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

2 x 96 (catalog no. 0594-0201)

20 x 96 (catalog no. 0594-0501)

The whole blood IFN-γ test measuring responses to ESAT-6, CFP-10, and TB7.7(p4) peptide antigens

IVD For in vitro diagnostic use

CE

REF 0594-0201, 0594-0501

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Contents

1. Intended Use

QuantiFERON-TB Gold (QFT®) 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 those peptide antigens that are associated with *Mycobacterium tuberculosis* infection.

QFT 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.

QFT 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 nontuberculous mycobacteria with the exception of *M. kansasii*, *M. szulgai*, and *M. marinum*.(1) Individuals infected with *M. tuberculosis* complex organisms usually have lymphocytes in their blood that recognize these and other mycobacterial antigens. This recognition process involves the generation and secretion of the cytokine, IFN-γ. The detection and subsequent quantification of IFN-γ forms the basis of this test.

The antigens used in QFT 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.(1–32) 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, 23) The QFT 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, but 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 QFT system uses specialized 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 QFT test is performed in two stages. First, whole blood is collected into each of the QFT blood collection tubes, which include a Nil tube, TB Antigen tube, and a Mitogen tube.

The Mitogen tube can be used with the QFT test as a positive control. This may be especially warranted where there is doubt as to the individual's immune status. The Mitogen tube may also serve as a control for correct blood handling and incubation.

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. If used, the plasma sample from the Mitogen tube serves as an IFN-γ positive control for each specimen tested. A low response to Mitogen (<0.5 IU/ml) indicates an indeterminate result when a blood sample also has a negative response to the TB antigens. This pattern may occur with insufficient lymphocytes, reduced lymphocyte activity due to improper specimen handling, 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 (if used).

Time Required for Performing Assay

The time required to perform the QFT 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
	(28 to 44 individuals)
	<1 hour labor
	Add 10 to 15 minutes for each extra plate

3. Components and Storage

Blood Collection Tubes*	300 tubes	200 tubes	100 tubes
Catalog no.	T0590-0301	0590-0201	T0593-0201
Number of preps	100	100	100
QuantiFERON Nil Tube (gray cap, white ring)	100 tubes	100 tubes	
QuantiFERON TB Antigen Tube (red cap, white ring)	100 tubes	100 tubes	
QuantiFERON Mitogen Tube (purple cap, white ring)	100 tubes		100 tubes
QFT Blood Collection Tubes Package Insert	1	1	1
High Altitude (HA) Blood Collection Tubes (for use between 1020 and 1875 meters)*	**300 tubes**	**100 tubes**	**100 tubes**
Catalog no.	T0590-0505	0590-0501	T0593-0501
QuantiFERON HA Nil Tube (gray cap, yellow ring)	100 tubes	100 tubes	
QuantiFERON HA TB Antigen Tube (red cap, yellow ring)	100 tubes	100 tubes	
QuantiFERON HA Mitogen Tube (purple cap, yellow ring)	100 tubes		100 tubes
QFT Blood Collection Tubes Package Insert	1	1	1

* Not all product configurations are available in every country. Please refer to QIAGEN customer care (details on www.qiagen.com) for more information on what configurations are available for ordering.

ELISA Components	2 Plate Kit ELISA	Reference Lab Pack
Catalog no.	0594-0201	0594-0501
Microplate Strips (12 x 8 wells) coated with murine anti-human IFN-γ monoclonal antibody	2 sets of 12 x 8-well Microplate Strips	20 sets of 12 x 8-well Microplate Strips
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)	10 x vials (8 IU/ml when reconstituted)
Green Diluent (contains bovine casein, normal mouse serum, 0.01% w/v Thimerosal)	1 x 30 ml	10 x 30 ml
Conjugate 100X Concentrate, lyophilized (murine anti-human IFN-γ HRP, contains 0.01% w/v Thimerosal)	1 x 0.3 ml (when reconstituted)	10 x 0.3 ml (when reconstituted)
Wash Buffer 20X Concentrate (pH 7.2, contains 0.05% v/v ProClin® 300)	1 x 100 ml	10 x 100 ml
Enzyme Substrate Solution (contains H_2O_2, 3,3′, 5,5′ Tetramethylbenzidine)	1 x 30 ml	10 x 30 ml
Enzyme Stopping Solution (contains 0.5M H_2SO_4)†	1 x 15 ml	10 x 15 ml
QFT ELISA Package Insert	1	1

† Contains sulfuric acid. See page 9 for precautions.

