FAQs for Health Professionals

QuantiFERON®-TB Gold
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Questions and answers

QuantiFERON-TB Gold (QFT®) 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, 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.

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 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 well contained by the host’s immune system. Hence, unlike active TB, individuals with LTBI are asymptomatic, and not contagious to others. However, this condition may progress or reactivate to active disease in the future. As the development of TB disease depends on a variety of risks and medical conditions, individuals with LTBI are commonly offered preventative therapy to prevent active disease from occurring. Preventative treatment is an important strategy to reduce TB morbidity and rates in many countries.

Active TB is a disease state of uncontrolled M. tuberculosis growth which 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 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 infectious.

QFT is an assay that detects TB infection by measuring the cell mediated immune response to TB-specific antigens. It can be used as a diagnostic aid for M. tuberculosis complex infection, whether active tuberculosis disease or LTBI, however, when using QFT in a person suspected of having active TB, it should not replace appropriate microbiological and molecular investigation. QFT cannot distinguish between active and latent TB infection and should therefore never be used as a sole diagnostic test.
What is the meaning of remote TB infection and can QFT distinguish between remote and recent infection?

The term remote infection is an ill-defined term that is increasingly being used in the TB community. For most, it appears that remote infection relates to old TB infection that may have been cleared by the individual, however, some may interpret it as meaning old TB infection that can still reactivate to TB disease. As with the TST, QFT cannot distinguish between remote and new infection. Individuals with LTBI are asymptomatic, and not contagious to others. However, this condition may progress or reactivate to active disease in the future. As the development of TB disease depends on a variety of risks and medical conditions, individuals with LTBI are commonly offered preventative therapy to prevent active disease from occurring. Preventative treatment is an important strategy to reduce TB morbidity and rates in many countries.

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 contagious disease.

Diagnosing LTBI, and preventive treatment, 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).

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 risks present. The purpose of TB screening is to find cases at an early asymptomatic phase that is 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 investigation 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 and preventive treatment of those at risk of developing disease in that setting.

Congregate settings may include:

- hospitals/healthcare institutions
- residential facilities
- prisons/correctional facilities
- renal dialysis units
- homeless shelters
- higher educational facilities
- military barracks
- certain settings of employment such as the mining industry.

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 multi-drug 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 list below).

Medical risks of reactivation

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

Chart 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. Similarly, patients with TB infection should be targeted for LTBI treatment before initiation of immune suppressive therapy.

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.

Is latent TB contagious?

No, TB in its latent form cannot spread. However, it can become active pulmonary TB, which is contagious, often before the individual is aware that they have it.

Doesn’t everybody in high incidence countries have latent TB?

No, this is a common misconception. 1 in 3 people worldwide is thought to be infected with LTBI, although there is significant variance in high incidence countries based on the demographics of the population being studied.

About QFT

What is QFT?

QuantiFERON-TB Gold (QFT) is an in vitro laboratory test that measures responses to TB-specific peptide antigens in whole blood. It is an indirect test for M. tuberculosis infection. A modern replacement to the tuberculin skin test (TST), QFT provides clinicians with an accurate, reliable and efficient tool for aiding the diagnosis of TB infection.

QFT is highly specific and sensitive; a positive result is strongly predictive of true infection with M. tuberculosis. However, like the TST and other Interferon-gamma release assays (IGRAs), QFT cannot distinguish between active tuberculosis disease and LTBI, and is intended for use in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations.

What is QFT’s intended use?

QFT is an in vitro laboratory diagnostic test using a whole blood specimen. It is intended for use as a diagnostic aid for M. tuberculosis complex infection, whether active tuberculosis disease or LTBI, and is intended for use in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations. Like the TST or any diagnostic aid, QFT should never be used as a stand-alone test for diagnosis or treatment of active tuberculosis, and a negative QFT result should be used with caution when the patient is suspected of having active TB, or is immunosuppressed. Like any other diagnostic aid, QFT cannot replace clinical judgement.
What are the clinical situations in which QFT can be used?

