides simulating two antigenic proteins that are specific for tuberculosis.

Unlike the in vivo TST, QFT-Plus is an in vitro test and does not induce boosting. However, the injection of the TST reagent can boost a QFT-Plus result if the TST is administered prior to QFT-Plus. Boosting may last up to a year and boosting is absent in those with a prior negative skin test indicating that the TST cannot sensitize an uninfected person. Moreover, QFT-Plus is not confounded by BCG vaccination and most common environmental NTM, whereas, the TST can be boosted by cross-reacting bacteria including BCG, and most of the common environmental bacteria.

There are numerous differences between the TST and QFT-Plus:

- The TST requires trained and often licensed clinic personnel to place and read the TST, whereas QFT-Plus requires a trained phlebotomist.
- The TST requires a person to return to have their test read 48 to 72 hours after administration. QFT-Plus requires only one visit to a healthcare provider for blood collection.
- The TST is subjective in its interpretation – in respect to both measuring the induration on the individual's arm and in deciding what cutoff to apply. QFT-Plus is an objective, laboratory-based, test with interpretation determined by analysis of ELISA data by QFT-Plus analysis software.
- Positive QFT-Plus results can be provided confidentially, whereas a positive inflammatory TST response can be a source of stigma since it is often visible, especially if redness accompanies the induration.
- Individuals can confound their own TST with something as simple as a hot shower or low-dose over-the-counter corticosteroid cream.
- Placement and reading of the TST must be manually entered into the medical record whereas the QFT laboratory result is computer generated and available electronically for e-medical records.

How long does it take to get QFT-Plus results?

This varies and depends on how frequently the laboratory in your area carries out the test. Results can be available within 24 hours, if samples are run daily. Unlike the TST, individuals do not need to return 2–3 days later in order to have the test read.

Does a prior TST influence a QFT-Plus result?

Yes, a prior TST can boost QFT results (15, 16). However, boosting does not occur in uninfected individuals because they do not have an established immune response to TB. Furthermore, the TST, despite its in vivo application, does not cause sensitization or establish a cell-mediated response and will therefore not cause subsequent boosting among persons without prior TB infection.

The implications of a boosted response are unknown, but thought to be the induction of a weak memory response from multiple points of stimulation. Andrews, et al. showed in his study in South African adolescents that QFT boosting usually resolved by one year (16). QFT has the distinct advantage over TST by not being a cause for boosting, which confounds assessments of new infection when doing serial testing. Boosting is a common phenomenon when a TST is repeated. Each TST can boost subsequent TST responses, due to remote TB infection, as well as infection with NTM or vaccination with BCG.

What is the minimum time necessary to wait between exposure to *M. tuberculosis* and QFT-Plus testing?

Available data suggests that QFT-Plus returns a positive result at least as quickly as the TST following recent infection.

The CDC guidelines on the use of QFT-Plus recommend that recent contacts that test QFT-Plus negative prior to 8 weeks after the end of exposure, be retested 8 to 10 weeks later; similar to the recommendations for the TST. Many other national guidelines recommend a similar approach. There are no adverse reports from this practice since IGRAs were FDA approved in the US in 2001.

Why do you include a positive control? How does this work?

The Mitogen tube is used as an IFN- γ positive control for each specimen tested. The Mitogen tube also serves as a control for correct blood handling and incubation. The mitogen used is phytohaemagglutinin (PHA), which stimulates T cells. While it is a direct activator of T cells, unpublished data suggest that the presence of other cells are also required for it to activate T cells.

A low IFN- γ response to Mitogen (<0.5 IU/ml) indicates an indeterminate result when a blood sample also has a negative response to the TB antigens. This pattern may occur with insufficient lymphocytes, reduced lymphocyte activity due to improper specimen handling or an inability of the patient's lymphocytes to generate IFN- γ .

What approvals does QFT-Plus have?

QFT-Plus is approved for use by the FDA and has been CE-marked, allowing it to be sold freely in the EU, as well as in the US.

What is the evidence supporting QFT and QFT-Plus?

There are currently over 1300 independent publications in numerous international journals that support the use of QFT and QFT-Plus in different clinical settings.

