QuantiFERON®-TB Gold

The Whole Blood IFN-gamma Test
Incorporating TB-Specific Antigens ESAT-6 and CFP-10

An Aid to Detect *M. tuberculosis* Infection

Catalogue Number: 0594 0201

PACKAGE INSERT

For *In Vitro* Diagnostic Use

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1. INTENDED USE

QuantiFERON®-TB Gold is an *in vitro* diagnostic test intended as an aid in the detection of infection with *Mycobacterium tuberculosis.*

2. SUMMARY AND EXPLANATION OF THE TEST

The QuantiFERON®-TB Gold test is a test for Cell Mediated Immune responses to *M. tuberculosis*-specific antigens. Individuals infected with *M. tuberculosis* complex organisms *(M. tuberculosis, M. bovis, M. africanum)* have lymphocytes in their blood that can recognize specific mycobacterial antigens. This recognition process involves the generation and secretion of the cytokine, interferon-γ (IFN-γ). The detection and subsequent quantification of IFN-γ forms the basis of this test.

The TB-Specific Antigens used in QuantiFERON®-TB Gold are ESAT-6 and CFP-10. Both of these antigens are absent from all BCG strains and from most non-tuberculosis mycobacteria, with the exception of *M. kansasii, M.szulgai and M. marinum*. Numerous studies have demonstrated the utility of these antigens in stimulating IFN-γ responses in T-cells from TB infected individuals but not from uninfected or BCG vaccinated people.

The QuantiFERON®-TB Gold test is supplied with TB-Specific Antigens (Catalogue number: 0596 0201), which are used to stimulate T-cells in whole heparinized blood. Following 16 to 24 hours of incubation, plasma is harvested and tested by ELISA to determine if IFN-γ has been produced in response to the TB-Specific Antigens, indicating TB infection.

Explanation and Principles of the Assay

The QuantiFERON®-TB Gold test assay detects CMI responses *in vitro* to tuberculosis infection by measuring IFN-γ harvested in plasma from whole blood incubated with TB-Specific Antigens. The QuantiFERON®-TB Gold laboratory test is performed in two stages. First, four aliquots of heparinized whole blood are incubated with either ESAT-6, CFP-10, Mitogen or Nil Control antigens.

Following a 16 to 24 hour incubation, plasma is removed and the amount of IFN-γ is quantified by enzyme-linked immunosorbent assay (ELISA). The QuantiFERON®-TB Gold ELISA uses a recombinant human IFN-γ standard, which has been assayed against a reference IFN-γ preparation (NIH Ref: Gxg01-902-535). Results for test samples are reported in International Units relative to this standard preparation.

A person is considered positive for *M. tuberculosis* infection if they have an IFN-γ response to either ESAT-6 or CFP-10 above the test cut-off.

The Mitogen-stimulated plasma sample serves as a positive control for each individual tested. However, a positive response to either of the TB-specific antigens ESAT-6 or CFP-10, without a response to Mitogen, is a valid result indicating infection. If a blood sample has a low IFN-γ response to Mitogen and both the TB-specific antigens, the test result is deemed "Indeterminate". The Nil sample adjusts for background, heterophile antibody effects, or non specific IFN-γ in blood samples.

3. REAGENTS AND STORAGE

Components

TB-Specific and Control Antigens - Catalogue number: 0596 0201

1. ESAT-6 TB-Specific Antigen *(contains 0.01% w/v Thimerosal)* (Red cap) — 1 x 6mL
2. CFP-10 TB-Specific Antigen *(contains 0.01% w/v Thimerosal)* (White cap) — 1 x 6mL
3. Mitogen Control *(contains 0.01% w/v Thimerosal)* (Purple cap) — 1 x 6mL
4. Nil Control *(contains 0.01% w/v Thimerosal)* (Grey cap) — 1 x 6mL

Components (ELISA) - Catalogue number: 0594 0201

1. Microplate strips coated with anti-human IFN–γ murine monoclonal antibody (*2 x 96 well plate*) — 24 x 8 wells
2. Human IFN-γ Standard — 1 x vial
   (contains recombinant human IFN-γ, bovine casein, 0.01 % w/v Thimerosal)

3. Green Diluent 1 x 30 mL
   (contains bovine casein, normal mouse serum, 0.01% w/v Thimerosal)

4. Conjugate 100X Concentrate (*Contains 0.01% w/v Thimerosal*) 1 x 0.3 mL
   (Anti-human IFN-γ HRP)

5. Wash Buffer 20X Concentrate (*contains 0.01% w/v Thimerosal)* 1 x 100mL

6. Enzyme Substrate Solution *(contains $H_2 0_2$)* 1 x 30mL

7. Enzyme Stopping Solution *(contains 0.5M H_2SO_4)* 1 x 15mL

Storage Instructions

Kit Reagents

- Store kit refrigerated at 2°C to 8°C.
- Always protect Enzyme Substrate Solution from direct sunlight.

