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Document Title: XVII. QuantiFERON-TB Gold In-Tube Test

Author	Effective Date	Supersedes Procedure #
Debra Jesien	11/11/2008	None

Reviewed/Revised by	Date Reviewed/Revised	Effective Date
Debra Jesien (Revised)	05/09/13	

Approval Signature	Approval Date
Dwight J. Hardy, PhD	5/30/2013

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AFB Lab	1	File	1

CHANGE CONTROL FORM

Document Title: XVII. QuantiFERON TB Gold In-Tube Test

Document Number: SH.CP.MC.AFB.0017.000 Version: from 5 To 6

Submitted By: Debra Jesien Date: 05/09/13

1. Check one:

New procedure ☐	New process ☐	New form ☐	New flow chart ☐
Revised procedure X	Revised process ☐	Revised form ☐	Revised flow chart ☐
New job aid ☐	New labels ☐		
Revised job aid☐	Revised label ☐		

Brief description of change:

1) Medical staff from within the SMH should be instructed to obtain QFT collection kits from 6th floor Hospital Stores. (x58211) A small supply of kits will be maintained in the AFB lab if for some reason the tubes are unavailable.
2) Medical staff from HH should be instructed to obtain QFT collection kits from HH Hospital Stores. (x16341) Collection kits may be sent/received via the HH courier.
3) QuantiFeron collection can be performed at 2 additional collection stations: Anthony Jordan Health Center and 777 S. Clinton Ave.
4) The AFB tech is responsible for checking that the volume in each QFT tube is sufficient. (Use the "Blank" provided at bench tube as a reference.) Volume of 0.8 -1.2 cc is critical for red and purple tubes. If volume in these tubes is not sufficient the test must be cancelled.
5) Green diluent, Conjugate and strips are interchangeable between kits of the same lot. All other reagents are interchangeable regardless of the lot.
6) Instructions for QFT testing based on a 4 point Standard curve, rather than the 8 point Standard curve previously used.
7) Procedure has been expanded to include instructions for running multiple specimens requiring >1 plate.
8) Examples of positive, negative, indeterminate and low-level positive (if repeat positive) reports have been included.

S. O. P. validation needed? (Circle one) (NO) YES If yes, attach validation sheet

2. Process validation needed? (Circle one) (NO) YES If yes, attach process validation documentation

3. Associated procedure and other documents (list those that need to be written or revised): _____

4. Table of Content (TOC) update needed? (Circle one) NO YES If yes, attach Updated TOC

Author: Debra Jesien Date: 11/11/08
Revised by: Debra Jesien Date: 5/9/13
Approved by: Dwight E. Hardy, PhD Date: 5/30/2013

QuantiFERON®-TB Gold In-Tube Test

I. Principles of the Procedure

The immune response to infection with *M. tuberculosis* is predominantly a Cell Mediated Immune (CMI) response that results in sensitization of T-cell lymphocytes specific to *M. tuberculosis* complex antigens. (*M. tuberculosis, M. bovis, M. africanum, M. microti, M. canetti*) Gamma interferon is a protein that is produced by sensitized T-cells upon stimulation with their specific antigen. These TB specific proteins, which are secreted by *M. tuberculosis* complex, stimulate a robust and detectable CMI response in infected people. They have been demonstrated to be specific for *M. tuberculosis* infection, and are unaffected by BCG vaccination and most non-tuberculous mycobacteria.

Using a standard ELISA format, the QuantiFERON®-TB Gold In-Tube (QFT-In Tube) assay detects CMI responses to tuberculosis infection by measuring gamma interferon produced in whole blood after incubation with synthetic peptides of the *M. tuberculosis* antigens.

Each QFT-In Tube result and its interpretation should be considered in conjunction with other epidemiological, historical, physical and diagnostic findings.

II. General Information

A. LIS Test Procedure Code: TBAG

B. Intended Use of QuantiFERON

1. Annual screening of HIV patients from SMH ID Clinic
2. The CDC[2] recommends testing for:

   a. patients who cannot be relied upon to return for reading of a Tuberculin Skin Test
   b. patients who have received BCG vaccine
   c. This test is NOT recommended for patients <5 years of age.

3. QFT-In Tube testing may also be useful in contact investigations, TB screening of health care workers, and in other instances in which the tuberculin skin test (TST) is currently used.

NOTE: The test is not intended for use in diagnosis of active disease, but should be used in the diagnosis of latent *Mycobacterium tuberculosis* infection. Persons with latent infections do not have active disease; they are asymptomatic and are not infectious. About 10 percent of those with latent tuberculosis infection will eventually develop TB

disease. The risk of developing disease is highest in those who are immunocompromised, especially those with HIV infection.

