January 2023

QuantiFERON®-TB Gold Plus (QFT®-Plus) Package Insert

2 x 96 (cat. no. 622130)

20 x 96 (cat. no. 622832)

The Whole Blood IFN-γ test measuring responses to ESAT-6 and CFP-10 peptide antigens

RX ONLY

IVD For in vitro diagnostic use

www.qiagen.com

REF 622130, 622832

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Contents

Intended Use

QuantiFERON-TB Gold Plus (QFT-Plus) is an in vitro diagnostic test using a peptide cocktail simulating ESAT-6 and CFP-10 proteins to stimulate cells in heparinized whole blood. Detection of interferon-γ (IFN-γ) by enzyme-linked immunosorbent assay (ELISA) is used to identify in vitro responses to these peptide antigens that are associated with *Mycobacterium tuberculosis* infection.

QFT-Plus 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.

Summary and Explanation of the Test

Tuberculosis is a communicable disease caused by infection with *M. tuberculosis* complex organisms (*M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. canetti* and *M. caprae*), which typically spreads to new hosts via airborne droplet nuclei from patients with pulmonary tuberculosis disease. A newly infected individual can become ill from tuberculosis within weeks to months, but most infected individuals remain well. Latent tuberculosis infection (LTBI), a non-communicable asymptomatic condition, persists in some, who might develop tuberculosis disease months or years later. The main purpose of diagnosing LTBI is to consider medical treatment for preventing tuberculosis disease. For more than 100 years, the tuberculin skin test (TST) was the only available method for diagnosing LTBI. Cutaneous sensitivity to tuberculin develops from 2 to 10 weeks after infection. However, some infected individuals, including those with a wide range of conditions hindering immune functions, but also others without these conditions, do not respond to tuberculin. Conversely, some individuals who are unlikely to have *M. tuberculosis* infection exhibit sensitivity to tuberculin and have positive TST results after vaccination with Bacille Calmette-Guérin (BCG) or infection with mycobacteria other than *M. tuberculosis* complex, or undetermined other factors.

LTBI must be distinguished from tuberculosis disease, a reportable condition that usually involves the lungs and lower respiratory tract but may also affect other organ systems. Tuberculosis disease is diagnosed from historical, physical, radiological, and mycobacteriological findings.

QuantiFERON-TB Gold Plus (QFT-Plus) test is the fourth generation in QuantiFERON-TB testing technology assessing cell-mediated response through a quantitative measurement of IFN-γ in a whole blood sample. QFT-Plus is a test that measures the cell-mediated immune (CMI) responses to peptide antigens that simulate mycobacterial proteins. These proteins, ESAT-6 and CFP-10, are absent from all BCG strains and from most non-tuberculosis mycobacteria with the exception of *M. kansasii*, *M. szulgai* and *M. marinum* (1). Individuals infected with *M. tuberculosis* complex organisms usually have lymphocytes in their blood that recognize these and other mycobacterial antigens. This recognition process involves the generation and secretion of the cytokine, IFN-γ. The detection and subsequent quantification of IFN-γ forms the basis of this test.

Tuberculin skin test and IGRA's tests are helpful but insufficient for diagnosing *M. tuberculosis* complex infection in sick patients – a positive result can support the diagnosis of tuberculosis disease; however, infections by other mycobacteria (e.g., *M. kansasii*) could also cause positive results. Other medical and diagnostic evaluations are necessary to confirm or exclude tuberculosis disease.

The antigens used in QFT-Plus are a peptide cocktail simulating the proteins ESAT-6 and CFP-10. Numerous studies have demonstrated that these peptide antigens stimulate IFN-γ responses in T cells from individuals infected with *M. tuberculosis* but generally not from uninfected or BCG-vaccinated persons without disease or risk for LTBI (1–32). However, medical treatments or conditions that impair immune functionality can potentially reduce IFN-γ responses. Patients with certain other mycobacterial infections might also be responsive to ESAT-6 and CFP-10, as the genes encoding these proteins are present in *M. kansasii*, *M. szulgai*, and *M. marinum* (1, 23).

In MTB infection, $CD4^+$ T cells play a critical role in immunological control through their secretion of the cytokine IFN-γ. Evidence now supports a role for $CD8^+$ T cells participating in the host defense to MTB by producing IFN-γ and other soluble factors, which activate macrophages to suppress growth of MTB, kill infected cells, or directly lyse intracellular MTB (33–35). IFN-γ producing MTB-specific $CD8^+$ cells have been detected in subjects with LTBI and with active TB (36–39). Moreover, ESAT-6 and CFP-10 specific $CD8^+$ T lymphocytes are described as being more frequently detected in subjects with active TB disease versus LTBI, and may be associated with a recent MTB exposure (40–42). In addition, MTB-specific $CD8^+$ T cells producing IFN-γ have also been detected in active TB subjects with HIV co-infection (43, 44) and in young children with TB disease (45).

QFT-Plus has two distinct TB antigen tubes: TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2). Both tubes contain peptide antigens from the MTB-complex-associated antigens, ESAT-6 and CFP-10. Both the TB1 tube and TB2 tubes contain peptides from ESAT-6 and CFP-10 that are designed to elicit CMI responses from $CD4^+$ T-helper lymphocytes; the TB2 tube contains an additional set of peptides targeted to the induction of CMI responses from CD8+ cytotoxic T lymphocytes.

Diagnostic testing for Mycobacterium tuberculosis using Interferon Gamma Release Assays should follow applicable published guidelines. QFT has been studied in adults and children identified at higher risk for TB infection, including individuals recently exposed to persons with active tuberculosis, persons living with HIV or otherwise immunocompromised, immigrants from high-burden countries, and residents or employees of high-risk congregate settings. (See WARNINGS and PRECAUTIONS below regarding use in co-morbid conditions that may affect immune function.) (46-84)

Links to the most recent American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children, as well as to other information regarding diagnostic testing for tuberculosis, are available at https://www.cdc.gov/tb/publications/guidelines/testing.htm.

Principles of the Assay

The QFT-Plus assay uses specialized blood collection tubes, containing peptide antigens that simulate *M. tuberculosis* proteins, which are used to collect whole blood. Incubation of the blood occurs in the tubes for 16 to 24 hours, after which, plasma is harvested and tested for the presence of IFN-γ produced in response to the peptide antigens.

The QFT-Plus test is performed in two stages. First, whole blood is collected into each of the QFT-Plus Blood Collection Tubes, which include a Nil tube, TB1 tube, TB2 tube and a Mitogen tube. Alternatively, blood may be collected in a single blood collection tube that contains lithium or sodium-heparin as the anticoagulant, and then transferred to QFT-Plus Blood Collection Tubes.

The QFT-Plus Blood Collection Tubes are shaken to mix antigen with the blood and should be incubated at 37°C ± 1°C as soon as possible, and within 16 hours of collection. Following a 16 to 24 hour incubation period, the tubes are centrifuged, the plasma is removed, and the amount of IFN-γ (IU/ml) is measured by ELISA. The QFT-Plus ELISA uses a recombinant human IFN-γ standard, which has been assayed against a reference IFN-γ preparation (NIH Ref: Gxg01-902-535). Results for test samples are reported in International Units per ml (IU/ml) relative to a standard curve prepared by testing dilutions of the standard supplied with the kit.

Heterophile (e.g., human anti-mouse) antibodies in serum or plasma of certain individuals are known to cause interference with immunoassays. The effect of heterophile antibodies in the QFT-Plus ELISA is minimized by the addition of normal mouse serum to the Green Diluent and the use of F(ab')2 monoclonal antibody fragments as the IFN-γ capture antibody coated to the microplate wells.

A QFT-Plus assay is considered positive for an IFN-γ response to either TB antigen tube that is significantly above the Nil IFN-γ IU/ml value. The plasma sample from the Mitogen tube serves as an IFN-γ 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 lymphocyte activity due to improper specimen handling, filling/mixing of the Mitogen tube, or inability of the patient's lymphocytes to generate IFN-γ. Elevated levels of IFN-γ in the Nil sample may occur with the presence of heterophile antibodies, or to intrinsic IFN-γ secretion. The Nil tube adjusts for background (e.g., elevated levels of circulating IFN-γ or presence of heterophile antibodies). The IFN-γ level of the Nil tube is subtracted from the IFN-γ level for the TB antigen tubes and the Mitogen tube.

Components and Storage

Kit contents

Blood collection tubes		200 / 4800 tubes	Dispenser pack	High Altitude (HA) 200 / 4800 tubes†	High Altitude (HA) Dispenser pack*
Catalog no.		**622536/622529**	**622433**	**623536/623529**	**623433**
Number of tests/pack		**50 /1200**	**25**	**50 / 1200**	**25**
QuantiFERON Nil Tube (gray cap, white ring)	Nil	50 / 1200 tubes	25 tubes	–	–
QuantiFERON TB1 Tube (green cap, white ring)	TB1	50 /1200 tubes	25 tubes	–	–
QuantiFERON TB2 Tube (yellow cap, white ring)	TB2	50 / 1200 tubes	25 tubes	–	–
QuantiFERON Mitogen Tube (purple cap, white ring)	Mitogen	50 / 1200 tubes	25 tubes	–	–
QuantiFERON Nil HA Tube (gray cap, yellow ring)	Nil	–	–	50 / 1200 tubes	25 tubes
QuantiFERON TB1 HA Tube (green cap, yellow ring)	TB1	–	–	50 / 1200 tubes	25 tubes
QuantiFERON TB2 HA Tube (yellow cap, yellow ring)	TB2	–	–	50 / 1200 tubes	25 tubes
QuantiFERON Mitogen HA Tube (purple cap, yellow ring)	Mitogen	–	–	50 / 1200 tubes	25 tubes

* See page 14 for precautions.

† HA = high altitude for use between 3350 and 6150 feet (1020 and 1875 meters). Refer to page 17 for more information.