Reconstituted and Used Solutions

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

4. WARNINGS AND PRECAUTIONS

Warnings

- QuantiFERON®-TB Gold has been evaluated for use with immunocompetent healthy adults with and without identified risk factors for tuberculosis infection.
- QuantiFERON®-TB Gold has not been assessed in individuals with lymphocyte counts outside the normal range and the effect of lymphocyte count on reliability is unknown.
- The performance of the QuantiFERON®-TB Gold test has not been evaluated in the following groups of individuals and it is not recommended for these population groups:
  1. Individuals who are immunocompromised such as those with HIV infection, AIDS, and transplant recipients.
  2. Persons with other clinical conditions that may compromise the immune system: 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).
  3. Individuals who are immunosuppressed such as those taking immunosuppressive drugs (e.g. corticosteroids, methotrexate, azathioprine, chemotherapy).
  4. Individuals under the age of 17 years.
  5. Pregnant women.

- **For *in vitro* diagnostic use.**
- **Handle human blood as if potentially infectious. Observe universal blood handling precautions (refer to NIH/CDC guidelines).**
- **Enzyme stopping solution is a strong acid.** Wipe spills up immediately and flush with water. If the stopping solution contacts the skin or eyes, flush with copious quantities of water and seek medical attention.

- **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 package insert may yield erroneous results. Please read the instructions carefully before use.
- Use only Heparin as blood anticoagulant. Other anticoagulants interfere with the assay.
- Use of Costar Tissue culture plates is recommended; other culture plates have not been validated.
- Blood samples should be transported to the laboratory at ambient temperature (22°C ± 5°C). Do not transport on ice or refrigerated.
- Blood must be incubated with stimulation antigens within 12 hours of collection; delay in incubation may cause false negative or indeterminate results.
- A negative QuantiFERON®-TB Gold result does not preclude the possibility of TB infection. The specimen may have been obtained prior to development of cellular immune response, sufficient lymphocytes may not be present in the blood sample collected, or handling of the specimen may have affected lymphocyte function.
- A positive QuantiFERON®-TB Gold 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.
- Some specimens may not yield a measurable IFN-γ response. This may result in low IFN-γ readings and cause indeterminate QuantiFERON®-TB Gold test results.

Precautions

- **Store kit components at 2°C to 8°C. Do not store kit at room temperature.**
- **Bring all components, except Conjugate 100X Concentrate to room temperature (22°C ± 5°C) before use.**
- **Store Conjugate 100X Concentrate at 2°C to 8°C at all times.**
- **Prepare fresh dilutions of the Kit Standard for each assay.**
- **Do not use kit if any reagent bottle shows signs of damage or leakage prior to use.**
- **Do not mix or use ELISA reagents from other QuantiFERON®-TB Gold kit batches.**
- **Do not use Kit Standard or Conjugate after three months from reconstitution.**
- **Discard unused reagents and biological samples in accordance with local, state and federal regulations.**
- **Do not use kit after the expiry date.**
- **Correct laboratory procedures should be adhered to at all times.**

5. SPECIMEN HANDLING

Blood Collection

Completely fill a blood collection tube **(minimum tube size 5mL)** containing heparin as the anticoagulant. Gently mix by inverting the tube several times to dissolve the heparin, and transport to the laboratory at ambient temperature (22°C ± 5°C). Blood should be incubated with stimulation antigens as soon as possible (and within 12 hours) after collection.

6. DIRECTIONS FOR USE

Time Required for Performing Assay

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

Draw blood	2 to 5 minutes per sample
Inititate incubation with antigens:	10 minutes (add 1 – 1.5 minutes per patient)
Incubation of blood with antigens:	16-24 hours (overnight)
Human IFN-γ ELISA stage:	Approx. 3 hours for 1 ELISA plate (<1 hour labor) (Add 10 – 15 minutes for each extra plate)

Stage One - Incubation of Blood

The following materials are required when setting up blood cultures. The TB-Specific and Control Antigens do not need to be brought to room temperature before use.

