

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



INDEX

| | |
|----------------------------------------------------------|-----------|
| 1. INTENDED USE | 2 |
| 2. SUMMARY AND EXPLANATION OF THE TEST | 2 |
| Principles of the Assay | 4 |
| Time Required for Performing Assay | 4 |
| 3. REAGENTS AND STORAGE | 5 |
| Blood Collection Tubes | 5 |
| ELISA Components | 5 |
| Materials Required (but not provided) | 5 |
| Storage Instructions | 6 |
| Blood Collection Tubes | 6 |
| Kit Reagents | 6 |
| Reconstituted and Unused Reagents | 6 |
| 4. WARNINGS AND PRECAUTIONS | 7 |
| Warnings | 7 |
| Precautions | 8 |
| 5. SPECIMEN COLLECTION AND HANDLING | 9 |
| 6. DIRECTIONS FOR USE | 10 |
| STAGE ONE - Incubation of Blood and Harvesting of Plasma | 10 |
| STAGE TWO - Human IFN- γ ELISA | 11 |
| 7. CALCULATIONS AND TEST INTERPRETATION | 15 |
| Generation of Standard Curve | 15 |
| Quality Control of Test | 16 |
| Interpretation of Results | 17 |
| 8. LIMITATIONS | 19 |
| 9. PERFORMANCE CHARACTERISTICS | 19 |
| 10. TECHNICAL INFORMATION | 22 |
| Indeterminate Results | 22 |
| Clotted Plasma Samples | 22 |
| ELISA Trouble Shooting | 23 |
| 11. BIBLIOGRAPHY | 26 |
| 12. TECHNICAL SERVICE | 29 |
| 13. ABBREVIATED TEST PROCEDURE | 30 |

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 heparinised 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

Tuberculosis is a communicable disease caused by infection with *M. tuberculosis* complex organisms (*M. tuberculosis*, *M. bovis*, *M. africanum*), 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 (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.

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

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-tuberculosis mycobacteria with the exception of *M. kansasii*, *M. szulgai* and *M. marinum*.¹ Individuals infected with *M. tuberculosis* complex organisms usually have lymphocytes in their blood that recognise 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.

The antigens used in QuantiFERON®-TB Gold IT are a peptide cocktail simulating the proteins ESAT-6, CFP-10 and TB7.7(p4). Numerous studies have demonstrated that these peptides antigens stimulate IFN- γ responses in T-cells from individuals infected with *M. tuberculosis* but generally not from uninfected or BCG vaccinated persons without disease or risk for LTBI.¹⁻³⁵ 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*.^{1,22} The QuantiFERON®-TB Gold IT test is both a test for LTBI and a helpful aid for diagnosing *M. tuberculosis* complex infection in sick patients. A positive result supports the diagnosis of tuberculosis disease; however, infections by other mycobacteria (e.g., *M. kansasii*) could also lead to positive results. Other medical and diagnostic evaluations are necessary to confirm or exclude tuberculosis disease.

Principles of the Assay

The QuantiFERON®-TB Gold IT system uses specialised blood collection tubes, which are used to collect whole blood. Incubation of the blood occurs in the tubes for 16 to 24 hours, after which, plasma is harvested and tested for the presence of IFN- γ produced in response to the peptide antigens.

The QuantiFERON®-TB Gold IT test is performed in two stages. First, whole blood is collected into each of the QuantiFERON®-TB Gold blood collection tubes, which include a Nil Control tube, TB Antigen tube, and a Mitogen Control tube.

The Mitogen tube is used with the QuantiFERON®-TB Gold IT test as a control for correct blood handling and incubation and serves as a control especially where there is doubt as to the individual's immune status and thus ability to respond in the test.

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

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 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 improper specimen handling, incorrect filling/mixing of the Mitogen tube, 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 tube is subtracted from the IFN- γ level for the TB Antigen tube and Mitogen tube.