QFT can be used for those being evaluated for possible *M. tuberculosis* infection, whether active disease or LTBI. According to the US Centers for Disease Control and Prevention (CDC), (4) QFT can be used in many situations:

**CDC Specific Recommendations**

- IGRAs may be used in place of (but not in addition to) a TST in all situations in which the CDC recommends TST as an aid in diagnosing *M. tuberculosis* infection.

- IGRAs is preferred for testing persons from groups that historically have poor rates of return for TST reading.

- IGRAs is preferred for testing persons who have received Bacille Calmette-Guerin (BCG) (as a vaccine or for cancer therapy).

- 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 (eg. 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 the relatively few published reports documenting the performance of IGRAs in young children. However use of an IGRA in conjunction with TST may increase diagnostic sensitivity in this age group.

- While routine testing with both TST and an IGRA is not recommended, results from both tests may be useful in the following situations when the initial test is NEGATIVE:
  - when the risk of infection, the risk of progression, and the risk of a poor outcome are increased (such as when persons with HIV infection, or children <5 years old are at increased risk for *M. tuberculosis* infection), or
  - when there is clinical suspicion of active tuberculosis (such as in persons with symptoms, signs, and/or radiographic evidence suggestive of active tuberculosis) and confirmation of *M. tuberculosis* infection is desired.

- While routine testing with both TST and an IGRA is not recommended, results from both tests may be useful in the following situations when the initial test is POSITIVE:
  - additional evidence of infection is required to encourage compliance (such as in foreignborn healthcare workers who believe their positive TST is due to BCG); or
  - in healthy persons who have a low risk of both infection and progression.

- Repeating an IGRA or performing a TST may be useful when the initial IGRA result is indeterminate and a reason for testing persists.
• Decisions should not be based on IGRA or TST results alone. A diagnosis of *M. tuberculosis* infection, and the decisions about medical or public health management should include epidemiological, historical, and other clinical information when using IGRA or TST results.

• Persons with a positive TST or IGRA result should be evaluated for the likelihood of *M. tuberculosis* infection, for risks of progression to tuberculosis disease if infected, and for symptoms and signs of tuberculosis disease.

• Neither an IGRA nor TST can distinguish LTBI from TB disease. A diagnosis of LTBI requires that tuberculosis disease be excluded by medical evaluation, which should include checking for suggestive symptoms and signs, a chest radiograph, and, when indicated, testing of sputum or other clinical samples for the presence of *M. tuberculosis*.

• In persons with symptoms, signs, or radiographic evidence of TB disease, and in those at increased risk of progression to tuberculosis disease if infected, a positive result with either an IGRA or TST may be taken as evidence of *M. tuberculosis* infection. However, negative IGRA or TST results are not sufficient to exclude infection in these persons, especially in those at increased risk of a poor outcome if disease develops, and clinical judgment dictates when and if further diagnostic evaluation and treatment are indicated.

• Both the standard qualitative test interpretation and the quantitative assay measurements should be reported, together with the criteria for test interpretation.

• As with the TST, IGRAs generally should not be used for testing persons who have a low risk of infection and a low risk of disease due to *M. tuberculosis*.

• IGRAs or TST should be used as aids in diagnosing infection with *M. tuberculosis*. These tests may be used for surveillance purposes or to identify persons who are likely to benefit from treatment.

• IGRAs should be performed and interpreted according to established protocols using FDA-approved test formats. IGRAs should be performed in compliance with Clinical Laboratory Improvement Amendment (CLIA) standards.

• For BCG-vaccinated persons who are not at increased risk for developing TB if infected, TST reactions <15mm may be reasonably discounted as false positives if the individual has a clearly negative IGRA result.

• If two different tests are performed, a positive result from either test should be taken as evidence of infection for those with suspected active TB or at high risk of progression.

Can QFT distinguish between active TB and LTBI?

Like the TST and other IGRA tests, QFT cannot distinguish between active TB and 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 measures cell-mediated immune (CMI) response in TB-infected individuals. T-cells of these individuals are sensitized to TB, and respond to stimulation with peptides simulating those expressed by the TB causing bacteria by secreting a cytokine called interferon-gamma (IFN-\(\gamma\)).