Materials Required But Not Provided

- 37°C incubator. CO_2 not required
- Calibrated variable volume pipets for delivery of 10 µl to 1000 µl with disposable tips
- Calibrated multichannel pipet capable of delivering 50 µl and 100 µl with disposable tips
- Microplate shaker
- Deionized or distilled water, 2 liters
- Microplate washer (automated washer recommended)
- Microplate reader fitted with 450 nm filter and 620 nm to 650 nm reference filter

Storage and Handling

Blood Collection Tubes

- Store blood collection tubes at 4°C to 25°C.

Kit Reagents

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

Reconstituted and Unused Reagents

For instructions on how to reconstitute the reagents, please see Section 6 (page 14)

- The reconstituted kit standard may be kept for up to 3 months if stored at 2°C to 8°C.

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

  Note the date on which 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

For in vitro diagnostic use

Warnings

- A negative QFT 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 QFT 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 QFT 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 nontuberculous mycobacteria, it is possible that a positive QFT 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 only.

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION: Handle human blood as if potentially infectious.
Observe relevant blood handling guidelines.

The following risk and safety phrases apply to components of the QuantiFERON-TB Gold ELISA.

QuantiFERON Enzyme Stopping Solution


Xi

Contains sulfuric acid: Irritant. Risk and safety phrases:* R36/38, S26-36/37/39

- **Green Diluent** contains normal mouse serum and casein, which may trigger allergic responses; avoid contact with skin.

For Chemical Emergency

Spill, Leak, Exposure, or Accident

Call CHEMTREC Day or Night

Within USA and Canada: 1-800-424-9300

Outside USA and Canada: +1-703-527-3887 (collect calls accepted)

Further information

Safety Data Sheets: www.qiagen.com/safety

- Deviations from the *QuantiFERON-TB Gold (QFT) ELISA 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 the Microplate Strips, Human IFN-γ Standard, Green Diluent, or Conjugate 100X Concentrate from different QFT kit batches. Other reagents (Wash Buffer 20X Concentrate, Enzyme Substrate Solution, and Enzyme Stopping Solution) can be interchanged between kits providing the reagents are

* R36/38: Irritating to eyes and skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.

within their expiration periods and lot details recorded. Discard unused reagents and biological samples in accordance with Local, State, and Federal regulations.

- Do not use the blood collection tubes or ELISA kit after the expiration date.
- Ensure that laboratory equipment such as plate washers and readers have been calibrated/validated for use.

5. Specimen Collection and Handling

QFT uses the following collection tubes:

1. QuantiFERON Nil tubes (gray cap with white ring; use between sea level and 810 m)
2. TB Antigen tubes (red cap with white ring; use between sea level and 810 m)
3. QuantiFERON Mitogen tubes (purple cap with white ring; use between sea level and 810 m)

High Altitude (HA) Tubes

4. QuantiFERON HA Nil tubes (gray cap with yellow ring; use between 1020 m and 1875 m)
5. HA TB Antigen tubes (red cap with yellow ring; use between 1020 m and 1875 m)
6. QuantiFERON HA Mitogen tubes (purple cap with yellow ring; use between 1020 m and 1875 m)

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 1 ml of blood by venipuncture directly into each of the QFT blood collection tubes. This procedure should be performed by a trained phlebotomist.**
   - Standard QFT blood collection tubes should be used up to an altitude of 810 meters. High Altitude (HA) QFT blood collection tubes should be used at altitudes between 1020 and 1875 meters.
   - If using QFT blood collection tubes outside these altitude ranges, or if low blood draw volume occurs, blood can be collected using a syringe, and 1 ml immediately 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 3 QFT tubes and adding 1 ml of blood to each (to the black mark on the side of the tube label). Replace the caps securely and mix as described below.
   - As 1 ml tubes draw blood relatively slowly, keep the tube on the needle for 2–3 seconds once the tube appears to have completed filling, to ensure that the correct volume is drawn.

     The black mark on the side of the tubes indicates the 1 ml fill volume. QFT blood collection tubes have been validated for volumes ranging from 0.8 to 1.2 ml. 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 QFT tubes being used.
   - Alternatively, blood may be collected in a single generic blood collection tube containing lithium heparin as the anticoagulant and then transferred to QFT tubes. **Only use lithium heparin** as a blood anticoagulant since other anticoagulants

interfere with the assay. Fill a blood collection tube (minimum volume 5 ml) and gently mix by inverting the tube several times to dissolve the heparin. Blood should be maintained at room temperature (22°C ± 5°C) before transfer to QFT tubes for incubation, which must be initiated within 16 hours of blood collection.