Time Required for Performing Assay

The time required to perform the QuantiFERON®-TB Gold IT assay is estimated below; the time of testing multiple samples when batched is also indicated:

37°C Incubation of blood tubes: 16 to 24 hours

ELISA: Approx. 3 hours for one ELISA plate

- <1 hour labor
- Add 10-15 minutes for each extra plate

3. REAGENTS AND STORAGE

Tuberculosis and Control Antigen Blood Collection Tubes

Catalogue Number: 0590 0301

- | | |
|---------------------------------|-------------|
| 1. Nil Control (Grey cap) | 100 x tubes |
| 2. TB Antigen (Red cap) | 100 x tubes |
| 3. Mitogen Control (Purple cap) | 100 x tubes |

NOTE: Tubes are also available in other configurations:

100 x Nil Control, 100 x TB Antigen tubes (Cat. No. 0590 0201)

100 x Mitogen Control tubes (Cat. No. 0593 0201)

ELISA Components – Catalogue Number: 0594 0201

- | | |
|----------------------------------------------|-------------|
| 1. Microplate strips | 24 x 8 well |
| 2. Human IFN- γ Standard, lyophilised | 1 x vial |
| 3. Green Diluent | 1 x 30mL |
| 4. Conjugate 100X Concentrate, lyophilised | 1 x 0.3mL |
| 5. Wash Buffer 20X Concentrate | 1 x 100mL |
| 6. Enzyme Substrate Solution | 1 x 30mL |
| 7. Enzyme Stopping Solution | 1 x 15mL |

Materials Required (but not provided)

- 37°C incubator. CO₂ not required.
- Calibrated variable-volume pipettes for delivery of 10 μ L to 1000 μ L with disposable tips.
- Calibrated multichannel pipette capable of delivering 50 μ L and 100 μ L with disposable tips.
- Microplate shaker.
- Deionised or distilled water - 2L.
- Microplate washer (automated washer recommended).
- Microplate reader fitted with 450nm filter and 620nm to 650nm reference filter.

Storage Instructions

Blood Collection Tubes

- Store blood collection tubes at 2°C to 25°C.
- The shelf life of the QuantiFERON®-TB Gold blood collection tubes is 15 months from the date of manufacture when stored at 2°C to 25°C.

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.

Reconstituted and Unused Reagents

For instructions on how to reconstitute the reagents, please see Section 6 (page 11).

- 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.*
- Once reconstituted, unused Conjugate 100X concentrate must be returned to storage at 2°C to 8°C and must also be used within 3 months.
 - *Note the date the Conjugate was reconstituted.*
- Working strength Conjugate must be used within 6 hours of preparation.
- Working strength Wash Buffer may be stored at room temperature for up to 2 weeks.

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 functions, incorrect handling of the blood collection tubes following venipuncture, incorrect performance of the assay, or other immunological variables.
- A positive QuantiFERON®-TB Gold IT result should not be the sole or definitive basis for determining infection with *M. tuberculosis*. Incorrect performance of the assay may cause false positive responses.
- A positive QuantiFERON®-TB Gold IT result should be followed by further medical evaluation and diagnostic evaluation for active tuberculosis disease (e.g., AFB smear and culture, chest x-ray).
- While ESAT-6, CFP-10 and TB7.7(p4) are absent from all BCG strains and from most known non-tuberculous mycobacteria, it is possible that a positive QuantiFERON®-TB Gold IT result may be due to infection by *M. kansasii*, *M. szulgai* or *M. marinum*. If such infections are suspected, alternative tests should be investigated.

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 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 quantities 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.
- 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.

5. SPECIMEN COLLECTION AND HANDLING

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

1. Nil Control (Grey cap).
2. TB Antigen (Red cap).
3. Mitogen Control (Purple cap).

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 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 blood collection tubes have been validated for volumes ranging from 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.
 - 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 tubes being used.
2. Mix the tubes by shaking **vigorously** the tube for 5 seconds ensuring that **the entire inner surface of the tube** has been coated with the blood.
 - Thorough mixing is required to ensure complete mixing of the blood with the tube’s contents.
3. Label tubes appropriately.
4. The tubes must be transferred to a 37°C incubator as soon as possible, and within 16 hours of collection. Do not refrigerate or freeze the blood samples.

