

QuantiFERON[®]-TB Gold

**The Whole Blood IFN-gamma Test
Measuring Responses to
ESAT-6 & CFP-10 Peptide Antigens**

Catalogue Number: 0598 0201

PACKAGE INSERT

For *In Vitro* Diagnostic Use



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1. INTENDED USE

QuantiFERON®-TB Gold is an *in vitro* diagnostic test using peptide cocktails simulating ESAT-6 and CFP-10 proteins to stimulate cells in heparinized whole blood. Detection of interferon- γ by ELISA is used to identify *in vitro* responses to ESAT-6 and CFP-10 that are associated with *Mycobacterium tuberculosis* infection.

- QuantiFERON®-TB Gold 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. Specimens for testing must be transported to laboratories to allow initiation of testing within 12 hr.

2. SUMMARY AND EXPLANATION OF THE TEST

The QuantiFERON®-TB Gold test is a test for 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 (*M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. canetti*) usually have lymphocytes in their blood that recognise these and other mycobacterial antigens. This recognition process involves the generation and secretion of the cytokine, interferon- γ (IFN- γ). The detection and subsequent quantification of IFN- γ forms the basis of this test.

Tuberculosis (TB) is a communicable disease caused by infection with *M. tuberculosis* complex, which typically spreads to new hosts via airborne droplet nuclei from patients with respiratory 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. Until recently the tuberculin skin test 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 skin test results after vaccination with Bacille Calmette-Guérin (BCG), infection with mycobacteria besides *M. tuberculosis* complex, or undetermined other factors.

LTBI must be distinguished from tuberculosis disease, a reportable condition which usually involves the lungs and lower respiratory tract, although most organ systems can be affected. Tuberculosis disease is diagnosed from historical, physical, radiological, histological, and mycobacteriological findings. The tuberculin skin test and QuantiFERON®-TB Gold 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 QuantiFERON®-TB Gold are peptide cocktails simulating the proteins ESAT-6 and CFP-10. Numerous studies have demonstrated that ESAT-6 and CFP-10 stimulate IFN- γ responses in T-cells from individuals infected with *M. tuberculosis* but usually not from uninfected or BCG vaccinated persons without disease or risk for LTBI.^{1-8, 10-17, 19-30} 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 both proteins are present in *M. kansasii*, *M. szulgai* and *M. marinum*.^{1,19}

Risk factors for *M. tuberculosis* infection include a current illness that is consistent with tuberculosis disease or historical, medical, or epidemiological predictors for tuberculosis disease or exposure to tuberculosis. These predictors include exposure at any time to persons with tuberculosis, employment in healthcare or other congregate settings (e.g., hospitals, jails, homeless shelters) that had clients with tuberculosis, residence in such congregate settings, birth in a country with highly prevalent tuberculosis or spending at least 2 cumulative months in such a country, and a chest radiograph with abnormalities consistent with healed tuberculosis. Individuals with impaired immune function, certain medical conditions, or recent contact with an infectious TB case are at high risk for progression to disease if infected with *M. tuberculosis*. Refer to the most recent CDC guidance (<http://www.cdc.gov/nchstp/tb/>) for detailed recommendations about diagnosing *M. tuberculosis* infection (including disease) and selecting persons for testing.

*Includes tuberculosis disease itself and infection with human immunodeficiency virus (HIV); refer to CDC guidance at <http://www.cdc.gov/nchstp/tb/>

Explanation and Principles of the Assay

The QuantiFERON®-TB Gold test is supplied with peptide cocktails simulating ESAT-6 and CFP-10 proteins, which are used to stimulate T-cells in whole heparinized blood during incubation. Following 16 to 24 hours of incubation, plasma is harvested and tested for the presence of IFN- γ produced in response to the peptide antigens.

The QuantiFERON®-TB Gold test is performed in two stages. First, four aliquots of heparinized whole blood are incubated with ESAT-6, CFP-10, Mitogen and Nil control antigens.

Following 16 to 24 hours incubation, plasma is removed from each well and the amount of IFN- γ measured by enzyme-linked immunosorbent assay (ELISA). The QuantiFERON®-TB Gold 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 (IU) relative to a standard curve prepared by testing dilutions of the secondary 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.¹⁸ The effect of heterophile antibodies in the QuantiFERON®-TB Gold 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.⁹

A test is considered positive for an IFN- γ response to either ESAT-6 or CFP-10 that is significantly above the Nil IFN- γ IU/mL value. The Mitogen-stimulated plasma sample serves as a IFN- γ positive control for each specimen tested. A low response to Mitogen (<0.5 IU/mL) is an indeterminate result when a blood sample also has a negative response to both ESAT-6 and CFP-10 peptide antigens. This pattern may occur with insufficient lymphocytes, reduced lymphocyte activity due to prolonged specimen transport or improper specimen handling, incorrect addition of the Mitogen, or inability of the patient's lymphocytes to generate IFN- γ . The Nil sample adjusts for background, heterophile antibody effects, or non specific IFN- γ in blood samples. The IFN- γ level of the Nil is subtracted from each of the levels for ESAT-6, CFP-10, and Mitogen. Elevated levels of IFN- γ in the Nil sample may occur with incorrect addition of antigens, with presence of heterophile antibodies, or to intrinsic IFN- γ secretion.

3. REAGENTS AND STORAGE

Tuberculosis and Control Antigens – Catalogue number: 0596 0201

1. Nil Control (*PBS contains 0.01% Thimerosal*) (Gray cap) 1 x 6mL
2. ESAT-6 Peptide Antigen (*contains 0.01% Thimerosal*) (Red cap) 1 x 6mL
A mixture of 7 peptides, each 8 µg/mL in PBS
3. CFP-10 Peptide Antigen (*contains 0.01% Thimerosal*) (White cap) 1 x 6mL
A mixture of 6 peptides, each 8 µg/mL in PBS
4. Mitogen Control (*contains 0.01% Thimerosal*) (Purple cap) 1 x 6mL
Phytohemagglutinin A, in PBS

Components (ELISA) – Catalogue number: 0594 0201

1. Microplate strips coated with anti-human IFN- γ murine monoclonal antibody (*24 x 8 well strips*) 2 x 96 well plates
2. Human IFN- γ Standard, lyophilized 1 x vial
(*contains recombinant human IFN- γ , bovine casein, 0.01 % w/v Thimerosal*) (8 IU/mL when reconstituted)
3. Green Diluent 1 x 30mL
(*contains bovine casein, normal mouse serum, 0.01% w/v Thimerosal*)
4. Conjugate 100X Concentrate, lyophilized 1 x 0.3mL
(*Murine Anti-human IFN- γ HRP, contains 0.01% w/v Thimerosal*) (when reconstituted)
5. Wash Buffer 20X Concentrate (*pH 7.2, contains 0.01 % w/v Thimerosal*) 1 x 100mL
6. Enzyme Substrate Solution 1 x 30mL
(*contains H₂O₂, 3,3',5,5' Tetramethylbenzidine*)
7. Enzyme Stopping Solution (*contains 0.5M H₂SO₄*) 1 x 15mL

Materials Required (but not provided)

- Sterile, 24 well culture plates. Costar brand plates are recommended and use of plates from other manufacturers has not been validated.
- Mechanical pipetting device and sterile graduated 5 or 10mL pipettes (1 pipette/patient).
- 37°C humidified incubator (with or without CO₂).

