IMPAACT and ACTG Cross-Network QFT-Plus Standard Operating Procedure

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Written By:	TB Quality Assurance Advisory Group		

	Network	Name, Title	Signature	Date
Approved by (Network):	IMPAACT / ACTG	**Grace Aldrovandi, MD** IMPAACT / ACTG Laboratory Center Principal Investigator		23 SEP 2019

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	Name, Title	Signature	Date
Review By (Laboratory):			

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1 Purpose

1.1 This Standard Operating Procedure describes a set of standard IMPAACT and ACTG Network requirements for collection, processing and storage of specimens for use with the QIAGEN QuantiFERON-TB Gold Plus (QFT-Plus) Interferon Gamma Release Assay (IGRA). The instructions provided fall within the requirements of both the CE marked and FDA approved versions of the package inserts. Some steps (e.g. processing times, incubation times) have been clarified, harmonized, or more strictly defined in this SOP, but remain within the original package insert requirements. Where language differs between the CE marked or FDA-approved version of the package insert, the FDA-approved package insert version takes precedent.

2 Scope

2.1 Due to the number of steps involved in sample processing, there are inherent preanalytical (and analytical) sources of variability. Preanalytical variation may be introduced in a variety of ways including: blood collection, ambiguous processing delays, differences in ambient shipping temperatures and the length of time of T cell stimulation to TB antigen (Banaei, et. al., 2016). This SOP has considered the aforementioned variabilities and standardized them, as much as possible, within the requirement of the FDA package insert. Network protocol-specific instructions supersede those in this SOP.

3 Intended Use

3.1 The QFT-Plus assay is an in vitro diagnostic test using a peptide cocktail simulating ESAT-6 and CFP-10 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.

3.2 QFT-Plus is an indirect test for *M. tuberculosis* infection and is intended for use in conjunction with other clinical and diagnostic evaluations.

4 Summary and Explanation of the Test

4.1 Tuberculosis is a communicable disease caused by infection with *M. tuberculosis* (MTB) complex organisms (*M. tuberculosis, M. bovis, M. africanum*), which typically spread to new hosts via airborne droplet nuclei from patients with respiratory tuberculosis disease.

4.2 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) or infection with mycobacteria other than *M. tuberculosis* complex, or undetermined other factors.

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

4.4 QFT-Plus is a test for cell-mediated immune (CMI) responses to peptide antigens that simulate mycobacterial proteins. These proteins, ESAT-6 and CFP-10, are absent from all BCG strains and from most nontuberculous mycobacteria with the exception of *M. kansasii*, *M. szulgai*, and *M. marinum*. Individuals infected with MTB-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-Plus are a peptide cocktail simulating the proteins ESAT-6 and CFP-10. Numerous studies have demonstrated that these peptide 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.

However, medical treatments or conditions that impair immune functionality can potentially reduce IFN-γ responses. Patients with certain other mycobacterial infections might also be responsive to ESAT-6 and CFP-10, as the genes encoding these proteins are present in *M. kansasii*, *M. szulgai*, and *M. marinum*. QFT-Plus 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.

4.5 QFT-Plus has two distinct TB antigen tubes: TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2). Both tubes contain peptide antigens from the MTB–complex–associated antigens, ESAT-6 and CFP-10. Whereas the TB1 tube contains peptides from ESAT-6 and CFP-10 that are designed to elicit CMI responses from CD4+ T-helper lymphocytes, the TB2 tube contains an additional set

of peptides targeted to the induction of CMI responses from CD8+ cytotoxic T lymphocytes. In the natural history of MTB infection, CD4+ T cells play a critical role in immunological control through their secretion of the cytokine IFN-γ. Evidence now supports a role for CD8+ T cells participating in the host defense to MTB by producing IFN-γ and other soluble factors, which activate macrophages to suppress growth of MTB, kill infected cells, or directly lyse intracellular MTB. MTB-specific CD8+ cells have been detected in subjects with LTBI and with active TB disease where IFN-γ producing CD8+ cells may be frequently found (36– 38). Moreover, ESAT-6 and CFP-10 specific CD8+ T lymphocytes are described as being more frequently detected in subjects with active TB disease versus LTBI, and may be associated with a recent MTB exposure. In addition, MTB-specific CD8+ T cells producing IFN-γ have also been detected in active TB subjects with HIV co-infection and in young children with TB disease.

