YALE-NEW HAVEN HOSPITAL	TITLE: QuantiFERON®-TB Gold ELISA on the DSX Soft Code: QFTG		DEPT OF LAB MEDICINE Policy and Procedure Manual DOCUMENT # IMM 203 Page 1 of 15
WRITTEN BY: Katelyn Marreiros, MLS (ASCP) Penny Smith, MT (ASCP)	**EFFECTIVE DATE:** February 7, 2013	**REVISION:** NEW	**SUPERCEDES:** NEW

I. Intended Use

QuantiFERON®-TB Gold In-Tube (QFTG) is an *in vitro* diagnostic test using a peptide cocktail simulating ESAT-6, CFP-10 and TB7.7(p4) proteins to stimulate cells in heparinized whole blood. Detection of interferon-γ (IFN-γ) by Enzyme-Linked Immunosorbent Assay (ELISA) is used to identify *in vitro* responses to these peptide antigens that are associated with *Mycobacterium tuberculosis* infection. QFTG 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.

II. Introduction

Tuberculosis is a communicable disease caused by infection with *M. tuberculosis* complex organisms, which typically spreads to new hosts via airborne droplet nuclei from patients with respiratory tuberculosis disease. A newly infected individual can become ill from tuberculosis within weeks to months, or can remain latently infected for years. Latent tuberculosis infection (LTBI), a non-communicable asymptomatic condition, persists in some, who might develop tuberculosis disease months or years later. The main purpose of diagnosing LTBI is to consider medical treatment for preventing tuberculosis disease. LTBI must be distinguished from tuberculosis disease, a reportable condition which usually involves the lungs and lower respiratory tract, although other organ systems may be affected. Tuberculosis disease is diagnosed from historical, physical, radiological, histological, and mycobacteriological findings.

Until recently the tuberculin skin test (TST) was the only available method for diagnosing LTBI. Cutaneous sensitivity to tuberculin develops from 2 to 10 weeks after infection. However, some infected individuals, including those with a wide range of conditions hindering immune functions, but also others without these conditions, do not respond to tuberculin. Conversely, some individuals who are unlikely to have *M. tuberculosis* infection exhibit sensitivity to tuberculin and have positive TST results after vaccination with bacille Calmette-Guérin (BCG), infection with mycobacteria other than *M. tuberculosis* complex, or undetermined other factors. The tuberculin skin test and QFTG are helpful but insufficient for diagnosing *M. tuberculosis* complex infection in sick patients: a positive result can support the diagnosis of tuberculosis disease; however, infections by other mycobacteria (e.g., *M. kansasii*) could also cause positive results. Other medical and diagnostic evaluations are necessary to confirm or exclude tuberculosis disease

III. Principles of the Procedure

QFTG 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 bacille Calmette-Guérin (BCG) strains and from most non-tuberculosis mycobacteria with the exception of *M. kansasii, M. szulgai* and *M. marinum.* Individuals infected with *M. tuberculosis* complex organisms (*M. tuberculosis, M. bovis, M. africanum, M. microti, M. canetti)* usually have lymphocytes in their blood that recognize these and other mycobacterial antigens. This recognition process involves the generation and secretion of the cytokine, IFN-γ. The detection and subsequent quantification of IFN-γ forms the basis of this test.

The test is performed in two stages. First, whole blood is collected into each of the QFTG blood collection tubes, which include a Nil Control tube, TB Antigen tube and a Mitogen tube. The tubes are shaken to mix antigen with the blood and should be incubated at 37°C ± 1° as soon as possible. Following a 16 to 24 hour incubation period, the tubes are centrifuged, the plasma is removed and the amount of IFN-γ (IU/mL) is measured by ELISA. The QFTG ELISA uses a recombinant human IFN-γ standard, which has been assayed against a reference IFN-γ preparation. Results for test samples are reported in International Units (IU) relative to a standard curve prepared by testing dilutions of the secondary standard supplied with the kit.

A test is considered positive for an IFN-γ response to the TB Antigen tube that is significantly above the Nil IFN-γ IU/mL value. The Nil sample adjusts for background, heterophile antibody effects, or non-specific IFN-γ in blood samples. The IFN-γ level of the Nil tube is subtracted from the IFN-γ level for the TB antigen tube and Mitogen tube. The Mitogen-stimulated plasma sample serves as an IFN-γ positive control for each specimen tested. A low response to Mitogen (<0.5 IU/mL) indicates an Indeterminate result when a blood sample also has a negative response to the TB antigens. This pattern may occur with insufficient lymphocytes, reduced lymphocyte activity due to prolonged specimen transport or improper specimen handling, including filling/mixing of blood tubes, or inability of the patient's lymphocytes to generate IFN-γ. Elevated levels of IFN-γ in the Nil sample may occur with the presence of heterophile antibodies, or to intrinsic IFN-γ secretion.