C. QFT Blood Collection Kits:
Antigens are dried onto the inner wall of tubes. Store at tubes at 4°C to 25°C.

Nil Control (Grey cap)
TB Antigen (Red cap)
Mitogen Control (Purple cap)
Special Collection Instruction Sheet

D. Availability of Test – ELISA portion of the test will be performed once per week, generally on Wednesday

1. FF Thompson Hospital (FFT)

- QuantiFERON specimens may come to us from FFT Hospital via courier Monday- Saturday, day or evening shift.
- These samples have already been incubated and centrifuged
- Leave the tubes in the bag. Place bag in AFB Bucket in the Set-up refrigerator.

2. ED and SMH Patients

- The test is orderable in e-records from "Facilities List". (Procedure code: TBAG)
- **Medical staff from within the SMH should be instructed to obtain QFT collection kits from 6th floor Hospital Stores. (x58211) A small supply of kits will be maintained in the AFB lab if for some reason the tubes are unavailable. Collection kits may be sent/received via the pneumatic tube system in MSR. (Microbiology Lab tube station is 102.)**
- **Medical staff should be instructed to read collection instructions thoroughly prior to collection of the sample. (Refer to section II. E. for Collection Instructions) Whenever possible, speak to the individual that will be collecting the sample and verbally go through the instructions, stressing that**

improper collection will lead to erroneous or indeterminate results.

- Once the samples have been collected they should be placed back into the bag and immediately returned to the Microbiology Lab.

- Dayshift: MSR staff will leave bagged specimens at room temperature.

- Evening/Night shift: MSR staff will pass samples through the Set-up window at a designated time in order to facilitate the controlled incubation period.

- Evening/Night Set-up tech: Shake each tube up and down 10 times to ensure that the entire inner surface of the tube has been coated with blood. Proper mixing is necessary to allow T-cells to contact antigen. (Mixture will be frothy.) Place the tubes in the designated rack, in an upright position in the 37°C Set-up incubator to be picked up by the AFB tech the next morning.

3. Highland Hospital

- **The test is orderable in e-records from "Facilities List". (Procedure code: TBAG)**

- **Medical staff from HH should be instructed to obtain QFT collection kits from HH Hospital Stores. (x16341) A small supply of kits will be maintained in the AFB lab if for some reason the tubes are unavailable. Collection kits may be sent/received via the HH courier.**

- **Medical staff should be instructed to read collection instructions thoroughly prior to collection of the sample. (Refer to section II. E. for Collection Instructions) Whenever possible, speak to the individual that will be collecting the sample and verbally go through the instructions, stressing that improper collection will lead to erroneous or indeterminate results.**

- **Once the samples have been collected they should be placed back into the bag and immediately returned to the SMH Microbiology Lab.**

- **Dayshift: SMH MSR staff will leave bagged specimens at room temperature.**

- **Evening/Night shift: SMH MSR staff will pass samples through the Set-up window at a designated time in order to facilitate the controlled incubation period.**

- **Evening/Night SMH Set-up tech: Shake each tube up and down 10 times to ensure that the entire inner surface of the tube has been coated with blood. Proper mixing is necessary to allow T-cells to contact antigen. (Mixture will be frothy.) Place the tubes in the designated rack, in an upright position in the 37°C Set-up incubator to be picked up by the AFB tech the next morning.**

3. ID Clinic

   - ID Clinic will maintain their own supply of collection kits

   - Clinic hours are Monday through Friday, 8:30 a.m. – 5:00 p.m.

   - Test is orderable in e-records from "Facilities List". (Procedure code: TBAG)

   - Immediately after collection the samples will be forwarded to the Microbiology Lab via pneumatic tube system.

   - Dayshift: MSR staff will leave bagged specimens at room temperature.

   - Evening/Night shift: MSR staff will pass samples through the Set-up window at a designated time in order to facilitate the controlled incubation period.

   - Evening/Night Set-up tech: Shake each tube up and down 10 times to ensure that the entire inner surface of the tube has been coated with blood. Proper mixing is necessary to allow T-cells to contact antigen. (Mixture will be frothy.) Place the tubes in the designated rack, in an upright position in the 37°C Set-up incubator to be picked up by the AFB tech the next morning.

4. Other Outpatient Locations
   Requests will frequently come from Allergy/Immunology and Rheumatology, but may be from other medical facilities as well.

- Collection can be performed at either of the following locations:

  SMH Outpatient Laboratory, room 1-1345
  Monday – Friday (6:30 a.m. – 6:00 p.m.)
  Saturday (9:00 a.m. – 1 p.m.)
  Phone: 585-275-2153

  400 Red Creek Drive
  Monday – Friday (8:00 a.m. – 5:00 p.m.)
  Phone: 585-487-1195

  Anthony Jordan Health Center
  82 Holland St.
  Monday (9:00a.m - 8:00 p.m.)
  Tue – Fri (9:00 a.m.- 5:00 p.m.)