Materials Provided

- QuantiFERON®-TB Gold Antigens ESAT-6 (Red cap) and CFP-10 (White cap)
- QuantiFERON®-TB Gold Mitogen control (Purple cap)
- QuantiFERON®-TB Gold Nil control (Grey cap)

Materials Required but not Provided

- Biohazard Cabinet Class II (recommended for safe handling of blood).
- Sterile, 24 well tissue culture plates.
- Mechanical pipetting device and sterile graduated 5 or 10mL pipettes (1 pipette/patient).
- 37°C humidified incubator (with or without CO_2).
- Calibrated variable-volume pipette capable of delivering 300-400µL with disposable tips.

- 1mL microtubes with caps in 96 well format racks or uncoated microtitre plates with plastic seals for plasma storage (22 patients/rack or plate)
- Microplate shaker. e.g. QuantiFERON Microplate Shaker (Cellestis Cat. No. 08500201) or equivalent.
- Protective clothing for handling potentially infectious blood.

Stage One - Procedure

1. Blood samples must be evenly mixed before aliquoting. Use a roller-rocker or gently invert tubes 20 times **immediately prior to dispensing.**

2. Dispense 1.0mL aliquots (one per test antigen and control) of heparinized whole blood from each subject into 4 wells of a 24 well tissue culture plate (see Figure 1 for recommended layout). Blood is best dispensed aseptically in a Biohazard cabinet using sterile pipettes to minimize the risk of contamination.

3. Prior to use, thoroughly mix the TB-Specific Antigens, Nil and Mitogen controls by inverting several times. Holding the dropper bottle vertically, carefully add **3 drops** of ESAT-6 TB-Specific Antigen, CFP-10 TB-Specific Antigen, Mitogen control and Nil control to the appropriate wells containing blood.

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

	Patient Sample Number					
	1	2	3	4	5	6
Nil Control ***(grey cap)***	○	○	○	○	○	○
ESAT-6 ***(red cap)***	○	○	○	○	○	○
CFP-10 ***(white cap)***	○	○	○	○	○	○
Mitogen ***(purple cap)***	○	○	○	○	○	○

4. Antigens must be mixed **THOROUGHLY** into the aliquoted blood. It is recommended to use a QuantiFERON Microplate Shaker at the following setting; Waveform. = 20, Amplitude = 9, Time = 1 minute, or an equivalent microplate shaker (mixing for 1 minute).

5. Incubate plates for 16-24 hours at 37°C in a humidified atmosphere.

- **Avoid stacking plates more than 2 high during incubation.**

6. Carefully remove approximately 200-300µL of plasma from above the sedimented red cells using a variable-volume pipette. Transfer the plasma into separate 1 mL microtubes in a 96 well format or an empty microtitre plate (to simplify addition to ELISA plate).

- **Use a new pipette tip for each plasma sample.**

- **Avoid harvesting blood cells with plasma. The assay will tolerate small quantities of cells, but if the harvested plasma sample is grossly contaminated with blood cells, centrifuge the sample to remove the cells.**

7. Plasmas can be stored at 2°C to 8°C for up to 14 days or at least 3 months at or below -20°C. Microtubes or microtitre plates should be sealed appropriately prior to storage to avoid evaporation.

- **Plasmas may clot during extended storage. If clots are present refer to TROUBLE SHOOTING section.**

Stage Two - Human IFN-γ ELISA

Materials Provided

- QuantiFERON®-TB Gold ELISA kit (Catalogue number: 0594 0201)

Materials Required but not Provided

- Calibrated variable-volume pipettes for delivery of 50µL, 300µL, 500µL and 5-120µL with disposable tips.

- Multichannel pipette capable of delivering 50µL and 100µL with disposable tips.
- Variable speed vortex.
- Timer.
- Measuring cylinder- 1L or 2L.
- Deionised or distilled water (ELISA quality)-2L.
- Microplate shaker. e.g. QuantiFERON Microplate Shaker (Cellestis Cat. No. 08500201) or equivalent.
- Microplate washer.
- Microplate reader fitted with 450nm and 620nm (or 650nm) filters.
- Protective clothing for handling potential infectious material.
- Reagent Reservoirs. (Polypropylene)

Stage Two - Preparation of Reagents

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

2. ELISA MICROTITER PLATE – READY TO USE

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

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

3. HUMAN IFN-γ STANDARD *Contains 0.01 % w/v Thimerosal*

Reconstitute the Human IFN-γ Kit Standard with the volume of deionised or distilled water as indicated on the label of the standard (ensure complete resolubilization and mix gently to minimize frothing). Dilution of the standard with the correct volume of water will produce a solution with a concentration of 8.0 IU/mL.