ELISA components

ELISA components	2-plate kit	Reference Lab Pack
Catalog no.	**622130**	**622832**
Microplate strips (12 x 8 wells) coated with murine anti-human IFN-γ monoclonal antibody	2 sets of 12 x 8 Microplate Strips	20 sets of 12 x 8 Microplate Strips
IFN-γ Standard, lyophilized (contains recombinant human IFN-γ, bovine casein, 0.01% w/v Thimerosal)	1 x vial (8 IU/ml when reconstituted)	10 x vials (8 IU/ml when reconstituted)
Green Diluent (contains bovine casein, normal mouse serum, 0.01% w/v Thimerosal)	1 x 30 ml	10 x 30 ml
Conjugate 100x Concentrate, lyophilized (murine anti-human IFN-γ HRP, contains 0.01% Thimerosal)	1 x 0.3 ml (when reconstituted)	10 x 0.3 ml (when reconstituted)
Wash Buffer 20x Concentrate (pH 7.2, contains 0.05% v/v ProClin® 300)	1 x 100 ml	10 x 100 ml
Enzyme Substrate Solution (contains H_2O_2, 3,3′,5,5′ Tetramethylbenzidine)	1 x 30 ml	10 x 30 ml
Enzyme Stopping Solution (contains 0.5 M H_2SO_4)*	1 x 15 ml	10 x 15 ml

* See page 14 for precautions.

Materials Required but Not Provided

Important: Make sure that all instruments used in this procedure have been checked and calibrated according to the manufacturer's recommendations.

- 37°C ± 1°C incubator (with or without CO_2)
- Calibrated variable-volume pipets for delivery of 10 µl to 1000 µl with disposable tips
- Calibrated multichannel pipet capable of delivering 50 µl to 100 µl with disposable tips
- Deionized or distilled water, 2 liters
- **Optional**: 1 ml microtubes with caps in 96-well format racks or uncoated microplates with plastic seals for plasma storage (22 patients/rack or plate)
- Centrifuge capable of centrifuging the blood tubes at least to 3000 RCF (*g*)

- Microplate shaker capable of speeds between 500 and 1000 rpm
- Microplate washer (for safety in handling plasma samples, an automated washer is recommended)
- Microplate reader fitted with 450 nm filter and 620 nm to 650 nm reference filter
- Variable speed vortex
- Timer
- Graduated cylinder, 1 liter or 2 liter
- Reagent reservoirs
- Plate lid

Storage and handling

Blood collection tubes

- Store blood collection tubes at 4°C to 25°C (40°F to 77°F).

ELISA kit reagents

- Store kit at 2°C to 8°C (36°F to 46°F).
- Always protect Enzyme Substrate Solution from direct sunlight.

Reconstituted and unused reagents

- For instructions on how to reconstitute the reagents, refer to "Stage 2: IFN-γ ELISA" (page 25).
- The reconstituted kit standard may be kept for up to 3 months if stored at 2°C to 8°C.

  Note the date the kit standard was reconstituted.
- The reconstituted Conjugate 100X Concentrate must be returned to storage at 2°C to 8°C and must also be used within 3 months.

  Note the date the conjugate was reconstituted.
- Working strength conjugate must be used within 6 hours of preparation.

- Working strength wash buffer may be stored at room temperature for up to 2 weeks.

Warnings and Precautions

For in vitro diagnostic use

Warnings

- A negative QFT-Plus result does not preclude the possibility of *M. tuberculosis* infection or tuberculosis disease: false-negative results can be due to stage of infection (e.g., specimen obtained prior to the development of cellular immune response), co-morbid conditions that affect immune function, incorrect handling of the blood collection tubes following venipuncture, incorrect performance of the assay, or other individual immunological variables. Heterophile antibodies or non-specific IFN-γ production from other inflammatory conditions may mask specific responses to ESAT-6 or CFP-10 peptides.
- A positive QFT-Plus result should not be the sole or definitive basis for determining infection with *M. tuberculosis*. Incorrect performance of the assay may cause false-positive QFT-Plus results.
- A positive QFT-Plus result should be followed by further medical evaluation for active tuberculosis disease (e.g., Acid Fast Bacilli smear and culture, chest X-ray).
- While ESAT-6 and CFP-10 are absent from all BCG strains and from most known nontuberculous mycobacteria, it is possible that a positive QFT-Plus result may be due to infection by *M. kansasii*, *M. szulgai*, or *M. marinum*. If such infections are suspected, alternative tests should be performed.
- A false-negative QFT-Plus result can be caused by incorrect blood sample collection or improper handling of the specimen affecting lymphocyte function. Please refer to "Specimen Collection and Handling" section, page 17, for correct handling of the blood specimens. Delay in incubation may cause false negative or indeterminate results, and other technical parameters may affect ability to detect a significant IFN-γ response.

- The effect of lymphocyte count on reliability of QFT-Plus results is unknown. Lymphocyte counts may vary over time for any individual person, and from person to person. The minimum number of lymphocytes required for a reliable test result has not been established and may also be variable.
- A positive QFT-Plus result can suggest and support the diagnosis of tuberculosis disease. ESAT-6 and CFP-10 are present in *M. tuberculosis*, but infections by other mycobacteria, including *M. kansasii*, *M. szulgai*, and *M. marinum* may also cause positive results. Other diagnostic evaluations (e.g., AFB smear and culture, chest X-ray) besides QFT-Plus are needed to confirm tuberculosis disease.
- The predictive value of a negative QFT-Plus result in immunosuppressed persons has not been determined.

Precautions

For in vitro diagnostic use only.

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs) available online in convenient and compact PDF format to view and print at **www.qiagen.com/safety**.

CAUTION: Handle human blood as if potentially infectious.

Observe relevant blood handling guidelines. Dispose of samples and materials in contact with blood or blood products in accordance with federal, state, and local regulations.

QuantiFERON Enzyme Stopping Solution

Contains: sulfuric acid. Warning! May be corrosive to metals. Causes skin irritation. Causes serious eye irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Enzyme Substrate Solution

Warning! Causes mild skin irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Green Diluent

Contains: tartrazine. Warning! May cause an allergic skin reaction. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Wash Buffer 20x Concentrate

Harmful to aquatic life with long lasting effects. Avoid release to the environment.

Further information

Safety Data Sheets: **www.qiagen.com/safety**

- Thimerosal is used as a preservative in some QFT-Plus reagents. It may be toxic upon ingestion, inhalation or skin contact.
- Deviations from the QuantiFERON-TB Gold Plus (QFT-Plus) Package Insert may yield erroneous results. Please read the instructions carefully before use.
- Do not use kit if any reagent bottle shows signs of damage or leakage prior to use.
- **Important:** Inspect vials prior to use. Do not use Conjugate or IFN-γ Standard vials that show signs of damage or if the rubber seal has been compromised. Do not handle broken vials. Take the appropriate safety precautions to dispose of vials safely. Recommendation: Use a vial de-crimper to open the Conjugate or IFN-γ Standard vials to minimize risk of injury from the metal crimp cap.
- Do not mix or use the Microplate strips, IFN-γ Standard, Green Diluent, or Conjugate 100x Concentrate from different QFT-Plus kit batches. Other reagents (Wash Buffer 20x Concentrate, Enzyme Substrate Solution, and Enzyme Stopping Solution) can be interchanged between kits providing the reagents are within their expiration periods and lot details recorded.
- Discard unused reagents and biological samples in accordance with Local, State, and Federal regulations.
- Do not use the QFT-Plus Blood Collection tubes or ELISA kit after the expiration date.
- Correct laboratory procedures should be adhered to at all times.
- Make sure that laboratory equipment such as plate washers and readers have been calibrated/validated for use.

Specimen Collection and Handling

Protocol: Specimen collection

QFT-Plus uses the following collection tubes:

Specimen collection tubes

- QuantiFERON Nil tubes (gray cap with white ring; use between sea level and 2650 feet (810 meters)
- QuantiFERON TB1 tubes (green cap with white ring; use between sea level and 2650 feet (810 meters)
- QuantiFERON TB2 tubes (yellow cap with white ring; use between sea level and 2650 feet (810 meters)
- QuantiFERON Mitogen tubes (purple cap with white ring; use between sea level and 2650 feet (810 meters)

High Altitude (HA) tubes

- QuantiFERON HA Nil tubes (gray cap with yellow ring; use between 3350 and 6150 feet (1020 and 1875 meters)
- QuantiFERON HA TB1 tubes (green cap with yellow ring; use between 3350 and 6150 feet (1020 and 1875 meters)
- QuantiFERON HA TB2 tubes (yellow cap with yellow ring; use between 3350 and 6150 feet (1020 and 1875 meters)
- QuantiFERON HA Mitogen tubes (purple cap with yellow ring; use between 3350 and 6150 feet (1020 and 1875 meters)

Antigens have been dried onto the inner wall of the blood collection tubes, so it is essential that the contents of the tubes be thoroughly mixed with the blood. For blood directly drawn into the QFT-Plus Blood Collection Tubes, the QFT-Plus Blood Collection Tubes must be transferred to a 37°C incubator as soon as possible and within 16 hours of collection. Alternatively, blood may be collected into a single lithium or sodium-heparin tube for storage prior to transfer to QFT-Plus Blood Collection Tubes and incubation. Blood specimens collected in lithium or sodium-heparin tubes can be stored up to 12 hours at room temperature (17–25°C) followed by transfer to QFT-Plus Blood Collection Tubes, or blood specimens in lithium or sodium-heparin tubes can be transferred to QFT-Plus Blood Collection Tubes directly after collection. Blood specimens in lithium or sodium-heparin tubes may also be stored at 2–8 °C for up to 48 hours prior to transfer to the QFT-Plus Blood Collection Tubes. Refer to section "Blood collection into a single lithium or sodium-heparin tube and then transfer to QFT-Blood Collection Tubes", page 20.

Important: Blood collection tubes (QFT-Plus Blood Collection Tubes for direct draw, or lithium sodium-heparin tubes when blood is collected into a single lithium or sodium-heparin tube initially) should be at room temperature (17–25°C/62.6 –77°F) at the time of blood collection.

Blood collection and hold time options

See Blood Collection Options below (Figures 1–3).

Direct draw into QFT-Plus Blood Collection Tubes

1. Label tubes appropriately.

   Make sure each tube (Nil, TB1, TB2 and Mitogen) is identifiable by its label or other means once the cap is removed.

   It is recommended to record the time and date of blood collection.

   Important: Blood collection tubes should be at room temperature 17–25°C (62.6–77°F) at the time of blood collection.