  777 S. Clinton Ave.
  Mon- Thurs (8:00 a.m. – 9:00 p.m., closed 5:30 p.m. - 6:00p.m.)
  Fri (8:00 a.m. - 5:30 p.m.)
  Sat (8:30 a.m. – 12:30 p.m.)

- Not all patient locations have the ability to order in e-records. Some samples may come with a paper requisition and need to be logged into the LIS by MSR. (Procedure code: TBAG)

E. Warnings and precautions

1. A negative QFT-In Tube result does not preclude the possibility of *M. tuberculosis* infection or disease.

2. The predictive value of a negative QFT-In Tube result in immunocompromised persons have not been determined.

3. A positive QFT-In Tube result should be followed by further medical evaluation for active tuberculosis disease. (e.g., AFB smear and culture, chest x-ray)

4. A positive QFT-In Tube result can suggest and support the diagnosis of tuberculosis disease, but **infections by other mycobacteria, including *M. kansasii, M. szulgai* and *M. marinum*, may also cause positive results.**

5. The performance of the QFT-In Tube has not been extensively evaluated with specimens from the following groups of individuals:

    a. Individuals who have altered or impaired immune function
    b. Individuals below 17 years of age
    c. Pregnant woman

E. **Collection of Sample (at Client site)**

1. One ml of blood is collected into **each** of the three specialized blood collection tubes. (The tubes are vacutainers and should automatically fill to 1 ml)

2. Immediately following collection, each tube must be mixed vigorously. Shake each tube up and down 10 times to ensure that the entire inner surface of the tube has been coated with blood. Proper mixing is necessary to allow T-cells to contact antigen. (Mixture will be frothy.)

3. Post collection tubes should be forwarded to the lab as quickly as possible. **Tubes must be received in the Microbiology Laboratory within 16 hours of collection, and incubated.** They must **not** be refrigerated or frozen.

F. **<u>Processing</u>- performed by SMH Mycobacteriology Lab, unless specimen is coming from a full service lab.**

1. Collect specimens from bottom shelf of Set-up incubator.

2. **Check that the volume in each tube is sufficient. (Use the "Blank" provided at bench tube as a reference.) Volume of 0.8 -1.2 cc is critical for red and purple tubes. If volume in these tubes is not sufficient the test must be cancelled.**

3. Access specimen in LIS and give preliminary status:
" ~Testing in progress." (Worklist: ZTBAG)

4. Incubate for 16 – 24 hours. Incubation is necessary to allow memory T-cells to respond to the antigens.

5. After incubation: Centrifuge with covered carriers, spin the tubes for **15 minutes at 3,000 RCF** to separate the plasma and the red cells. The blood collection tubes contain a gel plug that separates the plasma from the cells when centrifuged.

If the gel plug does not separate the cells from the plasma, the tubes should be re-centrifuged at a higher speed. After centrifugation, avoid mixing plasma and disturbing material on gel surface.

6. Following incubation and centrifugation, place tubes in pre-labeled rack in AFB refrigerator. Tubes can be stored at 2°C - 8°C for up to 28 days.

III. Materials, Reagents and Equipment

A. ELISA Components:
Store kit at 2˚C to 8˚C.

a. Microwell Strip Plate**:** 96 wells (12 strips of 8 wells each) coated with murine anti-human interferon monoclonal antibody
(Interchangeable only if same kit lot number.)

b. Human Interferon Standard, lyophilized: recombinant human interferon, bovine casein, 0.01% w/v Thimerosal
(Interchangeable regardless of kit lot number)

c. Green Diluent: bovine casein, normal mouse serum, 0.01% w/v Thimerosal **(Interchangeable only if same kit lot number)**

d. Conjugate 100X Concentrate, lyophilized: murine anti-human interferon HRP, 0901% w/v Thimerosal
(Interchangeable only if same kit lot number.)

e. Wash Buffer 20X Concentrate: pH 7.2, 0.01% w/v Thimerosal
(Interchangeable regardless of kit lot number.)

f. Enzyme Substrate Solution: H2O2 , Tetramethylbenzidine
Protect from direct sunlight.
(Interchangeable regardless of kit lot number.)

g. Enzyme Stopping Solution: 0.5M H2SO4
(Interchangeable regardless of kit lot number.)

B. Materials/Equipment:

1. 37˚C ± 1˚C incubator (with or without CO_2).
2. Calibrated variable-volume pipettes for delivery of 10-1000ul with disposable tips.

3. Calibrated multichannel pipette for delivery of 50ul and 100ul with disposable tips.
4. Microtiter plates with plastic seals
5. Centrifuge capable of centrifuging blood tubes at least to 3,000 RCF
6. Microplate shaker capable of speeds between 500 and 1,000 rpm.

