QFT®
QuantiFERON-TB Gold

Frequently Asked Questions

Health Professionals

cellestis

QuantiFERON®-TB Gold In-Tube (QFT®) is an innovative whole-blood test that measures the cell-mediated immune response of tuberculosis (TB) infected individuals.

Approved by the FDA—and CE marked—QFT, like the 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.

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 Package Inserts, available in up to 25 different languages, can be found at **www.cellestis.com.**

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Questions/Answers

About TB

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

Latent TB infection (LTBI) is when an individual carries the bacteria that cause TB in their body, but shows no symptoms. Such individuals are infected with *M. tuberculosis*, but do not have active TB disease. In individuals diagnosed with LTBI there is a chance that the bacteria may cause disease in the future; so they are likely to be offered treatment to prevent this from happening.

Active TB is when the person has symptoms (eg. cough, night sweats and weight loss). QFT can be used as an aid to diagnosing active TB disease, however it should not replace appropriate microbiological and molecular investigation. Culture remains the gold standard for confirming active TB disease.

What is the meaning of remote TB 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 has been cleared by the individual, however some may interpret it as meaning old TB infection that can still reactivate to TB disease.

What's the difference between Effector T-cells and Central Memory T-cells?

Effector T-cells are those present during infection and—for *M. tuberculosis*—are the main component of the immune response. These cells respond rapidly to stimulation with antigen and—among other things—secrete IFN-γ (importantly, within the 16 to 24 hour culture period used for QFT). For specific effector T-cells to be maintained, they require presence of the antigen they respond to. If a person clears their *M. tuberculosis* infection, such that there is no antigen to support maintenance of the effector T-cells, these cells will undergo apoptosis (cell death) or will differentiate into central memory T-cells.

Central memory T-cells are long-lived and are one of the immune system's methods for storing immune responses to past infections. If a person is re-infected with the organism that these central memory T-cells are specific for, they will differentiate into effector T-cells to rapidly fight the infection. However this process takes more than 24 hours. Central memory T-cells, when stimulated with their specific antigen *in vitro,* do not produce significant amounts of IFN-γ within the maximum 24 hour culture period used for QFT.[15]

The above differences in the ability of effector and central memory T-cells to produce IFN-γ when stimulated with antigen in the QFT assay account for why the QFT test detects current infection, but likely does not detect old/cured infection.

About QFT

What is QFT?

QuantiFERON-TB Gold is an *in vitro* laboratory test for detection of immune responses to tuberculosis (TB) infection in whole blood. It is an indirect test for *Mycobacterium tuberculosis* infection. A modern replacement to the tuberculin skin test (TST), QFT provides clinicians with an accurate, reliable and efficient tool for 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, 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 its 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.

What are the clinical situations in which QFT can be used?

QFT can be used in adults and children who are being evaluated for possible *M. tuberculosis*-complex infection, whether tuberculosis disease or LTBI. According to the US Centres 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.
- IGRA is preferred for testing persons from groups that historically have poor rates of return for TST reading.
- IGRA is preferred for testing persons who have received 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) with special considerations regarding conversions and reversions (see full guideline[1]). Two-step testing is not required since 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 foreign-born 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.

How long does it take to get the results back?

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

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 using clinical examination and/or a chest radiograph.

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-γ).

QFT uses peptides from three proteins made almost exclusively by *M. tuberculosis* and the other mycobacteria of the tuberculosis complex. 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*).[3]

Special blood collection tubes coated with these three TB antigenic proteins are used for blood collection and incubation of the patient's blood. IFN-γ 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 of infection. An 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 phagosomes of macrophages. During the latent phase of the infection when mycobacteria are largely within the macrophages, little—if any—antigen is expected to leave the macrophages and 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 series of immune responses that lead to the generation of specialized effector T-cells. Thus measurement of a CMI response rather than an antibody response to *M. tuberculosis* antigens provides a sensitive means of detecting LTBI.

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 single-step ELISA forms the basis of QFT.

How does QFT differ from the TST?

The tuberculin purified protein derivative (PPD) used in the TST is an undefined cocktail of proteins and protein fragments, of which some are specific for *M. tuberculosis* complex. But, the vast majority have homologs or are shared with many other bacteria, environmental mycobacteria and BCG vaccine strains. It is largely for this reason that the Mantoux 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-γ. The TST

measures response to PPD, a polyvalent antigenic mixture, whereas QFT measures response to a mixture of synthetic peptides simulating three specific antigenic proteins that are present in PPD.

Unlike the TST, QFT is not subject to boosting, is unaffected by the Bacille Calmette-Guérin (BCG) vaccination and most environmental non-tuberculous mycobacteria (except *M. kansasii*, *M. marinum*, and *M. szulgai*).

The TST is subjective in its interpretation—in respect to both measuring the bump on someone'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 computer software. 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 worker for blood collection.

Does a prior TST influence a QFT result?