2. For each patient, collect 1 ml of blood by venipuncture directly into each of the QFT-Plus Blood Collection Tubes. This procedure should be performed by a trained phlebotomist.
   a) As 1 ml tubes draw blood relatively slowly, keep the tube on the needle for 2–3 seconds once the tube appears to have completed filling. This will ensure that the correct volume is drawn.
   b) The black mark on the side of the tubes indicates the validated range of 0.8 to 1.2 ml. If the level of blood in any tube is outside of the indicator mark, a new blood sample should be obtained. Under or over-filling of the tubes outside of the 0.8 to 1.2 ml range may lead to erroneous results.
   c) If a "butterfly needle" is being used to collect blood, a "purge" tube should be used to ensure that the tubing is filled with blood prior to the QFT-Plus Blood Collection Tubes being used.
   d) QFT-Plus Blood Collection Tubes can be used up to an altitude of 2650 feet (810 meters) above sea level. HA QFT-Plus Blood Collection Tubes should be used at altitudes between 3350 and 6150 feet (1020 and 1875 meters).
   e) If using QFT-Plus Blood Collection Tubes outside these altitude ranges between 2650 and 3350 feet (810 and 1020 meters) or above 6150 feet (1875 meters), or if low blood draw volume occurs, users can collect blood with a syringe, and immediately transfer 1 ml to each of the 4 tubes. For safety reasons, this is best performed by removing the syringe needle, ensuring appropriate safety procedures, removing the caps from the 4 QFT-Plus Blood Collection Tubes and adding 1 ml of blood to each (to the black mark on the side of the tube label which indicates the validated range of 0.8 to 1.2 ml). Replace the caps securely and mix as described below. Ensure each tube (Nil, TB1, TB2 and Mitogen) is identifiable by its label or other means once the cap is removed.
3. Immediately after filling the tubes, shake them ten (10) times just firmly enough to make sure the entire inner surface of the tube is coated with blood. This will dissolve antigens on the tube walls.

   Over vigorous shaking may cause gel disruption and could lead to aberrant results.

4. Following labeling, filling and shaking, the tubes must be transferred to a 37°C ± 1°C incubator as soon as possible, and within 16 hours of collection. Prior to incubation, maintain tubes at room temperature [17–25°C (62.6–77°F)]. If QFT-Plus Blood Collection Tubes are not incubated at 37°C directly after blood collection and shaking, invert the tubes to mix 10 times prior to incubation at 37°C.
5. Incubate the QFT-Plus Blood Collection Tubes UPRIGHT at 37°C ± 1°C for 16 to 24 hours.

   The incubator does not require CO_2 or humidification.

Blood collection into a single lithium or sodium-heparin tube and then transfer to QFT-Plus Blood Collection Tubes

1. Blood may be collected in a single blood collection tube containing lithium or sodium-heparin as the anticoagulant and then transferred to QFT-Plus Blood Collection Tubes. Only use lithium or sodium-heparin as a blood anticoagulant because other anticoagulants interfere with the assay. 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 (62.6–77°F) at the time of blood collection.
2. Fill a lithium or sodium-heparin blood collection tube (minimum volume 5 ml) and gently mix by inverting the tube several times to dissolve the heparin. This procedure should be performed by a trained phlebotomist.
3. Hold time and temperature options for lithium or sodium-heparin tubes prior to transfer and incubation in QFT-Plus Blood Collection Tubes (See Figures 1–3 Blood Collection Options).

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

   Blood collected in lithium or sodium-heparin tube must be maintained at room temperature [17–25°C (62.6–77°F)] 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 or Sodium-Heparin Tube Refrigerated Storage and Handling

Important: Procedural steps a–d must be followed in sequence.

a) Blood drawn into a lithium or sodium-heparin tube may be held at room temperature (17–25°C) up to 3 hours after blood collection.

b) Blood drawn into a lithium or sodium-heparin tube may be refrigerated (2–8°C) for up to 48 hours.

c) After refrigeration, the lithium or sodium-heparin tube must equilibrate to room temperature (17–25°C) prior to transfer to QFT-Plus Blood Collection Tubes.

d) Aliquotted QFT-Plus Blood Collection Tubes should be placed in the 37°C incubator within 2 hours of blood transfer.

If QFT-Plus Blood Collection Tubes are not incubated at 37°C directly after transfer to QFT-Plus Blood Collection Tubes and shaking, 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 from a lithium or sodium-heparin tube 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 or sodium-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 as described below. Ensure each tube (Nil, TB1, TB2 and Mitogen) is identifiable by its label or other means once the cap is removed.

5. Mix tubes. Immediately after filling the QFT-Plus Blood Collection Tubes, shake them ten (10) times just firmly enough to make sure the entire inner surface of the tube is coated with blood. This will dissolve antigens on tube walls.

   Overly vigorous shaking may cause gel disruption and could lead to aberrant results.

6. Following labeling, filling and shaking, the tubes must be transferred to a 37°C ± 1°C incubator within 2 hours. If QFT-Plus Blood Collection Tubes are not incubated at 37°C directly after blood collection and shaking, invert the tubes to mix 10 times (10x) prior to incubation at 37°C. (See Figures 1–3, next page for blood collection options).

7. Incubate the QFT-Plus Blood Collection Tubes UPRIGHT at 37°C ± 1°C for 16 to 24 hours.

   The incubator does not require CO_2 or humidification.

Draw into QFT-Plus Blood Collection Tubes and hold at room temperature

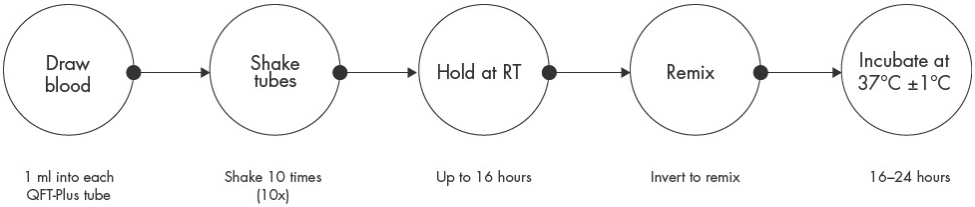


Figure 1. Blood collection option: Direct draw into QFT-Plus Blood Collection Tubes and hold at room temperature. The total time from blood draw in QFT-Plus Blood Collection Tubes to 37°C incubation must not exceed 16 hours.

Draw into lithium or sodium-heparin tube and hold at room temperature

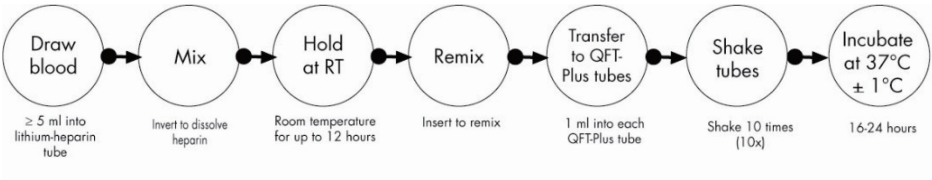
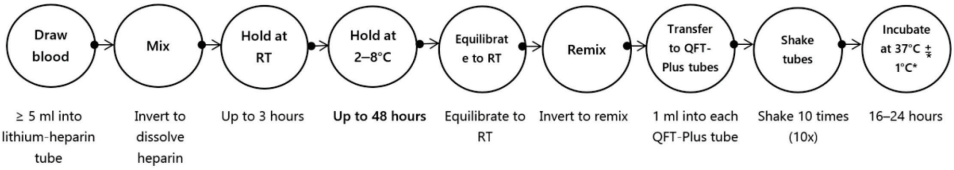


Figure 2. Blood collection option: Draw into lithium or sodium-heparin tube and hold at room temperature. The total time from blood draw in lithium or sodium-heparin tube to 37°C incubation must not exceed 12 hours.

Draw into lithium or sodium-heparin tubes and hold at 2–8°C



* Aliquotted QFT-Plus Blood Collection Tubes should be placed in a 37°C incubator within 2 hours of blood transfer to QFT-Plus Blood Collection Tubes.

Figure 3. Blood collection option: Draw into lithium or sodium-heparin tube and hold at 2–8°C. The total time from blood draw in lithium or sodium-heparin tube to 37°C incubation must not exceed 53 hours.

Directions for Performing ELISA

Time required for performing assay

In order to obtain valid results from the QFT-Plus assay, the operator needs to perform specific tasks within set times. Prior to use of the assay it is recommended that the operator plan each stage of the assay carefully to allow adequate time to perform each stage. The time required is estimated below; the time of testing multiple samples when batched is also indicated.

ELISA plate:

- Approximately 3 hours for one ELISA Plate
- <1 hour labor
- Add 10 to 15 minutes for each extra plate

Stage 1: Post-incubation of blood collection tubes and harvesting of plasma

Prior to harvesting plasma, samples in QFT-Plus Blood Collection Tubes must have been incubated at 37°C for 16–24 hours. The incubator does not require CO_2 or humidification. See "Protocol: Specimen collection" section, page 17.

Procedure

1. After incubation of the blood collection tubes at 37°C ± 1°C, tubes may be held between 4°C and 27°C for up to 3 days prior to centrifugation.
2. After incubation of the tubes at 37°C ± 1°C, harvesting of the plasma is facilitated by centrifuging tubes for 15 minutes at 2000 to 3000 RCF (*g*). The gel plug will separate the cells from the plasma. If this does not occur, the tubes should be re-centrifuged.

3. It is possible to harvest the plasma without centrifugation, but additional care is required to remove the plasma without disturbing the cells.
4. Plasma samples should only be harvested using a pipet.

   Important: After centrifugation, avoid pipetting plasma up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel.

   Plasma samples can be loaded directly from centrifuged blood collection tubes into the QFT-Plus ELISA plate.

   Plasma samples can be stored in centrifuged QFT-Plus Blood Collection Tubes for up to 28 days at 2°C to 8°C. Or harvested plasma samples can be stored for up to 28 days at 2°C to 8°C. Harvested plasma samples can also be stored below –20°C (preferably less than –70°C) for extended periods.

   For adequate test samples, harvest at least 150 µl of plasma.

Stage 2: IFN-γ ELISA

Refer to "Kit contents" and "Materials Required but Not Provided", page 11, for materials required to perform ELISA.