QFT uses peptides from three proteins made almost exclusively by \(M.\) \textit{tuberculosis} and the other mycobacteria of the tuberculosis complex. Those proteins are absent from \textit{all} BCG vaccine preparations and from most non-tuberculous mycobacteria (NTM) (with the exceptions of \(M.\) \textit{kansasii}, \(M.\) \textit{marinum}, and \(M.\) \textit{szulgai}) (5).

Special blood collection tubes coated with peptides from these three TB antigenic proteins are used for blood collection and incubation of the patient's blood. IFN-\(\gamma\) is released when the blood from infected individuals is incubated with the antigens (16–24 hours at 37°C). This is not the case for individuals free from infection. An ELISA laboratory test is used to detect and quantify the amount of IFN-\(\gamma\) that has been released.

Why measure interferon-gamma?

\(M.\) \textit{tuberculosis} is an intracellular pathogen primarily residing within macrophages. During the latent phase of the infection little—if any—antigen is expected to leave the macrophages to be available to B-cells to stimulate a humoral antibody response. However processed antigen is presented by infected macrophages to antigen-specific T-cells and triggers a cascade of immune responses leading to the generation of specialized effector T-cells, which will circulate in the individual's blood stream.

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

How does QFT differ from the TST?

\textbf{Sensitivity and specificity}

The tuberculin purified protein derivative (PPD) used in the TST is an ill-defined mix of proteins and protein fragments, of which some are specific for \(M.\) \textit{tuberculosis} complex. 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.

The TST assesses in vivo delayed-type hypersensitivity (Type IV), whereas QFT measures in vitro release of IFN-\(\gamma\). The TST measures response to PPD, a polyvalent antigenic mixture, whereas QFT measures responses to a well defined mix of synthetic peptides simulating three antigenic proteins that are specific for tuberculosis.

Unlike the TST, an uninfected individual is not subject to boosting with QFT. Moreover, QFT is not confounded by BCG vaccination and most common environmental NTM (except \(M.\) \textit{kansasii}, \(M.\) \textit{marinum}, and \(M.\) \textit{szulgai}).
Handling and interpretation

There are numerous differences between the TST and QFT:

- The TST requires skill in placing PPD, whereas QFT requires routine phlebotomy.
- The TST requires a person to return to have their test read 48 to 72 hours after administration. QFT 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 cut-off to apply. QFT is an objective, laboratory based, test with interpretation determined by analysis of ELISA data by QFT analysis software.
- Positive QFT 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.

How long does it take to get QFT results?

This varies and depends on how frequently the laboratory in your area carries out the test. Results can be available in 24 hours. 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 result?

There is some conflicting evidence that suggests that a prior TST can induce a positive QFT result in an uninfected individual. One paper, (6) based on the results from only 3 individuals, speculates that boosting does occur, but this has not been supported in much larger studies (7, 8). Reports by Leyten et al (7) and Richeldi et al (8) clearly demonstrate that a TST placed 3 days prior to QFT, and up to 12 weeks later does not induce positive responses in those uninfected. The largest study on the effect of the TST on a QFT response was part of a CDC/US Military study in Navy recruits. Data from this study, reviewed by the US FDA and presented in the QFT Package Insert, found that in 530 subjects tested twice, 4 to 5 weeks apart, the reproducibility of QFT was 98.5%. Five recruits changed from positive to negative and 3 became QFT positive.

The above findings are in agreement with the general knowledge of how an immune response is generated. It would not be expected that uninfected individuals would mount a primary cellular immune response to the extremely small amounts of the TB-specific antigens used in QFT that are present in the aqueous tuberculin injected. However, it is possible that even very small amounts of ESAT-6, CFP-10 and TB7.7 (p4) may be present in tuberculin and could boost responses of individuals infected with M. tuberculosis, but not from BCG vaccination. There is evidence of this possibility in the studies published to date. To avoid a boosted response, QFT should be administered concurrently, or no later than 3 days after a TST (9).

In contrast to QFT, boosting is a common phenomenon when a TST is repeated. Injection of tuberculin for the 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 testing?