- Calibrated variable-volume pipettes for delivery of 50µL, 300µL, 500µL and 5-120µL with disposable tips.
- Multichannel pipette capable of delivering 50µL and 100µL with disposable tips.
- 1mL microtubes with caps in 96 well format racks or uncoated microtitre plates with plastic seals for plasma storage (20 patients / rack or plate).
- Microplate shaker, (e.g., QuantiFERON Microplate Shaker). (Cellestis Cat. No. 0850 0201) or equivalent.
- Variable speed vortex.
- Timer.
- Measuring cylinder - 1L or 2L.
- Deionised or distilled water - 2L.
- Microplate washer (automated washer recommended).
- Microplate reader fitted with 450nm filter and 620nm (or 650nm) reference filter.
- Reagent Reservoirs.

Storage Instructions

Kit Reagents

- Store kit refrigerated at 2°C to 8°C.
- Always protect Enzyme Substrate Solution from direct sunlight.
- The shelf life of the QuantiFERON®-TB Gold ELISA kit is 3 years from the date of manufacture when stored at 2°C to 8°C.
- The shelf life of the QuantiFERON®-TB Gold Stimulation Antigens is 24 months from the date of manufacture when stored at 2°C to 8°C.

Reconstituted and Unused Reagents

For instructions on how to reconstitute the reagents, please see Preparation of Reagents in Section 6.

- The reconstituted **Kit Standard** may be kept for up to 3 months if stored at 2°C to 8°C.
 - *Always note the date the **Kit Standard** was reconstituted.*
- Once reconstituted, unused **100X Conjugate** must be returned to storage at 2°C to 8°C and must also be used within 3 months.
 - *Always 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.

4. WARNINGS AND PRECAUTIONS

Warnings

- A negative QuantiFERON®-TB Gold 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 which affect immune functions, or other individual immunological factors. Heterophile antibodies or non-specific IFN- γ production from other inflammatory conditions may mask specific responses to ESAT-6 or CFP-10 peptides.
- A false-negative QuantiFERON®-TB Gold result can be caused by incorrect blood sample collection or improper handling of the specimen affecting lymphocyte function. Blood must be incubated with stimulation antigens within 12 hours of collection; delay in incubation may cause false negative or indeterminate results, and other technical parameters may affect ability to detect a significant IFN- γ response.
- A positive QuantiFERON®-TB Gold result might not indicate infection with *M. tuberculosis*. Incorrect performance of the assay or reactivity to proteins present in other mycobacteria may cause false positive responses.
- A positive QuantiFERON®-TB Gold result should be followed by further medical and diagnostic evaluation for tuberculosis disease (e.g., AFB smear and culture, chest x-ray).
- The effect of lymphocyte count on reliability is unknown. Lymphocyte counts may vary from person to person. The minimum number required for a reliable result has not been established.
- While ESAT-6 and CFP-10 are absent from all BCG they are present in *M. tuberculosis* and also in *M. kansasii*, *M. szulgai* and *M. marinum*. Other diagnostic evaluations (e.g., AFB smear and culture, chest x-ray) besides QuantiFERON®-TB Gold are needed to confirm tuberculosis disease.

- QuantiFERON®-TB Gold has been evaluated with specimens from patients with culture confirmed active tuberculosis and from apparently healthy adults with and without identified risk factors for *M. tuberculosis* infection. The performance of the QuantiFERON®-TB Gold test has not been evaluated with specimens from the following groups of individuals:
 1. Individuals who have impaired or altered immune function such as those who have HIV infection or AIDS, those who have transplantation managed with immunosuppressive treatment or others who receive immunosuppressive drugs (e.g., corticosteroids, methotrexate, azathioprine, cancer chemotherapy), and those who have other clinical conditions: diabetes, silicosis, chronic renal failure, hematological disorders (e.g., leukemia and lymphomas), and other specific malignancies (e.g., carcinoma of the head or neck and lung).
 2. BCG-vaccinated and non-vaccinated individuals with a high likelihood of progression to TB disease.
 3. Individuals who subsequent to testing had progression to tuberculosis disease.
 4. Individuals with diseases other than tuberculosis.
 5. Individuals younger than age 17 years.
 6. Pregnant women.
- Indeterminate results have not been evaluated for association with any type of impaired immune function.

Precautions

- For *in vitro* diagnostic use.
- **Harmful: Enzyme Substrate Solution** contains 3,3',5,5' Tetramethylbenzidine that is harmful by ingestion, inhalation and skin contact. Skin and eye irritant. Mutagen. Use eye protection, wear gloves and handle as a potential carcinogen.
- **Harmful: Enzyme Stopping Solution** contains H₂SO₄ that is harmful by ingestion, eye contact, skin contact, and inhalation. Use eye protection, wear gloves and normal laboratory protective clothing. If the stopping solution contacts the skin or eyes, flush with copious quantities of water and seek medical attention.
- **Harmful: IFN- γ Standard and 100X Conjugate Concentrate** may be discomforting if ingested and may cause skin irritation. Wear gloves and normal laboratory protective clothing.
- **Handle human blood as if potentially infectious.** Observe universal blood handling precautions (refer NIH/CDC guidelines).
- **Thimerosal** is used as a preservative in some reagents. It may be toxic upon ingestion, inhalation or skin contact.
- **Green Diluent** contains normal mouse serum and casein, which may trigger allergic responses; avoid contact with skin.
- Deviations from the directions for use in the Package Insert may yield erroneous results. Please read the instructions carefully before use.
- **Use only Heparin** as blood anticoagulant. Other anticoagulants interfere with the assay.
- Use of Costar Tissue culture plates is recommended; other culture plates have not been validated.
- Blood samples should be transported to the laboratory at ambient temperature (22°C \pm 5°C). Do not transport on ice or refrigerated.
- Store kit components at 2°C to 8°C. Do not store kit at room temperature.
- Bring all ELISA components, except Conjugate 100X Concentrate, to room temperature (22°C \pm 5°C) before use.
- Store Conjugate 100X Concentrate at 2°C to 8°C at all times.
- Prepare fresh dilutions of the Kit Standard whenever you run the ELISA.
- Do not use kit if any reagent bottle shows signs of damage or leakage prior to use.

- Do not mix or use ELISA reagents from other QuantiFERON®-TB Gold kit batches.
- Do not use Kit Standard or Conjugate after three months from reconstitution.
- Discard unused reagents and biological samples in accordance with Local, State, and Federal regulations.
- Do not use kit after the expiry date.
- Correct laboratory procedures should be adhered to at all times.

5. SPECIMEN COLLECTION AND HANDLING

Fill a blood collection tube (**minimum volume 5mL**) containing heparin as the anticoagulant. Gently mix by inverting the tube several times to dissolve the heparin, and transport to the laboratory at ambient temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$). Blood should be incubated with stimulation antigens as soon as possible (as the IFN- γ response decreases with time) and culture with antigens **must** be initiated within 12 hours of blood collection.

6. DIRECTIONS FOR USE

Time Required for Performing Assay

In order to obtain valid results from the QuantiFERON®-TB Gold assay the operator needs to perform certain 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:

Initiate incubation with Antigens:	10 minutes (add 1 – 1.5 minutes per patient)
Incubation of blood with Antigens:	16-24 hours (overnight)
Human IFN- γ ELISA stage:	Approx. 3 hours for one ELISA plate (<1 hour labor) (Add 10 – 15 minutes for each extra plate)

Stage One – Incubation of Blood

The following materials are required when setting up blood cultures. The ESAT-6, CFP-10 and Control Antigens do not need to be brought to room temperature before use.