6. DIRECTIONS FOR USE

Stage One – Incubation of Blood and Harvesting of Plasma

Materials Provided

QuantiFERON®-TB Gold IT blood collection tubes (Refer to Section 3).

Materials Required (but not provided)

Refer to Section 3.

Procedure

1. If the blood is not incubated immediately after collection, **mixing of the tubes must be repeated immediately prior to incubation**, as described in Section 5.
2. Incubate the tubes **UPRIGHT** at 37°C for 16 to 24 hours. The incubator does not require CO₂ or humidification.
3. 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, harvesting of plasma is facilitated by centrifuging tubes for 15 minutes at 2000 to 3000 RCF (g). The gel plug will separate the cells from the plasma. If this does not occur, the tubes should be re-centrifuged at a higher speed.
 - It is possible to harvest the plasma without centrifugation, however, additional care is required to remove the plasma without disturbing the cells.
5. Plasma samples can be loaded directly from blood collection tubes into the QuantiFERON®-TB Gold 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 into microplate wells or racked microtubes in 96 well format and sealed to prevent spills and evaporation if samples are to be stored.
 - Plasma samples can be stored for up to 8 weeks at 2°C to 8°C or below –20°C (preferably less than –70°C) for extended periods.

Stage Two - Human IFN- γ ELISA

Materials Provided

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

Materials Required (but not provided)

Refer to Section 3.

Procedure

1. All plasma samples and reagents, except for Conjugate 100X Concentrate, must be brought to room temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) before use. Allow at least 60 minutes for equilibration.
2. 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 one strip for the QuantiFERON®-TB Gold Standards and sufficient strips for the number of subjects being tested (refer to Figure 2, page 14 for recommended plate layout). After use, retain frame and lid for use with remaining strips.

3. Reconstitute the freeze dried Kit Standard with the volume of deionised or distilled water indicated on the label of the Standard vial. Mix gently to minimise frothing and ensure complete solubilisation. Reconstitution of the Standard to the stated volume will produce a solution with a concentration of 8.0 IU/mL.

Note: The reconstitution volume of the Kit Standard will differ between batches.

Use the reconstituted Kit Standard to produce a 1 in 4 dilution series of IFN- γ in Green Diluent (GD) – refer to Figure 1. S1 (Standard 1) contains 4 IU/mL, S2 (Standard 2) contains 1 IU/mL, S3 (Standard 3) contains 0.25 IU/mL, and S4 (Standard 4) contains 0 IU/mL (GD alone). The standards should be assayed at least in duplicate.

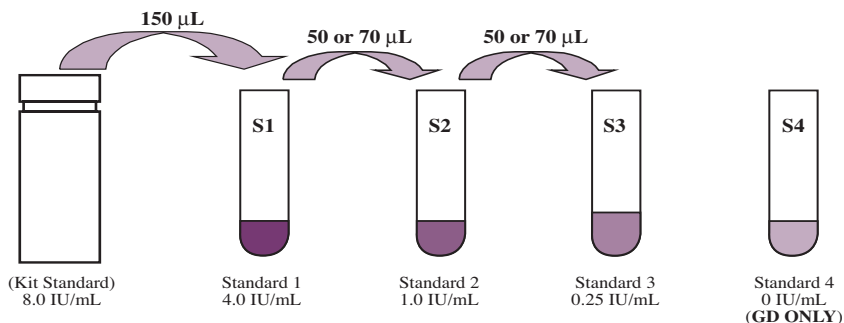
RECOMMENDED PROCEDURE FOR DUPLICATE STANDARDS

- Label 4 tubes "S1", "S2", "S3", "S4".
- Add **150 μ L** of GD to S1, S2, S3, S4.
- Add **150 μ L** of the Kit Standard to S1 and mix thoroughly.
- Transfer **50 μ L** from S1 to S2 and mix thoroughly.
- Transfer **50 μ L** from S2 to S3 and mix thoroughly.
- GD alone** serves as the zero standard (S4).

