QuantiFERON®-TB Gold

(In-Tube Method)

The Whole Blood IFN-gamma Test Measuring Responses to ESAT-6, CFP-10 & TB7.7 Peptide Antigens

PACKAGE INSERT

For *In Vitro* Diagnostic Use

cellestis

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1. INTENDED USE

QuantiFERON®-TB Gold In-Tube (IT) is an *in vitro* diagnostic test using a peptide cocktail simulating ESAT-6, CFP-10 and TB7.7(p4) 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.

QuantiFERON®-TB Gold IT 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.

2. SUMMARY AND EXPLANATION OF THE TEST

The QuantiFERON®-TB Gold IT test is a test for Cell Mediated Immune (CMI) responses to peptide antigens that simulate mycobacterial proteins. These proteins, ESAT-6, CFP-10 and TB7.7(p4), are absent from all BCG strains and from most non-tuberculous 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 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.

Tuberculosis is a communicable disease caused by infection with *M. tuberculosis* complex organisms, 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, or can remain latently infected for years. 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 (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), infection with mycobacteria other than *M. tuberculosis* complex, or undetermined other factors.

The tuberculin skin test and QuantiFERON®-TB Gold IT 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.

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

Numerous studies have demonstrated that the peptide antigens used in QuantiFERON®-TB Gold IT 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, CFP-10 and TB7.7(p4) as the genes encoding these proteins are present in *M. kansasii, M. szulgai* and *M. marinum.*[1]

Risk factors for *M. tuberculosis* infection include historical, medical or epidemiological predictors for tuberculosis disease or exposure to 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.

Principles of the Assay

The QuantiFERON®-TB Gold IT system uses specialized blood collection tubes, which are used to collect whole blood via venipuncture, that contain antigens representing certain *M. tuberculosis* proteins or controls. 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 QuantiFERON®-TB Gold IT test is performed in two stages. First, whole blood is collected into each of the QuantiFERON®-TB Gold IT blood collection tubes, which include a Nil Control tube, TB Antigen tube and a Mitogen tube.

The tubes are shaken to mix antigen with the blood and should be incubated at 37°C ± 1°C as soon as possible. Following a 16 to 24 hour incubation period, the tubes are centrifuged, the plasma is removed and the amount of IFN-γ (IU/mL) measured by ELISA. The

QuantiFERON®-TB Gold IT 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. The effect of heterophile antibodies in the QuantiFERON®-TB Gold IT 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 test is considered positive for an IFN-γ response to the TB Antigen tube that is significantly above the Nil IFN-γ IU/mL value. The Nil sample adjusts for background, heterophile antibody effects, or non-specific IFN-γ in blood samples. The IFN-γ level of the Nil tube is subtracted from the IFN-γ level for the TB Antigen tube and Mitogen tube. The Mitogen-stimulated plasma sample 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 prolonged specimen transport or improper specimen handling, including filling/mixing of blood tubes, 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.

3. REAGENTS AND STORAGE

Blood Collection Tubes

Catalog Number: 0590 0301

1.	Nil Control (Grey cap with white ring)	100 x tubes
2.	TB Antigen (Red cap with white ring)	100 x tubes
3.	Mitogen Control (Purple cap with white ring)	100 x tubes

High Altitude (HA) Blood Collection Tubes *(for use above 3,350 feet)*

Catalog Number: 0590 0501 (refer Section 5)

4.	Nil Control (Grey cap with yellow ring)	100 x tubes
5.	TB Antigen (Red cap with yellow ring)	100 x tubes
6.	Mitogen Control (Purple cap with yellow ring)	100 x tubes

ELISA Components

Catalog Number: 0594 0201

1.	Microplate strips coated with murine anti-human IFN-γ monoclonal antibody (24 x 8 well strips)	2 x 96 well plates
2.	Human IFN-γ Standard, lyophilized *(contains recombinant human IFN-γ, bovine casein, 0.01 % w/v Thimerosal)*	1 x vial (8 IU/mL when reconstituted)
3.	Green Diluent *(contains bovine casein, normal mouse serum, 0.01% w/v Thimerosal)*	1 x 30 mL
4.	Conjugate 100X Concentrate, lyophilized *(murine anti-human IFN-γ HRP, contains 0.01% w/v Thimerosal)*	1 x 0.3mL (when reconstituted)
5.	Wash Buffer 20X Concentrate *(pH 7.2, contains 0.01 % w/v Thimerosal)*	1 x 100mL
6.	Enzyme Substrate Solution *(contains H_2O_2, 3,3',5,5' Tetramethylbenzidine)*	1 x 30mL
7.	Enzyme Stopping Solution *(contains 0.5M H_2SO_4)*	1 x l5mL

Materials Required (but not provided)

- 37°C ± 1°C incubator (with or without CO_2).
- Calibrated variable-volume pipettes for delivery of 10µL to 1000µL with disposable tips.
- Calibrated 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 (26 patients / rack or plate) – not essential.
- Centrifuge capable of centrifuging the blood tubes at least to 3,000 RCF (g).
- Microplate shaker capable of speeds between 500 and 1,000 rpm.
- Deionised or distilled water - 2L.
- Microplate washer (for safety in handling plasma samples, an automated washer is recommended).
- Microplate reader fitted with 450nm filter and 620nm to 650nm reference filter.

- Variable speed vortex
- Timer
- Measuring cylinder – 1L or 2L
- Reagent reservoirs

Storage Instructions

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, please see Section 6.

- 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 **100X Conjugate** Concentrate must be returned to storage at 2°C to 8°C and must also be used within 3 months.
  - *Note the date the **100X 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 IT 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 function, or other individual immunological factors. Heterophile antibodies or non-specific IFN-γ production from other inflammatory conditions may mask specific responses to ESAT-6, CFP-10, or TB7.7 peptides.
- A false-negative QuantiFERON®-TB Gold IT result can be caused by incorrect blood sample collection or improper handing of the specimen affecting lymphocyte function. Blood tubes must be incubated at 37°C ± 1°C with stimulation antigens within 16 hours of collection; delay in incubation may cause false negative or indeterminate results (refer to the Technical Information section), and other technical parameters may affect ability to detect a significant IFN-γ response.
- Incorrect performance of the assay may cause false negative QuantiFERON®-TB Gold IT test results.
- Incorrect performance of the assay may cause false positive QuantiFERON®-TB Gold IT responses. A positive QuantiFERON®-TB Gold IT result should not be the sole or definitive basis for determining infection with *M. tuberculosis.*
- The effect of lymphocyte count on reliability of QuantiFERON®-TB Gold IT 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 QuantiFERON®-TB Gold IT result should be followed by further medical evaluation for active tuberculosis disease. (e.g., acid fast bacilli (AFB) smear and culture, chest x-ray)
- A positive QuantiFERON® -TB Gold IT result can suggest and support the diagnosis of tuberculosis disease. ESAT-6, CFP-10 and TB7.7(p4) 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 QuantiFERON®-TB Gold IT are needed to confirm tuberculosis disease.
- The predictive value of a negative QuantiFERON®-TB Gold IT result in immunosuppressed persons has not been determined.
- The performance of the USA format of the QuantiFERON®-TB Gold IT test has not been extensively 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. Individuals younger than age 17 years.
    3. Pregnant women.
- 'Standard' QuantiFERON®-TB Gold IT blood collection tubes are suitable for use at altitudes between sea level and 2,650 feet. To ensure that the correct volume of blood is drawn between altitudes of 3,350 and 6,150 feet, High Altitude (HA) tubes should be used. High Altitude tubes are distinguished from 'normal' tubes by the yellow ring in the cap.
- When collecting blood for the QuantiFERON®-TB Gold IT test at altitudes between 2,650 and 3,350 feet or above 6,150 feet, it is recommended to collect blood using a syringe as described in Section 5 to ensure that the correct volume of blood is added to the tubes.