Research indicates the new formulation of QFT-Plus has shown good correlation and performance compared to its predecessor (5, 8) with publications on sensitivity, specificity, performance in HIV-infected persons (6), immigrant students, healthcare workers and utility in monitoring treatment (7). Like its predecessors, a positive QFT result correlates better to TB risk and is a better predictor of true infection from *M. tuberculosis* compared to TST (8).

Sensitivity and specificity of QFT-Plus

The specificity of QuantiFERON-TB Gold Plus has consistently shown to be >97% in low-risk individuals (17). Specificity is the probability that the test indicates a person does not have the disease (or infection) when that person is disease free.

The sensitivity of QuantiFERON-TB Gold Plus is as high as 94% in individuals with active disease, but varies depending on the setting and extent of TB disease (17). Sensitivity is the probability that the test indicates a person has the disease (or infection) when in fact that person does have the disease.

The TST has traditionally been used to screen populations for LTBI but QFT is now the preferred test for epidemiological LTBI studies, infection control, host biomarker studies and vaccine trials around the world. However, there is no gold standard for diagnosing LTBI. All screening tests are designed to identify the possibility that a disease may be present and to prompt further evaluation in those who screen positive. For LTBI the only gold standard is the later development of active TB. QFT has been shown to be 2–4 times better than TST in detecting the individuals who will progress to active TB disease (18, 19), and this combined with its >99% specificity, provides confidence that QFT-Plus is detecting those truly infected.

Why is it important to have a test with high specificity?

Specificity is defined as the probability that the test indicates an individual does not have the disease, or infection, when in fact they are disease free. QFT-Plus has been shown to have >97–99% specificity compared to lower than 59% for the TST among BCG-vaccinated persons. In many low burden countries, targeted testing policies are in place to screen individuals for LTBI (such as those mentioned on page 7). Without high specificity in these low burden situations, there will often be more false-positive than true-positive results, and most people treated with latent TB drugs will be receiving drugs they do not need, with the potential for adverse side-effects from unnecessary therapy. Additionally, this wastes valuable resources (and funds) following up individuals who do not need treatment.

Can IGRA tests be used for infants and in the immunosuppressed?

Published data indicates that QFT performs as well in children as it does in adults and there is no apparent loss of performance in children under 5 years (16, 20–27). In South African infants, the negative predictive value for development of active TB within 2 years was 99% while those with high optical density (OD) QFT value over 4 IU/ml (TBAg-Nil) had an over 20% progression to disease (16). For detection of LTBI, QFT is as sensitive as the TST, and clearly more specific in BCG-vaccinated children (23, 28). In a study of children who lived in close contact with smear-positive adult TB patients, QFT-Plus detected more children infected with TB than did the TST. In this study, positive QFT-Plus results showed significant correlation with smear status of the infected adults, whereas TST did not (23). In a study comparing QFT-Plus with TST, QFT-Plus was more accurate than the TST in detecting who will progress to active TB disease with very high accuracy among pediatric contacts (16, 19). In another study, data on QFT indicated to be effective in children less than 2 years of age and in children with bacteriologically confirmed TB (the sensitivity of QFT was over 90%) (26). However, caution is always needed when interpreting a negative result in a young child suspected of having active TB.

Disclaimer: The performance of the USA format of the QFT-Plus test has not been extensively evaluated with specimens from individuals younger than age 17 years, as well as in the immunocompromised patient.

QFT and QFT-Plus in the Immunosuppressed/HIV-positive patients

Research studies show QFT-Plus has a sensitivity of 89% (excluding indeterminate results) even in patients with low CD4 counts (cells per microliter) as compared to QFT Gold In-Tube which yielded a sensitivity of 63%, and TST of 55% (6). Studies suggest that QFT is more sensitive and specific than the TST for detecting *M. tuberculosis* infection in HIV-positive people (29–31). QFT is generally accepted, based on the evidence as more sensitive than the TST for LTBI detection than the TST and is less impacted by low CD4 counts. All tests (IGRAs and TST) are impacted by low CD4 counts; however, QFT-Plus appears to be impacted less than prior QFT generations. Therefore, caution is always needed when interpreting a negative result when CD4 counts are less than <200/ μ l.