Materials Provided

- QuantiFERON®-TB Gold Nil Control (Gray cap).
- QuantiFERON®-TB Gold Antigens ESAT-6 (Red cap) and CFP-10 (White cap).
- QuantiFERON®-TB Gold Mitogen Control (Purple cap).

Materials Required (but not provided)

- Sterile, 24 well culture plates. Costar brand plates are recommended and use of plates from other manufacturers has not been validated.
- Mechanical pipetting device and sterile graduated 5 or 10mL pipettes (1 pipette/patient).
- 37°C humidified incubator (with or without CO₂).
- Calibrated variable-volume pipette capable of delivering 300-400µL with disposable tips.
- 1mL microtubes with caps in 96 well format racks or uncoated microtitre plates with plastic seals for plasma storage (20 patients / rack or plate).
- Microplate shaker, e.g., QuantiFERON Microplate Shaker (Cellestis Cat. No. 0850 0201) or equivalent.

Stage One – Preparation of Reagents

STIMULATION ANTIGENS – Ready to use *Contains 0.01% w/v Thimerosal*

Stimulation antigens do not need to be brought to room temperature before use. Use undiluted but mix thoroughly before use.

Stage One - Procedure

1. Blood samples must be evenly mixed before aliquoting. Use a roller-rocker or gently invert tubes 20 times **immediately prior to dispensing**.
2. Dispense 1.0mL aliquots (one per test antigen and control) of heparinized whole blood from each subject into 4 wells of a 24 well tissue culture plate (see Figure 1 for recommended layout). Blood is best dispensed aseptically in a Biohazard cabinet using sterile pipettes to minimize the risk of contamination.
3. Prior to use, mix each stimulation antigen well. Holding the dropper bottle vertically, carefully add **3 drops (125µL)** of each antigen to the appropriate wells containing blood.

FIGURE 1. Recommended layout for dispensing Blood and Stimulation Antigens into 24 Well Culture Plates

	Patient Sample Number					
	1	2	3	4	5	6
Nil Control (<i>gray cap</i>)	○	○	○	○	○	○
ESAT-6 (<i>red cap</i>)	○	○	○	○	○	○
CFP-10 (<i>white cap</i>)	○	○	○	○	○	○
Mitogen (<i>purple cap</i>)	○	○	○	○	○	○

4. Stimulation antigens must be mixed **THOROUGHLY** into the aliquoted blood (in covered plates) using the QuantiFERON Microplate Shaker at the following setting; Waveform = 20, Amplitude = 9, Time = 1 to 2 minutes.
5. Incubate covered plates for 16-24 hours at 37°C in a humidified atmosphere.
 - **Avoid stacking plates more than 2 high during incubation.**
6. Carefully remove approximately 200-300µL of plasma from above the sedimented red cells using a variable-volume pipette. Transfer the plasma into separate 1mL microtubes in a 96 well format or a disposable 96 well microtitre plate using the format outlined in Figure 2 (page 17), Recommended Sample Layout. Label sample racks appropriately.

- Use a new pipette tip for each plasma sample.
 - Avoid harvesting blood cells with plasma. The assay will tolerate small quantities of cells, but if the harvested plasma sample is grossly contaminated with blood cells, centrifuge the sample to remove the cells.
7. Plasmas can be stored at 2°C to 8°C for up to 14 days or at least 3 months at or below -20°C. Microtubes or microtitre plates should be sealed appropriately prior to storage to avoid evaporation. Freezing at or below -70°C is recommended to reduce the possibility of clot formation.
- Plasmas may clot during extended storage. If clots are present refer to TROUBLE SHOOTING section.

Stage Two - Human IFN- γ ELISA

Materials Provided

QuantiFERON®-TB Gold ELISA kit (Refer to Section 3).

Materials Required (but not provided)

- Calibrated variable-volume pipettes for delivery of 50 μ L, 300 μ L, 500 μ L and 5-120 μ L with disposable tips.
- Multichannel pipette capable of delivering 50 μ L and 100 μ L with disposable tips.
- Variable speed vortex.
- Timer.
- Measuring cylinder - 1L or 2L.
- Deionised or distilled water - 2L.
- Microplate shaker, e.g., QuantiFERON Microplate Shaker (Cellestis Cat. No. 0850 0201) or equivalent microplate shaker.
- Microplate washer (automated washer recommended).
- Microplate reader fitted with 450nm filter and 620nm (or 650nm) reference filter.
- Reagent Reservoirs (Polypropylene).

Stage Two - Preparation of Reagents

Allow all reagents except Conjugate 100X Concentrate to equilibrate at room temperature for at least 60 minutes before use.

1. ELISA MICROTITRE PLATE – READY TO USE

Remove strips that are not required from the frame, reseal in the foil pouch, and return to the refrigerator for storage until use.

Allow at least two strips for the QuantiFERON®-TB Gold Standards and one strip for every two individuals being tested. After use, retain frame and lid for use with remaining strips.

2. HUMAN IFN- γ STANDARD

Contains 0.01 % w/v Thimerosal

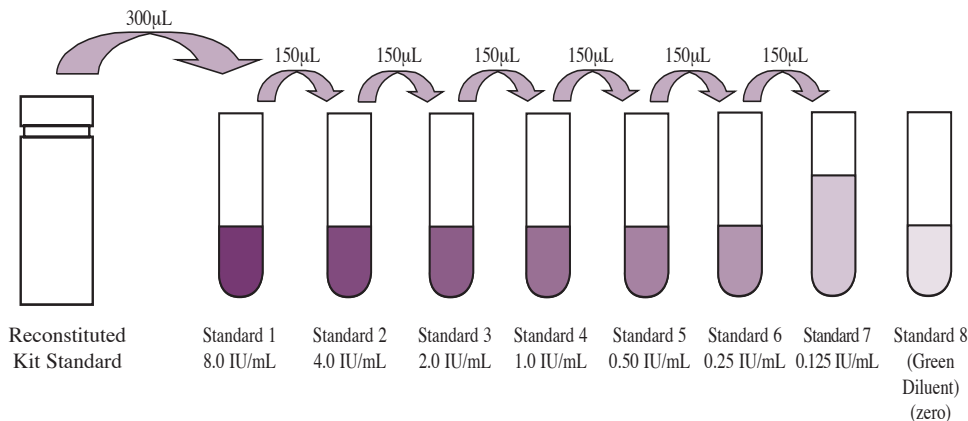
Reconstitute the Human Interferon- γ Kit Standard with the volume of deionized or distilled water AS INDICATED ON THE LABEL of the standard vial **ensuring complete resolubilization**. Mix gently to minimize frothing. Reconstitution of the standard to the correct volume will produce a solution with a concentration of 8.0 IU/mL.

Note: The reconstitution volume will differ between batches.

Use the reconstituted Kit Standard to produce a dilution series of 8 IFN- γ concentrations (refer figure on page 15).

If a dilution series other than that described is used, each laboratory should validate the alternative approach. All evaluations with the product were done with a 7-point standard curve (Standard 8, 0 IU/mL, not used for standard curve) and a high standard in the range of 8 to 19 IU/mL.

1. Add 300 μ L of the Kit Standard to a tube labelled as Standard 1.
2. Add 150 μ L of Green Diluent to 7 tubes (Labelled Standard 2 – Standard 8).
3. Perform serial dilutions by transferring 150 μ L of each Standard to the next tube. Mix each tube thoroughly before the next transfer.
4. The undiluted Kit Standard serves as the highest concentration (Standard 1).
5. Green Diluent serves as the Zero Standard (Standard 8).