There is no evidence to suggest that a prior TST can induce a positive QFT result in an uninfected individual. Reports by Leyton *et al* [23] and Richeldi *et al* [33] 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. One paper [24] based on the results from only 3 individuals speculates that boosting does occur, but this has not been supported in much larger studies.[23,33] 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 for uninfected individuals to 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*. There is some evidence of this possibility in the studies published to date, but the increases in QFT responses are only small (when present) and may be unrelated to prior TST.[23,25,33]

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, primarily in persons who have been infected with NTM or vaccinated 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 that 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.[31] In a contact investigation of Swiss military recruits, all who developed a QFT positive response (14 of 15) 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 soon 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 can be used with the QuantiFERON-TB Gold In-Tube test as an IFN-γ positive control for each specimen tested. This may be especially warranted where there is doubt as to the individual's immune status. 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-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, incorrect filling/mixing of the Mitogen tube, or inability of the patient's lymphocytes to generate IFN-γ.

What approvals does QFT have?

QFT is approved for use by the US Food and Drug Administration (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 500 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**, the online QuantiFERON library.

Sensitivity and specificity of QFT

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

The **specificity** of QuantiFERON-TB Gold In-Tube has consistently shown to be >99% in low risk individuals (QFT In-Tube Package Insert). Specificity is the probability that the test indicates a person does not have the disease when that person is disease free.

The **sensitivity** of QuantiFERON-TB Gold In-Tube is as high as 92% in individuals with active disease, but varies depending on the setting and extent of TB disease.[1] Sensitivity is the probability that the test indicates a person has the disease 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 6 times better than TST in detecting the individuals who will progress to

active TB disease [2], 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 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 western countries, targeted testing policies are in place to screen individuals who are at increased risk of having LTBI (such as those mentioned in the section above). Without high specificity in these situations there will often be more false positive than true positive results, and most people treated with Isoniazid (INH) will be receiving a drug 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 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.[5-8] For detection of LTBI, QFT is at least as sensitive (and certainly more specific) as the TST.[8] In a study of children who lived in close contact with smear-positive 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.[8]

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%).[7]

What is the sensitivity of QFT in HIV positive individuals?

The sensitivity of QFT (HIV/TB co-infected) is 63–85% vs 15–46% for the TST.[9,30]

In HIV/AIDS patients with CD4+ T-cell counts less than 100/µL, there may be an increase in the number of people who do not respond to the Mitogen positive control.[10] These people are deemed Indeterminate by QFT, which means a result cannot be given—which is appropriate if they do not have 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 <100/µL.[10,22,27] Data from the same studies suggest that QFT is more sensitive and specific than the TST for detecting *M. tuberculosis* infection in HIV positive people. 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.

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

   Or alternatively: At the collection site, incubate the tubes standing upright for 16 to 24 hours at 37ºC before shipping them to the laboratory at room temperature (or refrigerated) within 3 days.

Is there an order with respect to collecting the blood in the QFT tubes?

If you are only collecting blood for TB testing, it is advisable to collect 1 mL of blood in the Nil tube followed by the TB antigen tube and finally the Mitogen tube. However if you are also collecting blood for other tests at the same time, the correct order relative to these other tubes may depend on what other tests you are collecting blood for.

Why can filling of the tubes occur slowly?

The blood collection tubes have 1 mL of vacuum in 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—Cellestis) for altitudes between 1,020 m (3350 ft) and 1,875 m (6150 ft), or if outside of these altitudes, collect blood via a syringe and, using applicable safety precautions, add 1 mL to each of the three QFT tubes.

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 all of the tubes' contents, which are coated on the inner wall of the tubes. 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. This ensures that the entire inner surface of the tube has been coated with blood and leads to thorough mixing and integration of the tubes' contents into the blood. Tubes should be between 17 - 25ºC (63 - 77ºF) 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. If prolonged incubation does occur, caution needs to be exercised when interpreting the results. If there is doubt in the validity of the results (in relation to the clinical context) another sample needs to be collected and the test repeated.

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 (eg. 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 that the patient's immune system is not functioning well. 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 of them is increased under a different cut-off, then the other is decreased at the same time. As TB is generally a low prevalence disease in developed world settings, more emphasis was placed on high specificity when establishing the cut-off, with a target of at least 98%. However, sensitivity is also critical and thus a cut-off was chosen 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.[1]

The sensitivity of QuantiFERON-TB Gold In-Tube was shown to be 92.6%, while the specificity was 98.8%.[1] In this study the authors compared the earlier—liquid antigen—version of QFT with the In-Tube version and found that both tests had the same specificity, with the In-Tube version being significantly more sensitive.

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

Individuals displaying a response greater than or equal to 0.35 IU/mL above the Nil control, for the TB Antigen, are likely to be infected with *M. tuberculosis*. No 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 is highly likely to be indicative of *M. tuberculosis* infection. 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 healthcare workers). However, as is always the case, a positive result should be interpreted in light of all available information.