7. 2 L distilled water and measuring cylinder.
8. Automatic Microplate washer
9. Microplate reader fitted with 450nm filter and 620-650nm reference filter.
10. Variable speed vortex
11. Timer

IV. Safety Precautions

- Enzyme substrate solution contains tetramethylbenzidine which is harmful by ingestion, inhalation and skin contact.
- Enzyme stopping solution contains sulfuric acid which is harmful by ingestion, eye contact, skin contact and skin contact
- Conjugate 100X concentrate and Interferon standard may be discomforting if ingested and may cause skin irritation.
- Thimerosal may be toxic upon ingestion, inhalation or skin contact.
- Green diluent may trigger allergic responses; avoid skin contact.
- Observe relevant blood handling guidelines

V. Method

A. Stage One: Incubation of Blood and Harvesting of Plasma

- Refer to section II E/F

B. Stage Two: ELISA

(TAT ~ 3 hours for one ELISA plate)

1. Determine the number of plates that will be tested. Remove one set of positive and negative controls from the -85°C freezer **for each plate.**

2. All plasma samples and kit reagents, except for Conjugate 100X Concentrate, must be brought to room temperature before use. **Allow at least 60 minutes for equilibration.**

3. Prepare ELISA plate layout sheet.

4. Remove strips needed; return unused strips, reseal pouch and return to refrigerator. Allow 1 strip for the QFT Standards and sufficient strips for the number of subjects and controls being **tested <u>for each plate</u>.**

5. Reconstitute the Human Interferon Kit Standard with the volume of deionized/distilled water **AS INDICATED ON THE LABEL** of the vial ensuring complete resolubilization. (The reconstitution volume will differ between batches). Mix gently to minimize frothing.

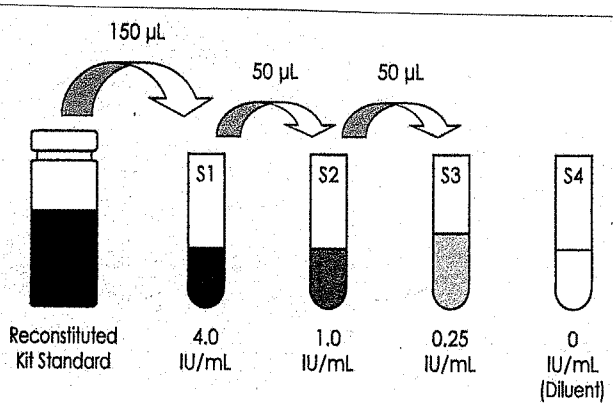
   Reconstitution of the standard to the correct volume will produce a solution with a concentration of 8.0 IU/ml. This standard is used to produce a dilution series of **4** IFN concentrations.

   When reconstituting the reagent, indicate the date on the reagent label. **The standard may be kept for up to 3 months if stored at 2°C to 8°C.**

6. Add **150 ul** of **Green Diluent** to each of **4** tubes, labeled as Standard **1** through Standard **4. <u>If using 2 plates double this amount.</u>**

7. Add **150 ul** of the **reconstituted Kit Standard** to the tube labeled as **Standard 1. <u>If using 2 plates double these amounts.</u>**

8. Change to **50 ul** pipet. Perform serial dilutions by transferring **50 ul** of each **Standard 1 to Standard 2** and from **Standard 2 to Standard 3**. Mix each tube before the next transfer. **(see Fig. 1) The undiluted Kit Standard serves as the highest concentration (Standard 1). The Green Diluent serves as the Zero Standard (Standard 4).**



9. Reconstitute freeze dried Conjugate 100X Concentrate with **300 ul** of deionized or distilled water and mix gently. **Indicate the date of reconstitution on the reagent label.** Return any unused conjugate 100X Concentrate to refrigerator immediately after use. **Once reconstituted this is good for 3 months if stored at 2˚C to 8˚C.**

   Working Strength Conjugate is prepared by diluting the required amount of reconstituted Conjugate 100X Concentration in Green Diluent as set out in the attached Table 1 – Conjugate Preparation.

   (HINT: To ensure that you have prepared enough working conjugate, add 1 to the actual number of strips that you will be using and follow guidelines on the chart below.)

   Working strength conjugate should be used within 6 hours of 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

10. Using the multichannel pipette, add **50 ul** of freshly prepared **Working Strength conjugate** to each ELISA well.

11. Add **50 ul** each of the **Standards 1-4**. The standards should be assayed in duplicate. **If multiple plates are being used standards must be run on each plate. Do not split a patient's samples between plates!**

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	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Pos Control G	Patient 1 P									
B	Std 2	Pos Control R	Patient 2 G									
C	Std 3	Pos Control P	Patient 2 R									
D	Std 4	Neg Control G	Patient 2 P									
E	Std 1	Neg Control R										
F	Std 2	Neg Control P										
G	Std 3	Patient 1 G										
H	Std4	Patient 1 R										

12. Following the plate layout, add **50 ul** of **test plasma** samples to appropriate wells. Each patient test sample requires 3 wells – one for each collection tube. Include a positive and negative control on each plate.