Procedure

1. All plasma samples and reagents, except for Conjugate 100x Concentrate, must be brought to room temperature (22°C ± 5°C [71.6°F ± 9°F]) before use. Allow at least 60 minutes for equilibration.
2. Remove ELISA plate strips that are not required from the frame, reseal in the foil pouch, and return to the refrigerator for storage until required.
3. Allow at least 1 strip for the QFT-Plus standards and sufficient strips for the number of subjects being tested (refer to Figure 5 for recommended plate format). After use, retain frame and lid for use with remaining strips.

a) Reconstitute the IFN-γ Standard with the volume of deionized or distilled water indicated on the label of the vial. Mix gently to minimize frothing and ensure that the entire content of the vial is completely dissolved. Reconstitution of the IFN-γ standard to the correct volume will produce a solution with a concentration of 8.0 IU/ml.

b) Using the reconstituted standard, prepare a dilution series of 4 IFN-γ concentrations (refer to Figure 4).

c) A standard curve should be generated with the following IFN-γ concentrations: S1 (Standard 1) contains 4.0 IU/ml, S2 (Standard 2) contains 1.0 IU/ml, S3 (Standard 3) contains 0.25 IU/ml, and S4 (Standard 4) contains 0 IU/ml (Green Diluent [GD] alone).

d) The standards must be assayed at least in duplicate.

e) Prepare fresh dilutions of the kit standard for each ELISA session.

Example of procedure for duplicate standards

Example of procedure for duplicate standards	
A	Label 4 tubes: S1, S2, S3, S4
B	Add 150 µl of GD to S1, S2, S3, S4
C	Add 150 µl of the kit standard to S1 and mix thoroughly
D	Transfer 50 µl from S1 to S2 and mix thoroughly
E	Transfer 50 µl from S2 to S3 and mix thoroughly
F	GD alone serves as the zero standard (S4)

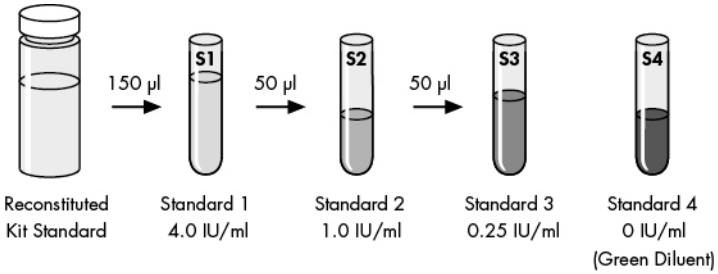


Figure 4. Preparation of standard curve dilution series.

4. Reconstitute lyophilized Conjugate 100x Concentrate with 0.3 ml of deionized or distilled water. Mix gently to minimize frothing and ensure that the entire content of the vial is completely dissolved.
   a) Working strength conjugate is prepared by diluting the required amount of reconstituted Conjugate 100x Concentrate in Green Diluent (Table 1).
   b) Working strength conjugate should be used within 6 hours of preparation.
   c) Return any unused Conjugate 100x Concentrate to 2°C to 8°C immediately after use.

Table 1. Conjugate preparation (working strength)

Number of strips	Volume of conjugate (100x concentrate)	Volume of Green Diluent
2	10 µl	1.0 ml
3	15 µl	1.5 ml
4	20 µl	2.0 ml
5	25 µl	2.5 ml
6	30 µl	3.0 ml
7	35 µl	3.5 ml
8	40 µl	4.0 ml
9	45 µl	4.5 ml
10	50 µl	5.0 ml
11	55 µl	5.5 ml
12	60 µl	6.0 ml

5. For plasma samples harvested from blood collection tubes and subsequently stored (refrigerated or frozen), thoroughly mix the stored sample before addition to the ELISA well.

   Important: If plasma samples are to be added directly from the centrifuged QFT-Plus Blood Collection Tubes, any mixing of the plasma should be avoided. At all times take care not to disturb material on the surface of the gel.

6. Add 50 µl of freshly prepared working strength conjugate to each ELISA plate well.
7. Add 50 µl of test plasma sample to appropriate wells (refer to recommended ELISA plate layout in Figure 5).
8. Finally, add 50 µl each of the Standards 1 to 4 to the appropriate plate wells (refer to recommended ELISA plate layout in Figure 5). The standards should be assayed in at least duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 N	3 N	5 N	7 N	9 N	S1	S1	13 N	15 N	17 N	19 N	21 N
B	1 TB1	3 TB1	5 TB1	7 TB1	9 TB1	S2	S2	13 TB1	15 TB1	17 TB1	19 TB1	21 TB1
C	1 TB2	3 TB2	5 TB2	7 TB2	9 TB2	S3	S3	13 TB2	15 TB2	17 TB2	19 TB2	21 TB2
D	1 M	3 M	5 M	7 M	9 M	S4	S4	13 M	15 M	17 M	19 M	21 M
E	2 N	4 N	6 N	8 N	10 N	11 N	12 N	14 N	16 N	18 N	20 N	22 N
F	2 TB1	4 TB1	6 TB1	8 TB1	10 TB1	11 TB1	12 TB1	14 TB1	16 TB1	18 TB1	20 TB1	22 TB1
G	2 TB2	4 TB2	6 TB2	8 TB2	10 TB2	11 TB2	12 TB2	14 TB2	16 TB2	18 TB2	20 TB2	22 TB2
H	2 M	4 M	6 M	8 M	10 M	11 M	12 M	14 M	16 M	18 M	20 M	22 M

Figure 5. Recommended ELISA plate layout. S1 (Standard 1), S2 (Standard 2), S3 (Standard 3), S4 (Standard 4). 1N (Sample 1. Nil Control plasma), 1 TB1 (Sample 1. TB1 plasma), 1 TB2 (Sample 1. TB2 plasma), 1M (Sample 1. Mitogen plasma.

9. Cover ELISA plate and mix the conjugate and plasma samples/standards thoroughly using a microplate shaker for 1 minute at 500 to 1000 rpm. Avoid splashing.
10. Cover ELISA plate and incubate at room temperature (22°C ± 5°C [71.6°F ± 9°F]) for 120 ± 5 minutes. ELISA plate should not be exposed to direct sunlight during incubation. Deviation from specified temperature range can lead to erroneous results.
11. During the ELISA plate incubation prepare working strength wash buffer. Dilute one part Wash Buffer 20x Concentrate with 19 parts deionized or distilled water and mix thoroughly. Sufficient Wash Buffer 20x Concentrate has been provided to prepare 2 liters of working strength wash buffer.
12. When ELISA plate incubation is complete, wash ELISA plate wells with 400 µl of working strength wash buffer. Perform wash step at least 6 times. An automated plate washer is recommended for safety reasons when handling plasma samples.

    Thorough washing is very important to the performance of the assay. Make sure each well is completely filled with wash buffer to the top of the well for each wash cycle. A soak period of at least 5 seconds between each cycle is recommended.

    Standard laboratory disinfectant should be added to the effluent reservoir, and established procedures followed for the decontamination of potentially infectious material.

13. Tap ELISA plate face down on absorbent (low-lint) towel to remove residual wash buffer. Add 100 µl of Enzyme Substrate Solution to each plate well, cover the plate and mix thoroughly for 1 minute at 500 to 1000 rpm using a microplate shaker.
14. Cover ELISA plate and incubate at room temperature (22°C ± 5°C [71.6°F ± 9°F]) for 30 minutes. ELISA plate should not be exposed to direct sunlight during incubation.
15. Following the 30 minute incubation, add 50 µl of Enzyme Stopping Solution to each plate well in the same order as the substrate was added and mix thoroughly at 500 to 1000 rpm using a microplate shaker.
16. Measure the Optical Density (OD) of ELISA plate wells within 5 minutes of stopping the reaction using a microplate reader fitted with a 450 nm filter and with a 620 nm to 650 nm reference filter. OD values are used to calculate results.

Calculations and Test Interpretation

QFT-Plus Analysis Software can be used to analyze raw data and calculate results. It is available at **www.qiagen.com**. Please make sure that the most current version of the QFT-Plus Analysis Software is used.

The software performs a Quality Control assessment of the assay, generates a standard curve and provides a test result for each subject, as detailed in "Interpretation of results", page 34. The software reports all concentrations greater than 10 IU/ml as ">10" as such values fall beyond the validated linear range of the ELISA.

As an alternative to using the QFT-Plus Analysis Software, results can be determined according to the following method.

Generation of standard curve and sample values

If QFT-Plus Analysis Software is not used

Determination of the standard curve and determination of sample IU/ml values require a spreadsheet program, such as Microsoft® Excel®, if the QFT-Plus software is not used.

Using a spreadsheet program:

1. Determine the mean OD values of the kit standard replicates on each plate.
2. Construct a $\log_{(e)}$–$\log_{(e)}$ standard curve by plotting the $\log_{(e)}$ of the mean OD (y axis) against the $\log_{(e)}$ of the IFN-γ concentration of the standards in IU/ml (x axis), omitting the zero standard from these calculations. Calculate the line of best fit for the standard curve by regression analysis.
3. Use the standard curve to determine the IFN-γ concentration (IU/ml) for each of the test plasma samples, using the OD value of each sample.

4. These calculations can be performed using software packages available with microplate readers, and standard spreadsheet or statistical software (such as Microsoft Excel). It is recommended that these packages be used to calculate the regression analysis, the coefficient of variation (%CV) for the standards, and the correlation coefficient (*r*) of the standard curve.

Sample calculation

If the following OD readings were obtained for the standards, the calculations using – $\log_{(e)}$ – would follow those in Table 2.

Table 2. Standard curve

Standard	IU/ml	OD values a and b	Mean OD	%CV	$\text{Log}_{(e)}$ IU/ml	$\text{Log}_{(e)}$ Mean (OD)
Standard 1	4	1.089, 1.136	1.113	3.0	1.386	0.107
Standard 2	1	0.357, 0.395	0.376	7.1	0.000	–0.978
Standard 3	0.25	0.114, 0.136	0.125	NA	–1.386	–2.079
Standard 4	0	0.034, 0.037	0.036	NA	NA	NA

The equation of the curve is y = 0.7885(X) – 0.9837, where "m" = 0.7885 and "c" = –0.9837. These values are used in the equation X = (Y–c)/m to solve for X. Based on the standard curve, the calculated correlation coefficient (*r*) = 1.000. NA: Not applicable.