2. **Immediately after filling the tubes, shake them ten (10) times just firmly enough to ensure that the entire inner surface of the tube is coated with blood, to dissolve antigens on tube walls.**
   - Tubes should be between 17°C – 25°C at the time of blood filling.
   - Over-energetic shaking may cause gel disruption and could lead to aberrant results.
   - If blood has been collected in heparin tube, samples must be evenly mixed before dispensing into QFT tubes. Ensure that the blood is thoroughly mixed by gentle inversion **immediately prior to dispensing**. Dispense 1.0 ml aliquots (one per QFT tube) into an appropriate Nil, TB Antigen, and Mitogen tube. This is best performed aseptically, **ensuring appropriate safety procedures**, removing the caps from the three QFT tubes and adding 1 ml of blood to each (to the black mark on the side of the tube label). Replace the tube caps securely and mix as described above.
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. **Following filling, shaking, and labeling, 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 the tubes at room temperature (22°C ± 5°C). Do not refrigerate or freeze the blood samples.**

6. Directions for Use

Stage 1 — Incubation of blood and harvesting of plasma

Materials provided

- QFT 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, re-mixing of the tubes by inverting 10 times must be performed immediately prior to incubation.
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. After incubation at 37°C, blood collection tubes may be held between 4°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 the 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, but additional care is required to remove the plasma without disturbing the cells.
5. Plasma samples should only be harvested using a pipet.
   - After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel.
   - Plasma samples can be loaded directly from centrifuged blood collection tubes into the QFT ELISA plate, including when automated ELISA workstations are used.
   - Plasma samples can be stored for up to 28 days at 2°C to 8°C or, if harvested, below –20°C for extended periods.
   - For adequate test samples, harvest at least 150 µl of plasma.

Stage 2 — Human IFN-γ ELISA

Materials provided

- QFT 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°C ± 5°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 1 strip for the QFT standards and sufficient strips for the number of subjects being tested (refer to Figures 2A and 2B for 3-tube and 2-tube formats, respectively). After use, retain frame and lid for use with remaining strips.
3. **Reconstitute the freeze dried kit standard with the volume of deionized or distilled water indicated on the label of the standard vial. Mix gently to minimize frothing and ensure complete solubilization. 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) (see 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	Recommended procedure for triplicate standards
a. Label 4 tubes "S1", "S2", "S3", "S4".	a. Label 4 tubes "S1", "S2", "S3", "S4".
b. Add **150 µl** of GD to S1, S2, S3, S4.	b. Add **150 µl** of GD to S1.
c. Add **150 µl** of the kit standard to S1 and mix thoroughly.	c. Add **210 µl** of GD to S2, S3, S4.
d. Transfer **50 µl** from S1 to S2 and mix thoroughly.	d. Add **150 µl** of the kit standard to S1 and mix thoroughly.
e. Transfer **50 µl** from S2 to S3 and mix thoroughly.	e. Transfer **70 µl** from S1 to S2 and mix thoroughly.
f. **GD alone** serves as the zero standard (S4).	f. Transfer **70 µl** from S2 to S3 and mix thoroughly.
	g. GD alone serves as the zero standard (S4).

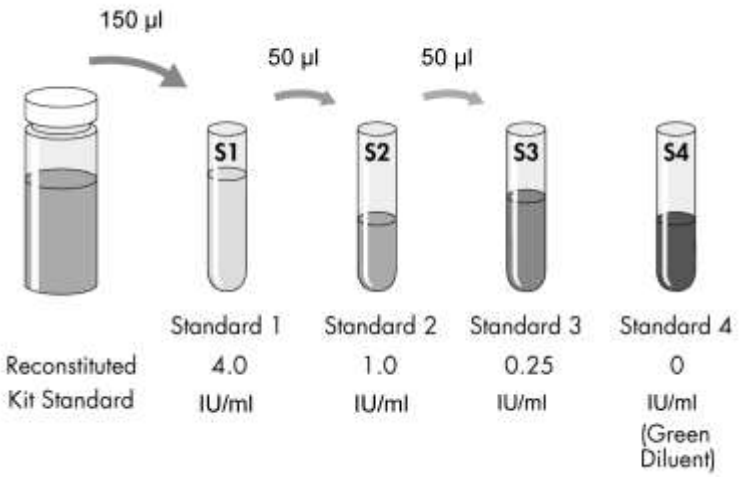


Figure 1. Preparation of standard curve. Prepare fresh dilutions of the kit standard for each ELISA session.

4. **Reconstitute freeze dried Conjugate 100X Concentrate with 0.3 ml 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
2	10 µl	1.0 ml
3	15 µl	1.5 ml
4	20 µl	2.0 ml
5	25 µl	2.5 ml
6	30 µl	3.0 ml
7	35 µl	3.5 ml
8	40 µl	4.0 ml
9	45 µl	4.5 ml
10	50 µl	5.0 ml
11	55 µl	5.5 ml
12	60 µl	6.0 ml

- 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. **For plasma samples harvested from blood collection tubes and subsequently frozen or stored for more than 24 hours prior to assay, thoroughly mix before addition to the ELISA well.**
   - If plasma samples are to be added directly from the centrifuged QFT tubes, any mixing of the plasma should be avoided.
6. **Add 50 µl of freshly prepared working strength conjugate to the required ELISA wells using a multichannel pipet.**
7. **Add 50 µl of test plasma samples to appropriate wells using a multichannel pipet (Refer to recommended plate layout on page 16 and 17, Figures 2A and 2B). Finally, add 50 µl each of the Standards 1 to 4.**

	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

Figure 2A. Recommended sample layout for Nil, TB Antigen, and Mitogen tubes (28 tests per plate).