- Prepare **fresh dilutions** of the Kit Standard for each ELISA run.
- The reconstituted Kit Standard may be kept for up to 3 months if stored at 2°C to 8°C. Always note the date the Kit Standard was reconstituted.

3. CONJUGATE

Contains 0.01 % w/v Thimerosal

Reconstitute freeze dried Conjugate 100X Concentrate with 0.3mL of deionized or distilled water. To **ensure complete resolubilization** of the Conjugate, mix thoroughly and gently to minimize frothing.

Working strength conjugate is prepared by diluting reconstituted Conjugate 100X Concentrate in Green Diluent as set out in Table 1 - Conjugate Preparation.

- **Mix thoroughly but gently to avoid frothing.**
- **Working strength conjugate should be used within 6 hours of preparation.**
- **Return any unused Conjugate 100X Concentrate to 2°C to 8°C immediately after use.**
- **Use only Green Diluent as it contains normal mouse serum to compete out effects of heterophile antibodies in plasma samples.**

TABLE 1. Conjugate Preparation

NUMBER OF STRIPS	VOLUME OF CONJUGATE 100X CONCENTRATE	VOLUME OF GREEN DILUENT
3	15 μ L	1.5mL
4	20 μ L	2.0mL
5	25 μ L	2.5mL
6	30 μ L	3.0mL
7	35 μ L	3.5mL
8	40 μ L	4.0mL
9	45 μ L	4.5mL
10	50 μ L	5.0mL
11	55 μ L	5.5mL
12	60 μ L	6.0mL

4. WASH BUFFER

Contains 0.01% w/v Thimerosal

Each plate (12 x 8 well strips) requires 1L of working strength wash buffer. Dilute one part Wash Buffer 20X Concentrate with 19 parts deionized or distilled water and mix thoroughly.

Stage Two – Procedure

1. All plasma samples and reagents, except for the Conjugate 100X Concentrate must be brought to room temperature (22°C \pm 5°C) before use. Allow at least 60 minutes for equilibration.
2. Reconstitute freeze dried Kit Standard and Conjugate 100X Concentrate.
3. Prepare dilutions of the reconstituted Kit Standard in Green Diluent to produce a dilution series of 8 IFN- γ concentrations for the preparation of the standard curve. Refer Preparation of Reagents - Human IFN- γ Standard (Pages 14 and 15). Green Diluent alone is used for the Zero Standard.
4. Prior to assay, plasmas should be vortexed to ensure that IFN- γ is evenly distributed throughout the sample.

- Dilute the required amount of Conjugate 100X Concentrate in Green Diluent according to the Conjugate Preparation Table (Table 1). Add 50µL of freshly prepared conjugate to the required ELISA wells using a multichannel pipette.
- Using a multichannel pipette, add 50µL of test plasma samples to appropriate wells containing conjugate (Refer to recommended plate layout below – Figure 2). Finally, add 50µL each of the Standards 1 to 8. **The standards should be assayed at least in duplicate.**

FIGURE 2. Recommended Sample Layout - Whole Plate

Row	1	2	3	4	5	6	7	8	9	10	11	12
A	1N	2N	3N	4N	5N	S1	S1	6N	7N	8N	9N	10N
B	1E	2E	3E	4E	5E	S2	S2	6E	7E	8E	9E	10E
C	1C	2C	3C	4C	5C	S3	S3	6C	7C	8C	9C	10C
D	1M	2M	3M	4M	5M	S4	S4	6M	7M	8M	9M	10M
E	11N	12N	13N	14N	15N	S5	S5	16N	17N	18N	19N	20N
F	11E	12E	13E	14E	15E	S6	S6	16E	17E	18E	19E	20E
G	11C	12C	13C	14C	15C	S7	S7	16C	17C	18C	19C	20C
H	11M	12M	13M	14M	15M	S8	S8	16M	17M	18M	19M	20M

S1–8 (S1: Standard 1, S2: Standard 2, S3: Standard 3, S4: Standard 4, S5: Standard 5, S6: Standard 6, S7: Standard 7, S8: Standard 8); 1N (Sample 1 Nil Control plasma); 1E (Sample 1 ESAT-6 plasma); 1C (Sample 1 CFP-10 plasma); 1M (Sample 1 Mitogen Control plasma)

- Cover each plate with a lid and mix the conjugate and plasma samples/standards thoroughly using the QuantiFERON® Microplate Shaker at the following setting; Waveform = 20, Amplitude = 6, Time = 1 minute, or an equivalent microplate shaker.
- Incubate covered plates at room temperature (22°C ± 5°C) for 120 ± 5 minutes.
 - Plates should not be exposed to direct sunlight during incubation.**
 - Deviation from specified temperature range can lead to erroneous results.**
- Wash wells with 300-400µL of working strength wash buffer for AT LEAST 6 cycles at room temperature (22°C ± 5°C). A fully automatic plate washer is recommended.

- **Thorough washing is very important to the performance of the assay. Ensure 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.**
10. Tap plates face down on an absorbent wipe to remove residual wash buffer. Add 100µL of Enzyme Substrate Solution to each well and mix thoroughly using the QuantiFERON® Microplate Shaker, adjusted to the settings described in Step 7.
- **Commence incubation time as substrate is added to the first well(s).**
11. Cover each plate with a lid and incubate at room temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) for precisely 30 minutes.
- **Plates should not be exposed to direct sunlight during incubation.**
 - **Deviation from specified temperature range can lead to erroneous results.**
12. Following the 30 minute incubation, add 50µL of Enzyme Stopping Solution to each well and mix by gentle agitation.
- **Enzyme stopping solution should be added to wells in the same order and at the same speed as the substrate in step 10.**
13. Read the Optical Density (OD) of each well within 5 minutes of terminating the reaction using a 450nm filter, with either a 620nm or 650nm reference filter. OD values are used to calculate results.

7. CALCULATIONS AND TEST INTERPRETATION

QuantiFERON®-TB Gold Analysis Software (Version 2.13 or later), used to generate curves and to calculate results, is available from Cellestis.

The software performs Quality Control of the assay, generates a standard curve and provides a test result from calculations performed on each plasma test, based on the method of interpretation (as detailed below).

As an alternative to using the QuantiFERON®-TB Gold Analysis Software, results can be determined according to the method as described on page 19.

Generation of Standard Curve and Sample Values

(if QuantiFERON®-TB Gold software not used)

Determination of the Standard Curve and determination of sample IU/mL values requires a spreadsheet program such as Microsoft Excel (Spreadsheet Program) if the QuantiFERON®-TB Gold software is not used.