5 Storage of QuantiFERON-TB Gold Plus tubes

5.1 Store QFT-Plus blood collection tubes at 4°C to 25°C. Check the expiration date of the tubes prior to use. Do not use QFT-Plus Blood Collection tubes after the expiration date.

5.2 Ensure that the QFT-Plus blood collection tubes are at room temperature 17-25°C (62.6-77°F) before dispensing blood per the indirect collection method described in Section 6.

6 Indirect Collection of blood specimen from participant

6.1 Collecting blood samples with a single tube provides greater flexibility and convenience: blood samples can be shipped in the generic tube without further processing at the collection site, and single tube blood collection offers laboratories greater control over correct specimen handling.

6.2 **IGRA testing performed for IMPAACT/ACTG Network studies must follow the indirect blood collection method in Lithium Heparin tubes.** Lithium Heparin is the only acceptable anticoagulant. Other anticoagulants will interfere with the assay.

6.3 Lithium Heparin blood collection tube must be at room temperature (17°C to 25°C [62.6–77°F]) at the time of blood collection.

6.4 Appropriately label Lithium Heparin blood collection tube, including the time and date of collection.

6.5 Collect 5 mLs of whole blood into a single blood collection tube containing Lithium Heparin. While each of the 4 QFT-Plus tubes requires 1 mL of whole blood, the additional 1 mL of blood ensures there is adequate total blood volume when dispensing into the QFT-Plus tubes.

6.6 Evenly mix the Lithium Heparin tube by gently inverting several times to completely dissolve the heparin.

7 Lithium-Heparin Tube Room Temperature Storage and Transport

7.1 Blood collected in lithium-heparin tube must be maintained at room temperature (17-25°C) for no more than 6 hours from the time of collection prior to transfer into QFT-Plus tubes and subsequent incubation.

7.2 The following times must be recorded for each sample:

- Blood draw time
- The time at which the Lithium Heparin sample reaches the lab
- The time at which the QFT-Plus tubes are placed into the incubator

8 Specimen Transport

8.1 Specimens must be transported to the testing laboratory **as soon as possible and within 6 hours of collection**. It is strongly recommended that arrangements are made with your local courier when a blood draw is expected, to ensure pick-up and delivery of the sample to the laboratory for processing within the required 6 hours.

8.2 Sample must be maintained at room temperature during transport, not exceeding 25°C. If ambient temperatures exceed 25°C then transport of the sample at 2-8°C is acceptable using cooler boxes and ice packs.

9 Processing of Samples

9.1 Label the top and the side of each of the four QFT-Plus Blood Collection tubes. Make sure each tube (Nil, TB1, TB2 and Mitogen) is identifiable by its label or other means once the cap is removed.

9.2 Immediately before transferring blood from the lithium heparin tube into the QFT-Plus tubes, ensure that the Lithium Heparin blood is evenly mixed by gentle inversion.

9.3 QFT-Plus tube must be at room temperature (17°C to 25°C [62.6–77°F]) at the time blood is added to the tubes. Ensure aseptic technique and appropriate safety procedures are followed.

9.4 Dispense blood into tubes in the following order: Nil, TB1, TB2 and Mitogen.

9.5 Dispense blood one tube at a time by removing cap from a single QFT-Plus tube and adding 1 mL of blood. Replace the cap securely before moving on to the next tube and use a new pipette tip to dispense blood into each subsequent tube. Check that each tube has the appropriate color cap.

9.6 Ensure that the volume of blood dispensed in each QFT-Plus tube does not go outside the boundaries set by the black markings on the tube. If you are pipetting 1 mL accurately, the blood will reach to the middle black mark.