- S1 (Standard 1), S2 (Standard 2), S3 (Standard 3), S4 (Standard 4)
- 1N (Sample 1. Nil plasma), 1A (Sample 1. TB Antigen plasma), 1M (Sample 1. Mitogen plasma)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1N	5N	9N	13N	17N	*S1*	*S1*	25N	29N	33N	37N	41N
B	1A	5A	9A	13A	17A	*S2*	*S2*	25A	29A	33A	37A	41A
C	2N	6N	10N	14N	18N	*S3*	*S3*	26N	30N	34N	38N	42N
D	2A	6A	10A	14A	18A	*S4*	*S4*	26A	30A	34A	38A	42A
E	3N	7N	11N	15N	19N	21N	23N	27N	31N	35N	39N	43N
F	3A	7A	11A	15A	19A	21A	23A	27A	31A	35A	39A	43A
G	4N	8N	12N	16N	20N	22N	24N	28N	32N	36N	40N	44N
H	4A	8A	12A	16A	20A	22A	24A	28A	32A	36A	40A	44A

Figure 2B. Recommended sample layout for Nil and TB Antigen tubes (44 tests per plate).

- S1 (Standard 1), S2 (Standard 2), S3 (Standard 3), S4 (Standard 4)
- 1N (Sample 1. Nil plasma), 1A (Sample 1. TB Antigen plasma)

8. **Mix the conjugate and plasma samples/standards thoroughly using a microplate shaker for 1 minute.**
9. **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.
10. **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 2 liters 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, lint-free towel to remove residual wash buffer. Add 100 µl 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°C ± 5°C) for 30 minutes.**
    - Plates should not be exposed to direct sunlight during incubation.
13. **Following the 30-minute incubation, add 50 µl 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 450 nm filter and with a 620 nm to 650 nm reference filter. OD values are used to calculate results.

7. Calculations and Test Interpretation

QFT Analysis Software is used to analyze raw data and calculate results. It is available from www.QuantiFERON.com. Please ensure that the most current version of the software is used.

The software performs a quality control assessment of the assay, generates a standard curve, and provides a test result for each subject, as detailed in the Interpretation of Results section.

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

Generation of Standard Curve

(if QFT Analysis Software is not used)

Determine the mean OD values of the kit standard replicates on each plate.

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

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 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 ≤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 QFT 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

QFT 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 QFT results (Tables 2 and 3).

Table 2. When Nil, TB Antigen, and Mitogen tubes are used

Nil (IU/ml)	TB Antigen minus Nil (IU/ml)	Mitogen minus Nil (IU/ml)*	QFT result	Report/Interpretation
≤8.0	< 0.35	≥ 0.5	Negative	*M. tuberculosis* infection NOT likely
	≥ 0.35 and < 25% of Nil value	≥ 0.5	Negative	*M. tuberculosis* infection NOT likely
	≥ 0.35 and ≥ 25% of Nil value	Any	Positive†	*M. tuberculosis* infection likely
	< 0.35	< 0.5	Indeterminate‡	Results are indeterminate for TB-Antigen responsiveness
	≥ 0.35 and < 25% of Nil value	< 0.5	Indeterminate‡	Results are indeterminate for TB-Antigen responsiveness
> 8.0§	Any	Any	Indeterminate‡	Results are indeterminate for TB-Antigen responsiveness

* 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.

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

‡ Refer to the "Troubleshooting" section for possible causes.

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

The magnitude of the measured IFN-γ level cannot be correlated to the stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease.