Available data suggests that QFT returns a positive result at least as quickly as the TST following recent infection. A Japanese study concluded that the standard 3 month follow-up used for the TST should be used for QFT. In that study, individuals were tested at the time of diagnosis of the index, and at 2, 3, 4 and 6 months. Of those who developed positive responses, 2 contacts were positive at the time of diagnosis of the index, 5 more were positive at 2 months and 1 more at 3 months (10). In a contact investigation of Swiss military recruits, 14 out of 15 contacts were positive when tested 8 weeks after exposure (11).

The CDC guidelines on the use of QFT recommend that recent contacts who test QFT 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.

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

The Mitogen tube is used as an IFN-\(\gamma\) 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-P (PHA), which is a non-specific stimulator of T-cells. While it is a direct activator of T-cells, unpublished data suggest that macrophages are also required for it to activate T-cells.

A low IFN-\(\gamma\) 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-\(\gamma\).

What approvals does QFT have?

QFT is approved for use by the FDA and has been CE marked, allowing it to be sold freely in the EU. The test has also been granted regulatory approval in Japan, Canada, Korea and many other countries. Some countries do not require regulatory approval of in vitro diagnostic tests.

What is the evidence supporting QFT?

- Over 1100 publications in numerous international journals support the use of QFT in different clinical settings.
- For a complete and up to date list of clinical papers and guidelines, please refer to [www.gnowee.net](http://www.gnowee.net), the online QuantiFERON library.
Sensitivity and specificity of QFT

What is the specificity and sensitivity of QuantiFERON-TB Gold?

The specificity of QuantiFERON-TB Gold has consistently shown to be >99% in low risk individuals (12). 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 is as high as 92% in individuals with active disease, but varies depending on the setting and extent of TB disease (12). 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, however there is no gold standard for diagnosing LTBI. All screening tests are designed to identify the possibility that a disease might 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 4 times better than TST in detecting the individuals who will progress to active TB disease (13), and this combined with its >99% specificity, provides confidence that QFT 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 has been shown to have >99% specificity compared to lower than 70% for the TST in some settings.

In many countries, targeted testing policies are in place to screen individuals who are at increased risk of having LTBI (such as those mentioned on page 7). Without high specificity in these 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.

QFT use in children and the immune–suppressed

Can IGRA tests be used for infants and children?

Evidence shows 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 (14-18). For detection of LTBI, QFT is as sensitive as the TST, and more specific (17). In a study of children who lived in close contact with smearpositive adult TB patients, QFT detected more children infected with TB than did the TST. Positive QFT results showed significant correlation with smear status of the infected adults, whereas TST did not (17). QFT has also been shown to be more accurate than the TST in detecting who will progress to active TB disease with very high accuracy among pediatric contacts (13).

Disclaimer: The performance of the USA format of the QFT test has not been extensively evaluated with specimens from individuals younger than age 17 years.
QFT has been shown to be effective in children less than 6 months of age and in children with bacteriologically confirmed TB (the sensitivity of QFT was 93%) (16). However, caution is always needed when interpreting a negative result in a young child suspected of having active TB.

What is the sensitivity of QFT in HIV positive individuals?

Studies suggest that QFT is more sensitive and specific than the TST for detecting *M. tuberculosis* infection in HIV positive people (19–21). In an HIV / TB co-infected population, the sensitivity of QFT is 63–85% compared to 15–46% for the TST (22, 23).

In HIV infected /AIDS patients with CD4⁺ T-cell counts less than 200/μl, there may be an increase in the number of people who do not respond to the Mitogen positive control (19, 24). These people are deemed indeterminate by QFT, which means a result cannot be given—which is appropriate if they do not have a sufficient immune response to measure. Most people with indeterminate responses due to low CD4⁺ T-cells counts test negative by the TST (which does not have a control for immune status). Indeterminate results are usually treated as negative in sensitivity estimates, meaning that in studies of patients with low CD4⁺ counts, lower sensitivity estimates will be reported.

What about indeterminate results in HIV positive individuals?