IV. Specimen Collection:

The QFTG system uses specialized blood collection tubes, which are used to collect whole blood via venipuncture, that contain antigens representing certain *M. tuberculosis* proteins or controls. Incubation of the blood occurs in the tubes for 16 to 24 hours, after which, plasma is harvested and tested for the presence of IFN-γ produced in response to the peptide antigens.

Collection: Collection tubes must be stored at 4°C to 25°C

1. Accession samples, **using test code QFTG**. Make sure to **enter collection time** as this test involves time sensitive processing.'

2. Collect 1 ml of patient blood into **each of 3 specialized** blood collection tubes, Grey cap (nil control), Purple cap (mitogen control tube) and Red cap (TB antigen tube**). Fill each tube to black mark (1ml). Tubes may fill slowly.**

3. If a butterfly is used, collect a purge tube first to ensure the tubing is filled with blood prior to the QFT tubes being issued.

4. Immediately after filling tubes, shake them 10 times just firmly enough to ensure the entire inner surface of the tube is coated with blood, to dissolve antigens on tube walls.
    a. Tube temperature should be between 17- 25°C (63 - 77°F) at the time of blood tube filling.
    b. **Overly vigorous shaking may cause gel disruption and could lead to aberrant results.**
    c. Label all 3 tubes appropriately. Record collection time.

5. Transport the sample at Room Temperature (17- 25°C). **Do Not Refrigerate or Freeze.**

Immunology Processing

1. Arrive the tubes in SOFT at the Immunology Aliquot tracking stop. Three labels will print out. Put the appropriate label on the corresponding QFTG tubes.
2. **Do Not Refrigerate tubes until after incubation.**
3. **Tubes must be incubated at 37°C within 16 hours of collection**.
    a. **Mix the tubes 10 times by gentle inversion.**
    b. Place in the **dry** incubator for **16-24 hours** in an **upright** position**.** Record time placed in the incubator on the Quantiferon Log. Keep in mind Immunology staffing and work hours when placing in the incubator. **Specimens must be removed from the incubator within a 24-hour time period.**

4. Check the Quantiferon Log daily (AM) for any samples that have been previously incubated between the 16-24 hour time frame. **Make sure to remove specimens no earlier then 16 hours and no later than 24-hours.**

5. **Spin samples at 2000-3000 rpm for 15 minutes**. The collection tubes contain a gel plug that separates the plasma from the cells. **If the plug does not move**, the tubes should be recentrifuged at a higher speed (3500 rpm).

6. **After centrifugation, do not aliquot. The samples are to be placed in the refrigerator for processing. Do not freeze. Stability at 2-8°C is 4 weeks.**

Note: If needed, the samples can be aliquotted and frozen at or below -20°C for up to 4 months but this is not routine practice.

V. Materials

A. Blood Collection Tubes

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

Can be used up until the expiration date

B. Reagents Supplied with Kit

1. Microplate strips coated with murine anti-human IFN-γ monoclonal antibody (2 x 96 well plates). Stable until the kit expiration date. Stored at 2-8°C.
2. Human IFN-γ Standard, lyophilized *(contains recombinant human IFN-γ, bovine casein, 0.01 % w/v Thimerosal)* (1 vial, 8 IU/mL when reconstituted). Unreconstituted standard is stable until the kit expiration date. Stored at 2-8°C.
   Preparation:
   Reconstitute with CLRW as indicated on the label. Mix gently. Note the date the **Kit Standard** was reconstituted.
   Note: The reconstitution volume will differ between lot numbers.
   Stability:
   The reconstituted **Kit Standard** may be kept for up to 3 months if stored at 2°C to 8°C.
   Note: The DSX will automatically create a standard curve that will be used to analyze the patient results and serve as quality control. The first two strips of the plate will represent Standards 1-8 in duplicate.
3. Green Diluent *(contains bovine casein, normal mouse serum, 0.01% w/v Thimerosal)* (1 x 30 mL bottle). Ready for use. Stable until the kit expiration date. Stored at 2-8°C.
4. Conjugate **100X** Concentrate, lyophilized *(murine anti-human IFN-γ HRP, contains 0.01% w/v Thimerosal)* (1vial 0.3mL when reconstituted).
   Preparation:
   Reconstitute freeze dried Conjugate 100X Concentrate with **0.3mL of CLRW water**. Mix gently to minimize frothing and ensure complete solubilization of the Conjugate. *Note the date the **100X Conjugate** was reconstituted.*
   Stability:
   The reconstituted 100X Conjugate must be returned immediately to storage at 2°C to 8°C and is stable for 3 months. *Note the date the **100X Conjugate** was reconstituted.*
   Working conjugate:
   Prepare when prompted by the DSX by diluting the required amount of reconstituted Conjugate 100X Concentrate in Green Diluent as set out in Table 1 (see DSX Preparation). Working conjugate is prepared fresh and must be used within 6 hours.
5. Wash Buffer 20X Concentrate *(pH 7.2, contains 0.01 % w/v Thimerosal)* (1x 100mL bottle).