13. Cover plate with lid and mix the conjugate and samples/standards on the microplate shaker for **1 minute at 550 rpm**.

14. Incubate covered plate in the dark at room temperature (22 degrees C +/- 5 degrees C) for **120 minutes.**

15. **Turn on plate reader.**

16. During incubation, prepare wash buffer as needed. Dilute one part Wash Buffer 20x Concentrate with 19 parts ddH2O and mix. **(use 50 ml wash buffer and 950 ml ddH20)**

    One full bottle of Wash Buffer 20x Concentrate into 1900 ml of H2O is sufficient to wash about 6 plates. Once prepared, indicate the preparation date/ expiration date on the bottle of Wash Buffer. **The prepared wash buffer may be stored at room temperature for 2 weeks.**

17. After incubation, wash wells at least 6 times with 400 ul of Working Strength wash buffer using the automated plate washer. See detailed instructions below:

ELx50 Automated Plate Washer Guide

Before Washing:

a. **Ensure there is wash buffer above the fill line. If necessary, prepare wash buffer per instructions.**
b. **Ensure there is deionized (Type I) water above the fill line. If the deionized water level is below the fill line add deionized water to the bottle.**
c. **Empty the waste bottle if necessary. Unscrew the entire lid of the waste bottle and empty the contents into the dirty sink with copious amounts of water. Screw the lid back on the waste bottle when complete.**
d. **Turn washer ON (power switch is located on the right side of the unit).**
e. **Disconnect the tubing from the deionized water bottle and connect to the top of the wash buffer bottle (gently hand-tighten the tubing attachment).**
f. **Press the MAIN MENU button.**
g. **Press RUN --- PRIME – press 02 for NEW PRIME BUFFER.**
h. **Press ENTER. Press START.**
i. **When Priming is complete, press ENTER – MAIN MENU or STOP then YES.**

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Washing:

a. **Each row (strip) must be a complete row of 8 wells.**
b. **Press RUN- Press WASH.**
c. **Type in the QFT assay wash # 05 – then press ENTER.**
d. **First Strip? Enter "01", press ENTER.**
e. **Enter number of strips, press ENTER.**
f. **Remove plate lid.**
g. **Open the translucent splash guard and place plate in carrier with well A-1 in the upper RIGHT hand corner. Ensure the plate is secured with the metal locking tab. Close the splash guard.**
h. **Press START.**
i. **When wash is finished- Press ENTER. Ensure there is no copious liquid remaining in the wells. If excess liquid is present blot the plate on a stack of paper towels.**
j. **For each additional plate to be washed: press MAIN MENU.**
Then repeat steps a-i for each additional plate to be washed.

AT END OF THE DAY:

a. **Disconnect the tubing from the top of the wash buffer bottle and connect tubing to the top of the deionized water bottle (gently hand-tighten the tubing attachment).**
b. **Press MAIN MENU- Press MAINTENANCE – then 05 for RINSE AND SOAK- press ENTER- press START. The washer will rinse the unit with deionized water then stop. After approximately 10 seconds the manifold will descend into deionized water in the washer trough.**
c. **Turn the washer off. The manifold will soak in deionized water until next use.**
d. **Once per month, run Alcohol through the tubing lines to disinfect (last Wednesday of each month):**

- **Add tubing to Alcohol bottle**
- **Press MAIN MENU**
- **Press MAINTENANCE**
- **Press O2 for Decontamination**
- **Press Enter, START, STOP, YES**
- **Reconnect tubing to ddH20**
- **Repeat steps a and b above.**

18. If there is residual wash buffer in the wells following final wash, tap place down on absorbent paper towel. Using the multichannel

pipette, add **100 ul of Enzyme Substrate Solution** to each well. (Liquid in wells will turn from yellow to blue color.)

19. Cover and mix for **1 minute at 550 rpm** on microplate shaker.

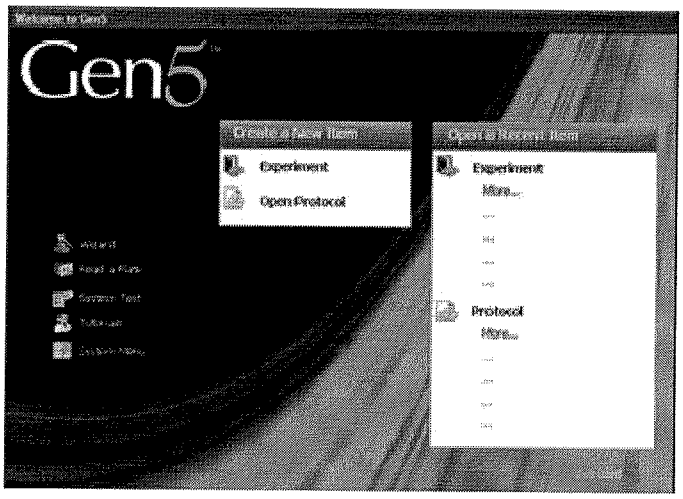
20. Incubate covered plate in the dark at room temperature (22 degrees C +/- 5 degrees) for **30 minutes**.