RECOMMENDED PROCEDURE FOR TRIPPLICATE STANDARDS

- Label 4 tubes "S1", "S2", "S3", "S4".
- Add **150 μ L** of GD to S1.
- Add **210 μ L** of GD to S2, S3, S4.
- Add **150 μ L** of the Kit Standard to S1 and mix thoroughly.
- Transfer **70 μ L** from S1 to S2 and mix thoroughly.
- Transfer **70 μ L** from S2 to S3 and mix thoroughly.
- GD alone** serves as the zero standard (S4).

FIGURE 1. Preparation of Standard Curve



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

- Reconstitute freeze dried Conjugate 100X Concentrate with 0.3mL of deionised or distilled water. Mix gently to minimize frothing and ensure complete solubilisation 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 |
|-----------------------------|-----------------------------------------------------|------------------------------------|
| 2 | 10µL | 1.0mL |
| 3 | 15µL | 1.5mL |
| 4 | 20µL | 2.0mL |
| 5 | 25µL | 2.5mL |
| 6 | 30µL | 3.0mL |
| 7 | 35µL | 3.5mL |
| 8 | 40µL | 4.0mL |
| 9 | 45µL | 4.5mL |
| 10 | 50µL | 5.0mL |
| 11 | 55µL | 5.5mL |
| 12 | 60µL | 6.0mL |

- Mix thoroughly but gently to avoid frothing.
 - Return any unused Conjugate 100X Concentrate to 2°C to 8°C immediately after use.
 - Use only Green Diluent.
5. Prior to assay, plasmas should be mixed to ensure that IFN- γ is evenly distributed throughout the sample.
 6. Add 50µL of freshly prepared Working Strength conjugate to the required ELISA wells using a multichannel pipette.
 7. Add 50µL of test plasma samples to appropriate wells using a multichannel pipette (Refer to recommended plate layout on page 14 – Figure 2). Finally, add 50µL each of the Standards 1 to 4.

FIGURE 2. Recommended Sample Layout for Nil, TB Antigen & Mitogen Tubes (28 tests per plate)

| Row | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A | 1N | 1A | 1M | S1 | S1 | S1 | 13N | 13A | 13M | 21N | 21A | 21M |
| B | 2N | 2A | 2M | S2 | S2 | S2 | 14N | 14A | 14M | 22N | 22A | 22M |
| C | 3N | 3A | 3M | S3 | S3 | S3 | 15N | 15A | 15M | 23N | 23A | 23M |
| D | 4N | 4A | 4M | S4 | S4 | S4 | 16N | 16A | 16M | 24N | 24A | 24M |
| E | 5N | 5A | 5M | 9N | 9A | 9M | 17N | 17A | 17M | 25N | 25A | 25M |
| F | 6N | 6A | 6M | 10N | 10A | 10M | 18N | 18A | 18M | 26N | 26A | 26M |
| G | 7N | 7A | 7M | 11N | 11A | 11M | 19N | 19A | 19M | 27N | 27A | 27M |
| H | 8N | 8A | 8M | 12N | 12A | 12M | 20N | 20A | 20M | 28N | 28A | 28M |

- S1 (Standard 1), S2 (Standard 2), S3 (Standard 3), S4 (Standard 4).
 - 1N (Sample 1. Nil Control plasma); 1A (Sample 1. TB Antigen plasma); 1M (Sample 1. Mitogen Control plasma).
- Mix the conjugate and plasma samples/standards thoroughly using a microplate shaker for 1 minute.
 - Cover each plate with a lid and incubate at room temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) for 120 ± 5 minutes.
 - Plates should not be exposed to direct sunlight during incubation.
 - During the incubation, dilute one part Wash Buffer 20X Concentrate with 19 parts deionised 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 **400 μL** of Working Strength wash buffer for at least 6 cycles. An automated 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.