Precautions

- **For *in vitro* diagnostic use only.**
- **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 H_2SO_4 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 amounts of water and seek medical attention.
- **Harmful: IFN-γ Standard** and **Conjugate 100X 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 relevant blood handling 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.
- Do not use kit if any reagent bottle shows signs of damage or leakage prior to use.
- Blood samples should be transported to the laboratory at ambient temperature (22°C ± 5°C, 72°F ± 9°F). Do not transport on ice or refrigerate.
- Do not mix or use ELISA reagents from other QuantiFERON®-TB Gold kit batches.
- Discard unused reagents and biological samples in accordance with Local, State, and Federal regulations.
- Do not use the blood collection tubes or ELISA kit after the expiry date.
- Correct laboratory procedures should be adhered to at all times.

5. SPECIMEN COLLECTION AND HANDLING

QuantiFERON®-TB Gold IT uses the following collection tubes:

1. Nil Control (Grey cap with white ring) (use between sea-level and 2,650 feet)
2. TB Antigen (Red cap with white ring) (use between sea-level and 2,650 feet)
3. Mitogen Control (Purple cap with white ring) (use between sea-level and 2,650 feet)

4. Nil Control (Grey cap with yellow ring) (use between 3,350 and 6,150 feet)
5. TB Antigen (Red cap with yellow ring) (use between 3,350 and 6,150 feet)
6. Mitogen Control (Purple cap with yellow ring) (use between 3,350 and 6,150 feet)

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. The tubes must be transferred to a 37°C ± 1°C incubator as soon as possible and within 16 hours of collection.

The following procedures should be followed for optimal results:

1. For each subject collect 1mL of blood by venipuncture directly into each of the QuantiFERON®-TB Gold IT blood collection tubes.

   - As 1mL tubes draw blood relatively slowly, keep the tube on the needle for 2-3 seconds once the tube appears to have completed filling, to ensure that the correct volume is drawn.

     The black mark on the side of the tubes indicates the 1mL fill volume. QuantiFERON®-TB Gold IT blood collection tubes are manufactured to draw 1mL ± 10% and perform optimally within the range of 0.8 to 1.2mL. If the level of blood in any tube is not close to the indicator line, it is recommended to obtain another blood sample or to collect blood via a syringe as described below. Under or over-filling of the tubes outside of the 0.8 to 1.2mL range may lead to erroneous results.

     Standard QFT-Gold IT blood tubes have been validated to draw between 0.8mL and 1.2mL at altitudes from sea-level to 2,650 feet. High Altitude (HA) tubes should be used at altitudes between 3,350 and 6,150 feet. If using the QuantFERON® blood collection tubes outside these altitude ranges (between 2,650 and 3,350 feet or above 6,150 feet), or if low blood draw volume does occur, blood can be collected using a syringe and 1mL transferred to each of the three tubes. For safety reasons, this is best performed by removing the syringe needle, ***ensuring appropriate safety procedures****, removing the caps from the three QFT-Gold IT tubes and adding 1mL of blood to each (to the*

black mark on the side of the tube label). Replace the tube caps securely and mix as described below.

- 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 QuantiFERON®-TB Gold IT tubes being used.

2. Mix the tubes by **SHAKING VIGOROUSLY** for 5 seconds (10X) to ensure that **the entire inner surface of the tube** has been coated with the blood.

   - Thorough mixing is required to ensure complete integration of the tube's contents into the blood.

3. Label tubes appropriately.

   - Ensure each tube (Nil, TB Antigen, Mitogen) is identifiable by its label or other means once the cap is removed.

4. 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 (22°C ± 5°C). Do not refrigerate or freeze the blood samples.

6. DIRECTIONS FOR USE

Time Required for Performing Assay

In order to obtain valid results from the QuantiFERON®-TB Gold IT 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:

37°C ± 1°C Incubation of blood tubes:	16 to 24 hours
ELISA:	Approx. 3 hours for one ELISA plate <1 hour labor Add 10 to 15 minutes for each extra plate

Stage One – Incubation of Blood and Harvesting of Plasma

Refer to Section 3 for materials required for blood incubation phase.

Procedure

1. If the blood is not incubated immediately after collection, **re-mixing of the tubes by vigorous shaking for 5 seconds must be repeated immediately prior to incubation**, as described in Section 5.2.
2. Incubate the tubes **UPRIGHT** at 37°C ± 1°C for 16 to 24 hours. The incubator does not require CO_2 or humidification.
3. Following 37°C ± 1°C incubation, blood collection tubes may be held between 2°C and 27°C for up to 3 days prior to centrifugation.
4. After incubation of the tubes at 37°C ± 1°C, centrifuge 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 at a higher speed.
5. Plasma samples can be loaded directly from blood collection tubes into the QuantiFERON®-TB Gold IT ELISA plate, especially when automated ELISA workstations are being used.
6. Alternatively, plasma samples can be stored prior to ELISA, either in the centrifuged tubes or collected into plasma storage containers. For example, harvest >150µL of plasma into microplate wells or racked microtubes in 96 well format and seal to prevent spills and evaporation if samples are to be stored.
   - Plasma samples can be stored for up to 28 days at 2°C to 8°C or below -20°C (preferably less than -70°C) for extended periods.
   - Plasmas may clot during extended storage. If clots are present refer to TROUBLE SHOOTING section.

Stage Two - Human IFN-γ ELISA

Refer to Section 3 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) before use. Allow at least 60 minutes for equilibration.

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

   Allow at least two strips for the QuantiFERON®-TB Gold Standards and sufficient strips for the number of subjects being tested (refer to Figure 2 for recommended plate format). After use, retain frame and lid for use with remaining strips.

3. **HUMAN IFN-γ STANDARD** ***(contains 0.01% w/v Thimerosal).*** Reconstitute the Human Interferon-γ Kit Standard with the volume of deionised 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 1 below).

   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).

i. Add 300µL of the Kit Standard to a tube labeled as Standard 1.

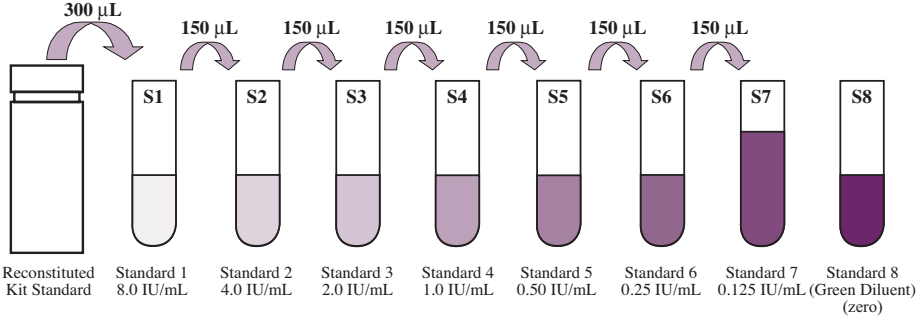
ii Add 150µL of Green Diluent to 7 tubes (labeled Standard 2 – Standard 8).

iii Perform serial dilutions by transferring 150µL of each Standard to the next tube. Mix each tube thoroughly before the next transfer.

iv The undiluted Kit Standard serves as the highest concentration (Standard 1).

v Green Diluent serves as the Zero Standard (Standard 8).