Using the Spreadsheet Program:

- Calculate the mean OD values of the Human IFN- γ Standard replicates on each plate for Standards 1 to 8, and the coefficient of variation (%CV) for the standards (%CV = 100 X Standard Deviation/Mean).
- Determine the natural logarithm ($\text{Log}_{(e)}$) of each mean OD value ($\text{LN}(x)$ in many Programs).
- Determine the $\text{Log}_{(e)}$ of each IU/mL value of the Human IFN- γ Standards 1 to 7. Do not use Standard 8 as a zero value cannot be converted to a $\text{Log}_{(e)}$ value.
- Enter the $\text{Log}_{(e)}$ of Mean OD in Spreadsheet Program cells beside the respective $\text{Log}_{(e)}$ of the IU/mL Standard.
- Determine the line of best fit using regression analysis.*† The line of best fit determined by regression analysis will have the equation $Y=m(X) + c$, where Y equals the $\text{Log}_{(e)}$ of the absorbance (OD) for each plasma sample, X is the $\text{Log}_{(e)}$ of the concentration of IFN- γ in IU/mL, m is the slope coefficient (X variable) and c is the Y-axis intercept of the curve. The c value can be negative.
- Ensure that the ELISA run meets Quality Control of Test specifications.
- For each patient test, determine $\text{Log}_{(e)}$ absorbance (Y-value) for the Nil, ESAT-6, CFP-10 and Mitogen test sample OD values.
- Using the transposed equation for the line of best fit ($X = (Y-c)/m$) and the calculated m and c values, determine the X-value ($\text{Log}_{(e)}$ IU/mL of IFN- γ) for each of the Y-values (OD).
- Determine the IFN- γ concentration (IU/mL) for the Nil, ESAT-6, CFP-10 and Mitogen test plasma samples, by converting the X-value using the antilog equation e^x .
- Subtract the Nil IU/mL value from the ESAT-6, CFP-10 and Mitogen values for each patient series of tests.

* Most spreadsheet programs offer a variety of methods for obtaining the line of best fit. Within Microsoft Excel the simplest method is selection of Data Analysis from the Tools menu and then selection of the Regression function. Insert the array of seven $\text{Log}_{(e)}$ OD as **the Y values** and the corresponding $\text{Log}_{(e)}$ IU/mL of the Standards as **the X values**. Finish the calculation, which will provide the Intercept value (c), Slope value (m) and Correlation Coefficient (r) into the location selected.

† It is also possible to use the graphing function of the Excel Program to plot the $\text{Log}_{(e)}$ of the mean Standard OD values against the $\text{Log}_{(e)}$ of the IFN- γ concentrations of the Standards and obtain the Standard curve regression. Select the Chart icon or select Chart from the Insert menu. Select a simple scatter plot Chart, and then proceed to select the Series option of data entry. Insert the array of seven $\text{Log}_{(e)}$ OD as the **Y values** and the seven corresponding $\text{Log}_{(e)}$ IU/mL of the Standards as the **X values**. Finish the chart and then right-click on any data point in the completed chart, ensuring the Data points are highlighted. Select the Add Trendline function, select Linear Regression and in the options tab ensure the Display Equation and Display R^2 functions are also selected. Finish and the equation $Y=m(X) + c$ values, and r value (Correlation Coefficient) will be displayed.

Sample Calculation

If the following OD readings were obtained for the Standards, the calculations would be:

Standard	IU/mL	OD Values a & b	Mean OD	%CV	$\text{Log}_{(e)}$ IU/mL	$\text{Log}_{(e)}$ Mean OD
Dilution 1	8	2.098, 2.084	2.091	0.5	2.079	0.738
Dilution 2	4	1.089, 1.136	1.113	3.0	1.386	0.107
Dilution 3	2	0.674, 0.704	0.689	3.1	0.693	-0.373
Dilution 4	1	0.357, 0.395	0.376	7.1	0.000	-0.978
Dilution 5	0.5	0.193, 0.215	0.204	7.6	-0.693	-1.590
Dilution 6	0.25	0.114, 0.136	0.125	Not Applicable	-1.386	-2.079
Dilution 7	0.125	0.082, 0.093	0.088	Not Applicable	-2.079	-2.436
Dilution 8	0	0.034, 0.037	0.036	Not Applicable	Not Applicable	Not Applicable

The equation of the curve is $y = 0.7786(X) - 0.9445$, where “m” = 0.7786 and “c” = -0.9445. 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) = 0.998.

Using the criteria specified in the Quality Control of Test section, the assay is determined to be valid.

The standard curve is used to convert the Antigen OD responses to International Units (IU/mL):

Antigen	OD Value	Log _(e) OD Value	X	e ^x (IU/mL)	Antigen - Nil (IU/mL)
Nil	0.029	-3.540	-3.334	0.04	-
ESAT-6	1.161	0.149	1.405	4.08	4.04
CFP-10	0.579	-0.546	0.511	1.67	1.63
Mitogen	1.783	0.578	1.956	7.07	7.03

IFN- γ values (in IU/mL) for ESAT-6, CFP-10 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 Test

The accuracy of test results is dependent on the ELISA generating a suitable standard curve. Therefore, results derived from the standards must be examined before test sample results can be interpreted.

For the ELISA test to be valid:

- **The correlation coefficient (r) calculated from the mean absorbance values of the standards must be ≥ 0.98 .**
- **Standard dilutions 1 to 5 must be within 15% of their individual mean OD values (% coefficient of variation (% CV) $\leq 15\%$).**
- **Replicate OD values for Standards 6 and 7 must not vary by more than 0.040 optical density units from their mean.**
- **The mean OD value for Standard 1 must be ≥ 1.200 .**

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 ≤ 0.15 . If the OD value is > 0.15 the plate washing procedures should be investigated. Replicate OD values for the Zero Standard must not vary by more than 0.040 optical density units from their mean.**

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 or -20°C. Recombinant IFN- γ is not recommended for establishing control standards.

Interpretation of Results

QuantiFERON®-TB Gold results are interpreted as follows:

NOTE: Diagnosing or excluding tuberculosis disease, and assessing the probability of LTBI, require a combination of epidemiological, historical, medical, and diagnostic findings that should be taken into account when interpreting QuantiFERON®-TB Gold results. See general guidance on the diagnosis and treatment of TB disease and LTBI (<http://www.cdc.gov/nchstp/tb/>).

ESAT-6 – Nil (<i>E</i>) CFP-10 – Nil (<i>C</i>) ¹ (IU/mL) ²	Mitogen – Nil ³ (IU/mL)	QuantiFERON®-TB Gold Result	Report/Interpretation
<i>E</i> and/or <i>C</i> ≥ 0.35 and $\geq 50\%$ of Nil Control IU/mL	Any	Positive (ESAT-6 and/or CFP-10 responsiveness detected)	<i>M. tuberculosis</i> infection likely See NOTE above
Both <i>E</i> and <i>C</i> < 0.35 and Nil ≤ 0.7	≥ 0.5	Negative (No ESAT-6 or CFP-10 responsiveness detected)	<i>M. tuberculosis</i> infection unlikely, but cannot be excluded especially when: 1. any illness is consistent with TB disease, 2. likelihood of progression to disease (e.g., because of immunosuppression) is increased See NOTE above
Both <i>E</i> and <i>C</i> < 0.35 and Any Nil result	< 0.5	Indeterminate Refer to Trouble Shooting Guide in Section 11	Results are indeterminate for ESAT-6 or CFP-10 responsiveness
Nil > 0.7 and both <i>E</i> and <i>C</i> $< 50\%$ of Nil Control IU/mL	Any		

¹ *E* is an abbreviation for ESAT-6 – Nil, and *C* is an abbreviation for CFP-10 – Nil.

² For a patient to be considered **POSITIVE**, EITHER OR BOTH of the individual CFP-10 – Nil and ESAT-6 – Nil (*E* or *C*) responses must be greater than or equal to 0.35 IU/mL.

³ Mitogen – Nil must be ≥ 0.5 IU/mL OR, either *E* or *C* must be ≥ 0.35 IU/mL and $\geq 50\%$ of Nil for a subject to have a Positive or Negative QuantiFERON®-TB Gold result.

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.

