QUANTIFERON-TB IN-TUBE

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PURPOSE

To provide instruction for performing Quantiferon-TB in-tube testing.

BACKGROUND

Clinical Significance

Tuberculosis is a communicable disease caused by infection with Mycobacterium tuberculosis complex. Latent tuberculosis infection (LTBI) persists in some individuals asymptomatically, who might develop TB months or years later. Until recently the Tuberculin skin test was the only method for diagnosing LTBI. Some infected individuals, such as those who have compromised immune systems, do not respond to tuberculin and show a false negative skin test. Conversely there are those who do respond to the skin test who are unlikely to have TB infection. Numerous studies have demonstrated that the peptide antigens used in Quantiferon TB Gold stimulate IFN responses in T-cells from those infected with M. tuberculosis but generally not from uninfected or BCG vaccinated persons without disease or risk for LTBI.

Methodology

Quantiferon-TB (QFT) Gold measures the cell-mediated immune responses to peptide antigens in individuals infected with Tuberculosis. QFT uses specially designed collection tubes that are coated with specific antigens (ESAT-6, CFP-10, and TB 7.7), along with a negative and positive control. Stimulation of whole blood with these antigens results in the production of gamma interferon (IFN-γ). An enzyme linked immunosorbent assay (ELISA) is used to measure the amount of IFN present.

SUPPLIES / EQUIPMENT

Quantiferon-TB Gold In-Tube blood collection tubes, by Cellestis. Stored at room temperature.
Gray top - NIL: Contains no antigens
Red top - ANTIGEN: coated with antigens ESAT-6, CFP-10 and TB 7.7
Purple top - MITOGEN: Serves as positive control of patient's immune response
Quantiferon -TB Gold ELISA kit, by Cellestis. Stored at 2°C-8°C
Microplate strips coated with murine anti-human IFN-γ monoclonal antibody (2 x 96 well plates)
Human IFN-γ Standard, lyophilized
Green Diluent
Conjugate 100x Concentrate, lyophilized
Wash Buffer 20x concentrate
Enzyme Substrate Solution

SPECIMEN

Collection

1. Label each of 3 tubes (Gray top, Red top, Purple top). The Purple top must be drawn first, Gray drawn second, and Red drawn last.

2. For each subject collect 1 ml of blood directly into each of the 3 collection tubes. The black mark on the side of the tube indicates the 1 ml fill volume. Keep the tube on the needle an extra 2-3 seconds after vacuum stops, to ensure correct volume. If a "butterfly needle" is being used to collect blood, a purge tube should be used to ensure adequate filling of the collection tubing.

3. Mix the tubes by shaking vigorously for at least 5 seconds. The inner surface of the tubes must be entirely coated with blood. Foaming of the blood is ideal.

4. Transfer as soon as possible, and within 16 hours of collection, to a 37°C incubator. Prior to transfer, hold the tubes at room temperature (22°C ± 5°C). DO NOT refrigerate or freeze the blood samples.

Sample Processing

1. Verify that specimens received are <16 hours old. Shake all tubes vigorously for at least 5 seconds immediately prior to incubation.

2. Incubate the tubes upright at 37°C ± 1°C for 16-24 hours. The incubator does not require CO2 or humidification.

3. Following incubation, centrifuge the tubes for 15 minutes at 2000-3000 RCF (g). ***Following incubation, tubes may also be held between 2°C and 27°C for up to 3 days prior to centrifugation.*

4. Verify that the gel plug has separated the cells from plasma, if this has not happened, re-centrifuge at a higher speed.

5. Plasma samples may be stored for up to 28 days at 2°C to 8°C or below -20°C (preferably less than -70°C) for extended periods. *Plasma samples may clot during extended storage. If clots are present, centrifuge samples to sediment clotted material and facilitate pipetting of plasma.*

REAGENTS

Reagent Preparation

1. Conjugate: Use a calibrated pipette to reconstitute Conjugate 100x Concentrate with 0.3ml deionized or distilled water. Mix gently to minimize frothing and ensure the conjugate is completely dissolved. Store immediately at 2-8°C. This will be used to make the Working Strength Conjugate. 100x Conjugate is stable for 3 months.

2. Standard: Use a calibrated pipette to reconstitute the Human IFN-γ Kit Standard with the volume of deionized or distilled water as indicated on the label. Mix gently to minimize frothing and ensure the standard is completely dissolved. The reconstituted Standard may be kept 3 months at 2-8°C.

3. Wash Buffer: Use a graduated cylinder to dilute one part Wash Buffer 20x Concentrate with 19 parts deionized or distilled water and mix thoroughly. Working strength wash buffer may be stored at room temperature for 2 weeks.

4. Evolis System Liquid: In the large 10L container (red cap) located below the instrument, add 2 ml Tween to 10L DI water using a graduated cylinder. Volume should be checked daily and refilled when it is approximately 3L.