FIGURE 1. Preparation of Standard Curve



- **Prepare fresh dilutions of the Kit Standard for each ELISA session.**

4. **CONJUGATE** ***(contains 0.01% w/v Thimerosal)***. Reconstitute freeze dried Conjugate 100X Concentrate with 0.3mL of deionized or distilled water. Mix gently to minimize frothing and ensure complete solubilization of the Conjugate.

   Working Strength conjugate is prepared by diluting the required amount of reconstituted Conjugate 100X Concentrate in Green Diluent as set out in Table 1 - Conjugate Preparation.

TABLE 1. Conjugate Preparation

NUMBER OF STRIPS	VOLUME OF CONJUGATE 100X CONCENTRATE	VOLUME OF GREEN DILUENT
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

- **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.**

5. Prior to assay, plasmas should be mixed thoroughly.

6. Add 50µL of freshly prepared Working Strength conjugate to each ELISA well.

7 Add 50µL of test plasma samples to appropriate wells (Refer to recommended plate layout below – Figure 2). Finally, add 50µL each of the Standards 1 to 8. The standards should be assayed in at least duplicate.

FIGURE 2. Recommended Sample Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	1N	1A	1M	*S1*	*S1*	9N	9A	9M	17N	17A	17M	25N
B	2N	2A	2M	*S2*	*S2*	10N	10A	10M	18N	18A	18M	25A
C	3N	3A	3M	*S3*	*S3*	11N	11A	11M	19N	19A	19M	25M
D	4N	4A	4M	*S4*	*S4*	12N	12A	12M	20N	20A	20M	26N
E	5N	5A	5M	*S5*	*S5*	13M	13N	13A	21N	21A	21M	26A
F	6N	6A	6M	*S6*	*S6*	14M	14N	14A	22N	22A	22M	26M
G	7N	7A	7M	*S7*	*S7*	15M	15N	15A	23N	23A	23M	
H	8N	8A	8M	*S8*	*S8*	16M	16N	16A	24N	24A	24M	

- 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); 1A (Sample 1. TB Antigen plasma); 1M (Sample 1. Mitogen Control plasma).

8. Mix the conjugate and plasma samples/standards thoroughly using a microplate shaker for 1 minute at 500 to 1,000 rpm.

9. Cover each plate and incubate 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.

10. **WASH BUFFER *(contains 0.01% w/v Thimerosal)***. During the incubation, 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 2L of Working Strength wash buffer.

Wash wells with **400µL** of Working Strength wash buffer. Perform wash step at least 6 times **(SEE NOTES BELOW)**. An automated plate washer is recommended for safety reasons when handling plasma samples.

- **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.**
- **When an automated plate washer is used, standard laboratory disinfectant should be added to the effluent reservoir, and established procedures followed for the decontamination of potentially infectious material.**

11. If there is residual wash buffer in the wells following the final wash, tap plates face down on absorbent towel to remove. Add 100µL of Enzyme Substrate Solution to each well and mix for 1 minute at 500 to 1,000 rpm using a microplate shaker.
12. Cover each plate with a lid and incubate at room temperature (22°C ± 5°C) for 30 minutes.
    - **Plates should not be exposed to direct sunlight during incubation.**
13. Following the 30 minute incubation, add 50µL of Enzyme Stopping Solution to each well and mix thoroughly.
    - **Enzyme Stopping Solution should be added to wells in the same order and at approximately the same speed as the substrate in step 11.**
14. Measure the Optical Density (OD) of each well within 5 minutes of stopping the reaction using a microplate reader fitted with a 450nm filter and with a 620nm to 650nm reference filter. OD values are used to calculate results.

7. CALCULATIONS AND TEST INTERPRETATION

QuantiFERON®-TB Gold IT Analysis Software (version 2.17 or later), used to analyze raw data and calculate results, is available free of charge from Cellestis (www.cellestis.com).

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 the Interpretation of Results section. 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 QuantiFERON®-TB Gold IT Analysis Software, results can be determined according to the following method:

Generation of Standard Curve and Sample Values

(if QuantiFERON®-TB Gold IT 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 QuantiFERON®-TB Gold IT software is not used.

Using the Spreadsheet Program:

1) 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).

2) Determine the logarithm ($Log_{(e)}$ or Log_{10}) of each mean OD value ($Log_{(e)}$ is LN(x) in many Programs).

3) Determine the Log 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 Log value.

4) Enter the Log of Mean OD in Spreadsheet Program cells beside the respective Log of the IU/mL Standard.

5) 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 Log of the absorbance (OD) for each plasma sample, X is the Log 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.

6) Ensure that the ELISA run meets Quality Control of Test specifications.

7) For each patient test, determine Log absorbance (Y-value) for the Nil, TB Antigen and Mitogen test sample OD values.

8) 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 (Log IU/mL of IFN-γ) for each of the Y-values (OD).

9) Determine the IFN-γ concentration (IU/mL) for the Nil, TB Antigen and Mitogen test plasma samples, by converting the X-value using the antilog equation e^X or 10^X for $Log_{(e)}$ and Log_{10} respectively.

10) Subtract the Nil IU/mL value from the TB Antigen and Mitogen values for each patient series of tests.

* Most spreadsheet programs offer a variety of methods for obtaining the values of 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 Log OD as **the Y values** and the seven corresponding Log 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 Log of the mean Standard OD values against the Log 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 Log OD as the **Y values** and the seven corresponding Log values of the 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 (using $Log_{(e)}$) would be:

Standard	IU/mL	OD Values a & b	Mean OD	%CV	$Log_{(e)}$ IU/mL	$Log_{(e)}$ 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	0.037	-3.297	-3.021	0.05	–
TB Antigen	1.161	0.149	1.405	4.08	4.03
Mitogen	1.783	0.578	1.956	7.07	7.02

IFN-γ values (in IU/mL) for the TB Antigen 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 ≥ 1.200.**
- **The % coefficient of variation (%CV) between replicates for Standards 1 to 5 must be ≤ 15%.**
- **Replicate OD values for Standards 6 to 8 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.**

The QuantiFERON®-TB Gold IT Analysis Software calculates and reports these quality control parameters.

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.**

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

QuantiFERON®-TB Gold IT results are interpreted using the following criteria:

<u>NOTE</u>: 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 QuantiFERON®-TB Gold IT results. See general guidance on the diagnosis and treatment of TB disease and LTBI (http://www.cdc.gov/nchstp/tb/).

Nil [IU/mL]	TB Antigen minus Nil [IU/mL]	Mitogen minus Nil [IU/mL][1]	QuantiFERON®-TB Gold IT Result	Report / Interpretation
≤ 8.0	≥ 0.35 and ≥ 25% of Nil value	Any	**Positive**[2]	*M. tuberculosis* infection likely
	< 0.35 OR ≥ 0.35 and < 25% of Nil value	≥ 0.5	**Negative**	*M. tuberculosis* infection NOT likely
		< 0.5	**Indeterminate**[3]	Results are indeterminate for TB Antigen responsiveness
> 8.0[4]	Any	Any		

[1] 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 QuantiFERON®-TB Gold IT software as >10 IU/mL.