Studies to date have shown that indeterminate QFT results are more prevalent in individuals with a CD4⁺ count <200/μl, especially when the CD4⁺ count is under 100/μl (19-21, 25) Indeterminate results are likely more frequent in HIV patients with active TB than LTBI (25). Individuals with a CD4⁺ count <100/μl are severely immune suppressed and the TST is also generally negative in these individuals, independent of infection status.

Disclaimer: The performance of the USA format of the QFT 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 procedure**

What are the steps in administering the test?

1. It is best to confirm arrangements for testing with a qualified laboratory, which can deliver the necessary sampling pack.

2. Draw a 1 ml sample of blood from a patient directly into each of the three blood collection tubes, following the manufacturer’s instructions. The black mark on the side of the tubes indicates the 1 ml fill volume. QFT blood collection tubes have been validated for volumes ranging from 0.8 ml and 1.2 ml. If the level of blood in any tube is not close to the indicator line, it is recommended to obtain another blood sample. As a guide, the picture on the left illustrates the approved fill range.

3. Assure delivery to the laboratory for incubation as soon as possible (and within 16 hours) after blood draw. Keep at room temperature (22±5°C) before incubation. Or, 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.

For comprehensive instructions for use, refer to the QFT ELISA package insert.
Do the QFT 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, TB Antigen, and then Mitogen.

Why can filling of the tubes occur slowly?

The 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 2,650 ft) the tubes will not draw sufficient blood (sufficient is close to the indicator line of the tube label). In these situations either use a high altitude QFT tube (QFT-HA) for altitudes between 1,020 m (3,350 ft) and 1,875 m (6,150 ft), or if outside of these altitudes, blood can be collected using alternate collection methods, as described in the QFT Package Insert.

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

As the tubes only collect 1 ml of blood, thorough mixing is essential to solubilize the tubes’ contents, 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-energetic shaking may cause gel disruption and could lead to aberrant results.

What is the effect of incubating the tubes for longer than the recommended time (ie. if accidently left over the weekend)?

Clinical studies conducted to develop the test cut-off for QFT incubated the tubes for 16–24 hours (as recommended in the QFT Package Insert). Incubating the tubes over 24 hours has not been validated by clinical studies and should be avoided. Another sample will need to be collected.

Interpretation of test results

How are QFT 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 result is an essential component. In some situations results are provided numerically (a value of 0.35 IU/ml and above is defined as a positive result), however the QFT test is a qualitative test of infection. Some pathology providers will choose to report QFT results as positive, negative, or indeterminate whereas others will also report IU/ml values.

- A positive QFT 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 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 immune suppression).
• 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.

How was the cut-off 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 cut-off, then the other is decreased at the same time. Thus a cut-off was selected that gave the best combination of sensitivity and specificity.

The primary test cut-off for QFT (TB antigen response – Nil ≥0.35 IU/ml) was established through Receiver Operator Characteristic (ROC) curve analysis of data from low risk BCG-vaccinated individuals for specificity, and from patients with culture confirmed *M. tuberculosis* infection for sensitivity (26).

Can the amount of IFN-γ measured be correlated to the stage or degree of TB infection?

Individuals displaying a response to the TB Antigen greater than or equal to 0.35 IU/ml above the Nil control, are likely to be infected with *M. tuberculosis*. No definitive correlation between their response to these antigens and the stage or degree of infection, their level of immune responsiveness, or their likelihood for progression to active disease can currently be made.

What constitutes a QFT conversion?

QFT 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, especially when there is a significant increase in quantitative values. Existing CDC guidelines define a QFT conversion as a ‘change from negative to positive’ (4). This definition is routinely used in situations where populations are serially screened with QFT (such as HCW 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 cut-point to define QFT 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—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 result (ie. no apparent risk factors), it is recommended to confirm the result by retesting the plasma samples in duplicate in the QFT ELISA and use the consensus from the 3 test results. From a medical management perspective, the CDC guidelines state ‘repeat testing, with either the initial test or a different test, may be considered on a case-by-case basis’, especially when there is a low probability of TB infection and risk of disease progression (4).
How reproducible are QFT results?