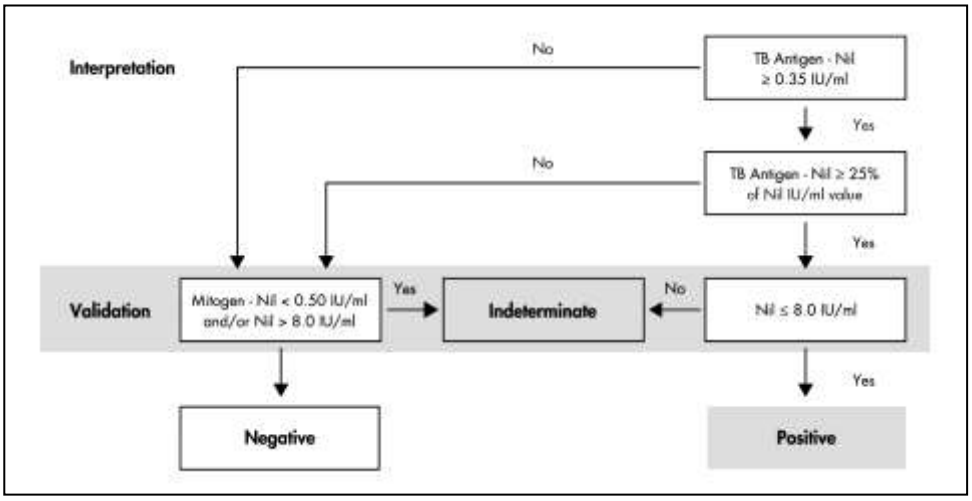


Figure 3. Interpretation flowchart where Nil, TB Antigen, and Mitogen tubes are used.

Table 3. When only QuantiFERON Nil and TB Antigen tubes used

Nil (IU/ml)	TB Antigen minus Nil (IU/ml)	QFT result	Report/Interpretation
≤8.0	< 0.35	Negative	*M. tuberculosis* infection NOT likely
	≥0.35 and < 25% of Nil value	Negative	*M. tuberculosis* infection NOT likely
	≥ 0.35 and ≥ 25% of Nil value	Positive*	*M. tuberculosis* infection likely
> 8.0†	Any	Indeterminate‡	Results are indeterminate for TB-Antigen responsiveness

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

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

‡ Refer to the "Troubleshooting" section for possible causes.

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.

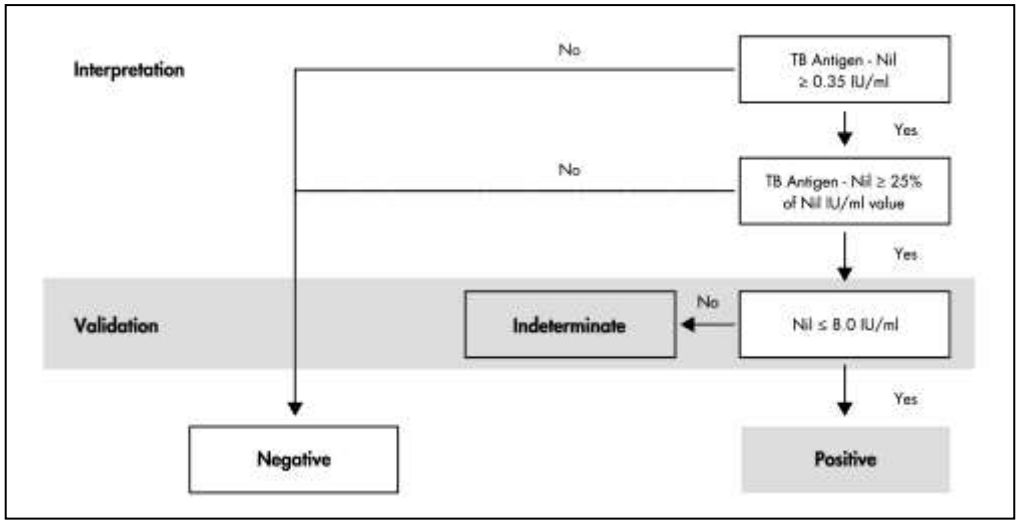


Figure 4. Interpretation flowchart when Nil and TB Antigen tubes are used.

8. Limitations

Results from QFT 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 *QuantiFERON-TB Gold (QFT) ELISA Package Insert*
- Excessive levels of circulating IFN-γ or presence of heterophile antibodies
- Longer than 16 hours between drawing the blood specimen and 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 QFT cannot be practically evaluated. Specificity of QFT 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 US study involving 866 volunteers, blood was drawn for QFT 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, QFT and TST results were available for 391. None were BCG vaccinated. A second specificity study was performed with QFT in low-risk individuals in Japan, approximately 90% of whom had received BCG vaccination. Results from the 2 specificity studies are shown in Table 4.

Table 4. QFT specificity: Results for persons with no reported risk for *M. tuberculosis* infection.

Study	BCG status (% vaccinated)	Total tested	No. QFT indeterminate	No. QFT positive/ no. valid tests	QFT specificity (95% CI)	No. TST positive / no. tested	TST* specificity (95% CI)
US (unpublished)	0%	391	1	3/390	99.2% (98–100)	6/391	98.5% (97–99)
Japan (15)	~90%	168	6	2/162	98.8% (95–100)	–	–
Total	–	559	7/559 (1.3%)	5/552	99.1% (98–100)	–	–

* Using 10 mm TST cut off in non-BCG-vaccinated people. TST specificity estimate is 99.1% if using a 15 mm cut off.

