Physician Update

QuantiFERON-TB Gold In-Tube Tuberculosis Blood Test Update

Interferon-Gamma Release Assay (IGRA) for Mycobacterium Tuberculosis Complex

Benefits

- Requires only one patient visit to draw a blood sample. IGRA s are recommended by the Centers for Disease Control and Prevention as preferred testing for patients who are unlikely to return for tuberculin skin test (TST) reading
- Higher specificity and similar or greater sensitivity when compared to TST
- May be used wherever a TST is currently used (with the exception of children <5 years), including health care worker screenings and in contact investigation for those who have had BCG vaccinations
- Does not cause the booster phenomenon seen with TST after repeated testing
- Provides an objective, reproducible result unaffected by subjective interpretation
- The increased specificity of the QFT-GIT test reduces the number of false-positive results and may estimate a lower prevalence of latent TB infection than the TST in low incidence countries such as the U.S.2, 3

Summary

PeaceHealth Laboratories offers QuantiFERON-TB Gold In-Tube (QFT-GIT) testing in addition to the traditional tuberculin skin test (TST).

QFT-GIT is an FDA-approved interferon-gamma release assay (IGRA) that measures interferon-gamma production in patients who are sensitized to TB antigens. Should active TB be suspected, culture (and/or PCR testing) of appropriate clinical specimens is still necessary for a definitive diagnosis.

As an alternative to the TST, the QFT-GIT can be used to detect latent TB infection in patients who have been vaccinated with BCG and have a positive TST.1, 4

The QFT-GIT test is indicated to detect latent infection with Mycobacterium tuberculosis (TB), not to diagnose or manage active tuberculosis.

About the Assay

The immune response to a TB complex infection is primarily cell-mediated. Part of the immune response results from the sensitization of T-cells to TB antigens. The T-cells retain “memory” to the TB antigens and, when stimulated, produce the cytokine interferon-gamma.

Testing Process

The QFT-GIT test is performed in two stages using three tubes:

Stage 1: Aliquots of heparinized whole blood are incubated with the M. tuberculosis-specific antigens, ESAT-6, CFP-10, and TB7.7 and compared to negative (“nil tube”) and positive (“mitogen”) control tubes to determine test validity. Following a 16-24 hour incubation, the plasma is harvested.

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Stage 2: The amount of interferon-gamma in the plasma samples is quantified by enzyme-linked immunosorbent assay (ELISA).

Interpretation
The QFT-GIT test result is interpreted by using quantitative cutoff points to determine positive, negative and indeterminate results. The in vitro production of interferon-gamma by patient T-lymphocytes upon recognition of TB-specific antigens is considered indicative of TB infection when above the cutoff and in the absence of inappropriate nil or mitogen responses.

INDETERMINATE RESULTS
The mitogen positive control is a general activator of T-lymphocytes and measures the normal interferon-gamma response of the blood. An indeterminate QFT-GIT test result due to failure of the mitogen positive control indicates that the TB infection status could not be determined because of impaired immune status or incorrect performance of the test due to incorrect blood handling or storage before incubation.

Failure of the nil control will also produce an indeterminate result and is due to a high background level of interferon-gamma in the blood (>8 IU/mL).

Indeterminate results obtained with QFT-GIT testing should be repeated with a fresh blood sample. Positive or negative results obtained at repeat testing should be accepted. Indeterminate results at repeat testing indicate that the TB infection status cannot be determined and other diagnostic procedures should be considered.

A metaanalysis has demonstrated the indeterminate rate among individuals tested with QFT-GIT to be 2.14%, rising to 4.42% when the analysis is limited to immunocompromised patients.

USING QUANTITATIVE VALUES AND REPEAT TESTING
The CDC recommends considering the quantitative results in interpreting QFT-GIT test results. Experience has shown that the lower negative values are more likely to be truly negative than values closer to the cutoff point.

Similarly, higher values are more likely to be truly positive than values closer to the cutoff point. In patients without risk factors for prior exposure of progression, retesting of low positive quantitative values (between 0.35 - 0.7) could be considered as they are more likely to revert to negative.

False Positive & False Negative Results
As with the TST, testing with IGRAs will result in false-positive results if they are used in low-prevalence populations.

Additionally, the TST will produce false positive results from cross-reactivity with many environmental mycobacteria. ESAT-6 and CFP-10 are present in M. kansasii, M. szulgai, and M. marinum. Sensitization to these specific organisms might contribute to the release of interferon-gamma in response to these antigens and cause false-positive QFT-GIT test results. Children <5 years old or immunosuppressed may have false negative QFT-GIT results.
CONVERSIONS WITH SERIAL TESTING OF HEALTH CARE WORKERS

The 2005 CDC guidelines for the QFT-GIT assay allows for replacement of the TST with QFT for annual testing of health care workers in the U.S. and this practice has since been widely adopted. An update in 2010 comments on the limitation of the IGRAs for serial testing stating that: “The criteria for interpreting changes in an IGRA that identify new infections remain uncertain.”