Use this time to create plate layout. QFT software to operate the Gen5CL microplate reader has been loaded onto the computer by Positive AFB bench.

Gen5CL QFT Operator Instructions

Double click on the Gen5CL program icon to open the program. After a brief load time, you will see the login screen. The default login is "Administrator" with the password **"admin".** The password is case-sensitive.

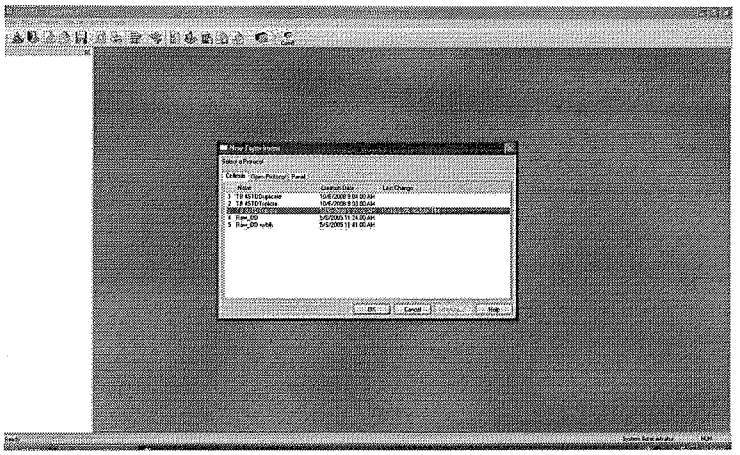
After logging in, you will be taken to the "Welcome" screen.



Under the "Create a New Item" menu, select "Experiment". You will see the following screen and then be prompted to select from the list of protocols that are currently installed. For QFT, the options are:

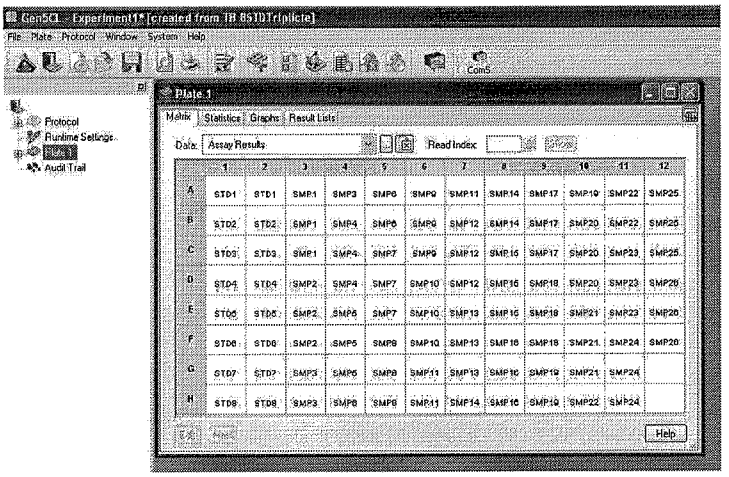
TB 4STDTriplicate
TB 8STDTriplicate

Note: "Duplicate" & "Triplicate" refers to the number of sample tubes per patient.



"TB 4STD triplicate" should be highlighted.

Once you click **"OK",** the experiment will be created with the selected protocol.

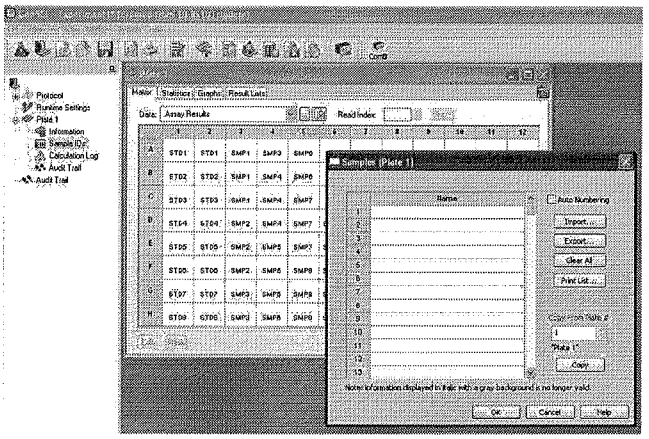


At this point, you can click on the **"+"** symbol on the far left column next to where it says **"Plate 1".** This will open the menu and allow you to edit the plate information and the sample IDs to match your run. Double click on "**Sample Ids**".

