# Test Definition: QFT4

QuantiFERON-TB Gold Plus, Blood

## Overview

### Useful For

Indirect test for *Mycobacterium tuberculosis* infection, to be used in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations

This test **is not recommended** for use for diagnosis of active tuberculosis infection.

### Special Instructions

• Mycobacterium tuberculosis Infection Determination by QuantiFERON-TB Gold Plus Collection and Processing Instructions

### Method Name

Chemiluminescence Immunoassay (CLIA)/Enzyme-Linked Immunosorbent Assay (ELISA) as appropriate

### NY State Available

Yes

## Specimen

### Specimen Type

Whole blood

### Ordering Guidance

This test **is not recommended** for use for diagnosis of active tuberculosis (TB) infection. It can be used as an aid to detect latent TB infection.

### Specimen Required

**Supplies:**

-QuantiFERON-TB Gold Plus Collection Kit (T794)

-QuantiFERON-TB Gold Plus HIGH ALTITUDE Collection Kit (T795)

**Collection Instructions:**

1. Special collection, incubation, and centrifugation procedures must be followed.
2. For blood collection options (1-tube collection or 4-tube collection) and specimen transport instructions, see *Mycobacterium tuberculosis* Infection Determination by Quanti-FERON-TB Gold Plus Collection and Processing Instructions (T688).

### Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

-General Request (T239)

-Infectious Disease Serology Test Request (T916)

-Microbiology Test Request (T244)
-Kidney Transplant Test Request

### Specimen Minimum Volume

4 mL: 1 mL per tube (4 tubes)

### Reject Due To

| | |
|---|---|
| Specimen submitted not following kit guidelines | Reject |
| Gross hemolysis | Reject |
| Gross lipemia | Reject |
| Gross icterus | Reject |

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Whole blood | Refrigerated | 28 days | QTBKIT |

## Clinical & Interpretive

### Clinical Information

Latent tuberculosis infection (LTBI) is a non-communicable, asymptomatic condition that persists for many years in individuals and may progress to active tuberculosis disease, particularly in patients who are immunosuppressed. The primary goal for diagnosis of LTBI is to initiate medical treatment in order to prevent progression to active disease. Historically, detection of LTBI has been done using the tuberculin skin test (TST). The TST has certain limitations, however, including subjective interpretation, limited sensitivity in immunosuppressed patients, and the possibility of false-positive results in individuals who have received the BCG vaccine or are infected with other mycobacteria.

The QuantiFERON-TB Gold Plus (QFT-Plus) test is an interferon (IFN)-gamma release assay (IGRA) that assesses the cell-mediated immune response to 2 *Mycobacterium tuberculosis* complex antigens, ESAT-6 and CFP-10, by measuring IFN-gamma levels in plasma. These 2 proteins are absent from all BCG strains and from most nontuberculosis mycobacteria with the exception of *Mycobacterium kansasii, Mycobacterium szulgai,* and *Mycobacterium marinum.* Individuals infected with *M tuberculosis* complex agents, including *M tuberculosis, Mycobacterium bovis, Mycobacterium africanum, Mycobacterium microti*, *Mycobacterium caprae*, and *Mycobacterium canetti,* usually have lymphocytes in their blood that recognize these specific antigens, and this recognition leads to the generation and secretion of IFN-gamma. This cytokine is subsequently detected and quantified using an IFN-gamma enzyme-linked immunosorbent assay.

In an *M tuberculosis* infection, CD4+ T cells play a critical role in immunological control through secretion of IFN-gamma. The prior version of the QFT-Plus assay, the QuantiFERON-TB Gold In-Tube (QFT-Gold) assay, only detected IFN-gamma

secreted from CD4+ T cells. Evidence now supports a role for CD8+ T cells in host defense against *M tuberculosis* infection by likewise producing IFN-gamma, but also by stimulating macrophages to suppress the growth of *M tuberculosis,* to kill infected cells, and to directly lyse intracellular *M tuberculosis* bacteria. IFN-gamma-producing *M tuberculosis* specific CD8+ T cells have been detected in subjects with LTBI and in patients with active TB. ESAT-6 and CFP-10 specific CD8+ T cells have also been frequently described in patients with active tuberculosis (TB) versus patients with LTBI and have been detected in HIV-positive patients and children with TB disease.

The QFT-Plus assay has 2 distinct TB antigen tubes: TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2). Both tubes contain peptide antigens from ESAT-6 and CFP-10 for stimulation of a CD4+ T-cell IFN-gamma response. However, the TB2 tube also contains an additional set of ESAT-6 and CFP-10 peptides specifically designed to stimulate a CD8+ T-cell response.