The value of IGRAs in the testing of health care workers was investigated in a 2012 review of over 50 studies and concluded that the use of IGRAs for serial testing is complicated by the lack of data on optimal cut-offs for serial testing and unclear interpretation and prognosis of conversions (new TB infections) and reversions.

This review found that a higher rate of subsequent conversions may be found with serial testing by QFT-GIT, and these serial testing results should be interpreted with caution by consideration for the amount of change in absolute interferon-gamma along with the relevant clinical information – especially for individuals with borderline results since these are most likely to change upon retesting.

IMPORTANT POINTS FROM THE CDC 2010 GUIDELINES

Repeat the Test
In contact investigations, negative QFT-GIT test results obtained prior to 8 weeks after the end of exposure typically should be confirmed by repeat testing 8-10 weeks after the end of exposure.

Children Under Five
TST is still preferred in children under 5 years old due to the lack of published reports documenting IGRA performance (QFT-GIT test) in young children.

Interpreting Test Results
TSTs and IGRAs are indirect tests that measure immunologic responses and are not direct tests that detect the causative organism or components of the organism. Public health and patient management decisions should not be based on TST or the QFT-GIT test results alone and should include epidemiological, medical history and other clinical information.

A positive TST or QFT-GIT test result suggests that M. tuberculosis infection is likely while a negative result suggests that infection is unlikely. In persons with symptoms, signs, or radiographic evidence of active tuberculosis, negative QFT-GIT results are not sufficient to exclude infection. This is especially true in those at increased risk for a poor outcome if disease develops. Clinical judgment should be used to decide when and if further diagnostic evaluation and treatment are needed.

Neither TST nor QFT-GIT testing can distinguish latent TB infection from active TB disease (see the Centers for Disease Control and Prevention Services’ “Latent Tuberculosis Infection: A Guide for Primary Health Care Providers” online at http://www.cdc.gov/tb/publications/LTBI/diagnosis.htm).

TST and QFT-GIT Recommendations
Routine testing with both TST and QFT-GIT is not recommended although results from both tests may be useful in some situations, such as with healthy persons who have a low risk of both infection and progression.

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 (e.g., in those persons with symptoms, signs and/or radiographic evidence) or who are at high risk of infection, progression and poor outcome (e.g., in persons with HIV infection or children <5 years).

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In healthy persons with a low likelihood of both *M. tuberculosis* infection and of progression to active tuberculosis if infected, a single positive TST or QFT-GIT result should not be taken as reliable evidence of *M. tuberculosis* infection. Because of the low probability of infection, a false-positive result is more likely. In this case, the likelihood of *M. tuberculosis* infection and of disease progression should be reassessed, and additional testing should be performed.

**QUESTIONS?**

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**ORDERING INFORMATION**

**58150:** QuantiFERON-TB Gold In-Tube

**Methodology:** Enzyme-linked Immunosorbent Assay (ELISA)

**Performed:** Monday–Friday

**Released:** Same day as tested (after 24 hour incubation)

**CPT Code:** 86480

**SPECIMEN REQUIREMENTS**

**Collect:** This test requires specialized collection tubes and handling. Please refer to our website test menu at www.peacehealthlabs.org, call Client Services at 800-826-3616 or contact your account representative.

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**REFERENCES**


QuantiFERON-TB Gold Collection Instructions

1. COLLECT BLOOD
   - Use Quantiferon Collection Kit #116768
   - Collect 1 mL into each tube. Tubes should be at room temperature (17-25°C) when filling.
   - Tubes fill slowly - hold tube on needle for 2–3 seconds after flow ceases
   - Fill each tube up to the black mark
   - Acceptable volume range is 0.8 mL to 1.2 mL
   - Do not underfill or overfill the tubes. Overfilled or underfilled tubes cannot be tested and will require recollection

2. SHAKE TUBES
   Immediately after filling tubes, shake ten (10) times, just firmly enough to ensure entire inner surface of tube is coated with blood, to solubilize antigens on tube wall.

3. LABEL TUBES
   When placing the patient label on the tubes, do not block the ability to confirm that the tube has been filled to the fill line. Do not cover the fill line with the patient label.

4. INCUBATE/SHIP
   Blood must be incubated as soon as possible and within 16 hours of collection. Select an option below:

<table>
<thead>
<tr>
<th>Option 1 – Incubate at Central Laboratory</th>
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<tbody>
<tr>
<td>- Label tubes as “NOT INCUBATED”</td>
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<tr>
<td>- Ship tubes to laboratory at 17-27°C for incubation. Blood must arrive at lab and be incubated within 16 hours of collection</td>
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<tr>
<th>Option 2 – Incubate at Collection Site</th>
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<tr>
<td>- If tubes are not incubated at 37°C soon after collection, re-shake tubes immediately prior to incubation</td>
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<tr>
<td>- Incubate tubes upright at 37°C for 16-24 hours</td>
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<tr>
<td>- Label tubes as “INCUBATED”</td>
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<tr>
<td>- Ship incubated tubes to testing laboratory at 4-27°C. (within 3 days, if not centrifuged)</td>
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<td>- If centrifugation is performed at collection site, ship centrifuged tubes refrigerated (2-8°C) within 28 days.</td>
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