Reproducibility has been studied in low risk individuals, those at high risk of infection, and in HIV infected subjects. Reproducibility of the test system from plasma to plasma and with multiple blood samples is part of the test validation for regulatory approval, and has been demonstrated as very high. Comparison of results obtained at 3 different laboratories, over 3 different days and with 3 different operators found variations of less than 8.7% CV (co-efficient of variation), on average in the IFN-γ response between testing sites, day of performance, between ELISA plates, and within ELISA plates.

An equally important clinical question is the reliability of the result when subjects are tested sequentially. Data from low risk individuals shows that reproducibility in such situations is very high (>98%). In an unpublished but FDA-reviewed study (see QFT Package Insert USA), of 530 Navy recruits, who were retested 4 to 5 weeks after initial QFT and TST testing, QFT reproducibility was 98.5% (522/530). Five (0.9%) individuals changed from positive to negative, while 3 (0.6%) changed from negative to positive and there was no evidence of the TST inducing positive QFT responses. In this same study TST reproducibility was lower—94.7% (520/549) if using a 5 mm cut-off and 97.4% (535/549) using a 10 mm cut-off, however there were 8 and 14 reversions, respectively.

A complicating factor in sequential testing is the period between testing. Short periods (a few weeks) and low TB risk environments allow less chance of infection in the intervening period or for natural or drug-induced resolution of the infection, which may decrease IFN-γ response to the TB antigens. Leyten et al demonstrated that reproducibility of results for both QFT positive and negative individuals was high when retested three days after having a skin test placed (7).

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 immune suppressed 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 IFNγ and/or IL-12. Others may develop active TB as a result of iatrogenic immune suppression, for example individuals taking anti-TNFα medications.

Studies evaluating the sensitivity of QFT in developed world settings (12, 16, 27) demonstrate a higher sensitivity for QFT than when evaluated in developing world populations (28–30). It is likely this reflects the variables mentioned above, almost all of which are more prevalent in the developing world.

It is important to note that QFT is a test for M. tuberculosis infection and is approved as a diagnostic aid for detection of M. tuberculosis infection (whether active TB disease or LTBI). Clinicians may use QFT to assist in the diagnosis of active TB (in conjunction with risk assessment, radiography and other medical and diagnostic evaluations). A negative QFT result in a person with obvious symptoms of active TB should by no means be considered definitive. 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. Studies show that IGRA perform equally well in each trimester with comparable results to non-pregnant women (31). When compared with the TST, QFT is more specific, and at least as sensitive in cross-sectional or longitudinal studies.

What should I do if the QFT 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 is meaningful, suggesting possible error in performing the test or immune suppression - 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 can enable the distinction between indeterminate results in those prone to immunosuppression and those that are truly QFT negative. In contrast, a negative TST does not differentiate between those individuals who cannot respond to the test due to immune suppression or incorrect test performance and those who have a truly negative TST.

How often does QFT yield an indeterminate result?

QFT indeterminate results generally occur very infrequently in healthy individuals. In clinical studies submitted to the FDA for approval of QFT, the indeterminate rate was less than 2% (12).

However, in populations where the level of immune suppression is high, indeterminate rates can be correspondingly higher (19, 21, 32–34). An indeterminate response in a highly immune-suppressed individual is appropriate as it indicates a measurable immune response is not present. In contrast, the TST would likely be 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 test with a new blood sample will result in a non-indeterminate QFT result, suggesting that the initial result may have been due to operational difficulties. 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 immune suppression and/or technical error are ruled out. However, such a response may be transient and retesting the individual after a period of a few weeks may result in a non-indeterminate test result.
Positive QFT results

Is a patient with a positive QFT response contagious?

The QFT test is both a test for LTBI and a helpful aid for diagnosing *M. tuberculosis* infection in sick patients where there is clinical suspicion of active TB disease. A positive result supports the diagnosis of TB disease; however, it does not differentiate between recently acquired or old infection, or between LTBI and active tuberculosis. If active TB is suspected, other diagnostic evaluations are necessary to confirm TB disease (e.g. culture of *M. tuberculosis*) and the patient should be considered at risk of transmitting TB disease.

A person with a positive QFT test result—but with no symptoms compatible with active TB—likely has LTBI and is not contagious. However, all people who return a positive QFT test result should undergo clinical evaluation for active TB before they can be assumed infectious or not.