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 using the consensus from the 3 test results. Additionally, when institutions change from screening using the TST to using QFT, given the superior accuracy of QFT there should be no surprise to find some people QFT positive who had historically been TST negative.

Are QFT results reproducible?

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 blood samples taken in duplicate 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 7% 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 version) of 530 Navy recruits, who were retested 4 to 5 weeks after an 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.

Additionally in HIV infected individuals, QFT results are highly reproducible. In a US study, only 3 of 206 specimens run in duplicate yielded discordant results.[22]

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. Leyton *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.[23] Thus there is no reason not to use QFT in a person who has been recently tested with TST.

What are the explanations of 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 of *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 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 mediations.

Studies evaluating sensitivity of QFT in developed world settings [1,7,18] demonstrate a higher sensitivity for QFT than when evaluated in developing world populations.[19-21] 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 meant as an aid to the diagnosis of active TB. Clinicians have many tools available to assist the diagnosis of active TB, with QFT providing a new one, which may have little benefit in cases of overt active TB. 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?

No definitive information is currently available on whether the results of IGRA tests are affected by pregnancy. However, there is no reason to suspect that QFT would be affected any more than is the TST.

What should I do if the QFT result is indeterminate?

When presented with an indeterminate result, you should repeat the test. However, an indeterminate QFT is meaningful, suggesting possible error in performing the test or immune suppression of the subjects, and thus should not be interpreted as a failed test. By including an internal positive control (Mitogen tube), QFT enables the distinction between indeterminate results 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 1.7%.[34]

However, in populations where the level of immune suppression is high, indeterminate rates can be correspondingly higher.[10,26-29] An indeterminate response in a highly immune suppressed individual is appropriate as it indicates a measureable 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 valid QFT result, suggesting that the initial result may have been due to operational difficulties. However, for an even smaller 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 valid 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 (eg. culture of *M. tuberculosis*) and the patient should be considered at risk of spreading 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.

What is the attitude to a QFT positive response without information about a recent contact?

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 (eg. *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?

The fact that QFT is more specific than the TST tells us that those with a QFT positive test result are very likely to be truly infected with *M. tuberculosis*. Therefore, as QFT has been shown to be at least as sensitive as the TST, simple logic tells us that those with QFT positive test results will be more likely to progress to active TB than those with TST positive test results—on a population basis. Recently, there has been significant growth in the body of evidence confirming that QFT accurately identifies individuals who will progress to active TB disease.

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 15%, more than 6 times greater than the 2.3% for the TST.[2] In this study, both TST and QFT were used in a TB contact investigation involving 601 individuals. 40% had a positive TST, but only 11% (66) of the exposed individuals were QFT positive and offered TB treatment—41 declined. Over the next two years, 6 of the 575 untreated individuals developed TB disease; QFT had detected all 6 and the TST only 5. There

were 181 contacts who were TST positive, but QFT negative, and none of these developed TB.

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.[16] This indicates that the risk of progression of QFT negative individuals in this BCG vaccinated population is low, even if they are TST positive.

All these studies suggest that with the use of QFT, doctors can now treat only a fraction of the individuals they would have had to based on the TST—with the knowledge that they are preventing TB disease.

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.

However, it has been postulated that those with higher QFT responses will be more likely to progress to active TB.[17] This is supported by the study of Diel *et al* which found that all individuals who subsequently developed TB disease had IFN-γ responses greater than 10 IU/mL soon after being infected.[2] Higuchi *et al* also found an association between magnitude of response and development of active TB.[16]

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 person'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.

To account for laboratory variations in performing the ELISA, the following recommendation appears in the QFT Package Insert:

"Where *M. tuberculosis* infection is not suspected, initially positive results can be confirmed by retesting the original plasma samples in duplicate in the QuantiFERON®-TB Gold IT ELISA. If repeat testing of one or both replicates is positive, the test result is considered positive."

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 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.[12-14] 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.[32]

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. This is supported by studies (as yet unpublished) showing that QFT responses become negative in a large percentage of successfully treated patients. 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.

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World Headquarters
Cellestis Limited
Email: info@cellestis.com
Tel: +61 3 8527 3500

North America / South America
Cellestis Inc.
Email: customer.service@cellestis.com
Tel: +1 661 775 7480 (outside USA)
Toll free: 800 519 4627 (USA only)

Europe / Middle East / Africa
Cellestis GmbH
Email: europe@cellestis.com
Tel: +49 6151 428 59 0

Asia / Pacific
Cellestis AP Pte Ltd
Email: asiapac@cellestis.com
Tel: +65 6322 0822

Australia / New Zealand
Cellestis International
Email: anz@cellestis.com
Tel: +61 3 8527 3500

Japan / Korea
Cellestis Asia KK
Email: jp.kr@cellestis.com

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