Stable until the kit expiration date. Stored at 2-8°C.

Preparation of working wash buffer:

The amount of wash solution required is dependent on the number of patient specimens to be run.

Add 50 mL of Wash Buffer to 950 mL of CLRW (Total Volume 1000mL)

Add 100 mL of Wash Buffer to 1900 mL of CLRW (Total Volume 2000mL)

Stability of Working Wash Buffer:

2 weeks when stored at Room Temperature.

6. Enzyme Substrate Solution *(contains H_2O_2, 3,3',5,5' Tetramethylbenzidine)* (1 x 30 mL bottle). Ready for use. Stable until the kit expiration date. Stored at 2-8°C.
7. Enzyme Stopping Solution (*contains 0.5M H_2SO_4*) (1 x 15 mL bottle)
   Ready for use. Stable until the kit expiration date. Stored at 2-8°C.

C. Materials required, but not provided

1. DSX (DYNEX Technologies)
2. DSX Reagent Tubes (INOVA)
3. Control/Calibrator Vials (INOVA)
4. Microdilution Tubes (INOVA)
5. DSX Reagent Tips (INOVA)
6. DSX Sample Tips (INOVA)
7. CLRW (Clinical Lab Reagent Water)

D. Quality Control

1. Pooled Nil, Antigen and Mitogen are to be run with every patient run.
   - Nil and Mitogen: pooled patient samples.
   - Antigen: Patient Mitogen diluted 1:20 with heparinized plasma.

   Stability

   4 months at -20°C.

VI. Assay Procedure

A. Assay Preparation:

1. The assay for one plate takes approximately 3 hours to complete. Add 10 to 15 minutes for each additional plate
2. Call a pending report by **Test** QFTG. Build a Tasklist by **Template** QFTG to define sample order.
3. 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.
4. The specimens are to be loaded in the collection tubes. After the samples are brought to room temperature, **spin at 3000 rpm for at least 5 minutes** to avoid extraneous particulate material from being aliquotted onto the DSX.

B. DSX Preparation

1. A DSX **worklist** must be created before the QFTG test can be run on samples. To create a worklist refer to the Soft Immunology Manual (IMM120). Choose DSX QFTG from the test menu. For general DSX operation, refer to DSX Automatic ELISA System (Imm17).
2. Load the tubes in the order listed below. The DSX will give an error message for the duplicate barcodes. Right click on the mouse over the highlighted ID and select manual entry. Change the last 2 digits as follows:
   Grey- Leave the Soft label extension (**1K**)
   Red- Change extension to **1R**
   Purple- Change extension to **1P**
3. When prompted by the DSX, remove strips that are not required from the frame, reseal in the foil pouch, and return to the refrigerator for storage until required.
4. Load the Standard and kit reagents as prompted.
   S8 is Green Diluent
   S1 is the reconstituted Human IFN-γ Standard.
   See below for working conjugate preparation:

TABLE 1. Working Conjugate Preparation

NUMBER OF STRIPS	VOLUME OF CONJUGATE 100X CONCENTRATE	VOLUME OF GREEN DILUENT
3	20 µL	2.0 mL
4	25 µL	2.5 mL
5	30 µL	3.0 mL
6	35 µL	3.5 mL
7	40 µL	4.0 mL
8	45 µL	4.5 mL
9	50 µL	5.0 mL
10	55 µL	5.5 mL
11	60 µL	6.0 mL
12	65 µL	6.5 mL

Working strength conjugate should be used within 6 hours of preparation.

5. When prompted add the required volume of Wash Buffer to position C.
   It is acceptable to combine wash buffer prepared on different dates as long as the original expiration date is used and the lot numbers are the same.

VII. Calculation of Results

QFTG Analysis Software is used to analyze raw data and calculate results. The software performs a Quality Control assessment of the assay, generates a standard curve and provides a test result for each subject, as detailed in the Interpretation of Results section. The software reports all concentrations greater than 10 IU/mL as ">10" as such values fall beyond the validated linear range of the ELISA.