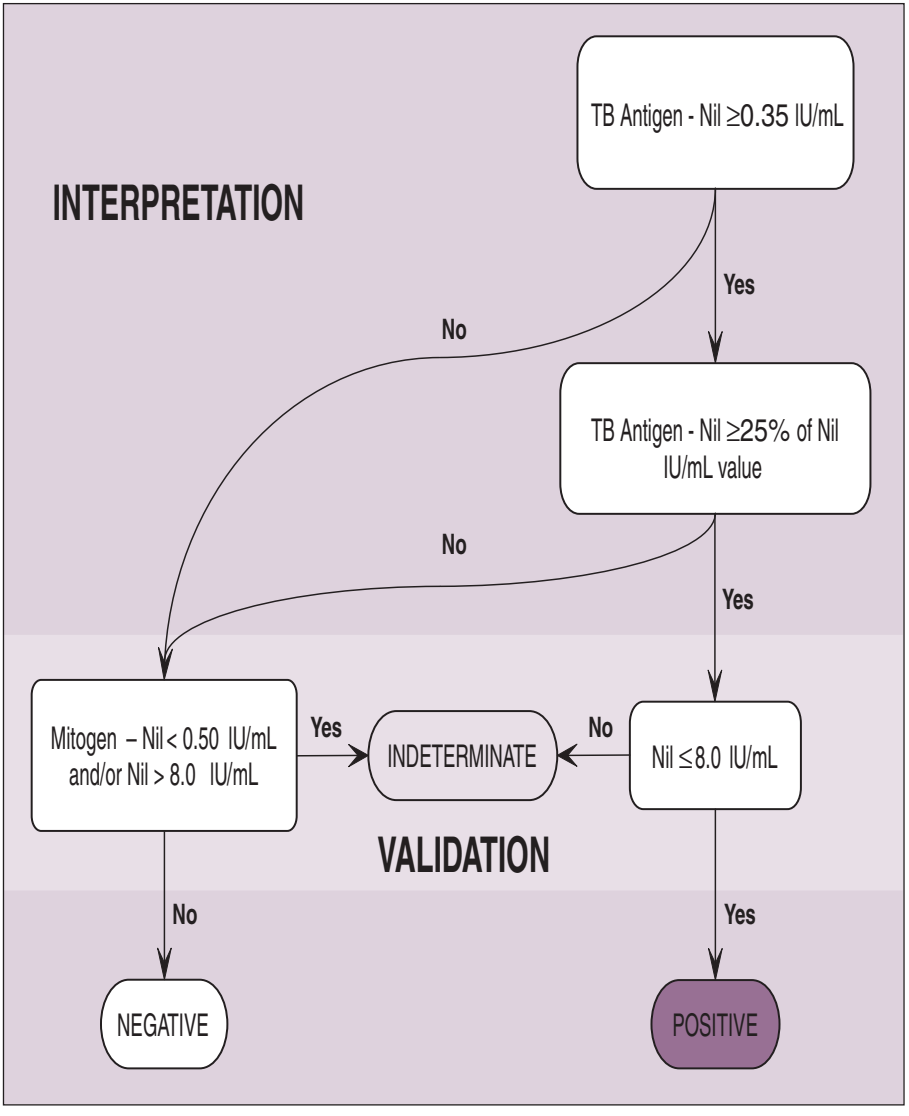
[2] Where *M. tuberculosis* infection is not suspected, initially positive results can be confirmed by retesting the original plasma samples in duplicate in the QuantiFERON®-TB Gold IT ELISA. If repeat testing of one or both replicates is positive, the test result is considered positive.

[3] Refer to Section 11 for possible causes.

[4] In clinical studies, less than 0.25% of subjects had IFN-γ levels of > 8.0 IU/mL for the Nil Control.

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 Antigen is greater than that to mitogen, which is possible as the level of mitogen does not maximally stimulate IFN-γ production by lymphocytes.

FIGURE 3. Interpretation Flow Diagram



8. LIMITATIONS

Results from QuantiFERON®-TB Gold IT testing must be used in conjunction with each individual's epidemiological history, current medical status, and results of 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 QuantiFERON®-TB Gold IT 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 Section 5. 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 to the QuantiFERON®-TB Gold IT tubes manually.

Unreliable or indeterminate results may occur due to:

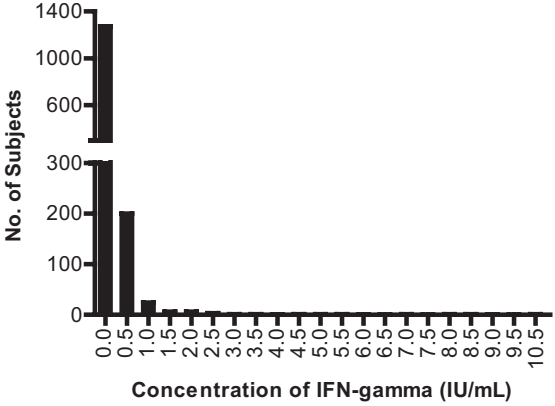
- Deviations from the procedure described in the Package Insert,
- Incorrect transport / handling of blood specimen,
- Excessive levels of circulating IFN-γ or presence of heterophile antibodies,
- Longer than 16 hours from blood specimen drawing to incubation at 37°C ± 1°C.

9. EXPECTED VALUES

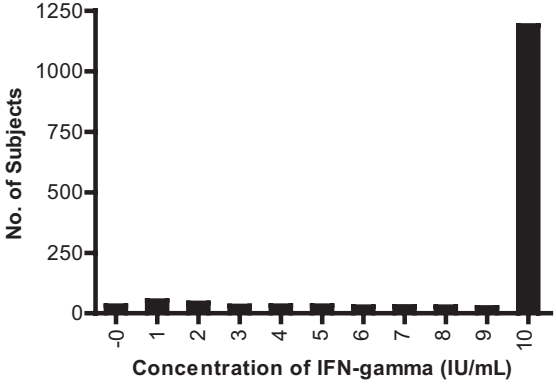
The range of IFN-γ responses to the TB Antigens and Control Antigens that have been observed in the clinical trials are shown in Figure 4.

FIGURE 4. Distribution of Nil, Mitogen and TB Antigen responses

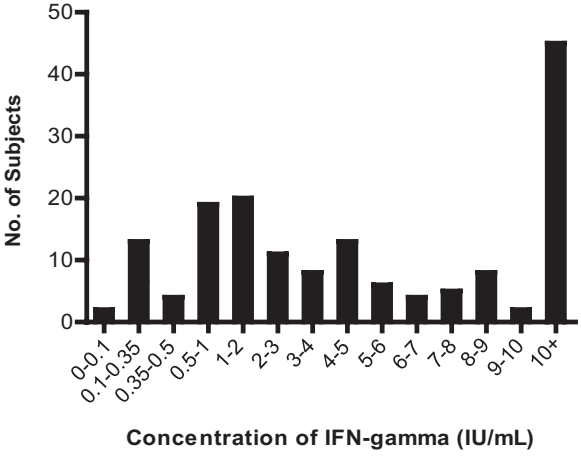
a) Distribution of Nil values from clinical studies (n=1,522). (Median 0.12 IU/mL)



b) Distribution of Mitogen –Nil values from clinical studies (n=1,522).
Results > 10 IU/mL are shown as 10 IU/mL.



c) Distribution of TB Antigen – Nil responses for culture-confirmed TB patients (n=160). Results > 10 IU/mL are shown as 10 IU/mL.