Using the criteria specified in "Quality control of the test", page 33, the assay is determined to be valid.

The standard curve (Table 2) is used to convert the Antigen OD responses to International Units (IU/ml).

Table 3. Sample calculation

Antigen	OD value	$Log_{(e)}$ OD value	X	e^X (IU/ml)	Antigen –Nil (IU/ml)
Nil	0.037	–3.297	–2.934	0.05	–
TB1	1.161	0.149	1.437	4.21	4.15
TB2	1.356	0.305	1.634	5.12	5.07
Mitogen	1.783	0.578	1.981	7.25	7.20

IFN-γ values (in IU/ml) for the TB1, TB2 and Mitogen are corrected for background by subtracting the IU/ml value obtained for the respective Nil control. These corrected values are used for interpretation of the test results.

Quality control of the test

The accuracy of test results is dependent on the generation of an accurate standard curve. Therefore, results derived from the standards must be examined before test sample results can be interpreted.

For the ELISA to be valid:

- The mean OD value for Standard 1 must be ≥0.600.
- The %CV for Standard 1 and Standard 2 replicate values must be ≤15%.
- Replicate OD values for Standard 3 and Standard 4 must not vary by more than 0.040 optical density units from their mean.
- The correlation coefficient (*r*) calculated from the mean absorbance values of the standards must be ≥0.98.
- If the above criteria are not met, the run is invalid and must be repeated.
- The mean OD value for the Zero Standard (Green Diluent) should be ≤0.150. If the mean OD value is > 0.150, the plate washing procedure should be investigated.

The QFT-Plus Analysis Software calculates and reports these quality control parameters.

Each laboratory should determine appropriate types of control materials and frequency of testing in accordance with Local, State, Federal, or other applicable accrediting organizations. External quality assessment and alternative validation procedures should be considered.

Note: Plasmas spiked with recombinant IFN-γ have shown reductions of up to 50% in concentration when stored at either 2°C to 8°C and –20°C. Recombinant IFN-γ is not recommended for establishing control standards.

Interpretation of results

Important: Diagnosing or excluding tuberculosis disease, and assessing the probability of LTBI, requires a combination of epidemiological, historical, medical, and diagnostic findings that should be taken into account when interpreting QFT-Plus results. See general guidance on the diagnosis and treatment of TB disease and LTBI at https://www.cdc.gov/tb/default.htm provided under section "US Centers for Disease Control and Prevention (CDC) Guidelines" below.

QFT-Plus results are interpreted using the following criteria (Table 4).

Table 4. Interpretation of QFT-Plus test results

Nil (IU/ml)	TB1 minus Nil (IU/ml)	TB2 minus Nil (IU/ml)	Mitogen minus Nil (IU/ml)*	QFT-Plus Result	Report/interpretation
≤8.0	≥0.35 and ≥25% of Nil	Any	Any	Positive†	*M. tuberculosis* infection likely
	Any	≥0.35 and ≥25% of Nil			
	<0.35 or ≥0.35 and <25% of Nil	<0.35 or ≥0.35 and <25% of Nil	≥0.50	Negative	*M. tuberculosis* infection NOT likely
	<0.35 or ≥0.35 and <25% of Nil	<0.35 or ≥0.35 and <25% of Nil	<0.50	Indeterminate‡	Likelihood of *M. tuberculosis* infection cannot be determined
>8.0§	Any				

* Responses to the Mitogen positive control (and occasionally TB Antigen) can be outside the range of the microplate reader. This has no impact on test results. Values >10 IU/ml are reported by the QFT-Plus software as >10 IU/ml.

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

‡ Refer to "Troubleshooting Guide", page 58 for possible causes.

§ In clinical studies, less than 0.25% of subjects had IFN-γ levels of >8.0 IU/ml for the Nil value.

The magnitude of the measured IFN-γ level cannot be correlated to stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease. A positive TB response in persons who are negative to Mitogen is rare, but has been seen in patients with TB disease. This indicates the IFN-γ response to TB antigens is greater than that to Mitogen, which is possible as the level of Mitogen does not maximally stimulate IFN-γ production by lymphocytes.

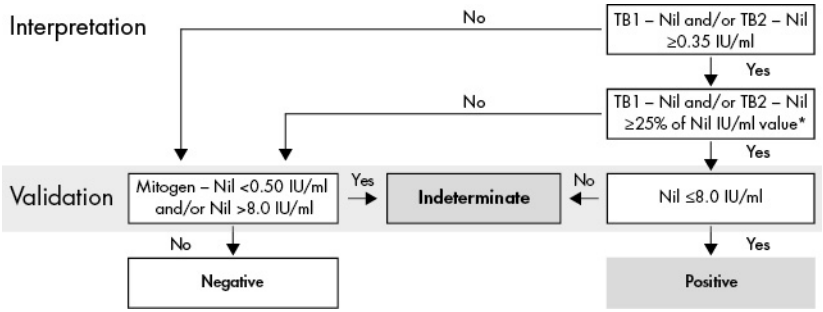


Figure 6. QFT-Plus test interpretation. *For TB1 minus Nil or TB2 minus Nil value to be valid, the ≥25% of Nil IU/ml value must be from the same tube as the original ≥0.35 IU/ml result.

Limitations

Results from QFT-Plus testing must be used in conjunction with each individual's epidemiological history, current medical status, and other diagnostic evaluations.

Individuals with Nil values greater than 8 IU/ml are classed as "Indeterminate" because a 25% higher response to TB Antigens may be outside the assay measurement range.

- The predictive value of a positive QFT-Plus result in diagnosing *M. tuberculosis* infection depends on the probability of infection, which is assessed by historical, epidemiological, diagnostic, and other findings.
- A diagnosis of LTBI requires that tuberculosis disease must be excluded by medical evaluation including an assessment of current medical and diagnostic tests for disease as indicated.
- A negative result must be considered with the individual's medical and historical data relevant to probability of *M. tuberculosis* infection and potential risk of progression to tuberculosis disease, particularly for individuals with impaired immune function. Negative predictive values are likely to be low for persons suspected to have *M. tuberculosis* disease and should not be relied on to exclude disease.
- The "Standard" and "High Altitude" blood collection tubes have both been calibrated for use at altitudes as specified in "Specimen Collection and Handling", page 17. Using either type of tube outside the recommended altitude ranges may result in an incorrect volume of blood draw and lead to an incorrect diagnostic outcome. If using the tubes outside the specified ranges of altitude, or if tubes do not fill to the indicator line, it is recommended to draw blood using a syringe and transfer the blood directly to the QFT-Plus Blood Collection Tubes manually or draw into a lithium or sodium-heparin tube followed by transfer to the QFT-Plus Blood Collection Tubes (see section "Blood collection into a single lithium or sodium-heparin tube and then transfer to QFT-Blood Collection Tubes", page 20).

Unreliable or indeterminate results may occur due to:

- Deviations from the procedure described in the package insert
- Incorrect transport/handling of blood specimen
- Elevated levels of circulating IFN-γ or presence of heterophile antibodies
- Exceeding validated blood times from blood specimen draw to incubation:
  - Blood samples collected directly into QFT-Plus Blood Collection Tubes stored longer than 16 hours at room temperature (17–25°C).
  - Blood samples collected in lithium or sodium-heparin tube stored longer than 12 hours at room temperature (17–25°C) prior to transfer to QFT-Plus Blood Collection Tubes.
  - Blood samples collected in lithium or sodium-heparin tube for refrigeration stored outside temperature and time ranges included in "Lithium or sodium-Heparin Tube Refrigerated Storage and Handling" procedure (page 21).

Performance Characteristics

Clinical studies

As there is no definitive standard test for confirming or excluding the diagnosis of LTBI, an estimate of sensitivity and specificity for QFT-Plus cannot be practically evaluated. Specificity of the QFT-Plus was approximated by evaluating false-positive rates in persons with low risk (no known risk factors) of tuberculosis infection. Sensitivity was approximated by evaluating groups of study subjects with culture-confirmed active TB disease. In addition, assay performance was evaluated for positive and negative rate in a population of healthy subjects with identified risk factors for tuberculosis infection (a mixed-risk population).

Specificity

A multi-center study evaluating the clinical specificity of the QFT-Plus was performed including 733 study subjects who were considered to have either low risk of *M. tuberculosis* infection or no risk factors for exposure to infection or disease. Demographic information and risk factors for TB exposure were determined using a standardized survey at the time of testing. The study was conducted at four independent sites, including one in the United States, two in Japan, and one in Australia. The QFT-Plus test was compared to the QuantiFERON-TB Gold-In-Tube (QFT) test. A summary of the clinical specificity performance data, stratified by study site and region is provided in Table 5. The performance results are based on the total number of valid tests. There were no indeterminate results.

Table 5. Interpretation of QFT-Plus test results

		Positive		Negative		Indeterminate		Specificity (95% CI)	
Site	**N**	**QFT**	**QFT-Plus**	**QFT**	**QFT-Plus**	**QFT**	**QFT-Plus**	**QFT**	**QFT-Plus**
United States									
(#1) USA-4	212	2	4	210	208	0	0	99.06% (210/212) (96.63–99.74)	98.11% (208/212) (95.25–99.26)
Japan									
(#2) JPN-3	106	1	2	105	104	0	0	99.06% (105/106) (94.85–99.83)	98.11% (104/106) (93.38–99.48)
(#3) JPN-1	216	3	5	213	211	0	0	98.61% (213/216) (96.00–99.53)	97.69% (211/216) (94.70–99.01)
Total Japan	322	4	7	318	315	0	0	98.76% (318/322) (96.85–99.52)	97.83% (315/322) (95.6–98.9)
Australia									
(#4) AU-3	199	8	9	191	190	0	0	95.98% (191/199) (92.27–97.95)	95.48% (190/199) (91.63–97.60)

The specificity of QFT-Plus in the US region was 98.11%, 97.83% in Japan and 95.48% in Australia. The overall specificity of the QFT-Plus was 97.27% (713/733). The specificity of QFT in the US region was 99.06%, 98.76% in Japan and 95.98% in Australia. The overall specificity of the QFT was 98.09% (719/733).

A breakdown of the results by TB antigen tube type and combinations thereof is shown to provide an example of expected results in a low-risk population (Table 6).