1) Minimize the DSX Software to display the desktop.

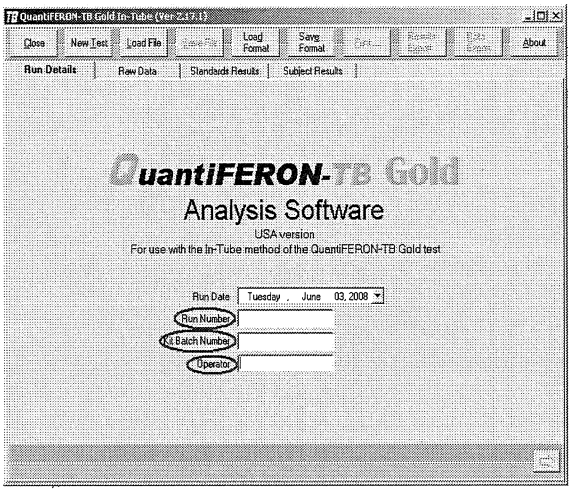
2) Double click the  icon on the desktop.

3) In the window that opens, select the name of the file that matches the name of the plate.

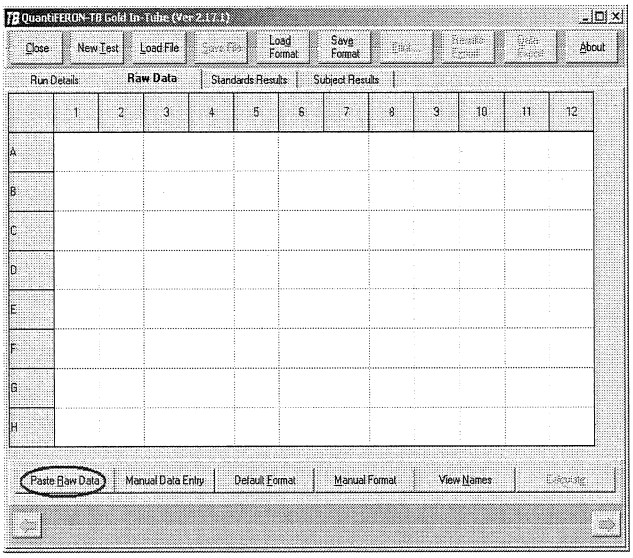
4) Another screen will open. Highlight the data, right click and select **Copy**

5) Double click on the  icon to open the TB-Gold software.

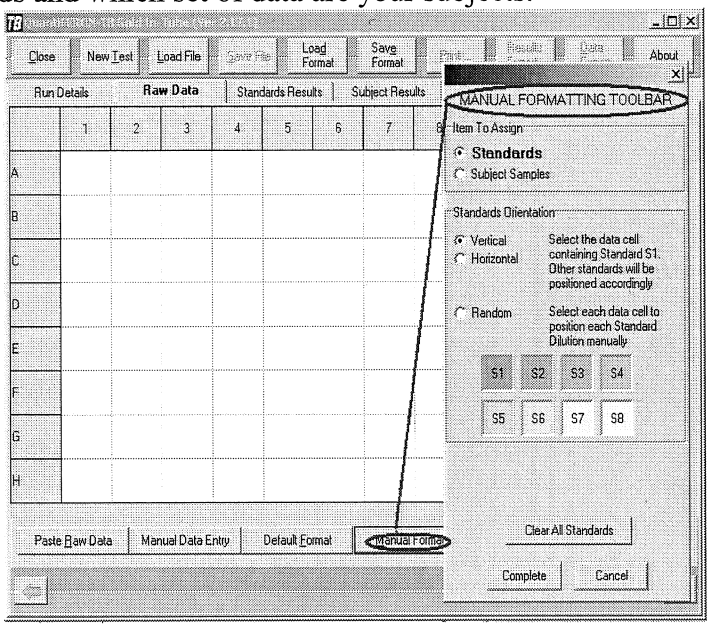
6) When opened, put in QFTG for Run Number, the kit lot and expiration date in Kit Batch Number and your initials in Operator.