8. LIMITATIONS

Results from QuantiFERON[®]-TB Gold testing must be used in conjunction with an understanding of individual epidemiological history, current medical status, and other diagnostic evaluations as indicated.

- The predictive value of a positive QuantiFERON[®]-TB Gold 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 condition 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. Additional medical and diagnostic evaluation may be needed. 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.

Care should be taken interpreting results when the IFN- γ level of the Nil sample is greater than 5 IU/mL. With such samples, a 50% higher response to either ESAT-6 and/or CFP-10 may be outside the assay measurement range. In clinical studies, only 3 of 1363 (0.2%) individuals tested had a detected IFN- γ level in the Nil plasma sample of greater than 5 IU/mL.

Unreliable or indeterminate results may occur due to:

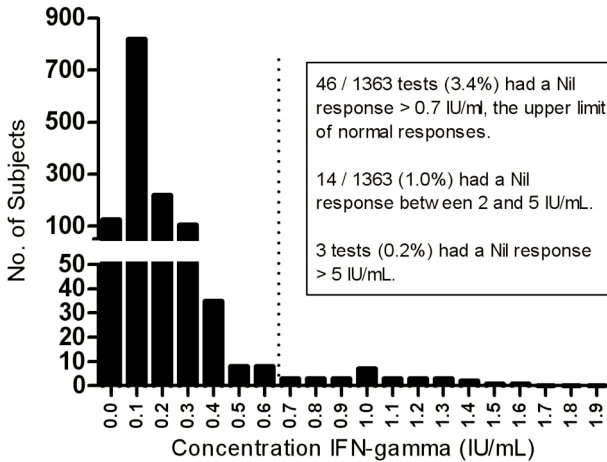
- Incorrect technique (e.g., incorrect incubation times or temperatures and other deviations from the recommended test procedure),
- Use of any anticoagulant other than heparin,
- Incorrect transport of blood specimens,
- Excessive levels of circulating IFN- γ or presence of heterophile antibodies,
- Longer than 12 hours from blood specimen drawing to incubation with antigens.

9. EXPECTED VALUES

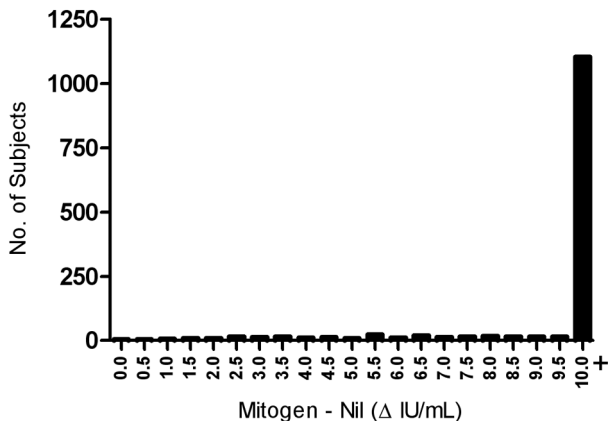
The range of IFN- γ responses to the TB-Specific and Control Antigens that have been observed during the clinical trials are shown in Figure 3. The pattern of response to ESAT-6 and CFP-10 peptides is shown in Figure 4.

FIGURE 3. Distribution of Nil, Mitogen and Antigen responses

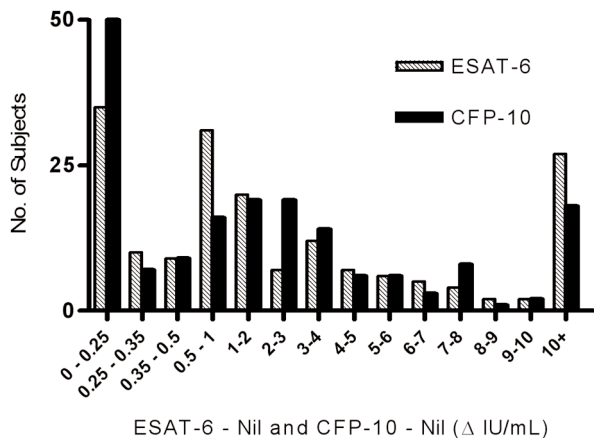
(a) Distribution of Nil values from clinical studies (n=1363). (Median 0.110 IU/mL)



(b) Distribution of Mitogen – Nil values from clinical studies (n=1366). Results $\geq \Delta$ 10 IU/mL are shown as 10 IU/mL.



(c) Distribution of QFT-Gold ESAT-6 – Nil and CFP-10 – Nil results for 178 culture confirmed TB patients. Results $\geq \Delta$ 10 IU/mL are shown as 10+ IU/mL.



(d) Distribution of QFT-Gold results for 178 culture confirmed TB patients. The higher response to either Δ ESAT-6 – Nil or Δ CFP-10 – Nil is shown for each subject. Results $\geq \Delta$ 10 IU/mL are shown as 10+ IU/mL. Data from Low Risk subjects are not shown (>98% had a response less than Δ 0.35 IU/mL, ref. Figure 4) unless they had a positive response (n=11, striped bar).

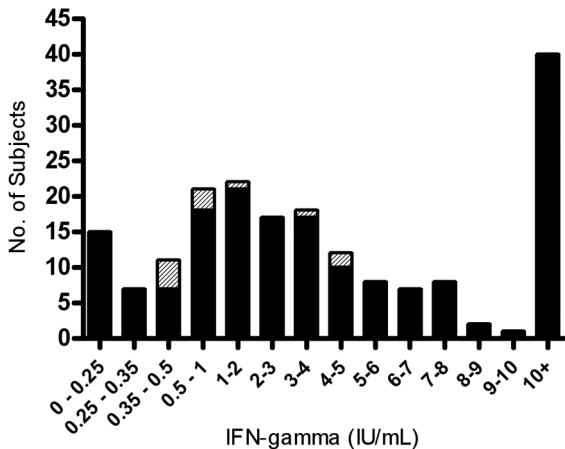


FIGURE 4. Pattern of response of 177 patients with culture confirmed *M. tuberculosis* to ESAT-6 and CFP-10 peptides in the QuantiFERON®-TB Gold assay. Note: one test had only CFP-10 response measured; this test is excluded.

ESAT-6 Peptides	CFP-10 Peptides	
	Positive	Negative
Positive	97 (54.8%)	23 (13.0%)
Negative	35 (19.8%)	22 (12.4%)

10. PERFORMANCE CHARACTERISTICS

Clinical Studies

As there is no definitive standard for latent tuberculosis infection (LTBI), an estimate of sensitivity and specificity for QuantiFERON®-TB Gold cannot be practically evaluated. In order to approximate sensitivity and specificity, QuantiFERON®-TB Gold has been evaluated in three groups of persons at low risk of tuberculosis infection (U.S.A., Australia, Japan), and three groups of patients with active TB disease (Japan, Australia, U.S.A.). Specificity of QuantiFERON®-TB Gold was approximated by evaluating false positive rates in the three groups of persons with low risk (no known risk factors) of tuberculosis infection. Sensitivity was approximated by evaluating the three groups of patients with culture-confirmed active TB disease.

