

QUANTIFERON-TB GOLD PLUS, BLOOD

Test ID: QFT4

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

METHOD: Enzyme-Linked Immunosorbent Assay (ELISA)

REFERENCE VALUES: Negative

SPECIMEN REQUIREMENTS:**Supplies:**

Standard Altitude: QuantiFERON-TB Gold Plus Collection Kit (T794)

High Altitude: QuantiFERON-TB Gold Plus High Altitude Collection Kit (T795)

Collection Instructions:

1. Special collection, incubation, and centrifugation procedures must be followed.
2. Prepare and transport specimen per QuantiFERON-TB (QFT) Gold Plus Collection and Processing Instructions (T688).

**Preferred and alternative collection and processing instructions are listed below.

Please note the attached directions are provided as information and will not apply until the test go live date.

Specimen Minimum Volume: 4 mL total (1 mL per tube)

SPECIMEN STABILITY INFORMATION:

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

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 *M kansasii*, *M szulgai*, or *M 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.

CPT CODE: 86480

DAY(S) SET UP: Monday through Friday; 9 a.m.

ANALYTIC TIME: 2 days

QUESTIONS: Contact your Mayo Medical Laboratories' Regional Manager or
Dunisha Messmer, MML Laboratory Technologist Resource Coordinator
Telephone: 800-533-1710

****Please note the attached directions are provided as information and will not apply until QFT4 test go live date.**



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

Blood Collection

Note: All collection tubes need to be at room temperature (17–27°C) at the time of draw.

A. Directly into 4 Tube QuantiFERON-TB Gold Plus Kit (Preferred)

1. Collect 1 mL of blood into each of 4 tubes.
 - **Tubes fill slowly.**
 - Use of a syringe may ensure correct blood volume.
 - When the tube is upright, blood must meet the small black mark on the label.
 - If butterfly needle is used, first collect other required tubes or use a “purge” tube to remove the air and then proceed with collecting the QTB tubes.
 - These tubes are manufactured to draw 1 mL of blood and perform optimally within the range of 0.8 to 1.2 mL. If the level of blood is outside of the black indicator line, another blood specimen should be collected (see figure 1).
2. Immediately shake the tubes firmly 10 times (see figure 2).
 - Entire inner surface of tubes must be coated with blood.
 - Overly energetic shaking may cause gel disruption and could lead to aberrant results.
 - Thorough mixing is required to ensure complete integration of the tube's contents into the blood.
3. Label tubes appropriately. The label should be placed below colored Quantiferon band so back window and black marks are visible on all 4 collection tubes.
4. Maintain tubes at room temperature (17°C–27°C) until incubation. Do not refrigerate or freeze.
5. Incubation of the 4 tubes must be started within 16 hours of collection.

B. Collection into a Single Lithium-Heparin Tube and Transfer to QFT-Blood Collection Tubes

1. Blood may be collected in a single blood collection tube containing lithium-heparin as the anticoagulant and then transferred to QFT-Plus blood collection tubes. Only use lithium-heparin as a blood anticoagulant because other anticoagulants interfere with the assay.
 - a. Label tubes appropriately. It is recommended to label the tube with the time and date of the blood collection.

Important: Blood collection tubes should be at room temperature (17–25°C) at the time of blood collection.
2. Fill a lithium-heparin blood collection tube (minimum volume 5 mL) and gently mix by inverting the tube several times to dissolve the heparin.

(Section B. continued on page 2)

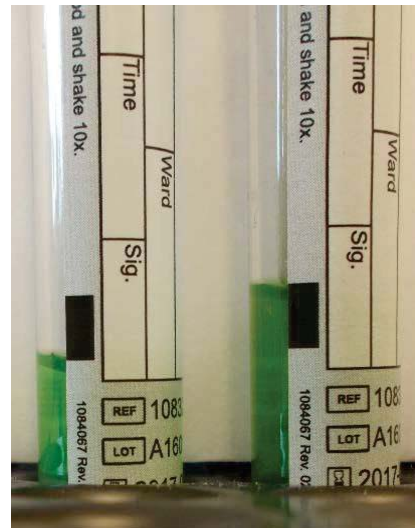


Figure1. Under- or over-filling of the tubes outside of the 0.8 mL (bottom of black mark) to 1.2 mL (top of black mark) range may lead to erroneous results. Specimens with volumes outside of black mark will be rejected.



Figure2. Entire inner surface of tube must be coated with blood.

3. Following blood collection into a lithium-heparin tube, the following are options for hold time and temperatures prior to transfer of blood into the 4 QFT-Plus tubes.

Option 1 – Lithium-Heparin Tube Room Temperature Storage and Handling

- a. Blood collected in lithium-heparin tube must be maintained at room temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) for no more than 12 hours from the time of collection prior to transfer to QFT-Plus blood collection tubes and subsequent incubation.

Option 2 – Lithium-Heparin Tube Refrigerated Storage and Handling Important:

Procedural steps a–d must be followed in sequence.

- a. Blood drawn into lithium-heparin tube must be held at room temperature ($17\text{--}25^{\circ}\text{C}$) between 15 minutes and 3 hours after blood collection.
- b. Blood drawn into lithium-heparin tube may be refrigerated ($2\text{--}8^{\circ}\text{C}$) for 16 to 48 hours.
- c. After refrigeration, lithium-heparin tube must equilibrate to room temperature ($17\text{--}25^{\circ}\text{C}$) prior to transfer to QFT-Plus blood collection tubes.
- d. Aliquoted QFT-Plus Blood Collection Tubes should be placed in the 37°C incubator within 2 hours of blood transfer.

Note: If QFT-Plus Blood Collection Tubes are not incubated at 37°C directly after transfer to QFT-Plus Blood Collection Tubes, invert the tubes to mix 10 times prior to incubation at 37°C . Total time from blood draw to incubation in QFT-Plus blood collection tubes should not exceed 53 hours.

4. Transfer of blood specimen to QFT-Plus blood collection tubes:
- a. Label each QFT-Plus blood collection tube appropriately. Ensure each tube (Nil, TB1, TB2 and Mitogen) is identifiable by its label or other means once the cap is removed. It is recommended to transfer the recorded time and date of blood collection from the lithium-heparin tubes to the QFT-Plus blood collection tubes.
- b. Samples must be evenly mixed by gentle inversion before dispensing into QFT-Plus blood collection tubes.
- c. Dispensing should be performed aseptically, ensuring appropriate safety procedures, removing the caps from the 4 QFT-Plus blood collection tubes and adding 1 mL of blood to each tube. Replace the tube caps securely and mix tubes well before incubation.



Figure 3. Shipping
(Ship QT B kit refrigerate: $2^{\circ}\text{--}8^{\circ}\text{C}$)

Incubation and Centrifugation

Notes: The following types of specimens will be rejected:

- Not incubated
- Not centrifuged
- Received in lithium-heparin tubes
- Incubation in waterbath or heat block

1. Incubate all 4 QFT-Plus tubes upright at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 16 to 24 hours.
2. Following incubation centrifuge for 15 minutes at 2,000–3,000 RCF (g).
 - Hemolyzed plasma is acceptable.
3. Place all 4 QFT-Plus tubes together in the supplied QT B Transport bag (see figure 3).
4. Store refrigerate 2°C to 8°C . Stable 28 days refrigerated.