9.7 Immediately shake each tube 10 times, just firmly enough to make sure the entire inner surface of the tube is coated with blood. This process will dissolve antigens on the tube walls. **Important:** Over vigorous shaking may cause gel disruption and could lead to aberrant results. Each tube must be shaken in the same manner. Differential shaking of each tube may also lead to aberrant results.

9.8 Record the date and time that the blood is added to the QFT-Plus Blood Collection Tubes.

9.9 Aliquoted QFT-Plus Blood Collection Tubes must be placed **UPRIGHT in a 37°C incubator** immediately after blood transfer and mixing. The incubator does not require CO2 or humidification. *Do not incubate using heat blocks or water bath.*

9.10 Incubate the QFT-Plus Blood Collection Tubes for 20 hours ± 2 hours (18-22 hours). It is critical to adhere to this incubation time, therefore laboratory staff must schedule workflow accordingly.

Important Note: *Total time from blood draw to incubation in QFT-Plus Blood Collection Tubes must not exceed 6 hours.*

10 Harvesting and Storage of Plasma from QFT-Plus Tubes

10.1 After incubation, remove the tubes from the incubator and keep tubes within 2-25°C. Record the date and time that the tubes were removed from the incubator.

10.2 Centrifuge tubes for 15 minutes at 2000 to 3000 RCF (g). The gel plug will separate the cells from the plasma. If this does not occur, the tubes should be re-centrifuged.

10.3 Plasma samples should only be harvested using a pipette. Important: After centrifugation, avoid pipetting plasma 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.

10.4 Plasma samples can be loaded directly from centrifuged blood collection tubes into the QFT-Plus ELISA plate. If plasma samples are not tested immediately, samples must be aliquoted and stored at -80°C. For adequate test samples, harvest at least 150 µl of plasma.

11 IFN- γ ELISA Procedure

11.1 Important Reminders:

- Perform either automated or manual ELISA as per **FDA version of the QuantiFERON-TB Gold Plus (QFT-Plus) package insert**.
- All ELISA mixing steps must be performed using a **microplate shaker set at 500-1000rpm for 1 minute.**

11.2 Time required for performing the assay:

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

11.2.1 ELISA plate:

- Approximately 3 hours for one ELISA Plate
- <1 hour labor
- Add 10 to 15 minutes for each extra plate

11.3 All plasma samples and reagents, except for Conjugate 100x Concentrate, must be brought to room temperature (22°C ± 5°C [71.6°F ± 9°F]) before use. Allow at least 60 minutes for equilibration.

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

11.5 Allow at least 1 strip for the QFT-Plus standards and sufficient strips for the number of subjects being tested (refer to Section 11.14 for recommended plate format). After use, retain frame and lid for use with remaining strips.

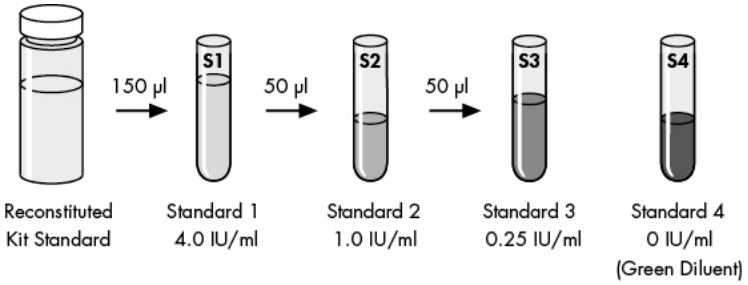
11.5.1 Reconstitute the IFN-γ Standard with the volume of deionized or distilled water indicated on the label of the vial. Mix gently to minimize frothing and ensure that the entire content of the vial is completely dissolved. Reconstitution of the IFN-γ standard to the correct volume will produce a solution with a concentration of 8.0 IU/ml.

Important note: The reconstitution volume of the kit standard will differ between batches.

11.5.2 Using the reconstituted standard, prepare a dilution series of 4 IFN-γ concentrations.

11.5.3 A standard curve should be generated with the following IFN-γ concentrations: S1 (Standard 1) contains 4.0 IU/ml, S2 (Standard 2) contains 1.0 IU/ml, S3 (Standard 3) contains 0.25 IU/ml, and S4 (Standard 4) contains 0 IU/ml (Green Diluent [GD] alone).