In a U.S.A. study with 900 military recruits, blood was drawn for QuantiFERON®-TB Gold when a TST was placed. Demographic and risk factors were gathered in a standard survey at the time of testing. Of the recruits with a QuantiFERON®-TB Gold test, 824 recruits also had TST results, had never been treated for *M. tuberculosis* infection and had completed surveys for assessing related risk. Of these, 548 related no risk factors for *M. tuberculosis* infection and had results available for both QuantiFERON®-TB Gold and the TST. Both QuantiFERON®-TB Gold and TST results were available for 276 subjects with reported risks for TB infection but who denied prior treatment for TB. Risks included: 1) birth or residence for > 1 month in a country with TB rates greater than 10/100,000 per year, 2) contact with a TB case, 3) residence or employment in a jail, prison, hospital, nursing home, homeless shelter, or drug rehabilitation center, and 4) prior diagnosis of TB. Of the 824 QFT tests, 17 were indeterminate (11 with no known risk and 6 with reported risk), while of the original 863 who agreed to participate and had blood drawn, 17 had a TST placed that was not read.

Specificity

For the U.S. military recruits with no reported risk, one was QuantiFERON®-Gold positive (and also TST-positive), 11 were indeterminate (10 had Nil >0.7 with <50% difference, and 1 had a Mitogen response <0.5 IU/mL), while five were TST-positive (>15mm). These recruits were all presumed non-infected based on information collected

in the survey. Extrapolated specificity for QuantiFERON®-TB Gold was 99.8% in the 537 low-risk group subjects with a valid QFT test result. For 92 low-risk individuals in Australia with valid test results specificity was estimated as 97.8%, while specificity was 98.1% in a group of BCG-vaccinated Japanese nursing recruits. The overall specificity of QuantiFERON®-TB Gold was 99.2%. These results are detailed in Table 2.

TABLE 2. QuantiFERON®-TB Gold specificity studies: Subjects with no reported risk for *M. tuberculosis* infection.

STUDY	BCG Status	Total tested	No. QFT-Gold Indeterminate	No. QFT-Gold Positive/No. Valid Tests (%)	QFT-Gold Extrapolated Specificity (95% CI)	No. TST Positive/No. Tested (15mm)	TST Extrapolated Specificity (95% CI)
Japanese nursing recruits (Mori et al, 2004) ¹⁹	100% vaccinated	216	7	4/209	98.1% (95.2-99.5)	36/113*	68.1%* (58.7-76.6)
Australian (Unpublished)	30% vaccinated	100	8	2/92	97.8% (92.4-99.7)	ND	ND
USA (Unpublished)	0% vaccinated	548	11	1/537	99.8% (99.0-100)	5/548	99.1% (97.9-99.7)
TOTAL				7/838	99.2% (98.3-99.7)		

* Used Japanese PPD; these results are not directly comparable with US-performed TST.

Sensitivity

While there is no definitive standard for latent tuberculosis infection (LTBI), culture findings from sputum or other specimen types are definitive for active TB disease. To extrapolate sensitivity of QuantiFERON®-TB Gold, TB-suspects from Japan, Australia, and the U.S. who were subsequently confirmed to have *M. tuberculosis* infection by culture were evaluated with QuantiFERON®-TB Gold. The Japanese group of tuberculosis patients had received either no treatment (80.5%), or less than 8 days of treatment prior to collecting blood for QuantiFERON®-TB Gold testing. The 41 Australian patients with active tuberculosis had received less than 2 weeks of chemotherapy or no treatment at the time of specimen collection. For the U.S. tuberculosis patients, all had received no treatment or less than 8 days of treatment prior to testing.

Table 3 summarizes findings from the three groups of *M. tuberculosis* culture positive patients. Overall sensitivity of QuantiFERON®-TB Gold for active tuberculosis is projected as 87.6% (156/178). Five patients (4 from the Japanese study and one from the U.S. study) had indeterminate test results.

TABLE 3. QuantiFERON®-TB Gold: Subjects with culture-confirmed infection of *M. tuberculosis*.

STUDY		No. QFT-Gold Indeterminate Tests	No. QFT-Gold Positive /No. Valid	QFT-Gold % Positive (95% CI) (5mm)	No. TST Positive/ No. Tested	TST % Positive (95% CI)
Japanese TB patients (Mori et al, 2004) ¹⁹		4**	102/114	89.5% (83.8-95.1)	50/77*	64.9%* (53.2-75.5)
Australian (Unpublished)	Pulmonary	0	20/24	83.3% (83.8-95.1)	ND	ND
	Extra-pulmonary	0	13/17	76.5% (50.1-93.2)	ND	ND
US TB patients		1***	21/23	91.3% (72.0-98.9)	19/24	79.2% (57.8-92.9)
TOTAL			156/178	87.6% (82.8-92.5)		

* Used Japanese PPD; TST results may not directly compare with US-approved PPD.

** 3 with Nil >0.7 IU and one with Mitogen <0.5 IU

*** 1 with Nil >0.7 IU

In the evaluation of US military recruits who had both TST and QuantiFERON®-TB Gold performed, 276 recruits related risks for TB infection and denied prior treatment for TB. Six of the QuantiFERON®-TB Gold results were indeterminate (4 had Nil >0.7 with <50% difference, and 2 had Mitogen response <0.5 IU/mL). Although TST is not a definitive standard for latent TB infection, TST is widely used as an initial screening test for both LTBI and active TB. Agreement between TST (≥10 mm) and QuantiFERON®-TB Gold for these is shown in Table 4.

TABLE 4. Comparison of QuantiFERON®-TB Gold and TST results for subjects with risk factors reported for LTBI.

	TST +	TST -	TOTAL
QFT-Gold +	4	0	4
QFT-Gold -	29	237	266
TOTAL	33	237	270

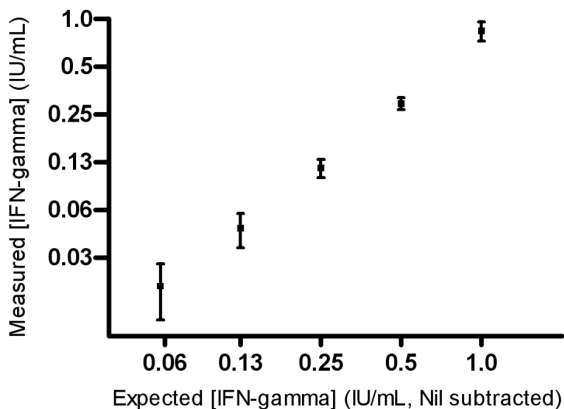
Patient Repeatability and effect of Tuberculin Skin Test on subsequent QuantiFERON®-TB Gold testing: As part of the U.S. military recruits study (predominantly no reported risk), a subset of the recruits were retested between 4 and 5 weeks after the original QuantiFERON®-TB Gold test and TST. QuantiFERON®-TB Gold results for 562 recruits were available at both time points. QuantiFERON®-TB Gold results agreed with earlier results 99.3% (558/562). Of the 5 initially QuantiFERON®-TB Gold positive recruits, 4 remained positive, and 3/562 changed from initially negative to positive at second test. Repeatability was (375/377; 99.5%) for the Low-Risk group and (183/185; 98.9%) for those with any reported risk for *M. tuberculosis* infection.

Assay Performance Characteristics

For measuring IFN- γ concentration by the QuantiFERON®-TB Gold ELISA, the method has been demonstrated to be linear from zero to 10 IU/mL within 10% difference in this interval. The QuantiFERON®-TB Gold ELISA shows no evidence of a high-dose hook effect (prozone effect) with IFN- γ concentrations of up to 10,000 IU/mL.