11. Tap plates face down on absorbent towel to remove residual wash buffer. Add 100µL of Enzyme Substrate Solution to each well and mix thoroughly using a microplate shaker.
12. Cover each plate with a lid and incubate at room temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) 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.
 - 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, used to analyse raw data and calculate results, is available from Cellestis.

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.

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

(if QuantiFERON®-TB Gold Analysis Software is not used)

Determine the mean OD values of the Kit Standard replicates on each plate.

Construct a $\log_{(e)}\text{-}\log_{(e)}$ standard curve by plotting the $\log_{(e)}$ of the mean OD (y-axis) against the $\log_{(e)}$ of the IFN- γ concentration of the standards in IU/mL (x-axis), omitting the zero standard from these calculations. Calculate the line of best fit for the standard curve by regression analysis.

Use the standard curve to determine the IFN- γ concentration (IU/mL) for each of the test plasma samples, using the OD value of each sample.

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

Quality Control of the Test

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

For the ELISA to be valid:

- **The mean OD value for Standard 1 must be ≥ 0.600 .**
- **The %CV for Standard 1 and Standard 2 replicate OD values must be $\leq 15\%$.**
- **Replicate OD values for Standard 3 and Standard 4 must not vary by more than 0.040 optical density units from their mean.**
- **The correlation coefficient (r) calculated from the mean absorbance values of the standards must be ≥ 0.98 .**

The QuantiFERON[®]-TB Gold 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.**

Interpretation of Results

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

NOTE: 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.

| Nil [IU/mL] | TB Antigen minus Nil [IU/mL] | Mitogen minus Nil [IU/mL] ¹ | QuantiFERON®-TB [IU/mL] | Report/Interpretation |
|--------------------|---------------------------------|-------------------------------------------|----------------------------------|------------------------------------------------------------|
| ≤ 8.0 | < 0.35 | ≥ 0.5 | Negative | <i>M. tuberculosis</i> infection NOT likely |
| | ≥ 0.35 and < 25% of Nil value | ≥ 0.5 | | |
| | ≥ 0.35 and ≥ 25% of Nil value | Any | Positive² | <i>M. tuberculosis</i> infection likely |
| | < 0.35 | < 0.5 | Indeterminate³ | Results are indeterminate for TB Antigen responsiveness |
| | ≥ 0.35 and < 25% of Nil value | < 0.5 | | |
| > 8.0 ⁴ | Any | Any | | |

¹ Responses to the Mitogen positive control (and occasionally TB Antigen) can be commonly outside the range of the microplate reader. This has no impact on test results.