11.5.4 Preparation of Standard Curve dilution series:



Reference: QuantiFERON-TB Gold Plus (QFT-Plus) Package Insert. July 2018

11.5.4 The standards must be assayed at least in duplicate.

11.5.5 Prepare fresh dilutions of the kit standard for each ELISA session.

11.5.6 Example of procedure for duplicate standards:

1. Label 4 tubes: S1, S2, S3, S4
2. Add 150 µl of GD to S1, S2, S3, S4
3. Add 150 µl of the kit standard to S1 and mix thoroughly
4. Transfer 50 µl from S1 to S2 and mix thoroughly
5. Transfer 50 µl from S2 to S3 and mix thoroughly
6. GD alone serves as the zero standard (S4)

11.6 Reconstitute lyophilized Conjugate 100x Concentrate with 0.3 ml of deionized or distilled water. Mix gently to minimize frothing and ensure that the entire content of the vial is completely dissolved.

11.7 Working strength conjugate is prepared by diluting the required amount of reconstituted Conjugate 100x Concentrate in Green Diluent (Table 1).

11.8 Working strength conjugate must be used within 6 hours of preparation.

11.9 Return any unused Conjugate 100x Concentrate to 2°C to 8°C immediately after use.

11.10 Conjugate preparation (working strength):

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	12.5 ml
12	60 µl	12.0 ml

11.11 Sample Preparation:

11.11.1 For plasma samples harvested from blood collection tubes and subsequently stored (frozen at -80°C): thoroughly mix the stored sample before addition to the ELISA well.

11.11.2 For plasma samples added directly from the centrifuged QFT-Plus Blood Collection Tubes: any mixing of the plasma should be avoided. At all times take care not to disturb material on the surface of the gel.

11.12 Add 50 µl of freshly prepared working strength conjugate to each ELISA plate well.

11.13 Add 50 µl of test plasma sample to appropriate wells (refer to recommended ELISA plate layout in Section 11.14).

11.14 Finally, add 50 µl each of the Standards 1 to 4 to the appropriate plate wells (refer to recommended ELISA plate layout in Section 11.14). The standards should be assayed in at least duplicate.

11.15 Recommended ELISA plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 N	3 N	5 N	7 N	9 N	S 1	S 1	13 N	15 N	17 N	19 N	21 N
B	1 TB1	3 TB1	5 TB1	7 TB1	9 TB1	S2	S2	13 TB1	15 TB1	17 TB1	19 TB1	21 TB1
C	1 TB2	3 TB2	5 TB2	7 TB2	9 TB2	S3	S3	13 TB2	15 TB2	17 TB2	19 TB2	21 TB2
D	1 M	3 M	5 M	7 M	9 M	S4	S4	13 M	15 M	17 M	19 M	21 M
E	2 N	4 N	6 N	8 N	10 N	11 N	12 N	14 N	16 N	18 N	20 N	22 N
F	2 TB1	4 TB1	6 TB1	8 TB1	10 TB1	11 TB1	12 TB1	14 TB1	16 TB1	18 TB1	20 TB1	22 TB1
G	2 TB2	4 TB2	6 TB2	8 TB2	10 TB2	11 TB2	12 TB2	14 TB2	16 TB2	18 TB2	20 TB2	22 TB2
H	2 M	4 M	6 M	8 M	10 M	11 M	12 M	14 M	16 M	18 M	20 M	22 M

S1 (Standard 1), S2 (Standard 2), S3 (Standard 3), S4 (Standard 4). 1N (Sample 1. Nil Control plasma), 1 TB1 (Sample 1. TB1 plasma), 1 TB2 (Sample 1. TB2 plasma), 1M (Sample 1. Mitogen plasma).

11.16 Cover ELISA plate and mix the conjugate and plasma samples/standards thoroughly using a microplate shaker for **1 minute at 500 to 1000 rpm**. Avoid splashing.