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

A positive QFT result is meaningful and even without history of recent contact indicates that *M. tuberculosis* infection is very likely. However, QFT does not differentiate between recently acquired or old infection, or between LTBI and active tuberculosis. Additionally, infections by other mycobacteria (e.g. *M. kansasii*) can also potentially lead to positive results. As with the TST, a positive QFT response should be not be interpreted in isolation but in conjunction with risk factors.

In this situation the person with a positive QFT result may have been infected some time ago and thus have a positive response. However, exposure to someone with active TB may not always be recognized by a person testing positive, and this is one of the factors to be taken into account by the clinician.

Does a positive QFT mean there is a greater risk of progressing to active TB than does a positive TST?

In a landmark study published in the American Journal of Respiratory and Critical Care Medicine, QFT had a predictive value for developing TB disease of 12.9%, more than 4 times greater than the 3.1% for the TST (13). In this study, both TST and QFT were used in a TB contact investigation involving 954 individuals. 66.3% (604) had a positive TST, but only 20.8% (198) of the exposed individuals were QFT positive. Of the QFT positive patients who completed preventative treatment (n=51) none progressed to active TB. The study followed patients for two years post testing, and 19 patients (all untreated) developed active TB disease. QFT had detected all 19 and the TST only 17 (cut-point 5 mm). There were 413 contacts who were TST positive, but QFT negative, and none of these developed TB.
Further, the progression rate was 28.6% (6 of 21) for QFT-positive children and significantly higher than 10.3% (13 of 126) for adults.

This German study builds on a previously published work by Higuchi et al which showed that after 3.5 years of follow-up, none of 91 QFT negative (but TST positive) contacts had developed TB disease [35]. This indicates that the risk of progression of QFT negative individuals in this BCG-vaccinated population is low, even if they are TST positive.

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

QFT is a qualitative (not quantitative) test of TB infection. With current knowledge, the magnitude of IFN-γ response cannot be correlated to stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease.

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

A QFT result above the population-derived cut-off (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 cut-off 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.” [12]

If, in serial testing, a person changes from a negative to a positive result, this result is an aid to the clinician making the final diagnosis and possible treatment decision. Ultimately, diagnosis and treatment decisions should be made in light of all available clinical and historical information. This is akin to interpretation of the TST when repeat test results are available.
Does a positive QFT result become negative after treatment, i.e. with Isoniazid therapy, and if so how quickly does this occur?

Among individuals who have had Isoniazid therapy for LTBI, data suggest that QFT responses decline with time but still remain above the test cut-off for a high proportion of individuals (37–39). Thus the current level of evidence does not support using QFT for monitoring the response to treatment for those with LTBI.

In individuals who are given multi-drug therapy for active TB, QFT responses appear to drop more significantly and many (up to 70%) do become QFT negative (unpublished). In one study, after eight months of treatment there was a significant decrease in QFT responses in all patients, with 57% (17/30) becoming QFT negative. Three of 13 patients with a positive response at the end of the eighth month continued to have microbiological isolation and absence of clinical improvement of disease (40).

While 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.

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

A positive QFT result is meaningful and, even in patients treated for active disease a long time ago, suggests *M. tuberculosis* infection is likely to be still present. However, as previously mentioned, QFT does not differentiate active disease from LTBI and may even remain positive for a considerable period of time in individuals who have cleared their infection. Overall, an individual who has been treated for active TB a long time ago and now tests positive by QFT may have been re-infected—or may still carry their old infection—and should be clinically evaluated for active TB.
References

References (continued)


QFT has been CE marked. QFT is approved by the US FDA

QFT is approved by FDA as an in vitro diagnostic aid for detection of Mycobacterium tuberculosis infection. It uses a peptide cocktail simulating ESAT-6, CFP-10 and TB7.7(p4) proteins to stimulate cells in heparinized whole blood. Detection of IFN-γ by ELISA is used to identify in vitro responses to these peptide antigens that are associated with M. tuberculosis infection. FDA approval notes that QFT 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 results alone cannot distinguish active TB from infection. 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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