Table 6. QFT-Plus specificity study results by TB antigen tube

Interpretation based on TB Antigen-Nil IU/ml in	TB1	TB2	QFT-Plus (positive by TB1 and/or TB2)*	Concordant positive TB1 and TB2 (alternate analysis)†
Positive	10	18	20	8
Negative	723	715	713	725
Indeterminate	0	0	0	0
Specificity (95% CI)	–	–	97.3% (713/733) (95.8–98.2)	–
Negativity rate (95% CI)	98.6% (723/733) (97.5–99.3)	97.5% (715/733) (96.2–98.4)	–	98.9% (725/733) (97.9–99.5)

* Interpretation based on a TB antigen – Nil value ≥0.35 IU/ml in both (TB1 and TB2) or either TB tube to fit the interpretation criteria for the QFT-Plus (TB1 or TB2) to be determined positive.

† Alternate analysis provided for information only.

In the subjects with low risk for TB infection, a total of 20/733 subjects returned a positive result. Of these, only 8 subjects returned a value of >0.35 IU/ml in both TB1 and TB2 tubes. A comparison of the QFT versus QFT-Plus assays was performed in the low-risk study cohort, and showed overall concordance of 97.5% (715/733), and a negative percent agreement of 98.3% (707/719).

Sensitivity

While there is no definitive standard test for LTBI, a suitable surrogate is microbiological culture of *M. tuberculosis* because infection with TB is a necessary precursor to disease.

A multi-center study evaluating the clinical sensitivity of the QFT-Plus was performed including 434 study subjects who presented with signs and symptoms of active *M. tuberculosis* disease confirmed by culture and/or PCR, and were on no TB treatment or with ≤14 days of treatment prior to blood collection. The study was performed at 7 independent sites including three sites in the United States, three sites in Japan, and one site in Australia. The QFT-Plus test was compared to the QuantiFERON-TB Gold in Tube (QFT) test. A summary of the clinical sensitivity performance data, stratified by study site, and region is provided in Table 7. The performance results are based on the total number of valid tests. The frequency of indeterminate results for the QFT and QFT-Plus was 2.3% (10/434) and 2.5% (11/434), respectively.

Table 7. Clinical sensitivity study performance summary stratified by site and overall

		Positive		Negative		Indeterminate		Sensitivity (n/N) (95% CI)	
Site	**N**	**QFT**	**QFT-Plus**	**QFT**	**QFT-Plus**	**QFT**	**QFT-Plus**	**QFT**	**QFT-Plus**
United States									
(#1) USA-5	15	13	13	2	2	0	0	86.67% (13/15) (62.12–96.26)	86.67% (13/15) (62.12–96.26)
(#2) USA-1	33	29	29	4	4	0	0	87.88% (29/33) (72.67–95.18)	87.88% (29/33) (72.67–95.18)
(#3) USA-4	5	5	5	0	0	0	0	100.0% (5/5) (56.55–100.0)	100.0% (5/5) (56.55–100.0)
Total United States	53	47	47	6	6	0	0	88.7% (47/53) (77.4–94.7)	88.7% (47/53) (77.4–94.7)
Japan									
(#4) JPN-2	76	72	67	1	3	3	6	98.63% (72/73) (92.64–99.76)	95.71% (67/70) (88.14–98.53)
(#5) JPN-3	99	97	98	2	1	0	0	97.98% (97/99) (92.93–99.44)	98.99% (98/99) (94.50–99.82)
(#6) JPN-1	177	159	157	12	15	6	5	92.98% (159/171) (88.14–95.94)	91.28% (157/172) (86.11–94.64)
Total Japan	352	328	322	15	19	9	11	95.63% (328/343) (92.91–97.33)	94.43% (322/341) (91.5–96.4)
Australia									
(#7) AU-2	29	27	29	1	0	1	0	96.43% (27/28) (82.29–99.37)	100.0% (29/29) (88.30–100.0)

The analysis above in the table does not include indeterminate results.

The sensitivity of QFT-Plus in the US region was 88.7%, 94.43% in Japan and 100.0% in Australia. The overall sensitivity of the QFT-Plus was 94.09% (398/423). The sensitivity of QFT in the US region was 88.7%, 95.63% in Japan and 96.43% in Australia. The overall sensitivity of the QFT was 94.81% (402/424).

A breakdown of the results by TB antigen tube type and combinations of tubes is shown to provide an example of expected results in a confirmed TB infected population (Table 8).

Table 8. QFT-Plus sensitivity study results by TB antigen tube

Interpretation based on TB Antigen-Nil IU/ml in	**TB1**	**TB2**	**QFT-Plus (positive by TB1 and/or TB2)**
Positive	388	397	398
Negative	32	26	25
Indeterminate	14	11	11
Sensitivity* (95% CI)	–	–	94% (398/423) (91.4–96.0)
Positivity rate* (95% CI)	92.4% (388/420) (89.4–94.6)	93.9% (397/423) (91.1–95.8)	–

* Excluding indeterminate values.

A comparison of the QFT and QFT-Plus assays was assessed in the culture confirmed active TB cohort (sensitivity study cohorts) and showed overall concordance of 95.9% and a positive percent agreement of 97.3% (391/402).

Performance in subjects with identified risk factors for a MTB infection (mixed-risk individuals)

A cohort of 601 individuals with mixed risk factors for TB infection (e.g., HIV positivity, history of treatment for active or latent TB, exposure to active TB case, HCW status, etc.) was assessed with both the QFT and QFT-Plus tests. Risk factors were identified using a standardized survey and individuals displayed no symptoms associated with active TB at the time of recruitment. Demographics and risk factors are reported in Table 9. In this population 68/601 (11.3%) subjects returned a positive QFT-Plus result, with a positive percent agreement (PPA) and negative percent agreement (NPA) of 98.44% and 99.07%, respectively (Table 10). In this cohort of 68 QFT-Plus positive subjects, a total of 62 subjects were positive by both TB1 and TB2 tubes, 2 subjects were positive by TB1 only, and 4 subjects were positive by TB2 only. No indeterminate results (0/601) were observed.

Table 9. Demographics and factors associated with risk of TB infection in a mixed cohort

Total subjects (601)		Number	Percentage
Gender	Male	539	89.7%
	Female	62	10.3%
Age (years)	Range	18–70	–
	Mean	46.7	
BCG vaccinated	Yes	15	2.5%
	No	586	97.5%
HIV positive or tested positive for HTLV viruses	Yes	12	2.0%
	No	589	98%
Previously diagnosed with active TB	Yes	11	1.8%
	No	590	98.2%
Had a positive Tuberculin Skin Test (TST)/Mantoux test for TB	Yes	47	7.8%
	No	554	92.2%
Ever been treated for active or latent TB	Yes	35	5.8%
	No	566	94.2%
Lived, worked or volunteered (>1 month) in a jail or prison	Yes	373	62.1%
	No	228	37.9%
Lived, worked or volunteered (>1 month) in a homeless shelter	Yes	525	87.4%
	No	76	12.6%
Healthcare worker	Yes	8	1.3%
	No	593	98.7%
Close contact of someone with or suspected of having active TB disease	Yes	9	1.5%
	No	592	98.5%

Table 10. Summary performance of QFT-Plus versus QFT in subjects with known risk factors for latent TB infection

		QFT		
		Positive (+)	**Negative (–)**	**Total**
QFT-Plus	**Positive (+)**	63	5*	68
	Negative (–)	1*	532	533
	Total	64	537	601

*All 6 discordant samples had IFN-γ levels of the TB Antigen tubes that were close to the assay cut-off.

The positive percent agreement (PPA) and negative percent agreement (NPA) between the results of the QFT and the QFT-Plus were as follows:

- PPA: 98.44% (63/64), 95%CI (91.67, 99.72)
- NPA: 99.07% (532/537), 95% CI (97.84, 99.60)

Table 11 below illustrates the performance of the QFT-Plus as compared to the QFT test in BCG vaccinated study subjects.

Table 11. Performance of the QFT-Plus as compared to the QFT Test in BCG-vaccinated study subjects (combined data from sensitivity, specificity, and LTBI study subjects)

		QFT		
		Positive (+)	**Negative (–)**	**Total**
QFT-Plus	**Positive (+)**	66	5	71
	Negative (–)	3	268	271
	Total	69	273	342*

*Two Sensitivity Study Subjects were excluded from the analysis due to indeterminate results.

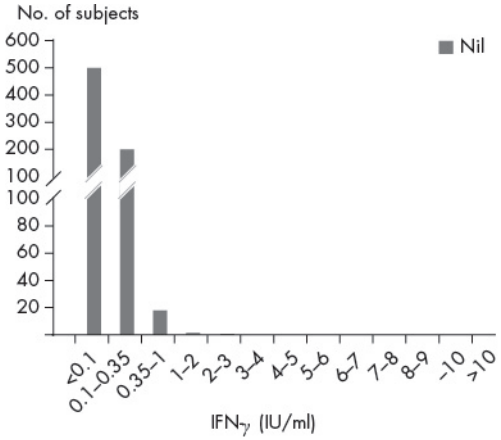
- PPA = 95.6% (66/69), 95%CI (87.98, 98.51)
- NPA = 98.2% (268/273), 95%CI (95.79, 99.22)

Expected Values

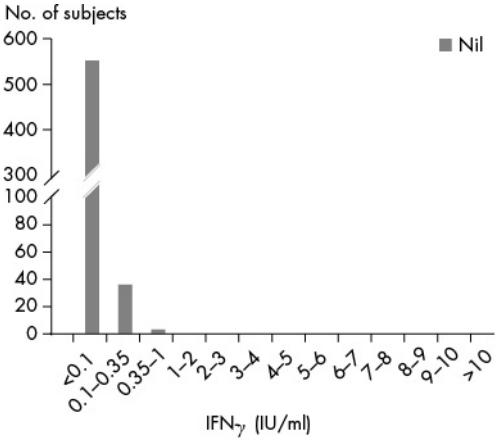
Observed response distributions — risk stratified

A range of IFN-γ responses to TB1, TB2, and control tubes were observed in clinical trials and stratified by risk of *M. tuberculosis* infection (Figure 7 through Figure 10). The mixed risk group consists of subjects representative of a general testing population, including subjects with and without risk factors for TB exposure, and where active TB is unlikely (i.e., LTBI).