11.17 Cover ELISA plate and incubate at room temperature (22°C ± 5°C [71.6°F ± 9°F]) for 120 ± 5 minutes. ELISA plate must not be exposed to direct sunlight during incubation. Deviation from specified temperature range and incubation time can lead to erroneous results.

11.18 During the ELISA plate incubation prepare working strength wash buffer. 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.

11.19 When ELISA plate incubation is complete, wash ELISA plate wells with 400 µl of working strength wash buffer. Perform wash step at least 6 times. An automated plate washer is recommended for safety reasons when handling plasma samples. Thorough washing is very important to the performance of the assay. Make sure 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.

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

11.21 Tap ELISA plate face down on absorbent (low-lint) towel to remove residual wash buffer.

11.22 Add 100 µl of Enzyme Substrate Solution to each plate well, cover plate with a lid and mix thoroughly for **1 minute at 500 to 1000 rpm** using a microplate shaker.

11.23 Cover ELISA plate and incubate at room temperature (22°C ± 5°C [71.6°F ± 9°F]) for 30 minutes. ELISA plate must not be exposed to direct sunlight during incubation.

11.24 Following the 30 minute incubation, add 50 µl of Enzyme Stopping Solution to each plate well in the same order as the substrate was added and mix thoroughly for 1 minute at 500 to 1000 rpm using a microplate shaker.

11.25 Measure the Optical Density (OD) of ELISA plate wells 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.

12 Calculations and Test Interpretation

12.1 Network laboratories are required to use the **Qiagen QFT-Plus Analysis Software** to analyze raw data and calculate results. The software is available at www.QuantiFERON.com. Please make sure that the most current version of the QFT-Plus Analysis Software is used.

12.2 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 “Interpretation of results”. The

standard curve is used to convert the Antigen OD responses to International Units (IU/ml). The software reports all concentrations greater than 10 IU/ml as ">10" as such values fall beyond the validated linear range of the ELISA.

12.3 IFN-γ values (in IU/ml) for the TB1, TB2 and Mitogen are corrected for background by subtracting the IU/ml value obtained for the respective Nil control. These corrected values are used for interpretation of the test results.

13 Quality control of the test

13.1 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.

13.2 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 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.
- 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.

13.3 The QFT-Plus Analysis Software calculates and reports these quality control parameters.

13.4 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.

13.5 Plasmas spiked with recombinant IFN-γ have shown reductions of up to 50% in concentration when stored at either 2°C to 8°C and –20°C. Recombinant IFN-γ is not recommended for establishing control standards.

14 Interpretation of Results

14.1 QFT-Plus results are interpreted using the following criteria:

Nil (IU/ml)	TB1 minus Nil (IU/ml)	TB2 minus Nil (IU/ml)	Mitogen minus Nil (IU/ml)*	QFT-Plus Result	Report/interpretation
≤8.0	≥0.35 and ≥25% of Nil	Any	Any	Positive†	*M. tuberculosis* infection likely
	Any	≥0.35 and ≥25% of Nil			
	<0.35 or ≥0.35 and <25% of Nil	<0.35 or ≥0.35 and <25% of Nil	≥0.50	Negative	*M. tuberculosis* infection NOT likely
	<0.35 or ≥0.35 and <25% of Nil	<0.35 or ≥0.35 and <25% of Nil	<0.50	Indeterminate‡	Likelihood of *M. tuberculosis* infection cannot be determined
>8.0§	Any				

** Responses to the Mitogen positive control (and occasionally TB Antigen) can be outside the range of the microplate reader. This has no impact on test results. Values >10 IU/ml are reported by the QFT-Plus software as >10 IU/ml.*