10. PERFORMANCE CHARACERISTICS

Clinical Studies

As there is no definitive standard for confirming or excluding the diagnosis of LTBI, an estimate of sensitivity and specificity for QuantiFERON®-TB Gold IT cannot be practically evaluated. Specificity of QuantiFERON®-TB Gold IT was approximated by evaluating false positive rates in the persons with low risk (no known risk factors) of tuberculosis infection. Sensitivity was approximated by evaluating groups of patients with culture-confirmed active TB disease.

Specificity

In a U.S. study involving 866 volunteers, blood was drawn for QuantiFERON®-TB Gold IT when a TST was placed. Demographic information and risk factors for TB were determined using a questionnaire at the time of testing. Of 432 volunteers with no known risk factors for *M. tuberculosis* infection, QuantiFERON®-TB Gold IT, QuantiFERON®-TB Gold (liquid antigen version) and TST results were available for 391, 418 and 424 respectively. None were BCG vaccinated.

Three low risk volunteers were QuantiFERON®-TB Gold IT positive (one was also TST-positive), one was indeterminate (Mitogen <0.5 IU/mL), whereas 6 were TST-positive using a 10mm cut off and 4 at ≥15mm. Therefore, specificity for QuantiFERON®-TB Gold IT was 99.2%. A comparison of results for QuantiFERON®-TB Gold IT, the liquid antigen version of QuantiFERON®-TB Gold, and the TST is shown in Table 2.

Table 3 shows the direct comparison of QuantiFERON®-TB Gold IT with TST responses (using a ≥15mm cut off) for the 383 subjects where results for both tests were available. Overall agreement, excluding the Indeterminate result, was 98.7% (377/382; 95% CI: 97.0% to 99.4%), with 25% (1/4; 95% CI: 4.6% to 69.9%) positive per cent agreement of QFT-Gold IT with the TST and 99.5% (376/378; 95% CI: 98.1% to 99.9%) negative per cent agreement.

TABLE 2. QuantiFERON®-TB Gold IT specificity: Comparison with other tests in subjects with low reported risk for *M. tuberculosis* infection.

Test	No. Test Not done	No. Test Indeterminate	No. Positive / Valid Tests	Specificity	95% Confidence Intervals
QuantiFERON®-TB Gold IT	41	1	3 / 390	(387/390) 99.2%	97.65% – 99.8%
QuantiFERON®-TB Gold (liquid antigen)	14	6	1 / 412	(411/412) 99.8%	98.4% – 100%
TST*	8	N/A	4 / 424*	(420/424) 99.1%	97.4% – 99.7%

*Using ≥15mm TST cut off. TST specificity estimate was 98.6% (95% CI: 96.8% - 99.4%) if using a 10mm cut off.

TABLE 3. Comparison of results for QuantiFERON®-TB Gold IT and TST in subjects with low reported risk for *M. tuberculosis* infection and where results for both tests were available.

		QFT-Gold IT Positive	QFT-Gold IT Negative	QFT-Gold IT Indeterminate	Total
TST (≥15mm)	Positive	1	3	0	4
	Negative	2	376	1	379
	Total	3	379	1	383

Sensitivity for active TB

TB-suspects from Australia, the U.S. and Japan who were subsequently confirmed by culture and/or nucleic acid amplification testing to have *M. tuberculosis* infection were tested to evaluate sensitivity of QuantiFERON®-TB Gold IT. While there is no definitive standard test for LTBI, a suitable surrogate is microbiological culture of *M. tuberculosis* since patients with disease are by definition infected. The patients had received less then 8 days of treatment prior to collecting blood for QuantiFERON®-TB Gold IT testing.

The Japanese sensitivity study was conducted at five general hospitals in the Tokyo region from February 2006 to November 2006. The study evaluated QuantiFERON®-TB Gold IT and QuantiFERON®-TB Gold (liquid antigen) in 100 patients with culture and/or PCR proven *M. tuberculosis* infection. Results for the two tests are shown in comparison in Table 4. For patients with either positive or negative results for both tests, sensitivity of QuantiFERON®-TB Gold IT was 93.5% (86/92; 95% CI: 86.5% to 97.0%) and that for QuantiFERON®-TB Gold was 83.7% (77/92; 95% CI: 74.8% to 89.9%). Overall agreement was 88.0% (81/92; 95% CI: 79.8% to 98.2%), with a positive per cent agreement of QuantiFERON®-TB Gold IT with QuantiFERON®-TB Gold of 98.7% (76/77; 95% CI: 93.0% to 99.8%), and negative per cent agreement of 33.3% (5/15; 95% CI: 15.2% to 58.3%).

TABLE 4. Agreement between QuantiFERON®-TB Gold IT and QuantiFERON®-TB Gold in TB patients from the Japanese sensitivity study.

		QuantiFERON®-TB Gold In Tube			
		Positive	Negative	Indeter*	TOTAL
QuantiFERON®-TB Gold (liquid antigen)	Positive	76	1	2	79 (79%)
	Negative	10	5	3	18 (18%)
	Indeter*	0	0	3	3 (3%)
	TOTAL	86 (86%)	6 (6%)	8 (8%)	100 (100%)

*Indeter = Indeterminate

The Australian study was conducted at one location (Monash Medical Centre, Clayton, Victoria) from July 2003 to November 2004. Twenty-seven patients with culture-proven *M. tuberculosis* infection were tested with both QuantiFERON®-TB Gold IT and QuantiFERON®-TB Gold (liquid antigen). All subjects had positive or negative results for both tests and the percentage with positive results was 88.9% (24/27; 95% CI: 71.9% to 96.2%) and 74.1% (20/27; 95% CI: 55.3% to 86.8%), respectively (Table 5). Overall agreement was 85.2% (23/27; 95% CI 67.5% to 94.1%) and there was 100% positive per cent agreement with QuantiFERON®-TB Gold results (20/20; 95% CI 83.9% to 100%) and 42.9% negative per cent agreement (3/7; 95% CI: 15.8% to 75.0%).

TABLE 5. Agreement between QuantiFERON®-TB Gold IT and QuantiFERON®-TB Gold in TB patients from the Australian sensitivity study.

		QuantiFERON®-TB Gold In Tube		
		Positive	Negative	TOTAL
QuantiFERON®-TB Gold (liquid antigen)	Positive	20	0	20 (74.1%)
	Negative	4	3	7 (25.9%)
	TOTAL	24 (88.9%)	3 (11%)	27 (100%)

There were no subjects with Indeterminate results for either test

The U.S. study was conducted at six locations from December 2002 to October 2005. Valid QuantiFERON®-TB Gold IT results were available for 61 patients with culture-confirmed *M. tuberculosis* infection.

Table 6 shows the agreement between QuantiFERON®-TB Gold IT and QuantiFERON®-TB Gold (liquid antigen) for the 50 patients where results were available for both tests (11 did not have the QuantiFERON®-TB Gold test performed). For patients with either positive or negative results for both tests, sensitivity of QuantiFERON®-TB Gold IT was 84.1% (37/44; 95% CI: 70.6% to 92.1%) and that for QuantiFERON®-TB Gold was 77.3% (34/44; 95% CI: 63.0% to 87.2%). Overall agreement was 93.2% (41/44; 95% CI: 81.8% to 97.7%), with a positive per cent agreement of QuantiFERON®-TB Gold IT with QuantiFERON®-TB Gold of 100% (34/34; 95% CI: 89.9% to 100%), and negative per cent agreement of 70.0% (7/10; 95% CI: 39.7% to 89.2%).