Use the reconstituted Kit Standard to produce a dilution series of 4 IFN-γ concentrations (refer figure on page 12).

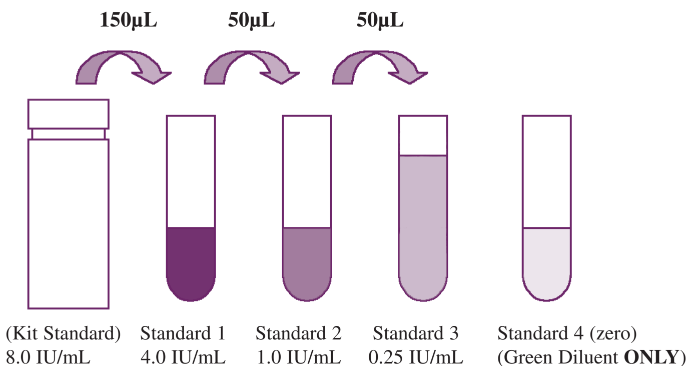
1. Add **150µL** of Green Diluent to 4 tubes (labelled Standard 1, 2, 3 and 4).

2. Add **150µL** of the Kit Standard to the tube designated as Standard 1 and mix thoroughly.

3. Transfer **50µL** from Standard 1 to the tube labelled Standard 2 and mix thoroughly.

4. Transfer **50µL** from Standard 2 to the tube labelled Standard 3 and mix thoroughly.

5. Green Diluent serves as the zero standard. (Standard 4)



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

4. CONJUGATE *Contains 0.01% w/v Thimerosal*

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

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

- **Mix thoroughly but gently to avoid frothing.**
- **Working strength conjugate should be used within 6 hours of preparation, although it is recommended to prepare the working solution as close as possible to the time that it is required for use.**
- **Return any unused Conjugate 100X Concentrate to 2°C to 8°C immediately after use.**
- **Use only Green Diluent as it contains normal mouse serum to compete out effects of heterophile antibodies in plasma samples.**

TABLE 1. CONJUGATE Preparation

NUMBER OF STRIPS	VOLUME OF CONJUGATE 100X CONCENTRATE	VOLUME OF GREEN DILUENT
1	5µL	0.5mL
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

5. WASH BUFFER *Contains 0.01% w/v Thimerosal*

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

Stage Two – Procedure

1. All plasma samples and reagents, except for the Conjugate 100X Concentrate must be brought to room temperature (22°C ± 5°C) before use. Allow at least 60 minutes for equilibration.

2. Reconstitute freeze dried Kit Standard and Conjugate 100X Concentrate.

3. Prepare dilutions of the reconstituted Kit Standard in Green Diluent to produce a dilution series of 4 IFN-γ concentrations for the preparation of the standard curve. Refer Preparation of Reagents - Human IFN-γ Standard (Pages 11, 12 and 13). Green Diluent alone is used for the zero standard.

4. Prior to assay, plasmas should be vortexed to ensure that IFN-γ is evenly distributed throughout the sample.

5. Dilute the required amount of Conjugate 100X Concentrate in Green Diluent according to the Conjugate Preparation Table (Table 1). Add 50µL of freshly prepared conjugate to the required ELISA wells using a multichannel pipette. (Refer to recommended plate layout below – Figure 2)

6. Using a multichannel pipette, add 50µL of test plasma samples to appropriate wells containing conjugate. Finally, add 50µL each of the Standards 1 to 4. **The standards should be assayed at least in duplicate.**

FIGURE 2. Recommended Sample Layout-Whole Plate

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

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

7. Mix the conjugate and plasma samples/standards thoroughly. It is recommended to use a QuantiFERON Microplate Shaker at the following setting; Waveform. = 20, Amplitude = 6, Time = 1 minute, or an equivalent microplate shaker for 1 minute.

8. Cover each plate with a lid 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.**

9. Wash wells with 300-400µL of working strength wash buffer for **AT LEAST 6 cycles** at room temperature (22°C ± 5°C). A fully automatic plate washer is recommended.

- **Thorough washing is very important to the performance of the assay. Ensure each well is completely filled with wash buffer to the top of the well for each wash cycle.**

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

10. Tap plates face down on an absorbent wipe to remove residual wash buffer. Add 100µL of Enzyme Substrate Solution to each well and mix thoroughly using the QuantiFERON Microplate Shaker, adjusted to the settings described in Step 7.