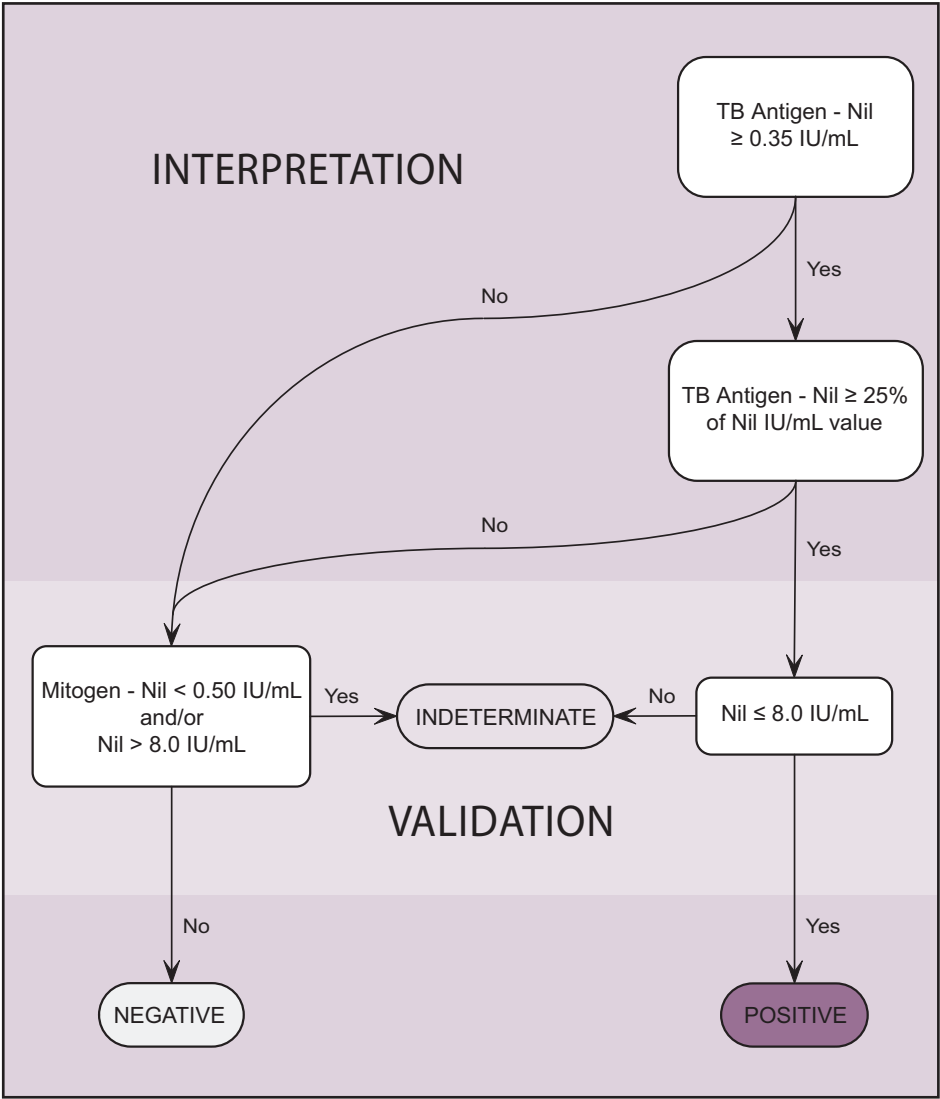
² Where *M. tuberculosis* infection is not suspected, initially positive results can be confirmed by retesting the original plasma samples in duplicate in the QuantiFERON®-TB Gold ELISA. If repeat testing of one or both replicates is positive, the individual should be considered test positive.

³ Refer to Trouble Shooting section 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.

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 other diagnostic evaluations.

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

Unreliable or indeterminate results may occur due to:

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

9. 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 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 standard survey at the time of testing. Of 432 volunteers with no known risk factors for *M. tuberculosis* infection, QuantiFERON®-TB Gold IT and TST results were available for 391. None were BCG vaccinated. A second specificity study was performed with QuantiFERON®-TB Gold IT in low risk individuals in Japan, approximately 90% who had received BCG vaccination. Results from both the specificity studies are shown in Table 2.

TABLE 2. QuantiFERON®-TB Gold IT specificity: Results for persons with no reported risk for *M. tuberculosis* infection.

| STUDY | BCG Status % Vaccinated | Total tested | No. QFT-G Indeterminate | No. QFT-G Positive / No. Valid Tests | QFT-G Specificity (95% CI) | No. TST Positive / No. Tested | TST* Specificity (95% CI) |
|---------------------|-------------------------|--------------|-------------------------|--------------------------------------|----------------------------|-------------------------------|---------------------------|
| USA (unpublished) | 0% | 391 | 1 | 3 / 390 | 99.2% (97.6-99.8) | 6 / 391 | 98.5% (96.5-99.4) |
| Japan (unpublished) | ~90% | 190 | 4 | 3 / 186 | 98.4% (95-99.6) | – | – |
| TOTAL | | 581 | 5 / 584 (0.9%) | 6 / 576 | 99.0% | – | – |

*Using a 10mm TST cut off. TST specificity estimate is 99.1% if using a 15mm cut off.