What about indeterminate results in HIV-positive individuals?

Research studies to date have indicated that indeterminate QFT results are more prevalent in individuals with a CD4 count <200/ μ l, especially when the CD4 count is under 100/ μ l (29–31, 26). Indeterminate results are likely more frequent in HIV patients with active TB than LTBI (32). Individuals with a CD4 count <100/ μ l are severely immunosuppressed and the TST is also generally negative in these individuals, independent of infection status.

Disclaimer: The performance of the USA format of the QFT-Plus test has not been extensively evaluated with specimens from individuals who have impaired or altered immune functions, such as those who have HIV infection or AIDS.

QFT-Plus procedure

What are the steps in administering the test?

It is best to confirm arrangements for testing with a qualified laboratory, which can deliver the necessary sampling pack. For comprehensive instructions for use, refer to the QFT ELISA Package Insert.

Blood collection workflow options

Option 1: Direct draw into QFT-Plus Blood Collection Tubes

- Draw a 1 ml sample of blood from a patient directly into each of the four blood collection tubes, following the manufacturer's instructions.
- Ensure delivery to the laboratory for incubation as soon as possible (and within 16 hours) after blood draw. Keep at room temperature ($22\pm 5^{\circ}\text{C}$) before incubation.
- Alternatively, at the collection site, incubate the tubes standing upright for 16 to 24 hours at 37°C before shipping them to the laboratory at room temperature (or refrigerated) within 3 days.

Option 2: Blood collection into a single lithium-heparin tube and then transfer to QFT-Blood Collection Tubes

- Draw a 6 ml blood sample into a lithium-heparin tube. Proceed with next steps according to arrangements made with your designated laboratory. These may include:
 - Workflow 1 – room temperature storage and handling: Ensure delivery of the lithium-heparin tube to the laboratory as soon as possible for aliquoting to QFT-Plus tubes and immediate incubation within 12 hours.
 - Workflow 2 – refrigerated storage and handling: After blood draw, leave at room temperature for between 15 minutes and 3 hours. Then, refrigerate at 4°C for up to 48 hours. Aliquoting to QFT-Plus tubes should be completed within 2 hours at room temperature prior to incubation.

Do the QFT-Plus tubes need to be collected in a specific order?

There is no specific order for blood collection, however a commonly used fill order is Nil, TB1, TB2 and then Mitogen.

Why can filling of the tubes occur slowly?

The QFT-Plus blood collection tubes are manufactured to draw a 1 ml sample into a 5 ml tube and therefore may fill slowly. In some locations at high altitudes (>810 m or 2650 ft) the tubes will not draw sufficient blood (sufficient is close to the indicator line of the tube label). Single lithium-heparin tube option can be used at high altitude, with the appropriate workflow designated by the processing laboratory.

Why it is necessary to shake the QFT-Plus tubes immediately after blood collection?

As the tubes only collect 1 ml of blood each, thorough mixing is essential to solubilize the tubes' contents, including heparin, which are coated on the inner wall. This is best achieved by shaking the tubes ten (10) times, just firmly enough to ensure the entire inner surface of the tube is coated with blood, immediately after filling tubes. Tubes should be between 17–25°C (63–77°F) at the time of blood filling. Over-vigorous shaking may cause gel disruption and could lead to aberrant results. For the single lithium-heparin tube option, standard phlebotomy practice should be followed.

Interpretation of test results

How are QFT-Plus test results interpreted?

Proper assessment of patients suspected of infection with TB takes into consideration a combination of epidemiological, historical, medical and diagnostic findings, of which the QFT Plus result is an essential component. The optical density readings are provided numerically (a value of 0.35 IU/ml and above is defined as a positive result). A positive QFT-Plus result suggests that current *M. tuberculosis* infection is likely. The result does not differentiate between recently acquired or old infection, or between LTBI and active tuberculosis.