- **Commence incubation time as substrate is added to the first well(s).**

11. Cover each plate with a lid and incubate at room temperature (22°C ± 5°C) for precisely 30 minutes.

- **Plates should not be exposed to direct sunlight during incubation.**

- **Deviation from specified temperature range can lead to erroneous results.**

12. Following the 30 minute incubation, add 50µL of Enzyme Stopping Solution to each well and mix by gentle agitation.

- **Enzyme stopping solution should be added to wells in the same order and at the same speed as the substrate in step 10.**

13. Read the Optical Density (OD) of each well within 5 minutes of terminating the reaction using a 450nm filter, with either a 620nm or 650nm reference filter. OD values are used to calculate results.

7. DATA ANALYSIS AND TEST INTERPRETATION

QuantiFERON®-TB Gold Analysis Software, to analyse raw data and to calculate results is available from Cellestis.

The software performs Quality Control of the assay, generates a standard curve and reports a test result for each sample, based on the approved method of interpretation (as detailed below)

As an alternative to using the QuantiFERON®-TB Gold Analysis Software, data can be analysed according to the method below.

Generation of Standard Curve

Determine the mean OD values of the Human IFN-γ Standard replicates on each plate.

Construct a log-log standard curve by plotting the log of the mean absorbance against the log of the IFN-γ concentration of the standards in IU/mL on the x-axis (omit the zero standard from these calculations). Calculate the line of best fit for the standard curve by regression analysis.

To determine the IFN-γ concentration (IU/mL) for each of the test plasma samples, use the standard curve to read off the IFN-γ concentration (IU/mL) from the OD value of each sample.

These calculations can be generated automatically using standard 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 Test

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

For the ELISA test to be valid:

- **The correlation coefficient (r) calculated from the mean absorbances values of the standards must be ≥ 0.98.**
- **Replicate OD values for Standards 3 and 4 (Green Diluent) must not vary by more than 0.040 optical density units from their mean.**
- **Standard dilutions 1 and 2 must be within 15% of their individual mean OD values (% coefficient of variation (CV) ≤ 15%).**
- **The mean OD value for Standard 1 must be ≥ 0.700.**

If the above criteria are not met the run is invalid and must be repeated.

- **The mean OD value for the Zero Standard (Green Diluent) should be ≤ 0.15. If the OD value is > 0.15 the plate washing procedures should be investigated.**

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.

Calculation of Results

IFN-γ values (in IU/mL) for ESAT-6, CFP-10 and Mitogen are corrected for background by subtracting the value obtained for the respective Nil control. These corrected values are used for interpretation of the test results.

Interpretation of Results

QuantiFERON®-TB Gold results are interpreted as follows:

Mitogen-Nil IU/mL	ESAT-6 - Nil and / or CFP-10 - Nil IU/mL	Result	Interpretation
≥ 0.5	≥ 0.35	QuantiFERON®-TB Gold Positive	*M. tuberculosis* infection likely
< 0.5	≥ 0.35	QuantiFERON®-TB Gold Positive	*M. tuberculosis* infection likely
≥ 0.5	< 0.35	Negative	*M. tuberculosis* infection not likely
< 0.5	< 0.35	Indeterminate	Result not obtained

Mitogen - Nil must be ≥ 0.5 IU/mL AND/OR either ESAT-6 - Nil or CFP-10 - Nil must be ≥ 0.35 IU/mL for a subject to have a valid QuantiFERON®-TB Gold result.

QuantiFERON®-TB Gold test results can only be interpreted from specimens capable of generating detectable levels of IFN-γ. The Mitogen Positive Control generally elicits the greatest IFN-γ response of the 4 samples from each blood specimen.

Under most circumstances the Nil Control will not generate IFN-γ above 1.0 IU/mL. The IFN-γ level of the Nil Control is considered background and is subtracted from the other results for that blood specimen.

In some cases the Mitogen Positive Control OD value will be above the limit of the Microplate reader, this has no impact on the test interpretation.

The cut-off for the QuantiFERON®-TB Gold test is 0.35 IU/mL above the Nil control for either ESAT-6 or CFP-10 stimulated plasma samples. Individuals displaying a response to either TB-Specific Antigen above this cut-off are likely to be infected with *M. tuberculosis.*

In low risk populations where the probability of true infection is low, it is recommended that samples which are initially positive should be retested in duplicate using the original plasma samples. If repeat testing of one or both duplicate tests are positive, the subject is considered positive. If an inadequate volume of sample is available, another blood sample can be drawn for a repeat assay.