Sensitivity for active TB

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

Table 5 summarizes findings from the 3 groups of *M. tuberculosis* culture-positive patients. The overall sensitivity of QFT for active TB disease was 89% (157/177).

Table 5. QFT: Subjects with culture-confirmed *M. tuberculosis* infection.

Study	No. QFT positive / no. valid tests	QFT sensitivity (95% CI)
Japan TB patients (15)	86/92	93% (86–97%)
Australian	24/27	89% (70–97%)
US	47/58	81% (68–90%)
Total	157/177	89% (83–93%)

Diagnosis of LTBI

A number of studies have been published which demonstrate the performance of QFT in various populations at risk of LTBI. The principle findings of some selected studies are shown in Table 6.

Table 6. Selected published studies on QFT in populations at risk of LTBI.

Study	Total tested	Outcomes and findings
Healthcare workers in India (Pai, et al 2005) (26)	726	Setting of very high TB rates. 40% QFT positive and 41% TST positive at 10 mm. High concordance with TST, no effect of BCG on either side. Both tests related to risk factors of age and period of work in healthcare.
Danish HIV+ patients (Brock, et al 2006) (5)	590	Overall prevalence of LTBI by QFT was 4.6% (27/590) in HIV+ persons. Positive results were associated with TB risks. Two QFT-positive subjects progressed to active TB within 1 year. Indeterminate responses (n=20. 3.4%) were significantly associated with a CD4 count <100/µl.
Hospitalized children in India (Dogra, et al 2006) (10)	105	Children in whom TB was suspected or who had a history of TB contact were tested with QFT and TST; 10.5% QFT positive and 9.5% TST positive at 10 mm. Agreement between tests was 95.2% overall and 100% in non-BCG vaccinated.
Contact investigations in Germany (Diel, et al 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 positive, respectively. QFT 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 QFT) and the QFT test. These studies include use of the test(s) in contacts of active TB cases (9, 11, 19, 25), children (6-10, 25, 28), HIV-positive patients (2, 5, 20), healthcare workers (13, 26, 32), immune suppressed patients (3, 4, 22, 23, 27, 30, 31), as well as TB suspects (7, 8, 10, 18), and low-risk individuals (15).

Repeatability and effect of TST on subsequent QFT testing

As part of the US specificity study, a subset of the volunteers was retested between 4 and 5 weeks after the original QFT test and TST. QFT 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 QFT 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 between drawing of the blood and incubation at 37°C
- Storage of blood outside the recommended temperature range (17°C to 27°C)
- Insufficient mixing of blood collection tubes
- Incomplete washing of the ELISA plate

If technical issues are suspected with the collection or handling of the blood samples, repeat the entire QFT 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 the samples to sediment clotted material and facilitate pipetting of plasma.

Troubleshooting Guide

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

ELISA troubleshooting

Non-specific color development

Possible cause	Solution
a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b) Cross-contamination of ELISA wells	Take care when pipetting and mixing sample to minimize risk.
c) Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100X Concentrate are used within three months of the reconstitution date.
d) Enzyme Substrate Solution is contaminated	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.
e) Mixing of plasma in QFT tubes before harvesting	After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel.

Low optical density readings for standards

Possible cause	Solution
a) Standard dilution error	Ensure dilutions of the Kit Standard are prepared correctly as per the QFT ELISA Package Insert.
b) Pipetting error	Ensure pipets are calibrated and used according to manufacturer's instructions.
c) Incubation temperature too low	Incubation of ELISA should be performed at room temperature (22°C ± 5°C)
d) Incubation time too short	Incubation of the plate with the conjugate, standards and samples should be for 120 ± 5 minutes. The Enzyme Substrate Solution is incubated on the plate for 30 minutes.
e) Incorrect plate reader filter used	Plate should be read at 450 nm with a reference filter between 620 and 650 nm.

f) Reagents are too cold	All reagents, with the exception of the Conjugate 100X Concentrate, must be brought to room temperature prior to commencing the assay. This takes approximately one hour.
g) Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100X Concentrate are used within 3 months of the reconstitution date.

High background

Possible cause	Solution
a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b) Incubation temperature too high	Incubation of the ELISA should be performed at room temperature (22°C ± 5°C).
c) Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100X Concentrate are used within 3 months of the reconstitution date.
d) 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
a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b) Standard dilution error	Ensure dilutions of the standard are prepared correctly as per the QFT ELISA Package Insert.
c) Poor mixing	Mix reagents thoroughly by inversion or gentle vortexing prior to their addition to the plate.
d) Inconsistent pipetting technique or interruption during assay set up	Sample and standard addition should be performed in a continuous manner. All reagents should be prepared prior to commencing the assay.