Note: If 2 plates are to being used, click on icon at top of screen (picture of plate with + sign on top).

Will display:

"**Number _**" (enter number of plates that you want to ADD)
Click on **Plate1** on side bar, fill in data as below, **close, YES**
Click on **Plate 2** on side bar, fill in data as below, **close, YES**



Sample IDs:

Enter patient details.
Click OK to save
Close
YES

After doing this you can see the plate layout with the sample IDs by selecting the "**Layout**" option on the drop-down arrow.

After the details have been entered, select the "**Read Plate**" option at the top of the screen (second icon from the end, select Plate 1 or Plate 2, as appropriate) Enter **number** of patient samples in box toward bottom of screen. Click the "**READ**" button near the bottom of the screen.

Note: If for some reason the LIS is not communicating with the reader you will get an error message. Shut down and restart LIS, then recheck communication. If still not communicating consult reader manual. (page 19. "Comport not recognized")

The "**Save as**" box will appear. Select "**OK**".

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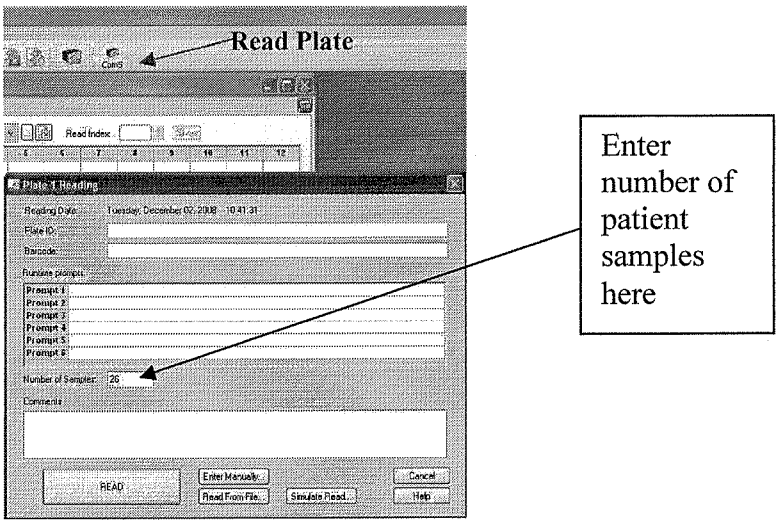
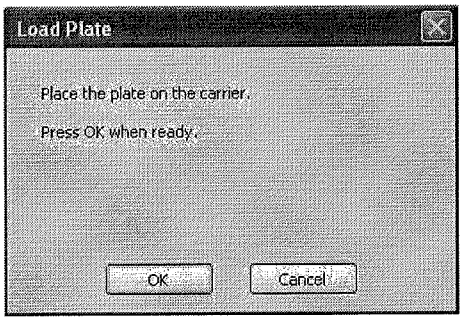


21. Following incubation **add 50 ul** (multichannel pipette) of **Enzyme Stopping Solution** to each well and tap (~20 seconds) to mix. Do not use shaker.

22. Measure the Optical Density of each well within **5** minutes of adding the stopping solution using the microplate reader fitted with a 450nm filter and with a 620-650nm reference filter.

When prompted, **remove the lid of the plate** and load the microplate onto the plate carrier. **Always load the plate with the "A" in the top left corner.** Click "OK".



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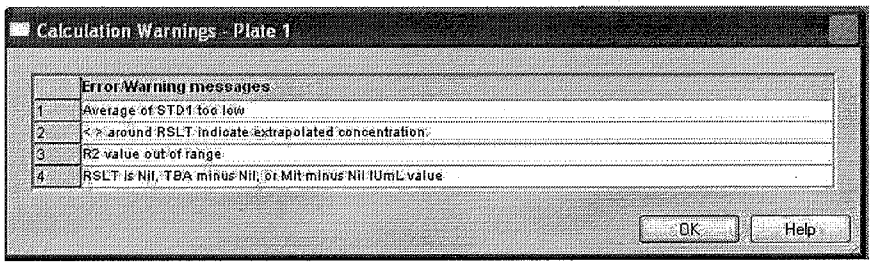
After the plate is read, warning messages will pop up on the screen. The warning messages will indicate if any of the standards have failed the QC requirements. Hit **"OK"** to close the warning message box.



Click on **ASSAY RESULTS** from drop down arrow on the blue bar. Select printer icon from top toolbar at top of screen. This will print out the full report. Note that a full report with results will still print even if the ELISA failed the QC criteria. The warning messages are listed on every page of the report.