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.

Quality Control in the laboratory should be maintained by using correct laboratory procedures at all times and adhering to the instructions in the Package Insert.
The accuracy of test results is dependent on the ELISA generating a suitable standard curve. Therefore, results derived from the standards must be examined before test sample results can be interpreted.

Physicians Instructions

Although QuantiFERON®-TB Gold has high sensitivity (89%) for active tuberculosis, a positive QuantiFERON®-TB Gold result does not necessarily indicate the presence or absence of active tuberculosis disease. Other diagnostic procedures, such as X-ray examination of the chest and microbiological examination of sputum should be used when TB disease is suspected.

Due to the absence of the TB-Specific Antigens from all strains of BCG vaccines,[1] a positive QuantiFERON®-TB Gold result cannot be due to prior BCG vaccination.

However both ESAT-6 and CFP-10 are present in *M. kansasii, M. marinum* and *M. szulgai*[1] and people with these mycobacterial infections are likely to be positive in QuantiFERON®-TB Gold. If such infections are suspected, rather than TB, alternative tests such as mycobacterial culture should be investigated.

The intended use of the QuantiFERON®-TB Gold test is as an aid to the detection of TB infection. Individuals who are positive in the QuantiFERON®-TB Gold test have *in vitro* evidence of a cellular immune response to TB-Specific Antigens. The treatment options for a patient considered to have TB infection are at the discretion of the physician and should be based on all the available laboratory results, patient history, and information.

For further information refer to the Cellestis website: www.cellestis.com or contact Cellestis.

Sample Calculations

If the following OD readings were obtained, the Mean OD and %CV would be:

	IU/mL	OD Readings	Mean OD	%CV
Standard Dilution 1	4.0	1.278, 1.285	1.282	0.39
Standard Dilution 2	1.0	0.349, 0.377	0.363	5.5
Standard Dilution 3	0.25	0.131, 0.139	0.135	N/A
Standard Dilution 4	0	0.071, 0.075	0.073	N/A

Based on the Standard curve, the calculated Correlation coefficient (r) = 0.998

Using the criteria specified in the Validation of Test Performance section the assay is determined to be valid.

Antigen OD responses			
ESAT-6	1.086	Mitogen	1.689
CFP-10	0.532	Nil	0.025

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

ESAT-6	3.450 IU/mL	Mitogen	5.944 IU/mL
CFP-10	1.432 IU/mL	Nil	0.033 IU/mL

Calculations for determining QuantiFERON results:

Mitogen - Nil = 5.911 IU/mL   Therefore valid test

ESAT-6 - Nil = 3.417 IU/mL

CFP-10 - Nil = 1.399 IU/mL

In this case the subject's IFN-γ response to both ESAT-6 and CFP-10 is greater than the 0.35 IU/mL test cut-off. This result is interpreted as positive indicating the likelihood of infection with *M. tuberculosis.*

8. LIMITATIONS

False results may occur due to:

1. Incorrect technique
2. Use of any anticoagulant other than heparin
3. Incorrect transport of blood specimens
4. Excessive levels of circulating IFN-γ
5. Longer than 12 hours from blood specimen drawing
6. Incorrect incubation times or temperatures
7. Expired reagents or reconstituted components
8. Other deviations from the recommended test procedure

The effects of the TST on subsequent QuantiFERON®-TB Gold results has not been evaluated. Boosting or suppression of the TB-specific antigen response is theoretically possible.

Heterophilic, (e.g., human anti-mouse) antibodies in the serum or plasma of certain individuals are known to cause interference with many immunoassays.[8] The effect of heterophile antibodies in the QuantiFERON®-TB Gold ELISA is neutralized 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 microtitre wells. [5] Studies during the development of the test showed that 12 of 201 (6%) plasma samples tested demonstrated high levels of heterophile antibody interference. The addition of 20% normal mouse serum to the Green Diluent abrogated this interference.[5] It should be noted that subtraction of the IFN-γ value for the Nil sample from all other values prior to test interpretation provides an internal control for any possible residual heterophile antibody interference.

9. EXPECTED VALUES

The following range of IFN-γ responses to the TB-Specific and Control Antigens have been observed during the clinical trials conducted:

Nil:	0 to 4 IU/mL (mean 0.16 IU/mL; n=220)
ESAT-6:	0 to >15* IU/mL
CFP-10:	0 to >15* IU/mL
Mitogen:	0 to >15* IU/mL

* Maximum concentration that can be estimated from the standard curve.