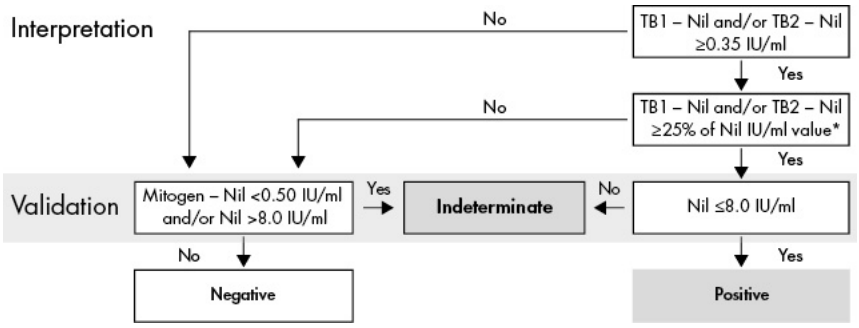
† Where M. tuberculosis infection is not suspected, initially positive results can be confirmed by retesting the original plasma samples in duplicate in the QFT-Plus ELISA. If repeat testing of one or both replicates is positive, the test result is considered positive.

‡ Refer to "Troubleshooting Guide", page 58 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.

14.2 The magnitude of the measured IFN-γ level cannot be correlated to stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease. A positive TB response in persons who are negative to Mitogen is rare, but has been seen in patients with TB disease. This indicates the IFN-γ response to TB antigens is greater than that to Mitogen, which is possible as the level of Mitogen does not maximally stimulate IFN-γ production by lymphocytes.

14.3 QFT-Plus Test Interpretation Flowchart:



**For TB1 minus Nil or TB2 minus Nil value to be valid, the ≥25% of Nil IU/ml value must be from the same tube as the original ≥0.35 IU/ml result.*

14.4 QFT-Plus Results of "Indeterminate" per the interpretation criteria should be repeated with the original samples. If the repeated result remains "Indeterminate", another blood sample may be requested within the study screening window as allowed by the study protocol.

15 Limitations

15.1 Results from QFT-Plus 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 TB Antigens may be outside the assay measurement range.

15.2 The performance of the USA format of the QFT-Plus test has not been extensively evaluated with specimens from the following groups of individuals:

- Individuals who have impaired or altered immune functions, such as those who have HIV infection or AIDS, those who have transplantation managed with immunosuppressive treatment or others who receive immunosuppressive drugs (e.g., corticosteroids, methotrexate, azathioprine, cancer chemotherapy), those who have other clinical conditions, such as diabetes, silicosis, chronic renal failure, and hematological disorders (e.g., leukemia and lymphomas), or those with other specific malignancies (e.g., carcinoma of the head or neck and lung)
- Individuals younger than age 17 years
- Pregnant women

15.3 The predictive value of a positive QFT-Plus result in diagnosing *M. tuberculosis* infection depends on the probability of infection, which is assessed by historical, epidemiological, diagnostic, and other findings.

15.4 A diagnosis of LTBI requires that tuberculosis disease must be excluded by medical evaluation including an assessment of current medical and diagnostic tests for disease as indicated.

15.5 A negative result must be considered with the individual's medical and historical data relevant to probability of *M. tuberculosis* infection and potential risk of progression to tuberculosis disease, particularly for individuals with impaired immune function. Negative predictive values are likely to be low for persons suspected to have *M. tuberculosis* disease and should not be relied on to exclude disease.

15.6 Unreliable or indeterminate results may occur due to:

- Deviations from the procedure described in the package insert
- Incorrect transport/handling of blood specimen
- Elevated levels of circulating IFN-γ or presence of heterophile antibodies
- Exceeding validated blood times from blood specimen draw to incubation:
- Blood samples collected directly into QFT-Plus Blood Collection Tubes stored longer than 16 hours at room temperature (17–25°C).
- Blood samples collected in lithium-heparin tube stored longer than 12 hours at room temperature (17–25°C) prior to transfer to QFT-Plus Blood Collection Tubes.
- Blood samples collected in lithium-heparin tube for refrigeration stored outside temperature and time ranges.

References

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2. Qiagen. QuantiFERON-TB Gold Plus (QFT-Plus) Package Insert (USA Version). July 2018.
3. Qiagen. QuantiFERON-TB Gold Plus (QFT-Plus) Package Insert (CE-marked). February 2015.