A



B



C

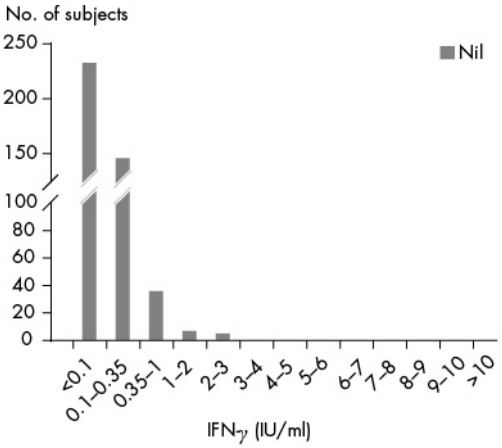
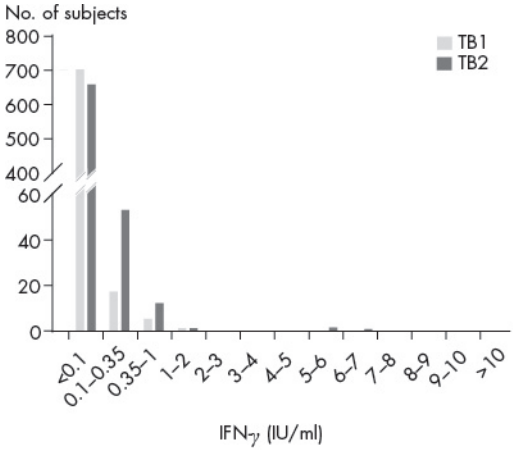
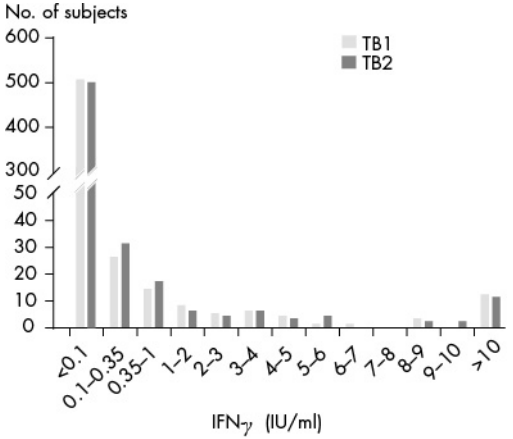


Figure 7. Distribution of Nil. **A** Distribution of Nil values in a low-risk population (n=744). **B** Distribution of Nil values in a mixed-risk population (n=601). **C** Distribution of Nil values in a population with culture-confirmed *M. tuberculosis* infection (n=416).

A


No. of subjects
800
700
600
500
400
60
40
20
0
TB1
TB2
<0.1
0.1–0.35
0.35–1
1–2
2–3
3–4
4–5
5–6
6–7
7–8
8–9
9–10
>10
IFN-γ (IU/ml)

B


No. of subjects
600
500
400
300
50
40
30
20
10
0
TB1
TB2
<0.1
0.1–0.35
0.35–1
1–2
2–3
3–4
4–5
5–6
6–7
7–8
8–9
9–10
>10
IFN-γ (IU/ml)

C

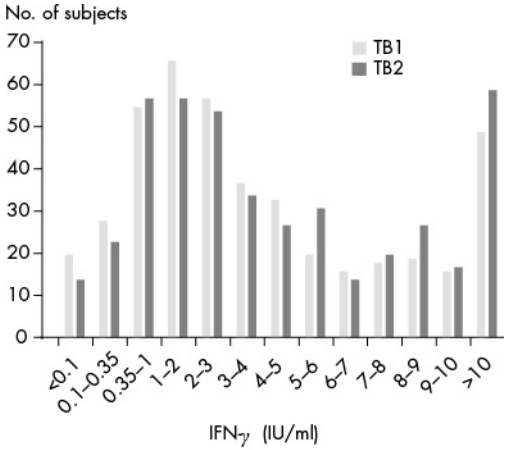
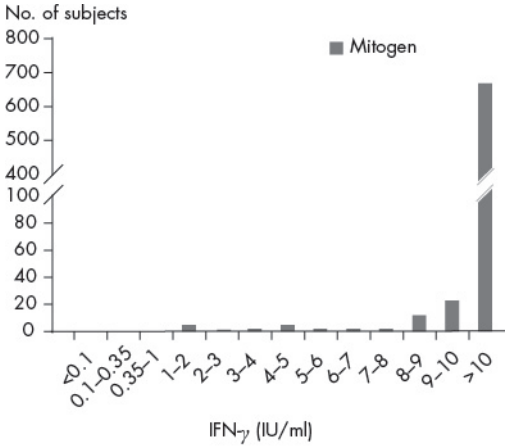
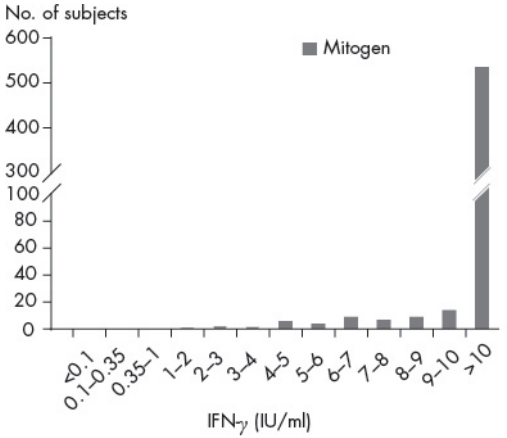


Figure 8. Distribution of TB1 and TB2 (nil subtracted). A Distribution of TB1 and TB2 (nil subtracted) values in a low-risk population (n=744). **B** Distribution of TB1 and TB2 (nil subtracted) values in a mixed-risk population (n=601). **C** Distribution of TB1 and TB2 (nil subtracted) values in a population with culture-confirmed *M. tuberculosis* infection (n=416).

A


No. of subjects
Mitogen
800
700
600
500
400
100
80
60
40
20
0
<0.1
0.1–0.35
0.35–1
1–2
2–3
3–4
4–5
5–6
6–7
7–8
8–9
9–10
>10
IFN-γ (IU/ml)

B


No. of subjects
Mitogen
600
500
400
300
100
80
60
40
20
0
<0.1
0.1–0.35
0.35–1
1–2
2–3
3–4
4–5
5–6
6–7
7–8
8–9
9–10
>10
IFN-γ (IU/ml)

C

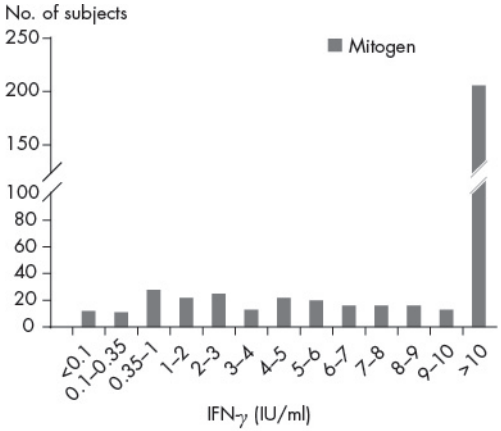


Figure 9. Distribution of Mitogen (nil subtracted). A Distribution of Mitogen (nil subtracted) values in a low-risk population (n=744). **B** Distribution of Mitogen (nil subtracted) values in a mixed-risk population (n=601). **C** Distribution of Mitogen (nil subtracted) values in a population with culture-confirmed *M. tuberculosis* infection (n=415).

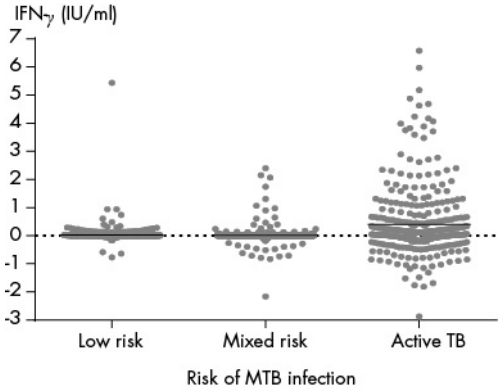


Figure 10. Observed difference between TB1 and TB2 values (nil subtracted), stratified by risk. Includes data from the Mixed-risk cohort study to show differences between low risk, active risk and mixed-risk cohorts. This data analysis included a mixed-risk cohort with known risk factors. Therefore, from the low risk cohort n=733, from the mixed-risk cohort n=588 and from the active TB cohort n=357. The quantitative difference in IU/ml for each subject was obtained by subtracting the TB1 value from the TB2 value.

Assay Performance Characteristics

A study was conducted to assess the linearity of the QFT ELISA using a 4-point standard curve. Two panels each of 11 contrived plasma samples were tested. The contrived samples were prepared by spiking negative plasma samples with either a 10 IU/ml IFN-γ concentrated plasma sample (High Pool), or a 1.5 IU/ml IFN-γ concentrated plasma sample (Low Pool) in a dilution series.

Weighted linear regression analysis of the calculated mean across replicates versus expected values (based on the dilution factor) was performed for both High and Low Pools.

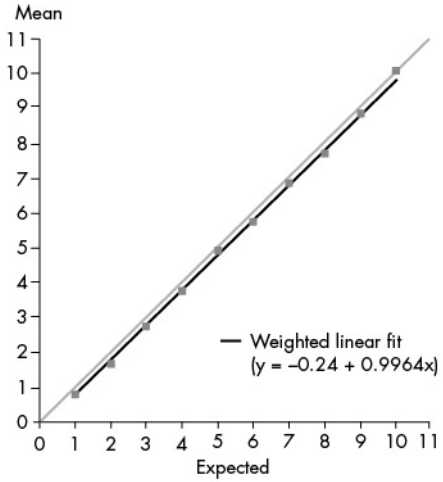


Figure 11. Illustration of Linearity Study Regression Analysis – High Pool Mean = –0.24 +0.9964•Expected.

Analysis of samples of the High Pool demonstrated linearity across the range of 0.79 IU/ml to 10 IU/ml with deviation from linearity ≤6.1%.