Sensitivity for active TB

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

Table 3 summarizes findings from the two groups of *M. tuberculosis* culture positive patients. Overall sensitivity of QuantiFERON®-TB Gold IT for active TB disease was 89% (48/54).

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

| STUDY | | Disease Confirmed By | No. QFT-Gold Positive / No. Valid Tests | QFT-Gold Sensitivity (95% CI) |
|-----------------------------------------|-----------------|----------------------|-----------------------------------------|-------------------------------|
| Japan TB Patients Validation study | | Culture | 24 / 27 | 89% (72-96%) |
| Australian TB Patients Validation Study | Pulmonary | Culture | 7 / 10 | 70% (40-89%) |
| | Extra-Pulmonary | | 17 / 17 | 100% (82-100%) |
| TOTAL | | | 48 / 54 | 89% (78-95%) |

Diagnosis of LTBI

A number of studies have been published which demonstrate the performance of QuantiFERON®-TB Gold IT in various populations at risk of LTBI. The principle findings of some selected studies are shown in Table 4.

TABLE 4. Selected published studies on QuantiFERON®-TB Gold IT in populations at risk of LTBI.

| STUDY | Total tested | Outcomes and Findings |
|------------------------------------------------------------------|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Indian HCW (Pai <i>et al</i> 2005) ²⁷ | 726 | Setting of very high TB rates. 40% QFT-Gold IT positive cf 41% TST positive at 10mm. High concordance with TST, no effect of BCG on either test. Both tests related to risk factors of age and period of work in healthcare. |
| Danish HIV (Brock <i>et al</i> 2006) ⁵ | 590 | Overall prevalence of LTBI by QFT-Gold IT was 4.6% (27/590) in HIV+ persons. Positive results were associated with TB risks. Two QFT-Gold IT positive subjects progressed to active TB within one year. Indeterminate responses (n=20, 3.4%) were significantly associated with a CD4 count <100 / μ L. |
| Hospitalized Children (Dogra <i>et al</i> 2006) ¹⁰ | 105 | Children in whom TB was suspected or had a history of TB contact were tested with QFT-Gold IT and TST. 10.5% QFT-Gold IT positive cf 9.5% TST positive at 10mm. Agreement between tests was 95.2% overall and 100% in non-BCG vaccinated. |
| German Contacts (Diel <i>et al</i> 2006) ⁹ | 309 | Close contacts of 15 different index cases were tested. 51% were BCG vaccinated, 27% foreign born. 70% of BCG vaccinates and 18% of non-vaccinated were TST positive (5mm), whereas 9% and 11% were QFT-Gold IT positive, respectively. QFT-Gold IT was associated with TB risk. TST was only associated with BCG vaccination. |

Many more publications describe the performance of the less-sensitive liquid antigen version of QuantiFERON®-TB Gold (the precursor to QuantiFERON®-TB Gold IT) and the QuantiFERON®-TB Gold IT test. These studies include use of the test(s) in contacts of active TB cases^{7,9,10,13,14,17,20,25}, children^{10,23,25}, HIV positive^{5,18}, healthcare workers^{16,27,28}, incarcerated²⁹, as well as TB suspects^{7,22,31} and low risk individuals^{17,22}.

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 were rested between 4 and 5 weeks after the original QuantiFERON®-TB Gold IT test and TST. QuantiFERON®-TB Gold IT results for 260 recruits were available at both time points and the level of agreement was 99.6% (259/260). A prior TST did not induce positive QuantiFERON®-TB Gold IT responses.

10. TECHNICAL INFORMATION

Indeterminate Results

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

- Longer than 16 hours from blood draw to incubation at 37°C,
- Storage of blood outside the recommended temperature range (22°C ± 5°C),
- 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 a new blood specimen. 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.