VI. Calculations and Test Interpretation

A. Calculations

- The software performs a quality control assessment of the assay, generates a standard curve which is used to convert the Antigen OD responses to International Units, providing a numerical test result for each patient sample based on the values from their Nil, TB Antigen and Mitogen tubes.
- Warning/error messages will be generated in the event that the run fails and **"Invalid ELISA Test Result" will print at the bottom of the first paragraph on the first page of the printout.**
- **After each run and prior to reporting results, check:**
  - **The mean OD value for Standard 1 must be ≥0.600.**
  - **The % CV between replicates for Standards 1 and 2 must be ≤15%.**
  - **Replicate OD values for Standards 3 and 4 must not vary by more than 0.040 optical density from their mean.**
  - **Correlation coefficient (R) calculated from mean absorbance values of standards must be ≥0.98.**

- The numerical result for each patient (in IU/ml) must be manually resulted in the LIS. Concentrations greater than 10 IU/ml must be reported as >10 IU/ml as such values fall beyond the validated linear ranges of the ELISA.
- Values <0.00 IU/ml are reported as 0.00 IU/ml.

B. Test Interpretation

QuantiFERON®-In Tube results are based on the amount of gamma interferon that is released in response to the antigen.

All results must be reviewed by a supervisor prior to reporting.

1) **Positive QuantiFERON Results:**

a. **Positive patient results >1.0 IU/ml may be reported as positive without the need for further testing.**

"Patient Gamma Interferon result: ____

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

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

NOTE: Diagnosing or excluding tuberculosis disease, and assessing the probability of latent tuberculosis infection requires a combination of epidemiological, historical, medical, and diagnostic findings that should be taken into account when interpreting QuantiFERON-TB Gold results.

False positive results may occur in patients with a history of exposure to M. kansasii, M. marinum or M. szulgai.

Test Method: Cell Stimulation/ Semi-Quantitative ELISA
Organism Positive for Gamma Interferon"

b. **Positive patient samples ≥ 0.35 IU/mL but ≤ 1.0 IU/ml should be retested in <u>duplicate</u> the following week.**

If **both** repeat tests are >0.35 IU/ml, the initial positive report has been verified. **<u>Report as above 1a).</u>**

If **both** repeat tests are <0.35 IU/ml, the initial positive result has NOT been verified. **<u>Report as negative</u>.**

If **only one** of the repeat tests is >0.35 IU/ml, the initial positive report has been verified, but **report as**:

"NOTE: The patient result is __ IU/ml which is slightly above the cutoff for positive interpretation. Per the manufacturer's package insert, the patient specimen was re-tested in duplicate with the following results: ___IU/ml and ___IU/ml, and is interpreted as positive. In such cases, repeat testing with a new specimen may be warranted.

"Patient Gamma Interferon result: ____

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

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

NOTE: Diagnosing or excluding tuberculosis disease, and assessing the probability of latent tuberculosis infection requires a combination of epidemiological, historical, medical, and diagnostic findings that should be taken into account when interpreting QuantiFERON-TB Gold results.

False positive results may occur in patients with a history of exposure to M. kansasii, M. marinum or M. szulgai.

Test Method: Cell Stimulation/ Semi-Quantitative ELISA
Organism Positive for Gamma Interferon"

2) **Negative Result:**

"Patient Gamma Interferon result: ____

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

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

NOTE: Diagnosing or excluding tuberculosis disease, and assessing the probability of latent tuberculosis

infection requires a combination of epidemiological, historical, medical, and diagnostic findings that should be taken into account when interpreting QuantiFERON-TB Gold results.

False positive results may occur in patients with a history of exposure to M. kansasii, M. marinum or M. szulgai.

Test Method: Cell Stimulation/ Semi-Quantitative ELISA"

3) **Indeterminate Result:**

"Patient Gamma Interferon result =
Indeterminate due to negative mitogen response.

Negative mitogen response may occur either due to T-cell anergy or mishandling of the specimen draw tubes.

Test Method: Cell Stimulation/Semi-Quantitative ELISA"

VII. Preparing and Maintaining Controls

A. Patient samples that have demonstrated 0.0 IU readings may be used as negative controls.

B. Patient samples that have demonstrated >2 IU readings may be used as positive controls.

C. For each sample to be used as a control aliquot 0.55 ul from red,

gray and purple tubes and place into microcentrifuge tubes. Label each tube appropriately.

example: label side of tube positive Mitogen, put "P" on cap
label side of tube positive Nil, put "G" on cap
label side of tube positive TB, put "R" on cap

Reference
[1]QuantiFERON®- TB Gold In Tube, Cellestis Inc. USA, Valencia, CA, package insert
[2] MMWR, 2010, vol 59:RR-5
[3]ELX50 Operator's Manual, Washer
[4] ELX800 Operator's Manual, Reader

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