TABLE 6. Agreement between QuantiFERON®-TB Gold IT and QuantiFERON®-TB Gold in TB patients from the U.S. sensitivity study where results were available for both tests.

		QuantiFERON®-TB Gold In Tube			
		Positive	Negative	Indeter*	TOTAL
QuantiFERON®-TB Gold (liquid antigen)	Positive	34	0	0	34 (68%)
	Negative	3	7	0	10 (20%)
	Indeter*	2	3	1	6 (12%)
	TOTAL	39 (78%)	10 (20%)	1 (2%)	50 (100%)

*Indeter = Indeterminate

Agreement between QuantiFERON®-TB Gold IT and the TST (using a 5mm cut off) is shown in Table 7 for the 57 patients who had valid results available for both tests (4 of the 61 with QuantiFERON®-TB Gold IT results had invalid TSTs; one was not read and 3 were read prior to the 48 to 72 hour window defined in the protocol).

For patients with either positive or negative results for both tests, sensitivity of QuantiFERON®-TB Gold IT was 81.5% (44/54; 95% CI: 69.2% to 89.6%) and that for the TST was 77.8% (42/54; 95% CI: 65.1% to 86.8%). Overall agreement was 81.5% (44/54; 95% CI: 69.2% to 89.6%), with a positive per cent agreement of QuantiFERON®-TB Gold IT with TST of 90.5% (38/42; 95% CI: 77.9% to 96.2%), and negative per cent agreement of 50% (6/12; 95% CI: 25.4% to 74.6%).

TABLE 7. Agreement between QuantiFERON®-TB Gold IT and the TST in TB patients from the U.S. sensitivity study.

		QuantiFERON®-TB Gold In Tube			
		Positive	Negative	Indeter*	TOTAL
TST (5mm cut off)	Positive	38	4	2	44 (77.2%)
	Negative	6	6	1	13 (22.8%)
	TOTAL	44 (77.2%)	10 (17.5%)	3 (5.3%)	57 (100%)

*Indeter = Indeterminate

Table 8 summarizes findings from the three clinical studies of *M. tuberculosis* culture positive patients. Overall sensitivity of QuantiFERON®-TB Gold IT for active TB disease was 88.7% (157/177; 95% CI: 83.2% to 92.6%). Eleven patients (3 from the U.S. study and 8 from the Japanese study) had Indeterminate test results.

TABLE 8. QuantiFERON®-TB Gold IT: Summary of results from clinical studies of subjects with culture-confirmed *M. tuberculosis* infection.

STUDY	QuantiFERON®-TB Gold IT			QuantiFERON®-TB Gold (liquid antigen)			TST (5mm)*	
	Pos	Neg	Ind	Pos	Neg	Ind	Pos	Neg
Australian	24	3	0	20	7	0	–	–
USA	47	11	3	34	10	6	60	19
Japanese	86	6	8	78	14	8	–	–
Overall Sensitivity	89% (157/177)			81% (132/163)			76% (60/79)	

Pos – Positive; Neg – Negative; Ind – Indeterminate
* In the U.S. study of 86 *M. tuberculosis* patients, TST results were missing for 4 and invalid for 3.

Comparative performance of QuantiFERON®-TB Gold IT in people with risk factors for LTBI

A multicenter study in the U.S. evaluated contacts of TB patients with QuantiFERON®-TB Gold IT, QuantiFERON®-TB Gold, and the TST. Valid data for both QuantiFERON®-TB Gold tests were available for 377 and for QuantiFERON®-TB Gold IT and the TST, 403 subjects. Data were collected as to the extent of exposure of the contacts to their respective index cases and also their risk factors for *M. tuberculosis* infection prior to their known exposure (Table 9).

TABLE 9. Demographics and factors associated with prior risk of *M. tuberculosis* infection of TB contacts.

	Number	%
Total subjects	346	100
Gender		
Male	192	55.5%
Female	154	44.5%
Age (years)		
< 20 years	36	10.4%
20 - 39 years	120	34.7%
40 - 59 years	122	35.3%
60 + years	68	19.7%
Ethnicity		
White-Hispanic	121	35.0%
White-Non-Hispanic	110	31.8%
African-American	72	20.7%
Asian-American	41	11.8%
Other	2	0.6%
BCG vaccinated		
Yes	91	26.3%
No	214	61.8%
Unknown	41	11.8%
TB rate in country of birth*		
0 to 20 per 100,000	203	58.7%
20 to 100 per 100,000	84	24.3%
>100 per 100,000	59	17%

* As per WHO published rates for 1990.

Agreement between the two versions of the QuantiFERON®-TB Gold test is shown in Table 10 for those subjects with results available for both tests. For patients with either positive or negative results for both tests, overall agreement was 91.3% (316/346; 95% CI: 87.9% to 93.9%), with a positive per cent agreement of QuantiFERON®-TB Gold IT with QuantiFERON®-TB Gold of 81.7% (58/71; 95% CI: 71.2% to 89.0%), and negative per cent agreement of 93.8% (258/275; 95% CI: 90.3% to 96.1%). Indeterminate results were seen for the QuantiFERON®-TB Gold IT test in 8/377 (2.1%) of subjects, compared with 27/377 (7.2%) for QuantiFERON®-TB Gold.

TABLE 10. Agreement between QuantiFERON®-TB Gold IT and QuantiFERON®-TB Gold in contacts of TB patients.

		QuantiFERON®-TB Gold In Tube			
		Positive	Negative	Indeter*	TOTAL
QuantiFERON®-TB Gold (liquid antigen)	Positive	58	13	0	71 (18.8%)
	Negative	17	258	4	279 (74%)
	Indeter*	8	15	4	27 (7.2%)
	TOTAL	83 (22%)	286 (75.9%)	8 (2.1%)	377 (100%)

*Indeter = Indeterminate

Agreement between QuantiFERON®-TB Gold IT and the TST (using a 5mm cut off) is shown in Table 11 for the 403 subjects who had valid results available for both. For those with either positive or negative results for both tests, overall agreement was 80.9% (321/397; 95% CI: 76.7% to 84.4%), with a positive per cent agreement of QuantiFERON®-TB Gold IT with TST of 55.3% (73/132; 95% CI: 46.8% to 63.52%), and negative per cent agreement of 93.6% (248/265; 95% CI: 90.0% to 96.0%).

TABLE 11. Agreement between the TST and QuantiFERON®-TB Gold IT in contacts of TB patients.

		QuantiFERON®-TB Gold In Tube			
		Positive	Negative	Indeter*	TOTAL
TST (5mm cut off)	Positive	73	59	0	132 (32.8%)
	Negative	17	248	6	271 (67.2%)
	TOTAL	90 (22.3%)	307 (76.2%)	6 (1.5%)	403 (100%)

*Indeter = Indeterminate

Association of test results and risk factors for *M. tuberculosis* infection (both recent and prior risks) was analyzed by multiple logistic regression. Positive results for both QuantiFERON®-TB Gold IT and the TST were significantly associated with a high rate of tuberculosis in the country of birth or a country visited for more than one month, as were White-Hispanic race and Asian-American race in comparison with African-American

and White non-Hispanic races, and age greater than 60 years. Analysis of discordant results between these two tests by multiple logistic regression demonstrated a significant association between TST-positive and QuantiFERON®-TB Gold IT-negative results in those BCG vaccinated. Table 12 shows the comparative test results for those that reported no BCG vaccination and those who reported vaccination (or unknown status). A similar rate of TST-negative discordance was found in both groups (4.2% and 4.5%), but a much larger rate of TST-positive/QuantiFERON®-TB Gold IT-negative results was found in the BCG vaccinated cohort (30.3% versus 7.0%).