10. PERFORMANCE CHARACTERISTICS

Clinical Studies

QuantiFERON®-TB Gold has been evaluated in two studies of specificity in persons at low risk of tuberculosis infection, a study of sensitivity in active disease, a study correlating QuantiFERON®-TB Gold results to risks of infection in Healthcare Workers in a Hospital setting, and two studies evaluating persons in contact with a case of active tuberculosis. Other studies are ongoing.

Studies conducted in Japan have shown QuantiFERON®-TB Gold to have a specificity of at least 98% in BCG vaccinated individuals who had no identified risk factors for TB exposure (n = 213). The epidemiologically estimated rate of latent TB infection (LTBI) in the population tested was 2%, in accordance with the 98% specificity seen. Similar specificity was seen in a study of 99 low-risk individuals in Australia, where sensitivity was estimated at 97%. This study highlighted the value of retesting positive samples in low-risk individuals, as retesting positive samples in duplicate increased specificity.

Due to the absence of a definitive standard for LTBI, a perfect estimate of the sensitivity of QuantiFERON®-TB Gold for LTBI cannot be determined. However, for active TB disease a definitive marker does exist; culture of *M. tuberculosis* from sputum or other bodily samples. Therefore, to obtain an estimate of sensitivity, TB-suspects, subsequently proven to have TB infection by culture of *M. tuberculosis* (n = 118), were tested with QuantiFERON®-TB Gold. Sensitivity for active TB was 89% (105/118). The TB patients tested had received either no treatment (80.5%), or less than 7 days of

TB treatment prior to testing, to avoid any confounding affects of treatment on the immune response. Skin testing was 66% sensitive in this group. QuantiFERON®-TB using tuberculin was 82% sensitive in this study, in accord with previous reports. Other studies have reported that QuantiFERON®-TB testing is much more sensitive in detecting untreated active disease than skin-testing[10].

The sensitivity of QuantiFERON®-TB Gold for LTBI may be higher than the 89% determined for active TB patients. Up to 25% of subjects with active TB disease have reduced CMI responses to TB antigens (prior to treatment),[12] and IFN-γ responses (the responses measured by QuantiFERON®-TB Gold) are usually stronger in healthy contacts of active TB cases (i.e. those with LTBI).[11,18,21,25] This is also the case for responses measured by conventional skin-testing. In a study following exposure to an active TB case conducted in Europe in a population without BCG vaccination, with very low risk of pre-existing TB or of sensitization to non-tuberculous mycobacteria, results with skin-testing and QuantiFERON®-TB Gold were extremely similar, and closely matched the exposure of the individuals to the index TB case.

QuantiFERON®-TB Gold appears to function well in persons of all ages. Data from 110 culture confirmed TB patients were stratified by age (decades). A significant decline in test sensitivity was found for the Mantoux test (cut-off 5 mm of induration) between 20 and 80+ years of age ($p = 0.015$). However there was no significant decline with age for the QuantiFERON®-TB Gold test ($p = 0.35$), suggesting that measurement of *in vitro* IFN-γ responses is more appropriate in the elderly than measurement of DTH.

Studies in healthcare workers in Japan have demonstrated a significant correlation between positive QuantiFERON®-TB Gold responses and risk factors for TB exposure such as, length of employment in the healthcare industry, working with TB infected patients, and chest x-ray evidence of minimal TB. Two separate contact investigations in Europe and the USA studies have shown strong correlation with degree of exposure to the TB source case and positive QuantiFERON®-TB Gold results.

A number of other studies investigating the utility of the QuantiFERON®-TB Gold test in different settings are currently underway worldwide. These studies are investigating the performance of the test in infants, children, HIV positive people, and immunocompromised individuals.