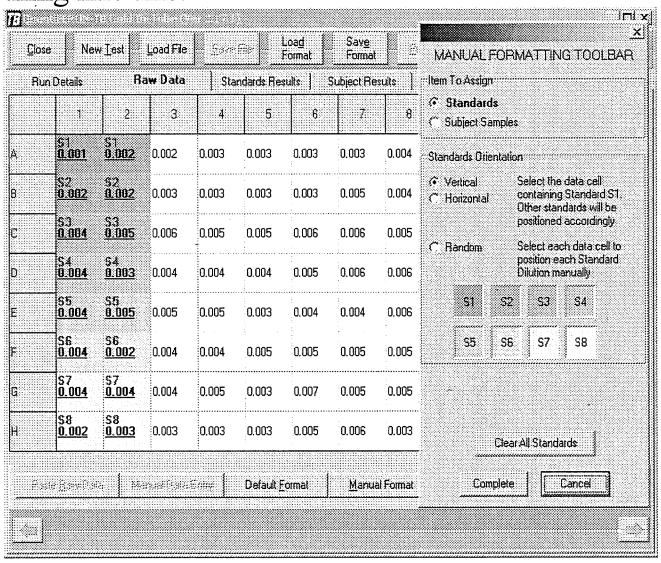
7) Click on **Raw Data** to open up the table to Paste the data from the Excel spreadsheet. Click on **Paste Raw Data** in the bottom left corner.



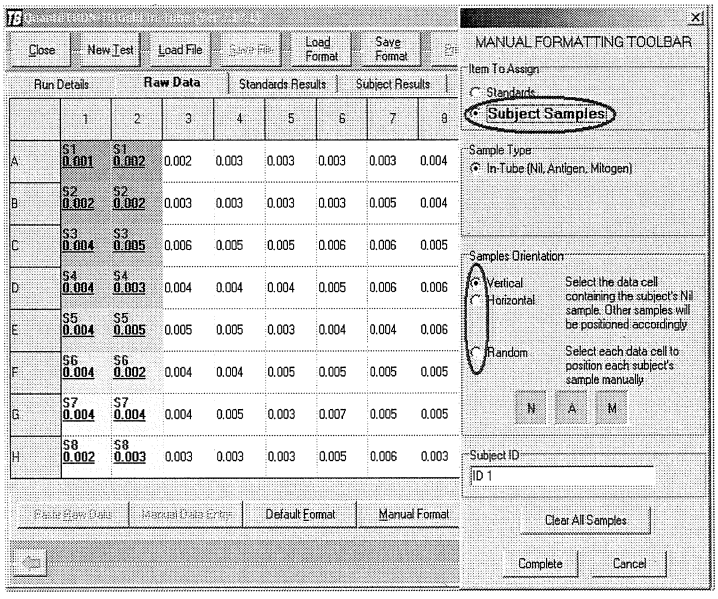
8) Click on the **Manual Format** button at the bottom of the window. Another window called the **Manual Formatting Toolbar** will open up. With this window, you will designate which set of data are your standards and which set of data are your subjects.



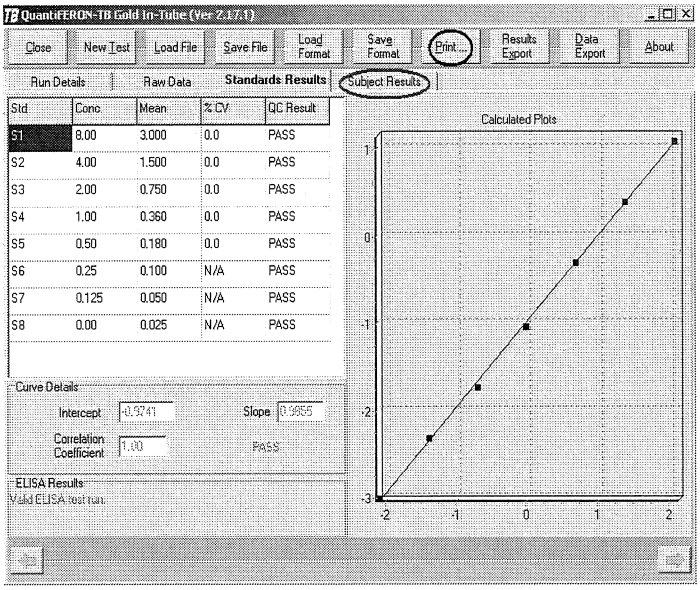
For Standards, keep in vertical format and click on the first two rows with your mouse. Your table should look something like this:



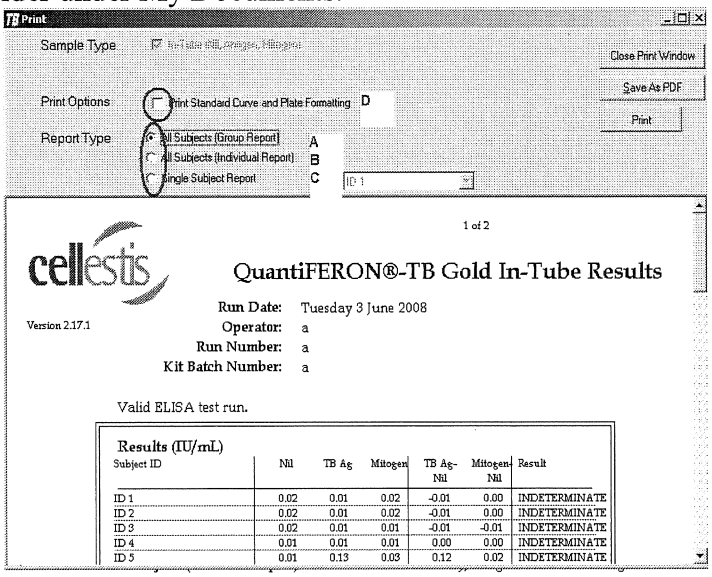
At this point, you will now designate your samples. Keep your Sample Orientation as vertical. In the subject ID box, type in the patient's order number in place of ID 1. Then click on A3 to designate their set of tubes. (A3-C3 should turn a color). When ID 2 appears in the Subject ID box, type in the second patient's ID number. Then click D3 to designate their set of three tubes. Continue until your plate data is completed. When finished, press the Complete button followed by the Calculate button.



9) The Standard Results page should resemble what is below. This shows the results of the standard curve and the QC for the ELISA. The QC results will tell you pass or fail. From here, the **Subject Results** can also be viewed.



10) To **Print**, click on the tab in the center of the window. Check off the box that says "Print Standard curve and plate formatting". For report type, select **All Subjects (Group Report)**. This prints all the results together on the first page; on the second page, prints the standard curve and the raw OD values of all patients. Save the data as a PDF with the date of the run in the QuantiFERON PDF folder under My Documents.



Any positive patients need to be repeated! RESULTS NEED TO BE ENTERED INTO SOFT AND QC INTO FOLDER ON THE L DRIVE!

VIII. Quality Control

A. Internal Controls

1. The mean OD value for Standard 1 must be ≥ **1.200**.
2. The % coefficient of variation (%CV) between replicates for Standards 1 to 5 must be ≤ **15%.**
3. Replicate OD values for Standards 6 to 8 must not vary by more than **0.040** optical density units from their mean.
4. The correlation coefficient (r) calculated from the mean absorbance values of the standards must be ≥ **0.98.**
5. The mean OD value for Standard 8 (Green Diluent) should be ≤ **0.150**. If the mean OD value is > 0.150 the plate washing procedure should be investigated.

The QFTG Analysis Software calculates and reports these quality control parameters. If the above criteria are not met, the run is invalid and must be repeated.

B. External Controls

1. The external TB-Nil value must be > 1.00 IU/mL
2. The external Nil value must be < 0.50 IU/mL
3. The external Mitogen value must be >5.00 IU/mL

IX. Interpretation and Reporting of Results

A. Interpretation

QFTG results are interpreted using the following criteria:

Table 2. Interpretation of Results

	NIL (IU/mL)	TB Ag – NIL (IU/mL)	Mitogen – NIL (IU/mL)
Positive	≤ 8.0	≥ 0.35 and ≥ 25% of NIL	Any #
Negative	≤ 8.0	< 0.35 OR ≥ 0.35 and < 25% of NIL	≥ 0.5
Indeterminate	≤ 8.0	< 0.35 OR ≥ 0.35 and < 25% of NIL	< 0.5
Indeterminate	>8.0	Any #	Any #

All positive samples are to be repeated on the next run. If the repeated test result does not agree with the initial result, repeat a third time. To be acceptable two out of the three results must agree.

B. Reporting of results

1. Numerical results are manually entered into SOFT by Tasklist.
2. Indeterminate Results are entered as Indeterminate
3. Results greater than 10 IU/mL are reported as >10.00 IU/mL.
4. Results less than 0.1 IU/mL are reported as <0.10 IU/mL.
5. Have a second technologist check the results and sign off.
6. Keep a hard copy of results in the Quantiferon binder in addition to scanning all documents onto the O Drive in the Immunology Folder under "QFTG Runs."

The following comment will be included on all results:
INTERPRETIVE INFORMATION:

0.34 IU/mL or less: M. tuberculosis infection unlikely, but cannot be completely excluded.

0.35 IU/mL or greater: M. tuberculosis infection (latent) or disease (active) likely.

Indeterminate: Results are Indeterminate for TB Antigen responsiveness. Indeterminate results may be caused by insufficient lymphocytes or inability of the patient's lymphocytes to generate IFN-γ, reduced lymphocyte activity due to prolonged specimen transport or improper specimen handling, the presence of heterophile antibodies, or to intrinsic IFN-γ secretion.