- A negative QFT-Plus result suggests that *M. tuberculosis* infection is unlikely but cannot be excluded, especially when the illness is consistent with tuberculosis disease or the likelihood of progression to disease is increased (e.g., because of immunosuppression).
- In rare cases, results cannot be interpreted as the blood cells have not responded to a positive control stimulant. This indicates the sample may have been mishandled (delays in sending samples or over-/under-filling of specimen tubes) or may be related to the immune system of the individual being tested. These results are called 'indeterminate'; TB infection can neither be excluded nor confirmed. Such persons are usually TST-negative. Repeat testing is recommended. The timing of retesting may best be delayed if live viral vaccination, acute immunosuppression from concurrent illness or immunosuppressive medication is suspected as the cause of the indeterminate result.

How was the cutoff value of ≥ 0.35 IU/ml established?

As expected for any diagnostic test, there is a trade-off between sensitivity and specificity, so that if one is increased under a different cutoff, then the other is decreased at the same time. Thus a cutoff was selected that gave the best combination of sensitivity and specificity.

What constitutes a QFT-Plus conversion?

QFT-Plus is highly specific, thus a change from negative to positive in a person with known exposure to tuberculosis is likely to be indicative of *M. tuberculosis* infection. Existing CDC guidelines define a QFT-Plus conversion as a "change from negative to positive" (4). This definition is routinely used in situations where populations are serially screened with QFT-Plus (such as healthcare worker screening programs in the USA). However, as is always the case, a positive result should be interpreted in light of all available information. In regards to using the 0.35 IU/ml (TB Antigen minus Nil) dichotomous cutoff to define QFT-Plus conversion, the CDC guidelines state specifically that "using this lenient criterion to define IGRA conversion might produce more conversions than are observed with the more stringent criteria applied to TSTs".

It should also be noted that the specificity of QFT-Plus – although much higher than for the TST – is not absolute and, therefore, there is the possibility of an occasional false-positive result. As suggested in the Package Insert, for anyone with an unexpected positive QFT-Plus result (i.e., no apparent risk factors), it is recommended to confirm the result by retesting the plasma samples in duplicate in the QFT-Plus ELISA and use the consensus from the three test results. From a medical management perspective, the 2016 ATS guidance and 2010 CDC guidelines recommend repeat testing to increase the specificity, with either the initial test or a different test, when there is a low probability of TB infection and risk of disease progression (4, 33).

Why would I see false-negative results in patients with active TB?

Individuals who progress to active TB do so because their immune system cannot control their infection. This can result from a large infectious exposure to *M. tuberculosis*. It may also be due to individuals having an impaired immune response, typical for malnourished individuals, those with advanced TB, those who are severely immunosuppressed or whose immune function has altered. Some individuals may develop active TB as a result of a genetic deficiency in their immune system, such as an inability to produce sufficient IFN- γ and/or IL-12. Others may develop active TB as a result of iatrogenic immune suppression, for example individuals taking anti-TNF- α medications.

It is important to note that QFT-Plus is a test for *M. tuberculosis* infection and is approved as a diagnostic aid for indirect detection of *M. tuberculosis* infection (whether active TB disease or LTBI). Clinicians may use QFT-Plus to assist in the diagnosis of active TB (in conjunction with risk assessment, radiography and other medical and diagnostic evaluations). A negative QFT-Plus result in a person with obvious symptoms of active TB should by no means be used to rule out active disease. Culture of *M. tuberculosis* remains the gold standard for confirming a diagnosis of active TB.

Are the results affected by pregnancy?

There is no clinical evidence to show that results of IGRA tests are affected by pregnancy in low-burden countries, such as the US. When compared with the TST, QFT-GIT is more specific, and at least as sensitive in cross-sectional or longitudinal studies (34).

Disclaimer: The performance of the USA format of the QFT-Plus test has not been extensively evaluated in pregnant women.

What should I do if the QFT-Plus result is indeterminate?