To test inter-assay and inter-operator variability of the QuantiFERON®-TB Gold ELISA, 9 different plasma samples were tested by two separate operators on different days in two different laboratories. Individual plasma samples were spiked with natural IFN- γ to give a concentration of approximately 1 IU/mL and these were then serially diluted in normal plasma. Three replicates of each plasma dilution were tested by each operator. Pooling all data at each dilution, the mean IU/mL of IFN- γ detected and the 95% Confidence Intervals are shown in Figure 5. The 9 different normal plasma samples used as diluent for the serial dilutions were also tested in the ELISA. The 95% Confidence Intervals around the mean response (0.030 IU/mL) for the Nil plasma samples were 0.019 to 0.041 IU/mL. The mean value for each Nil plasma dilution has been deducted from their respective dilution samples containing IFN- γ to obtain the level of IFN- γ in the sample under test in Figure 5.

FIGURE 5. Mean and 95% Confidence Intervals for plasma samples containing different concentrations of IFN- γ up to 1.0 IU/mL. Two different operators tested three replicates of each of 9 dilution series of samples on different days at separate laboratories (each point represents the mean of 54 different determinations).



11. TECHNICAL INFORMATION

Trouble Shooting

Indeterminate results

Indeterminate QuantiFERON[®]-TB Gold results can result from:

- Mitogen – Nil value being less than 0.5 IU/mL and both ESAT-6 – Nil and CFP-10 – Nil are < 0.35 IU/mL, and where the Nil is \leq 0.7 IU/mL,
- Nil value being greater than 0.7 IU/mL and both the ESAT-6 – Nil and CFP-10 – Nil being < 50% of Nil value.

Such results should be uncommon and may be due to a number of technical factors:

- Storage of blood outside of the 12 hour time limit or outside the recommended temperature range ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$).
- Insufficient mixing of antigens with whole blood.
- Errors in addition of antigens to aliquots of blood.
- Pipetting errors.
- Incomplete washing of the ELISA plate.
- Miscalculation of ELISA standard curve.

If technical factors are suspected with the collection, handling or stimulation of blood samples, it is not possible to repeat QuantiFERON®-TB Gold testing without requesting a new blood specimen. Repeating the ELISA testing of stimulated plasmas can be done if washing error or other procedural error with the ELISA test is suspected. Indeterminate tests that result from low Mitogen or high Nil backgrounds would not be expected to change on repeat unless there was an error with the ELISA testing. Indeterminate results should be reported as such. Physicians may choose to redraw a blood specimen or perform other procedures as appropriate.

Difficulties that may be encountered in performing the assay include:

1. Incorrect washing of the ELISA plate.
2. Clot formation in plasma samples that have been stored frozen for an extended period of time. Clotted material can block multichannel pipette tips.
3. Very lipemic samples. Fatty deposits can block multichannel pipette tips.
4. Plasma samples with high levels of IFN- γ that give OD values above the limit of the ELISA reader (Off-scale). Unless occurring in the Nil control sample, this has no effect on the interpretation of the test if the response to the Nil control sample is ≤ 0.7 IU/mL. High values (> 0.7 IU/mL) for Nil controls did occur in clinical trials; if the Nil control sample is > 0.7 IU/mL the test result is Indeterminate unless the response to ESAT-6 and/ or CFP-10 is ≥ 50 % above the respective Nil control value. An off-scale Mitogen IFN- γ response is a valid test if the Nil control sample is ≤ 0.7 IU/mL.

How to Deal with Clotted Plasma Samples

Firstly, centrifuge thawed samples in tube racks at 500g for 2 minutes to remove any plasma in the neck of the tubes. Carefully remove the tube cap band. **Care should be taken to avoid cross-contamination of samples.**

Mix each plasma tube by vortexing at moderate-high speed in a stop-start fashion, 3-5 times, with care. The purpose of this treatment is to facilitate sedimentation. For ease of handling, transfer mixed tubes to the same location in an empty rack. **Do not mix up tubes.**

Centrifuge the samples again at 500g for 2 minutes to sediment clotted material. Care should be taken not to disturb pelleted material after centrifugation. Perform ELISA.

12. U.S. CENTERS FOR DISEASE CONTROL AND PREVENTION GUIDELINES

SUMMARY

On May 2, 2005, a new in vitro test, QuantiFERON[®]-TB Gold (QFT-G, Cellestis Limited, Carnegie, Victoria, Australia), received final approval from the U.S. Food and Drug Administration as an aid for diagnosing Mycobacterium tuberculosis infection. This test detects the release of interferon-gamma (IFN- γ) in fresh heparinized whole blood from sensitized persons when it is incubated with mixtures of synthetic peptides representing two proteins present in M. tuberculosis: early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10). These antigens impart greater specificity than is possible with tests using purified protein derivative as the tuberculosis (TB) antigen. In direct comparisons, the sensitivity of QFT-G was statistically similar to that of the tuberculin skin test (TST) for detecting infection in persons with untreated culture-confirmed tuberculosis (TB). The performance of QFT-G in certain populations targeted by TB control programs in the United States for finding latent TB infection is under study. Its ability to predict who eventually will have TB disease has not been determined, and years of observational study of substantial populations would be needed to acquire this information. In July 2005, CDC convened a meeting of consultants and researchers with expertise in the field to review scientific evidence and clinical experience with QFT-G. On the basis of this review and discussion, CDC recommends that QFT-G may be used in all circumstances in which the TST is currently used, including contact investigations, evaluation of recent immigrants, and sequential-testing surveillance programs for infection control (e.g., those for health-care workers). This report provides specific cautions for interpreting negative QFT-G results in persons from selected populations. This report is aimed at public health officials, health-care providers, and laboratory workers with responsibility for TB control activities in the United States.

A copy of the full guideline is available at

<http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5415a4.htm>, or contact Cellestis (Section 14).

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14. TECHNICAL SERVICE

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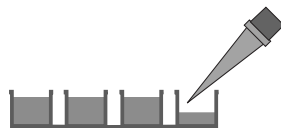
15. ABBREVIATED TEST PROCEDURE

STAGE I - INCUBATION OF BLOOD

1. Draw 5 - 10mL blood into heparin tubes.



2. Aliquot 1mL heparinized whole blood.



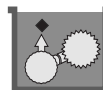
3. Add Nil, ESAT-6, CFP-10 and Mitogen Control.



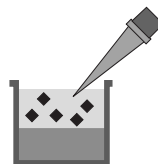
4. Thoroughly mix blood and antigens together.



5. Incubate overnight.

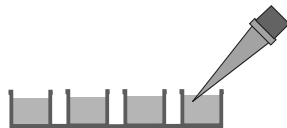
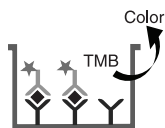
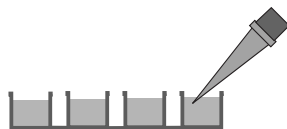
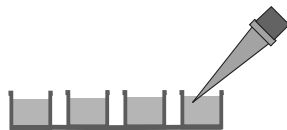
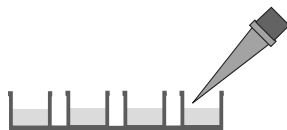


6. Harvest plasmas.



STAGE 2 - HUMAN IFN- γ ELISA

1. Prepare Conjugate in Green Diluent and add 50 μ L to ELISA wells.
2. Add 50 μ L test plasmas (simultaneously for each patient) and 50 μ L standards to wells.
3. Incubate for 120 minutes at room temperature.
4. Wash wells at least 6 times.
5. Add 100 μ L Enzyme Substrate Solution to wells.
6. Incubate for 30 minutes at room temperature.
7. Add 50 μ L Stop Solution to wells.
8. Read results at 450 /620 (or 650) nm.



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