For the most up-to-date information regarding use of IGRAs, refer to the most recent guidelines on the Diagnosis of Tuberculosis in Adults and Children from the American Thoracic Society, the Infectious Diseases Society of America, the Centers for Disease Control and Prevention.(1)

## Reference Values

Negative

## Interpretation

A single positive result by this test should not be used solely to diagnose latent tuberculosis (TB). Results should be used in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations.

Positive:
Interferon-gamma (IFN-gamma) response to *Mycobacterium tuberculosis* antigens detected, suggesting infection with *M tuberculosis*. Positive results in patients at low-risk for TB should be interpreted with caution and repeat testing on a new sample should be considered as recommended by the 2017 American Thoracic Society, the Infectious Diseases Society of America, the Centers for Disease Control and Prevention (ATS/IDSA/CDC) Clinical Practice Guidelines for Diagnosis of Tuberculosis in Adults and Children.(1) False-positive results may occur in patients with prior infection with *Mycobacterium marinum*, *Mycobacterium szulgai*, or *Mycobacterium kansasii*.

Negative:
No IFN-gamma response to *M tuberculosis* antigens was detected. Latent infection with *M tuberculosis* is unlikely. A single negative result does not exclude infection with *M tuberculosis*. In patients at high risk for *M tuberculosis* infection, a second test should be considered in accordance with the 2017 ATS/IDSA/CDC Clinical Practice Guidelines for Diagnosis of Tuberculosis in Adults and Children.(1)

Indeterminate due to Low Mitogen Value:
Indeterminate results due to a low IFN-gamma level in the mitogen (positive control) tube. This may occur due to a low lymphocyte count, reduced lymphocyte activity, or inability of the patient's lymphocytes to generate IFN-gamma.

Indeterminate due to High Nil value:
Indeterminate results due to a high level of IFN-gamma in the Nil (negative control) tube. This may occur due to heterophile antibody effects or nonspecific, circulating IFN-gamma in the patient's blood sample. Repeat testing on a new specimen is suggested.

## Cautions

A negative QuantiFERON-TB Gold Plus (QFT-Plus) result does not preclude the possibility of *Mycobacterium tuberculosis* infection or tuberculosis disease. False-negative results can be due to the stage of infection (eg, specimen obtained prior to the development of cellular immune response), comorbid conditions that affect immune functions, incorrect handling of the blood collection tubes following venipuncture, or other individual immunological factors. Additionally, heterophile antibodies or nonspecific interferon-gamma (IFN-gamma) production from other inflammatory conditions may mask specific responses to ESAT-6 or CFP-10 peptides.

A delay in incubation may cause false-negative or indeterminate results, and other technical parameters may affect the ability to detect a significant IFN-gamma response.

A positive QFT-Plus result should not be the sole or definitive basis for determining infection with *M tuberculosis*. Positive results should be followed by further medical evaluation for active tuberculosis disease (eg, acid-fast bacilli smear and culture, chest X-ray).

While ESAT-6 and CFP-10 are absent from all bacille Calmett-Guerin (BCG) strains and from most known nontuberculous mycobacteria, it is possible that a positive QFT-Plus result may be due to infection with *Mycobacterium kansasii, Mycobacterium szulgai,* or *Mycobacterium marinum*. If such infections are suspected, alternative tests should be performed.

The effect of lymphocyte count on reliability is unknown. Lymphocyte counts may vary over time for any individual person and from person to person. The minimum number required for a reliable result has not been established and may also be variable.

The predictive value of a negative QFT-Plus result in immunosuppressed patients has not been determined.

For healthcare personnel or patients who require baseline tuberculosis (TB) testing (at onboarding or entry into facilities) at the same time they are set to receive a COVID-19 messenger RNA (mRNA) vaccine, the Centers for Disease Control and Prevention recommends the following:
-Perform TB symptom screening on all healthcare personnel or patients.
-If using interferon-gamma release assays (IGRA), collect blood prior to COVID-19 mRNA vaccination.
-If using tuberculin skin test (TST), place prior to COVID-19 mRNA vaccination.
-If COVID-19 mRNA vaccination has already occurred, defer TST or IGRA until 4 weeks after completion of 2-dose COVID-19 mRNA vaccination.