When presented with an indeterminate result, physicians may choose to redraw a specimen or perform other procedures, as appropriate. However, an indeterminate QFT-Plus is meaningful, suggesting possible error in performing the test or immunosuppression – particularly in patients with known or suspected immunosuppression, chronic disease, malnutrition or on medications known to decrease immunity. By including an internal positive control (Mitogen tube), QFT-Plus is better at distinguishing between indeterminate results in those prone to immunosuppression and those that are QFT-Plus-negative. In contrast, a negative TST cannot differentiate between individuals who

are truly negative and those who cannot respond to the test due to immunosuppression or incorrect test performance. Due to lack of internal controls in the TST, false negative results are more likely to occur.

How often does QFT-Plus yield an indeterminate result?

QFT-Plus indeterminate results generally occur very infrequently in healthy individuals. In clinical studies for QFT-Plus, the indeterminate rate was less than 2.5% for active TB (17).

However, in populations where the level of immunosuppression is high, past studies of QFT show that indeterminate rates can be correspondingly higher (29, 31, 34–37). An indeterminate response in a highly-immunosuppressed individual is appropriate, as it indicates a measurable immune response is not present. In contrast, the TST which does not have a control could be falsely negative in such individuals – thus not providing any real measure of their infection status.

What is the meaning of Mitogen negative responses in healthy individuals?

In a very small proportion of individuals, indeterminate QFT results may be obtained despite the subject being apparently healthy and immune competent. In most instances, repeating the QFT Plus test with a new blood sample will result in a non-indeterminate QFT result, suggesting that the initial result may have been due to technical reasons (e.g., under-shaking or overfilling of the mitogen tube). However, for a very small proportion of subjects, the repeat test may also be indeterminate. In these rare cases, the reason for the indeterminate result is unclear if immunosuppression and/or technical error are ruled out. However, such a response may be transient and retesting the individual after several weeks may result in a non-indeterminate test result.

Positive QFT-Plus results

Is a patient with a positive QFT-Plus response contagious?

The QFT-Plus test is both a test for LTBI and can be a helpful aid for diagnosing *M. tuberculosis* disease however, the result should never be used to determine contagion or active disease. When a positive result is obtained, a chest x-ray and medical evaluation is necessary to determine whether contagious disease may be present. Microbiological tests should be ordered if the evaluation suggests active TB, a contagious condition. In contrast, if the chest x-ray is normal and the medical evaluation is unremarkable, the patient likely has LTBI, an asymptomatic and noncontagious state of TB infection.

How should a positive QFT-Plus response, without information about a recent contact, be interpreted?

As tuberculosis is an airborne yet cryptic infection, the overwhelming majority of individuals with LTBI do not know how they became infected and cannot confirm contact to persons with active TB. Hence, if the patient has an indication for testing and has a positive result, they should be assumed infected and undergo work up with a chest x-ray and clinical evaluation.

Can the level of a positive QFT-Plus result be used to give an indication of the likelihood of active disease in the future?

QFT-Plus is a qualitative (not quantitative) test of TB infection; With current knowledge from QFT, a higher magnitude of IFN- γ response has correlated with higher rates of disease progression. QFT-Plus research studies are underway to examine the correlation of disease progression with the CD8 IFN- γ response (TB2–TB1 tubes), as well as overall quantitative values.

Can you explain the occasional change in QFT-Plus results for people with responses close to the cutoff when the test is repeated?

A QFT-Plus result, either to TB1, TB2, or both, above the population-derived cutoff (0.35 IU/ml) is meaningful and suggests likely *M. tuberculosis* infection. But on an individual basis, small changes in the level of response between two different testing points should be expected. These changes may be due to the inherent variability of the test itself (<15% CV), variation in the individual's immune response over time, perhaps a laboratory artefact, or a change in their infection status. However, it is difficult to determine if small changes around the test's cutoff are meaningful. For example, a change from 0.34 IU/ml to 0.36 IU/ml is unlikely to have clinical significance, but probably suggests that the person has not changed infection status between testing points.