TABLE 12. Agreement between the TST and QuantiFERON®-TB Gold IT in BCG vaccinated and unvaccinated contacts of TB patients.

		QuantiFERON®-TB Gold IT	
Unvaccinated (n=214)		**Positive**	**Negative**
TST (5mm)	**Positive**	31 (14.5%)	15 (7.0%)
	Negative	9 (4.2%)	159 (74.3%)
BCG vaccinated or unknown (n=132)			
TST (5mm)	**Positive**	29 (22.0%)	40 (30.3%)
	Negative	6 (4.5%)	57 (43.2%)

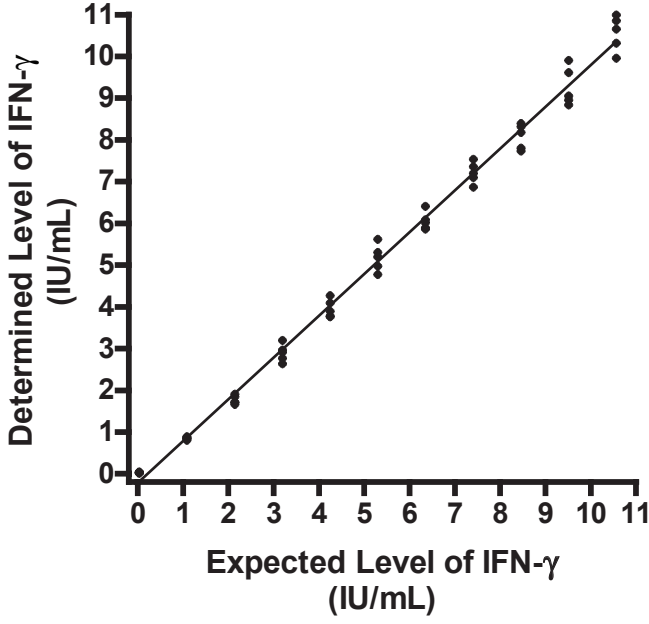
QuantiFERON®-TB Gold IT has been evaluated with specimens from patients with culture confirmed active tuberculosis and from apparently healthy adults with and without identified risk factors extensively for *M. tuberculosis* infection. Samples from HIV infected individuals in a Danish study indicates those with a CD4 count > 100/µL can provide QuantiFERON®-TB Gold IT results.[5] Individuals on immunosuppressive medication (corticosteroids, methotrexate, azithoprine) have shown positive results in QuantiFERON®-TB Gold IT testing.[23]

Repeatability and effect of TST on subsequent QuantiFERON®-TB Gold IT testing

As part of the U.S. specificity study, a subset of the volunteers was retested between 4 and 5 weeks after the original QuantiFERON®-TB Gold IT test and TST. QuantiFERON®-TB Gold IT results for 530 recruits were available at both time points and the level of agreement was 98.5% (522/530). Five subjects initially positive were negative at retest, and three subjects initially negative had positive results at retest. There was no evidence for the TST inducing positive QuantiFERON®-TB Gold IT responses.

The method for measuring IFN-γ concentration by the QuantiFERON®-TB Gold IT ELISA has been demonstrated to be linear from zero to 10 IU/mL (Figure 5). The linearity study was performed by placing 5 replicates of 11 plasma pools of known IFN-γ concentrations randomly on the ELISA plate. The QuantiFERON®-TB Gold IT ELISA shows no evidence of a high-dose hook (prozone) effect with concentrations of IFN-γ of up to 100,000 IU/mL.

FIGURE 5. Linearity profile of QuantiFERON®-TB Gold IT ELISA determined from testing 5 replicates of 11 plasma samples of known IFN-γ concentrations. The linear regression line has a slope of 1.002 ± 0.011 and a correlation coefficient of 0.99.



Intra- and inter-assay imprecision (%CV) of the QuantiFERON®-TB Gold IT ELISA was estimated by testing twenty plasma samples with varying IFN-γ concentrations in replicates of 3, in 3 laboratories, on 3 non-consecutive days, by 3 operators. Thus each sample was tested 27 times, in 9 independent assay runs. One sample was a nil control and had a calculated IFN-γ concentration of 0.08 (95%CI 0.07 - 0.09) IU/mL. Of the

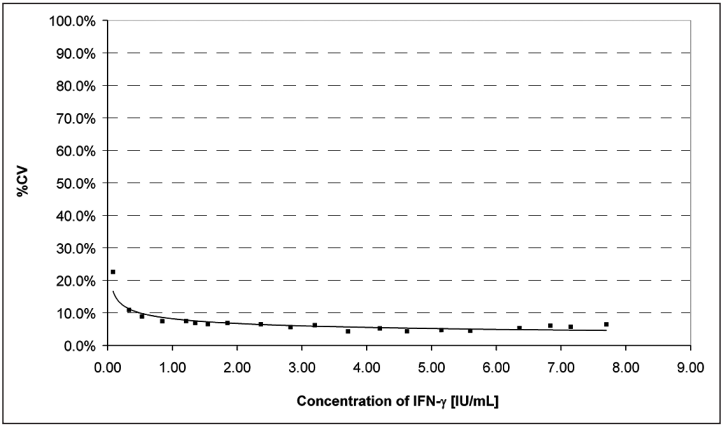
remaining 19 plasma samples, the range of concentrations was 0.33 (0.31 - 0.34) to 7.7 IU/mL (7.48 - 7.92).

Within run or intra-assay imprecision was estimated by averaging the %CVs for each test plasma containing IFN-γ from each plate run (n=9) and ranged from 4.1 to 9.1%CV. The average within run %CV (±95%CI) was 6.6% ± 0.6%. The zero IFN-γ plasma averaged 14.1%CV.

Total or inter-assay imprecision was determined by comparing the 27 calculated concentrations of IFN-γ for each plasma and ranged from 6.6 to 12.3%CV. The overall average %CV (±95%CI) was 8.7% ± 0.7%. The zero IFN-γ plasma showed a 26.1%CV. This level of variation is to be expected because the calculated concentration of IFN-γ is low and variation around a low estimate of concentration will be larger than that for higher concentrations.

The precision profile for QuantiFERON®-TB Gold IT ELISA is shown in Figure 6 and indicates that imprecision does not increase with higher concentrations of IFN-γ.

FIGURE 6. Precision profile of QuantiFERON®-TB Gold IT ELISA determined from testing 20 plasma samples in triplicate, on 3 non-consecutive days, at 3 laboratories and by 3 operators. The trendline is a calculation of least squares fit.



The reproducibility of the QuantiFERON®-TB Gold IT test was determined using blood samples from five volunteers with known tuberculosis or suspicion of tuberculosis and 3 known not to have any risk of tuberculosis exposure. Three lots of QuantiFERON®-TB Gold IT blood collection tubes and three ELISA kits were tested at three laboratories.