An assay procedure video and solutions to most technical problems can be found on Gnowee™ by registering directly at www.gnowee.net for online access. Product information and technical guides are available free of charge from QIAGEN, via your distributor, or by visiting www.QuantiFERON.com.

11. Bibliography

A comprehensive list of QFT references is located on Gnowee — the QuantiFERON reference library, available at www.gnowee.net.

1. Andersen, P. et al. (2000) Specific immune-based diagnosis of tuberculosis. Lancet **356**, 1099.
2. Balcells, M.E. et al. (2008) A comparative study of two different methods for the detection of latent tuberculosis in HIV-positive individuals in Chile. Int. J. Infect. Dis. **12**, 645.
3. Bartalesi, F. et al. (2009) QuantiFERON-TB Gold and TST are both useful for latent TB screening in autoimmune diseases. Eur. Respir. J. **33**, 586.
4. Bocchino, M. et al. (2008) Performance of two commercial blood IFN-gamma release assays for the detection of *Mycobacterium tuberculosis* infection in patient candidates for anti-TNF-alpha treatment. Eur. J. Clin. Microbiol. Infect. Dis. **27**,907.
5. Brock, I. et al. (2006) Latent tuberculosis in HIV positive, diagnosed by the *M. tuberculosis* specific interferon-gamma test. Respir. Res. **7**, 56.
6. Chun, J.K. et al. (2008) The role of a whole blood interferon gamma assay for the detection of latent tuberculosis infection in bacille Calmette-Guerin vaccinated children. Diagn. Microbiol. Infect. Dis. **62**, 389.
7. Connell, T.G. et al. (2008) A three-way comparison of tuberculin skin testing, QuantiFERON-TB gold and T-SPOT.TB in children. PLoS ONE **3**, e2624. doi: 10.1371/journal.pone.0002624.
8. Detjen, A.K. et al. (2007) Interferon-gamma release assays improve the diagnosis of tuberculosis and nontuberculous mycobacterial disease in children in a country with a low incidence of tuberculosis. Clin. Infect. Dis. **45**, 322.
9. Diel, R. et al. (2009) Comparative performance of tuberculin skin test, QuantiFERON-TB-Gold In-Tube assay, and T-Spot.TB test in contact investigations for tuberculosis. Chest **135**, 1010.
10. Diel, R. et al. (2008) Predictive value of a whole-blood IFN-γ assay for the development of active TB disease. Am. J. Respir. Crit. Care Med. **177**, 1164.
11. Diel, R. et al. (2006) Tuberculosis contact investigation with a new, specific blood test in a low-incidence population containing a high proportion of BCG-vaccinated persons. Respir. Res. **7**, 77.
12. Dogra, S. et al. (2007) Comparison of a whole blood interferon-gamma assay with tuberculin skin testing for the detection of tuberculosis infection in hospitalized children in rural India. J. Infect. **54**, 267.
13. Drobniewski, F. et al. (2007) Rates of latent tuberculosis in health care staff in Russia. PLoS Med. **4**, e55.
14. Gerogianni, I. et al. (2008) Whole-blood interferon-gamma assay for the diagnosis of tuberculosis infection in an unselected Greek population. Respirology **13**, 270.
15. Harada, N. et al. (2008) Comparison of the sensitivity and specificity of two whole blood interferon-gamma assays for *M. tuberculosis* infection. J. Infect. **56**, 348.
16. Higuchi, K. et al. (2009) Comparison of performance in two diagnostic methods for tuberculosis infection. Med. Microbiol. Immunol. **198**, 33.
17. Kang, Y.A. et al. (2005) Discrepancy between the tuberculin skin test and the whole-blood interferon gamma assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA **293**, 2756.

18. Katiyar, S.K. et al. (2008) Use of the QuantiFERON-TB Gold In-Tube test to monitor treatment efficacy in active pulmonary tuberculosis. Int. J. Tuberc. Lung Dis. **12**, 1146.

19. Kipfer, B. et al. (2008) Tuberculosis in a Swiss army training camp: contact investigation using an Interferon gamma release assay. Swiss. Med. Wkly. **138**, 267.

20. Luetkemeyer, A. et al. (2007) Comparison of an interferon-gamma release assay with tuberculin skin testing in HIV-infected individuals. Am. J. Respir. Crit. Care Med. **175**, 737.

21. Mackensen, F. et al. (2008) QuantiFERON TB-Gold - A new test strengthening long-suspected tuberculous involvement in serpiginous-like choroiditis. Am. J. Ophthalmol. **146**, 761.

22. Manuel, O. et al. (2007) Comparison of Quantiferon-TB Gold with tuberculin skin test for detecting latent tuberculosis infection prior to liver transplantation. Am. J. Transplant. **7**, 2797.