“Where *M. tuberculosis* infection is not suspected, initially positive results can be confirmed by retesting the original plasma samples in duplicate in the QFT ELISA. If repeat testing of one or both replicates is positive, the individual should be considered test positive.” (38)

For individuals undergoing serial testing and who have values near the cut point, changes from a negative to a positive result, or vice-versa may occur. When, this happens, the final diagnosis and management decisions by the clinician should include the likelihood of interim TB exposure, new health conditions that may affect the immune response, and medical risks that increase the risk of disease progression. Ultimately, diagnosis and treatment decisions should be made in light of all available clinical and historical information. This is similar to interpretation of the TST when repeat test results are available.

Does a positive QFT-Plus result become negative after treatment, i.e. with isoniazid therapy, and if so how quickly does this occur?

In prior studies using QFT-Gold In-Tube, among individuals who have had isoniazid therapy for LTBI, data suggest that responses decline with time but still remain above the test cutoff for a high proportion of individuals (39–41). Thus, the current level of evidence does not support using QFT Plus for monitoring the response to treatment for those with LTBI.

In individuals who are given multidrug therapy for active TB, a drop in QFT response (or a change to a negative result) with treatment is often observed, it is currently not known if this has any association with clearance of *M. tuberculosis* (see next question) or prognosis of the patient. However, a small QFT-Plus study undertaken in Japan among active pulmonary patients showed that the CD8 response of the TB2 tube continues to decline throughout treatment whereas, the CD4 response declined initially, but not significantly after 3 months (41). However, more evidence is needed and not all patients with active TB have a significantly higher CD8 response initially.

What does a positive QFT-Plus result mean in patients treated for active disease a long time ago?

A positive QFT-Plus result in someone with prior treatment for TB disease may be due to a variety of reasons. It could simply represent a memory response, continued presence of *M. tuberculosis* infection or reinfection. It is unclear at this time whether the presence of a high CD8 response will help in stratifying the patient's risk of future disease. However, as previously mentioned, QFT-Plus does not differentiate active disease from LTBI and may even remain positive for a considerable period of time in individuals who have a clinical response to treatment. Overall, an individual who has been treated for active TB a long time ago and now tests positive by QFT-Plus may have been re infected – or may still carry their old infection – and should be clinically evaluated for active TB.

Should I expect the TB2 tube quantitative value to always be higher than the TB1 response? Can you explain why the TB1 response may be higher on occasion?

The TB2 antigen tube of QFT-Plus has additional peptides optimized for stimulating CD8 cells specific for TB compared to the TB1 tube that contains only CD4 optimized antigens. Although one might expect a consistently larger response from TB2 because there are more antigens, this is not necessarily the case because the CD8 response should be self-limited in the TB immune response and CD8 T cell effector cell function should be more readily detected when intracellular TB burden is higher. Hence, near equal responses among the TB1 and TB2 tubes should be expected in remote TB infection where intracellular burden may be low and/or controlled, as opposed to recent infection. Treatment of active TB or LTBI or low-burden TB disease conditions such as TB lymphadenitis or pleural disease may also be conditions where CD4 responses dominate. In the registration trials and recent publications by Barcellini, et al. on QFT-Plus, the isolated CD8 response was calculated by subtracting the quantitative values of TB1 from TB2 and found to be enhanced in the following conditions:

- Frequently in active untreated pulmonary tuberculosis (11, 38).
- Among some persons with higher risk for TB exposure (38).
- Among some contacts who had higher association to cumulative exposure and being European-born (as opposed to being born in higher burden settings) (8).

Possible causes of noticeably higher TB1 than TB2 tube values:

- Technical error.
- Biological variability of gamma interferon between tubes when high background Nil values are present.
- CD8 suppressor cell presence.

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QFT-Plus is an in vitro diagnostic aid for detection of *Mycobacterium tuberculosis* infection. It uses a peptide cocktail simulating ESAT-6 and CFP-10 proteins to stimulate cells in heparinized whole blood. Detection of interferon- γ (IFN- γ) by ELISA is used to identify in vitro responses to these peptide antigens that are associated with *Mycobacterium tuberculosis* infection. QFT-Plus is an indirect test for *M. tuberculosis* infection (including disease) and is intended for use in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations.

QFT Package Inserts, available in multiple languages, as well as up-to-date licensing information and product-specific disclaimers can be found at www.QuantiFERON.com.

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