This is a qualitative test. The TB antigen IU/mL value should not be used to monitor disease progression or response to therapy. Diagnosing or excluding tuberculosis disease, and assessing the probability of latent TB, requires a combination of epidemiological, historical, clinical, and diagnostic findings that should be taken into account when interpreting QuantiFERON-TB results. An overall Negative result does not completely rule out TB infection. An overall Positive result does not differentiate active from latent tuberculosis The TB antigen IU/mL value is required for documentation on certain government reporting forms (e.g., Form I-693), but this value should not be used to monitor disease progression or response to therapy.

X. Reference Range

<0.35 IU/mL

XI. Analytic Measuring Range (AMR)

0.10 to 10 IU/ML

XII. Limitations of the Procedure

- Results from QFTG testing must be used in conjunction with each individual's epidemiological history, current medical status, and results of other diagnostic evaluations.
- Individuals with Nil values greater than 8 IU/mL are classed as "Indeterminate" because a 25% higher response to TB Antigens may be outside the assay measurement range.
- The predictive value of a positive QFTG result in diagnosing *M. tuberculosis* infection depends on the probability of infection, which is assessed by historical, epidemiological, diagnostic, and other findings.
- A diagnosis of LTBI requires that tuberculosis disease must be excluded by medical evaluation including an assessment of current medical and diagnostic tests for disease as indicated.
- A negative result must be considered with the individual's medical and historical data relevant to probability of *M. tuberculosis* infection and potential risk of progression to tuberculosis disease, particularly for individuals with impaired immune function. Negative predictive values are likely to be low for persons suspected to have *M. tuberculosis* disease and should not be relied on to exclude disease.
- Unreliable or indeterminate results may occur due to:
  - Deviations from the procedure described in the Package Insert,
  - Incorrect transport / handling of blood specimen,
  - Excessive levels of circulating IFN-γ or presence of heterophile antibodies,
  - Longer than 16 hours from blood specimen drawing to incubation at 37°C ± 1°C.
- The predictive value of a negative QFTG result in immunosuppressed persons has not been determined.
- The performance of the USA format of the QFTG test has not been extensively evaluated with specimens from the following groups of individuals:
  a. Individuals who have impaired or altered immune function such as those who have HIV infection or AIDS, those who have transplantation managed with immunosuppressive treatment or others who receive immunosuppressive drugs (e.g. corticosteroids, methotrexate, azathioprine, cancer chemotherapy), and those who have other clinical conditions: diabetes, silicosis, chronic renal failure, hematological disorders (e.g., leukemia and lymphomas), and other specific malignancies (e.g., carcinoma of the head or neck and lung).
  b. Individuals younger than age 17 years
  c. Pregnant women

XIII. Assay Validation

Correlation:

Correlation was performed by comparing the results of 30 samples previously run at Clinical Laboratory Partners (CLP) by the same ELISA method.

Negative Agreement was 100% (15 of 15).
Positive Agreement was 100% (15 of 15).
Overall Agreement was100% (30 of 30).

Intra-run Precision:

Intra-assay performance was evaluated by testing of 2 specimens, a Negative and a Strong Positive, 4 times on a single run. All results were comparable to that of the manufactures precision and reproducibility studies.

Negative: CV % N/A (Values were 0, 0, 0 and 0.01)
Strong Pos: CV 5.0%

Inter-run Precision:

Inter-assay performance was evaluated by testing of 2 specimens, a Negative and a Strong Positive on 4 different runs. All results were comparable to that of the manufactures precision and reproducibility studies.

Negative: CV % N/A (Values were 0, 0.01, 0.01 and 0.11)
Strong Pos: CV 9.9%

Reference Range Verification:

The reference range, suggested by the manufacturer, of <0.35 IU/mL, was verified by running 23 patient samples from our YNHH population. Of the 20 samples run, all were negative and fell within the <0.35 IU/mL range. These results are within allowable error for reference range verification.

Cap Survey Results:

Currently there are no CAP proficiency surveys available for this assay. Future proficiency testing will take place by comparing results from reference laboratories in which this assay is performed.