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 Colour Development

| POSSIBLE CAUSE | SOLUTION |
|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Incomplete washing of the plate. | Wash the plate at least 6 times with 400 μ L/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used. |
| Cross-contamination of ELISA wells. | Take care pipetting and mixing sample to minimise 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 colouration 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 is 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 400 μ L/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used. |
| 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 colouration 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 400 μ L/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used. |
| 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. |

Assay procedure video and solution to most technical problems can be found on the Product Information and Technical Guide CD-ROM available free of charge from Cellestis or via your distributor.

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12. TECHNICAL SERVICE

For technical service please contact:

| | | |
|-----------------------------------------|-------------------|---------------------------------------|
| Cellestis International Pty Ltd: | Phone: | +61 3 9571 3500 |
| | Fax: | +61 3 9571 3544 |
| | E-mail: | quantiferon@cellestis.com |
| | Website: | www.cellestis.com |
| Cellestis Inc.: (USA) | Phone: | +1 661 775 7480 |
| | Fax: | +1 661 775 7479 |
| | Toll free: | 800 519 4627 |
| | Email: | customer.service@cellestis.com |
| Cellestis GmbH: (Europe) | Phone: | +49 6151 428 59 - 0 |
| | Fax: | +49 6151 428 59 - 110 |
| | Email: | europe@cellestis.com |

13. ABBREVIATED TEST PROCEDURE

STAGE 1 – BLOOD INCUBATION

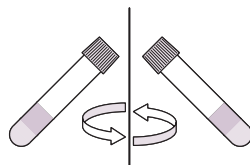
1. Collect patient blood into blood collection tubes and **SHAKE** vigorously to mix.



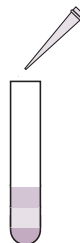
2. Incubate tubes **upright** at 37°C for 16 to 24 hours.



3. Following incubation, centrifuge tubes for 15 minutes at 2000 to 3000g 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.

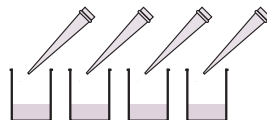


2. Reconstitute the Kit Standard to 8.0 IU/mL with distilled or deionised water. Prepare four (4) standard dilutions.

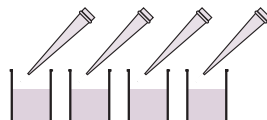


3. Reconstitute freeze-dried Conjugate 100X Concentrate with distilled or deionised water.

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



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

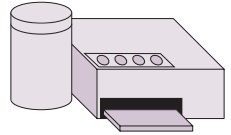


6. Incubate for 120 minutes at room temperature.

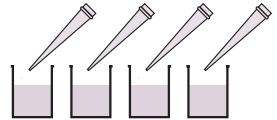


STAGE 2 – IFN- γ ELISA (Continued)

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



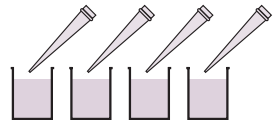
8. Add 100 μ L Enzyme Substrate Solution to wells. Mix using shaker.



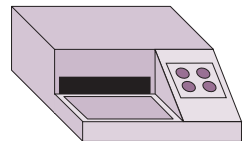
9. Incubate for 30 minutes at room temperature.



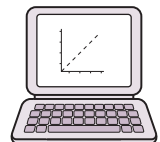
10. Add 50 μ L Stop Solution to all wells. Mix using shaker.



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



12. Analyse Results.





Manufactured for:

Cellestis Limited (Australia), Cellestis Inc. (USA) and Cellestis GmbH (Europe)

1046A Dandenong Road, Carnegie, Victoria, 3163, Australia

Phone: (Australia) +61 3 9571 3500; (USA) 800 519 4627; (Europe) +49 6151 428 59-0

E-mail: quantiferon@cellestis.com

Website: www.cellestis.com

Doc. No. CA05990301A

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Authorised Representative:

Priory Analysts Ltd

PO Box 4030

Milton Keynes

MK 13 0ZG

United Kingdom