A large number of published clinical studies (using methods other than QuantiFERON) have demonstrated the utility of measuring IFN-γ responses to the ESAT-6 and/or CFP-10 antigens used in QuantiFERON®-TB Gold for the detection of TB infection.[11,18,21,23,25] These studies have generally used testing systems that are complex to perform, requiring lymphocyte purification and enumeration and are thus unsuited for routine diagnostic application. Nevertheless, the data supports the findings from studies of the QuantiFERON®-TB Gold test;, *in vitro* IFN-γ response to TB-specific antigens is an accurate and specific means of detecting both LTBI and active TB infection. A selection of references to the most relevant of these publications has been included in the bibliography.[1-26]

Assay Performance Characteristics

Studies have demonstrated that the IFN-γ response of blood specimens tested by QuantiFERON®-TB Gold decreases with the length of time blood is stored prior to incubation with stimulation antigens. For some QuantiFERON®-TB Gold positive individuals, this decrease may be significant (>50%) within the 12 hour recommended timeframe. For a small number of individuals (generally those with a IFN-γ response close to the cut-off) the QuantiFERON®-TB Gold test result may alter from positive to negative, depending on the time of initiation of incubation post blood sample collection. Storage of blood samples for longer than the recommended 12 hours or outside of the quoted temperature range (22°C ± 5°C) can lead to erroneous results.

The length of incubation of blood samples with the QuantiFERON®-TB Gold stimulation antigens was shown to be optimal between 16 and 24 hours.

Studies with plasma samples from donors have demonstrated that natural IFN-γ is stable for at least 14 days when stored at 2°C to 8°C, or for at least 3 months when stored at or below -20°C. When re-testing samples note that the test result may differ by ±15% (CV of the test); samples close to the diagnostic threshold may alter their diagnostic outcome.

The shelf life of the QuantiFERON®-TB Gold ELISA kit is 3 years from the date of manufacture when stored at 2°C to 8°C.

The shelf life of the QuantiFERON®-TB Gold Stimulation Antigens is currently 12 months from the date of manufacture when stored at 2°C to 8°C.

The analytical range of the QuantiFERON®-TB Gold ELISA for detecting IFN-γ (Sample value less Nil Control) is between zero and 15 IU/mL. No prozone or "hook-effect" has been observed for the ELISA at concentrations of IFN-γ up to 10,000 IU/mL.

The analytical sensitivity of the QuantiFERON®-TB Gold ELISA is 0.05 IU/mL above the negative control (Nil) plasma sample for an individual.

11. TECHNICAL INFORMATION

Trouble Shooting

Difficulties that may be encountered in performing the assay include:

1. Clot formation in plasma samples that have been stored frozen for an extended period of time. Clotted material can block multichannel pipette tips.
2. Very lipemic samples. Fatty deposits can block multichannel pipette tips.
3. Plasma samples with high levels of IFN-γ give OD values above the limit of the ELISA reader. Unless these high levels are associated with the Nil control sample, this has no effect on the interpretation of the test.

How to deal with Clotted Plasma Samples

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

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

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

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13. TECHNICAL SERVICE

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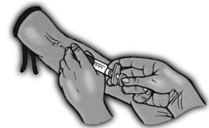
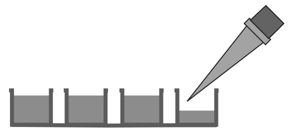
14. ABBREVIATED TEST PROCEDURE

STAGE I - INCUBATION OF BLOOD

1. Draw 10mL blood into heparin tubes.

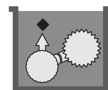




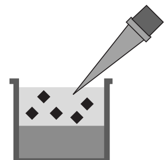
2. Aliquot 1mL heparinized whole blood.

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

4. Thoroughly mix blood and antigens together.



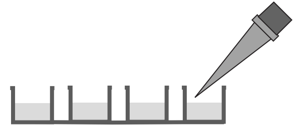
5. Incubate overnight.



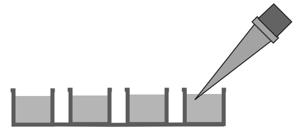
6. Harvest plasmas.

STAGE 2 - HUMAN IFN-γ ELISA

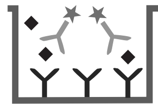
1. Prepare Conjugate in Green Diluent and add 50µL to ELISA wells.



2. Add 50µL test plasmas (simultaneously for each patient) and 50µL standards to wells.

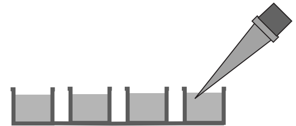


3. Incubate for 120 minutes at room temperature.

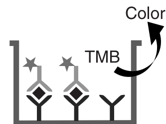


4. Wash wells at least 6 times.

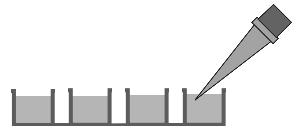
5. Add 100µL Enzyme Substrate Solution to wells.



6. Incubate for 30 minutes at room temperature.



7. Add 50µL Stop Solution to wells.



8. Read results at 450 /620 (or 650) nm.

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