XV. Ordering Information

Description	Catalog Number	Price
Quantiferon-TB Gold In-Tube Multipack Kit: Includes 2 x cat # 0590-0301 (200 Nil, TB Antigen, and Mitogen blood collection tubes) and 3 x cat # 0594-0201 (Elisa Kits) Runs up to 174 samples with 26 additional sets of tubes.	0599-0401	$4,100.00
Items sold seperately		
Quantiferon-TB Gold ELISA: Runs up to 58 Samples	0594-0201	$670.00
Quantiferon-TB Gold Tubes: Nil, TB Antigen and Mitogen blood collection tubes. 100 each	T0590-0301	$1,045.00
Quantiferon-TB Gold Unitized Dispenser Pack:Nil, TB Antigen and Mitogen blood collection tubes packaged in a dispenser. 4 dispensers x 25 ct. single patient packs	T0597-0403	$1,045.00
Quantiferon-TB Gold Dispenser Pack:1 dispenser x 25 ct. single patient packs	0597-0401	$400.00
Quantiferon-TB Gold Reference Lab Pack: contains the components equivalent to 10 ELISA kits in one convenient package for less packaging material and lower shipping rates.	0594-0501	$6,700.00
Quantiferon-TB Gold Human IFN γ Standard: 1 vial of standard	2139-0420	$95.00

XIV. References

Quantiferon-TB Gold Package Insert, US05990301 Edition, July 2011. Cellestis, Inc., Valencia, CA.

XVI. Appendix

203-A	QuantiFERON®-TB Gold ELISA on the DSX Quiz
203-B	QuantiFERON®-TB Gold ELISA on the DSX Checklist
203-C	QuantiFERON®-TB Gold ELISA on the DSX Quick Reference Guide (QRG)
203-Q	QuantiFERON®-TB Gold ELISA on the DSX Quality control Chart

Quantiferon Gold TB
Doc# IMM 203-B
Training Checklist
Initial ☐ 6 Months ☐

Trainee_____ Training Date(s)_____

	YES/NO
1. Knows proper specimen requirements and sample processing	_____
2. Knows proper specimen handling and incubation	_____
3. Read the procedure and understands test principle	_____
4. Can properly set up worklist on DSX and perform a run	_____
5. Is familiar with kit components and reagent storage and preparation	_____
6. Can navigate software for result analysis	_____
7. Knows how interpret results: negative, positive or indeterminate	_____
8. Passed the training quiz	_____

Training Completed _____

Signature _____ Verified By _____
Learning Technologist Teaching Technologist

Quick Reference Guide: QuantiFERON®-TB Gold ELISA on the DSX

Doc# IMM 203-C

Quality Control:

ELISA Controls

- The mean OD value for Standard 1 must be ≥ **1.200**.
- The % coefficient of variation (%CV) between replicates for Standards 1 to 5 must be **≤ 15%.**
- Replicate OD values for Standards 6 to 8 must not vary by more than **0.040** optical density units from their mean.
- The correlation coefficient (r) calculated from the mean absorbance values of the standards must be ≥ **0.98.**
- The 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.

External Controls

- The external TB-Nil value must be > 1.00 IU/mL
- The external Nil value must be < 0.50 IU/mL
- The external Mitogen value must be >5.00 IU/mL

Interpretation of Results:

	NIL	**TB Ag – NIL**	**Mitogen – NIL**
Positive	≤ 8.0	≥ 0.35 and ≥ 25% of NIL	Any #
Negative	≤ 8.0	< 0.35 OR ≥ 0.35 and < 25% of NIL	≥ 0.5
Indeterminate	≤ 8.0	< 0.35 OR ≥ 0.35 and < 25% of NIL	< 0.5
Indeterminate	>8.0	Any #	Any #

Quick Reference Guide: QuantiFERON®-TB Gold ELISA on the DSX

Doc# IMM 203-C

Working Conjugate Preparation

NUMBER OF STRIPS	VOLUME OF CONJUGATE 100X CONCENTRATE	VOLUME OF GREEN DILUENT
3	20 µL	2.0 mL
4	25 µL	2.5 mL
5	30 µL	3.0 mL
6	35 µL	3.5 mL
7	40 µL	4.0 mL
8	45 µL	4.5 mL
9	50 µL	5.0 mL
10	55 µL	5.5 mL
11	60 µL	6.0 mL
12	65 µL	6.5 mL

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February 7, 2013

Signature Approval for Annual Review
Name: QuantiFERON®-TB Gold ELISA on the DSX
Siemens BNII Nephlometer

Document #: IMM 203

Name (Print)	Title	Signature	Date of Review	Revision Page and Section # (Use Procedure Review Log to document staff review)	Issue Date for Training if Applicable	Effective Date for Use
TEODORICO LEE	LAB MANAGER	Teodorico Lee	2/7/13	NEW		2/7/13
BRIAN SMITH	LAB DIRECTOR	B Smith	2/7/13	NEW		2/7/13