23. Matulis, G. et al. (2007) Detection of latent tuberculosis in immunosuppressed patients with autoimmune diseases performance of a *Mycobacterium tuberculosis* antigen specific IFN-gamma assay. Ann. Rheum. Dis. **67**, 84.

24. Mirtskhulava, V. et al. (2008) Prevalence and risk factors for latent tuberculosis infection among health care workers in Georgia. Int. J. Tuberc. Lung Dis. **12**, 513.

25. Nakaoka, H. et al. (2006) Risk for tuberculosis among children. Emerging Infect. Dis. **12**, 1383.

26. Pai, M. et al. (2005) Mycobacterium tuberculosis infection in health care workers in rural India: comparison of a whole-blood, interferon-g assay with tuberculin skin testing. JAMA **293**, 2746.

27. Ponce de Leon, D. et al. (2008) Comparison of an interferon-gamma assay with tuberculin skin testing for detection of tuberculosis (TB) infection in patients with rheumatoid arthritis in a TB-endemic population. J Rheumatol. **35**, 776.

28. Richeldi, L. et al. (2008) Prior tuberculin skin testing does not boost QuantiFERON-TB results in paediatric contacts. Eur. Respir. J. **32**, 524.

29. Rothel, J.S. and Andersen, P. (2005) Diagnosis of latent *Mycobacterium tuberculosis* infection: is the demise of the Mantoux test imminent? Expert Rev. Anti Infect. Ther. **3**, 981.

30. Schoepfer, A.M. et al. (2008) Comparison of interferon-gamma release assay versus tuberculin skin test for tuberculosis screening in inflammatory bowel disease. Am. J. Gastroenterol. **103**, 2799.

31. Silverman, M.S. et al. (2007) Use of an interferon-gamma based assay to assess bladder cancer patients treated with intravesical BCG and exposed to tuberculosis. Clin. Biochem. **40**, 913.

32. Stebler, A. et al. (2008) Whole-blood interferon-gamma release assay for baseline tuberculosis screening of healthcare workers at a Swiss university hospital. Infect. Control Hosp. Epidemiol. **29**, 681.

12. Technical Service

For technical service please contact:

www.QuantiFERON.com

Asia-Pacific ■ techservice-ap@qiagen.com

Europe ■ techserviceQFT-eu@qiagen.com

Middle East / Africa ■ techserviceQFT-eu@qiagen.com

USA / Canada ■ techservice-na@qiagen.com

Latin America (not including Brazil or Mexico) ■ techservice-latam@qiagen.com

Mexico ■ techservice-MX@qiagen.com

Brazil ■ techsebr@qiagen.com

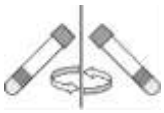
13. Abbreviated Test Procedure

Stage 1 — blood incubation

1. Collect patient blood into blood collection tubes and mix by shaking them ten (10) times just firmly enough to ensure that the entire inner surface of the tube has been coated with blood, to dissolve antigens on tube walls.

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 3000g RCF (g) to separate the plasma and the red cells.



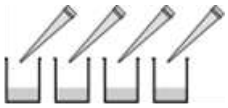
4. After centrifugation, avoid pipetting up and down or mixing the plasma by any means prior to harvesting. At all times, take care not to disturb the material on the surface of the gel.

Stage 2 — IFN-γ ELISA

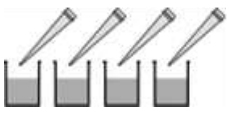
1. Equilibrate ELISA components, with the exception of the Conjugate 100X Concentrate, to room temperature (22°C ± 5°C) for at least 60 minutes.

2. Reconstitute the kit standard to 8.0 IU/ml with distilled or deionized water. Prepare four (4) standard dilutions.
3. Reconstitute freeze-dried Conjugate 100X Concentrate with distilled or deionized water.

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



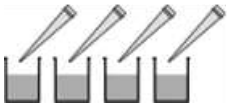
5. Add 50 µl of test plasma samples and 50 µl standards to appropriate wells. Mix using shaker.



6. Incubate for 120 ± 5 minutes at room temperature.

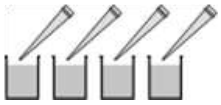
7. Wash wells at least 6 times with 400 µl/well of wash buffer.

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



9. Incubate for 30 minutes at room temperature.

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



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

12. Analyze results.

Significant Changes

Significant changes in this Edition (1075115 Rev. 01) of the QFT ELISA Package Insert are summarized in the table below:

Section	Page	Change(s)
3. Warnings and Precautions	9-10	Amendment to the use of certain ELISA components between kit lots.
12. Technical Service	31	New email addresses for Technical Service.

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