Therefore, a total of 27 diagnostic determinations were made for each volunteer and 216 in total for all volunteers. Overall, diagnostic reproducibility was 97.2% (95%CI: 94.1% to 98.7%) where the diagnostic status of all 8 volunteers was correctly interpreted for 210 of 216 determinations. The samples from one volunteer were close to the cut off and accounted for all variation.

Analytical reproducibility was estimated using the individual IU/mL values of IFN-γ determined for each assayed plasma sample. All concentrations greater than 10 IU/mL are simply reported as ">10" as they fall beyond the linear range of most ELISA readers. This is consistent with how the QuantiFERON®-TB Gold IT software reports results. Sixty-four per cent (87/135) of TB Antigen minus Nil values for the 5 positive volunteers, and 73% of Mitogen minus Nil values for all 8 volunteers, were reported to be >10 IU/mL, respectively. For the assessment of analytical reproducibility, these values were deemed to have 10 IU/mL.

The overall average %CV for Nil, TB Antigen minus Nil, and Mitogen minus Nil values were 37.9% (due to the low values for the Nil; ±95%CI: 4.8%), 7.3% (3.2%) and 7.0% (3.1%), respectively. The TB Antigen minus Nil analysis only included data from the 5 volunteers with tuberculosis.

11. TECHNICAL INFORMATION

Indeterminate Results

Indeterminate results are uncommon and may be related to the immune status of the individual being tested,[5] but may also be related to a number of technical factors:

- Longer than 16 hours from blood draw to incubation at 37°C ± 1°C,
- Storage of filled blood collection tubes outside the recommended temperature range (22°C ± 5°C) prior to 37°C ± 1°C incubation,
- Insufficient mixing of blood collection tubes,
- Incomplete washing of the ELISA plate

If technical issues are suspected with the collection or handling of blood samples, repeat the entire QuantiFERON®-TB Gold IT 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. Indeterminate tests that result from low Mitogen or high Nil values 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 specimen or perform other procedures as appropriate.

Difficulties that may be encountered in performing the assay include:

1. Incorrect washing of the plate.
   - Automated plate washers were used during the validation of all clinical studies performed in the U.S.
2. Clot formation in plasma samples that have been stored frozen for an extended period of time. Clotted material can block 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 at > 8 IU/mL, this has no effect on the interpretation of the test.

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.

ELISA Trouble Shooting

Non-specific Color Development

POSSIBLE CAUSE	SOLUTION
Incomplete washing of the plate	Wash the plate at least 6 times with 400µL/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.
Cross-contamination of ELISA wells.	Take care pipetting and mixing sample to minimize risk.
Kit / Components have expired.	Ensure kit is used within the expiry date. Ensure reconstituted Standard and Conjugate 100X Concentrate are used within three months of the reconstitution date.
Enzyme Substrate Solution is contaminated.	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.

Low Optical Density Readings for Standards

POSSIBLE CAUSE	SOLUTION
Standard dilution error.	Ensure dilutions of the Kit Standard are prepared correctly as per the Package Insert.
Pipetting error.	Ensure pipettes are calibrated and used according to manufacturer's instructions.
Incubation temperature too low.	Incubation of the ELISA should be performed at Room Temperature, 17°C to 27°C.
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.
Incorrect plate reader filter used.	Plate should be read at 450nm with a reference filter of between 620 and 650nm.
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 one hour.
Kit / Components have expired.	Ensure kit is used within the expiry date. Ensure reconstituted Standard and Conjugate 100X Concentrate are used within three months of the reconstitution date.

High Background

POSSIBLE CAUSE	SOLUTION
Incomplete washing of the plate	Wash the plate at least 6 times with 400µL/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.
Incubation temperature too high.	Incubation of the ELISA should be performed at Room Temperature, 17°C to 27°C.
Kit / Components have expired.	Ensure kit is used within the expiry date. Ensure reconstituted Standard and Conjugate 100X Concentrate are used within three months of the reconstitution date.
Enzyme Substrate Solution is contaminated.	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.

Non-linear Standard Curve and Duplicate Variability

POSSIBLE CAUSE	SOLUTION
Incomplete washing of the plate	Wash the plate at least 6 times with 400µL/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.
Standard dilution error.	Ensure dilutions of the Standard are prepared correctly as per the Package Insert.
Poor mixing.	Mix reagents thoroughly by inversion or gentle vortexing prior to their addition to the plate.
Inconsistent pipetting technique or interruption during assay set-up.	Sample and standard addition should be performed in a continuous manner. All reagents should be prepared prior to commencing the assay.

12. US CENTERS FOR DISEASE CONTROL AND PREVENTION GUIDELINES

The following summary of the CDC guidelines relates to the liquid antigen version of QuantiFERON®-TB Gold. Although clinical studies have demonstrated commensurate performance between the two versions of QuantiFERON®-TB Gold (liquid antigen and IT), new guidelines that reflect current experience with interferon-gamma assays may be issued.

As updated guidelines for QuantiFERON®-TB Gold IT become available from the CDC, they will be made available on the Cellestis website (www.cellestis.com) and incorporated in this Package Insert.

Current CDC publications on diagnosis and treating tuberculosis are available at: http://www.cdc.gov/nchstp/tb/

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.*

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14. TECHNICAL SERVICE

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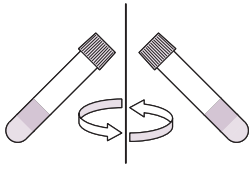
15. ABBREVIATED TEST PROCEDURE

STAGE 1 – BLOOD INCUBATION

1. Collect patient blood into blood collection tubes and **SHAKE** vigorously for 5 seconds (10X) to mix. (Repeat shaking just prior to incubation if not incubated immediately after collection).

2. Incubate tubes **UPRIGHT** at 37°C ± 1°C for 16 to 24 hours.



3. Following incubation, centrifuge tubes for 15 minutes at 2000 to 3000 RCF (g) to separate the plasma and the red cells.

4. Following centrifugation, harvest plasma sample from each tube for IFN-γ quantification.

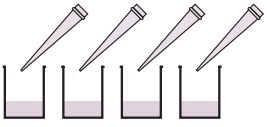
STAGE 2 – IFN-γ ELISA

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

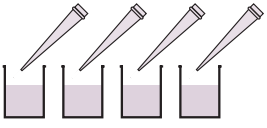
2. Reconstitute the Kit Standard to 8.0 IU/mL with distilled or deionised water. Prepare eight (8) standard dilutions.

3. Reconstitute freeze-dried Conjugate 100X Concentrate with distilled or deionised water.

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



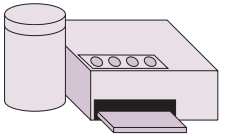
5. Add 50µL of test plasma samples and 50µL of standards to appropriate wells. Mix using shaker.



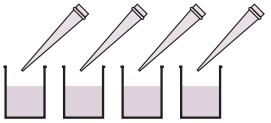
6. Incubate for 120 minutes at room temperature.

STAGE 2 – IFN-γ ELISA (Continued)

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

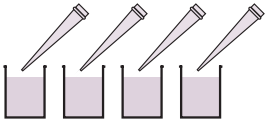


8. Add 100µL Enzyme Substrate Solution to wells. Mix using shaker.

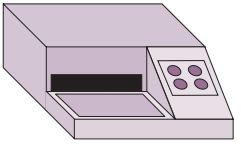


9. Incubate for 30 minutes at room temperature.

10. Add 50µL Stop Solution to all wells. Mix using shaker.



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



12. Analyze Results.

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