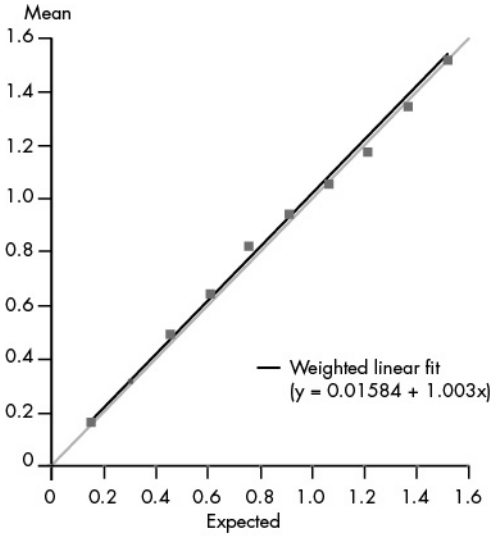


Figure 12. Illustration of Linearity Study Regression Analysis – Low Pool Mean = 0.016 +1.003•Expected

Analysis of samples of the Low Pool demonstrated linearity across the range of 0.17 IU/ml to 1.52 IU/ml with deviation from linearity ≤6.2%.

The combined study data demonstrated linearity across the range of 0.17 IU/ml to 10 IU/ml with deviation ≤6.2%. Deviation from linearity using the 4-point standard curve was observed to be less than 7%.

A multi-center study reproducibility study was conducted to evaluate performance of the QFT-Plus across study sites with multiple operators. This was a prospective study conducted at three external testing sites and one collection site. A total of 32 positive and 34 negative (determined by the currently marketed QFT test) study subjects were enrolled. The study subjects were comprised of healthcare workers in the United States. The study subjects represented group with mixed risk for TB exposure due to their occupation or as foreign born healthcare workers originating from a location with at TB rate exceeding 50/100,000.

Three LiHep blood collection tubes were obtained from each study subject at the collection site. The LiHep blood collection tubes were then transferred to each of three testing sites where they were aliquoted into two sets of QFT-Plus Blood Collection Tubes (QFT-Plus TB1, TB2, Mitogen, and Nil) then tested in accordance with the QFT-Plus assay procedure. At each site at least two operators ran the two tests per study subject independently. Each operator was blinded to the results obtained by the other operator and blinded to the QFT test result of the study subject.

There were six results generated across all three testing sites per each of the 66 study subjects, resulting in a total of 396 data points. A summary of the reproducibility summary results are provided in Table 12.

Table 12. Reproducibility study results summary – within site %agreement of qualitative results between operators; N = 66 patient samples

Site 1 – 2 Operators	Site 2 – 2 Operators	Site 3 – 3 Operators
64/66 = 96.97%	64/66 = 96.97%	59/66 = 89.39%
Agreement of Qualitative Results of Tube Set 1 and Tube Set 2	Agreement of Qualitative Results of Tube Set 1 and Tube Set 2	Agreement of Qualitative Results of Tube Set 1 and Tube Set 2

The qualitative percent agreement across all study sites is 94.7% (375/396). In this calculation, the total number of test results in agreement (375) includes, those instances where there is agreement of all 6 results, agreement of 5 out of 6 results, agreement of 4 out of 6 results, and agreement of 3 out 6 results, combined.

Technical Information

Indeterminate results

Indeterminate results are uncommon and may relate to the immune status of the individual being tested (5), but may also be related to a number of technical factors (e.g., inappropriate handling/storage of blood collection tubes, incomplete ELISA plate washing) if the above instructions for use are not followed.

If technical issues are suspected with the reagent storage, blood collection or handling of the blood samples, repeat the entire QFT-Plus test with new blood specimens. Repeating the ELISA testing of stimulated plasmas can be performed if inadequate washing or other procedural deviation with the ELISA test is suspected. Physicians may choose to redraw a specimen or perform other procedures as appropriate.

Clotted plasma samples

Should fibrin clots occur with long-term storage of plasma samples, centrifuge samples to sediment clotted material and facilitate pipetting of plasma.

Lipemic plasma samples

Care should be exercised when pipetting lipemic samples as fatty deposits can block pipet tips.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see the technical information provided at **www.qiagen.com**. For contact information, see the back cover.

		Comments and suggestions
ELISA troubleshooting		
Nonspecific color development		
a)	Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b)	Cross-contamination of ELISA wells	Take care while pipetting and mixing sample to minimize risk.
c)	Kit/components have expired	Ensure kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100X Concentrate are used within three months of the reconstitution date.
d)	Enzyme Substrate Solution is contaminated	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.
e)	Mixing of plasma in in QFT-Plus Blood Collection Tubes before harvesting	After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel.
Low optical density readings for standards		
a)	Standard dilution error	Ensure dilutions of the Kit Standard are prepared correctly as per this Package Insert.
b)	Pipetting error	Ensure pipets are calibrated and used according to manufacturer's instructions.
c)	Incubation temperature too low	Incubation of the ELISA should be performed at room temperature (22°C ± 5°C).
d)	Incubation time too short	Incubation of the plate with the conjugate, standards and samples should be for 120 ± 5 minutes. The Enzyme Substrate Solution should be incubated on the plate for 30 minutes.

		Comments and suggestions
e)	Incorrect plate reader filter used	Plate should be read at 450 nm with a reference filter of between 620 and 650 nm.
f)	Reagents are too cold	All reagents, with the exception of the Conjugate 100X Concentrate, must be brought to room temperature prior to commencing the assay. This takes approximately 1 hour.
g)	Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted Standard and Conjugate 100X Concentrate are used within 3 months of the reconstitution date.
High background		
a)	Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required. A soak time of at least 5 seconds between cycles should be used.
b)	Incubation temperature too high	Incubation of the ELISA should be performed at room temperature (22°C ± 5°C).
c)	Kit/components have expired	Ensure that the kit is used within the expiry date. Ensure reconstituted standard and Conjugate 100X Concentrate are used within three months of the reconstitution date.
d)	Enzyme Substrate Solution is contaminated	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.
Nonlinear standard curve and duplicate variability		
a)	Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required. A soak time of at least 5 seconds between cycles should be used.
b)	Standard dilution error	Ensure dilutions of the standard are prepared correctly as per this Package Insert.
c)	Poor mixing	Mix reagents thoroughly by inversion or gentle vortexing prior to their addition to the plate.
d)	Inconsistent pipetting technique or interruption during assay setup	Sample and standard addition should be performed in a continuous manner. All reagents should be prepared prior to commencing the assay.

US Centers for Disease Control and Prevention (CDC) Guidelines

Diagnostic testing for *Mycobacterium tuberculosis* using Interferon Gamma Release Assays should follow applicable published guidelines, including when testing in populations such as children, pregnant women, and HIV-infected or otherwise immunocompromised individuals. (Also see WARNINGS and PRECAUTIONS above regarding use in co-morbid conditions which affect immune function). Links to the most recent American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children, as well as to other information regarding diagnostic testing for tuberculosis, are available at https://www.cdc.gov/tb/publications/guidelines/testing.htm.

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A comprehensive list of QFT references is located in the QuantiFERON reference library, available at: **www.gnowee.net.**

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Symbols

The following symbols may appear on the packaging and labeling:

Symbol	Symbol definition
	Sufficient for n sample preparations
	For in vitro diagnostic use
	Lot number
	Catalog number
	Global Trade Item Number
	Use by
	Temperature limitation
	Consult instructions for use
	Do not reuse
	Keep away from sunlight

Contact Information

For technical assistance and more information, please call toll-free 800-362-7737, see our Technical Support Center at **www.qiagen.com/contact** or contact one of the QIAGEN Technical Service Departments (see back cover or visit **www.qiagen.com**).

Abbreviated ELISA Test Procedure

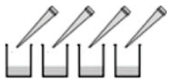
1. Equilibrate ELISA components, with the exception of the Conjugate 100x Concentrate, to room temperature for at least 60 minutes.

2. Reconstitute the Kit Standard to 8.0 IU/ml with distilled or deionized water. Prepare four (4) standard dilutions.

3. Reconstitute freeze-dried Conjugate 100x Concentrate with distilled or deionized water.

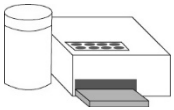
4. Prepare working strength conjugate in Green Diluent and add 50 µl to all wells.

5. Add 50 µl of test plasma samples and 50 µl standards to appropriate wells. Mix using shaker.



6. Incubate for 120 minutes at room temperature.

7. Wash wells at least 6 times with 400 µl/well of wash buffer.

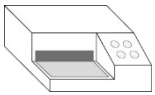


8. Add 100 µl Enzyme Substrate Solution to wells. Mix using shaker.

9. Incubate for 30 minutes at room temperature.

10. Add 50 µl Enzyme Stopping Solution to all wells. Mix using shaker.

11. Read results at 450 nm with a 620 to 650 nm reference filter



12. Analyze results.

Significant Changes

Significant changes in this edition of the QF-TB Gold Plus Package Insert are summarized in the table below:

Revision Level and Date	Section	Page	Change(s)
Rev. 09 2023/01	Specimen Collection and Handling	21-24	Added sodium-heparin tube as an alternative blood collection tube
Rev. 08 2022/06	Components and Storage, Kit component/ELISA components	10-11	Remove references to package insert (will now be online).
Rev. 07 2021/12	Materials Required but Not Provided	12	Added Plate Lid
	Specimen Collection and Handling	20	Define room temperature as17–25°C (62.6–77°F)
	Limitations	36, 68	Removal of Various Populations Limitations such as, Individuals who have impaired or altered immune functions, Individuals younger than age 17 years, Pregnant women based on FDA approval.
			Additional references added to "Reference" section.
Rev. 06 2019/11	Materials Required but Not Provided	12	Added Plate Lid
	Specimen Collection and Handling	20	Define room temperature as17–25°C (62.6–77°F)
Rev. 05 2019/04	Table 9 Various	46 Various	Updated data. Update lithium-heparin tube storage conditions post blood draw.
Rev. 04 2018/07	Components and Storage	10	Added catalog numbers related to new tube kits
	Precautions	15	New GHS information

Revision Level and Date	Section	Page	Change(s)
Rev. 03 2018/06	Precautions	15	New GHS information
Rev. 02 2017/08	Cover	Front Cover	Replaced Rx Only text with RX Only Symbol.
	Further Information	16	Removed MAT Symbol. Added Important note related to damaged vials and crimp cap safety.
Rev. 01 2017/06	N/A	N/A	Original Revision.

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