## Clinical Reference

1. Lewinsohn DM, Leonard MK, LoBue PA, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of tuberculosis in adults and children. Clin Infect Dis. 2017;64(2):111-115
2. Moon HW, Gaur RL, Tien SSH, Spangler M, Pai M, Banaei N. Evaluation of QuantiFERON-TB Gold Plus in health care workers in a low-incidence setting. J Clin Micobiol. 2017;55(6):1650-1657
3. Telisinghe L, Amofa-Sekyi M, Maluzi K, et al. The sensitivity of the QuantiFERON-TB Gold Plus assay in Zambian adults with active tuberculosis. Int J Tuberc Lung Dis. 2017;21(6):690-696
4. Theel ES, Hilgart H, Breen-Lyles M, et al. Comparison of the QuantiFERON-TB Gold Plus and QuantiFERON-TB Gold In-Tube Interferon Gamma Release Assays in patients at risk for tuberculosis and in health care workers. J Clin Microbiol. 2018;56(7):e00614-e00618. doi:10.1128/JCM.00614-18

## Performance

### Method Description

Primary method:
The LIAISON QuantiFERON-TB Gold Plus detects the analyte interferon-gamma (IFN-gamma) by direct, sandwich chemiluminescence immunoassay. Anti IFN- gamma monoclonal (mouse) antibodies are used for coating magnetic particles (solid phase) and anti IFN-gamma monoclonal (mouse) antibodies are linked to an isoluminol derivative forming an isoluminol-antibody conjugate. Binding between the monoclonal antibodies and isoluminol in the conjugate is mediated by a biotin-streptavidin immunocomplex.

During the first incubation, analyte IFN-gamma present in calibrators, samples, or controls will bind to the solid phase and conjugated monoclonal antibodies and form a sandwich. Any unbound material in the test sample is removed with a wash cycle. During the second incubation, assay buffer W is added to reduce nonspecific binding, followed by a second wash cycle.

Next, the starter reagents are added and a flash chemiluminescence reaction is induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of the amount of analyte IFN-gamma present in calibrators, samples, or controls.(Package insert: LIAISON QuantiFERON-TB Gold Plus. DiaSorin; EN-200/077-002, 01/2022)

As needed:
The QuantiFERON-TB Gold Plus test uses specialized blood collection tubes, which are used to collect whole blood via venipuncture. Two of the 4 collection tubes contain antigens representing certain *Mycobacterium tuberculosis* proteins and 2 of the tubes are controls (Nil and mitogen). TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2) both contain peptide antigens from the *Mycobacterium tuberculosis* (MTB)-complex-associated antigens ESAT-6 and CFP-10 and are designed to elicit a cell-mediated immunity (CMI) response from CD4+ T-helper lymphocytes. The TB2 tube contains an additional set of peptides targeted to the induction of a CMI response from CD8+ cytotoxic T lymphocytes. The tubes are immediately shaken after collection to mix antigen with the blood and then incubated at 37 degrees C + or - 1 degree C within 16 hours from collection. The tubes are incubated for 16 to 24 hours, and then plasma is tested for the presence of IFN-gamma produced in response to the peptide antigens. The amount of IFN-gamma is measured by enzyme-linked immunosorbent assay in IU/mL.

A test is considered positive for an IFN-gamma response to the TB antigen cocktail when results are significantly above the negative control value. The Nil tube adjusts for background, heterophile antibody effects, or nonspecific IFN-gamma in blood samples. The IFN-gamma level of the Nil tube is subtracted from the IFN-gamma level for the TB1 and TB2 antigen tubes and Mitogen tube. The mitogen-stimulated plasma sample serves as an IFN-gamma positive control for each specimen tested. A low 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 activity due to prolonged specimen transport, or improper specimen handling, including filling/mixing of blood tubes or inability of the patient's lymphocytes to generate IFN-gamma. Elevated levels of IFN-gamma in the Nil tube may occur with the presence of heterophile antibodies or due to intrinsic IFN-gamma secretion.(Package insert: QuantiFERON-TB Gold Plus (QFT-Plus). Qiagen; 06/2022)

### PDF Report
No

### Day(s) Performed
Monday through Friday

### Report Available
2 to 5 days

### Specimen Retention Time
Until testing is complete

### Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

## Fees & Codes

### Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

### Test Classification
This test has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

### CPT Code Information
86480

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| QFT4 | QuantiFERON-Tb Gold Plus, B | 71775-1 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| QFTQ2 | QuantiFERON-Tb Gold Plus Result | 71773-6 |
| DEXQE | TB1 Ag minus Nil Result | 64084-7 |
| DEXQF | TB2 Ag minus Nil Result | 88517-8 |
| DEXQG | Mitogen minus Nil Result | 71774-4 |
| DEXQH | Nil Result | 71776-9 |