PROCEDURE STEPS

Load Samples

1. Place bar-coded sample tubes into the T-rack, with labels facing outward. Each specimen contains 3 tubes and should be loaded in the order of Gray, Red, Purple (Nil, Antigen, Mitogen). Only load the rack in groups of 3. DO NOT split the specimens among more than one T-rack. *Caution: rack can easily be tipped, spilling specimens or introducing bubbles. Always use rack holder and transport using both hands.*

2. Slide the T-rack with patient specimens onto the available rails, indicated by the Red LED light. “Patient Editor” screen will display. If nothing happens, remove T-rack and try again. Load using a steady motion, not too slow or too fast.

3. Ensure all bar codes have been read and are listed in Patient Editor.

4. Click on the drop-down arrow under Assay and select Quantiferon-TB. Drag mouse thru all the patient specimens so they are all selected for the assay.

5. Click on “Close”.

6. Repeat steps 1-5 for each T-rack to be loaded.

Create Worklist

1. Click on *New Worklist* icon from toolbar.

2. View plates by clicking “+” on the tree to expand it. Select “OK”.

3. Enter the Lot Number of the current Cellestis kit.

4. Worklist screen appears. Click on “+” to view Worklist. Click on Required Reagents and Print.

5. Use this list to prepare reagents. The Evolis lists the required volume for each reagent, based on how many test samples were loaded.

6. Click on the green ”Start” button on the menu bar. The load screen window will appear displaying allocated resource on the right side, required disposable tips and the required wash buffer bottles.

Load Standards and Reagents

1. Transfer the prepared Standard into a 2-ml Sarstedt tube, place this tube into a 13x75mm polypropylene tube, and put into the 3-rack.

2. Load 6 empty 13x75 mm polypropylene tubes into the 3-rack. Slide rack onto next available rail.

3. Specimen Diluent (Green Diluent): No dilution necessary. Pour the required amount into a V-Vial. Place in first position on Metal #1 Rack.

4. Working Conjugate: Remove vial of 100x conjugate from refrigerator and prepare (into a V-Vial) the required amount of conjugate into Green Diluent as per the following table:

Number of Strips	Volume of Conjugate 100x Concentrate	Volume of Green Diluent
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

   Put this vial into Position 2 of the reagent rack. *The Working Conjugate must be used within 6 hours. Be sure to put 100x Conjugate back in the refrigerator as soon as possible.*

5. Substrate: Transfer the required volume into a V-Vial, place into 3rd position of the reagent rack.

6. Stop Solution: Transfer the required volume into a V-Vial, place into position 4 of the reagent rack. When all 4 reagents have been loaded, slide rack onto next two available rails.

NOTE: If the number of specimens requires more than one plate, the user is prompted to load 6 additional empty tubes for the Standard Dilution. All 12 tubes can be on one 3-rack.

Allocate Resources

1. Load tips according to display. To load full racks, click “Refill Partial Tip Racks.”
   - Gold indicates a full 300 ml rack
   - Gray indicates a full 1100 ml rack
   - Red indicates a partial rack

2. Verify that the reagents, standard, and dilution tubes are all showing up in the appropriate positions on the screen. If not, click on the reagent and drag it to the correct position. The reagents locations MUST match up with this screen.

3. Check the Wash Buffer bottle and the DI Water bottle, making sure the volume is sufficient (according to Reagent Requirement list) and that they are in the proper position.

Start Worklist

1. When all resources are properly loaded, click “OK”.

2. The machine will prompt you when it is time to load the plate. Open the foil package and remove any test strips that won't be used for the assay. Reseal package completely, making sure the desiccant is inside.

3. Place the plate onto a silver plate carrier. Make sure all the strips are flat in the tray. Load onto Plate loading dock. Instrument will begin processing the plate.

4. The operator should observe the instrument until the plate has been inoculated completely and has moved to the incubator. After this point it should be checked periodically until the run is complete.

Storage

1. When the Evolis has completed sampling the specimen, Standard, and Standard dilutions, it is indicated by a blinking red light on the appropriate rail. These racks can now be removed from the loading deck. DO NOT OPEN top cover during pipetting, wait until the plate has gone to incubation.

2. Patient specimens should be kept 7 days at 2-8°C.

RESULTS

The instrument will print O.D. values (raw data) for each specimen, and will determine if the run passed, based on certain "Validation Criteria." The Cellestis Data Analysis Software (QFT-Gold IT v. 2.17) takes the Log of the OD Values, subtracts the Nil value from the Antigen and Mitogen values, and determines if the specimen is Positive, Negative or Indeterminate.

1. Review the Validation Criteria on Page 1 and ensure it has all passed (can also see "V.C. passed" on the upper right corner of each page.

2. Review the list of patient data and look for any flags. If a result was not calculated, there will be **** in the result section for that tube.

3. This data needs to be exported from Flex-E into Cellestis Data Analysis Software (QFT-Gold IT v 2.17).
   - Click on Utilities
     - Export Results
     - Yes
   - Ok

4. Minimize screen

5. On the Desktop, double click on "TB GOLD:QFT v.2.17.3". Do not minimize this application.

6. On the Desktop, double click on "Flex-E results to TB Gold v.4.8".
   - A disclaimer will pop up. Click OK.
   - Click on "Browse".
   - Find the exported file (.txt) under TBG folder (c:/Flex-E/TBG).
     - Drop down "look in:" and click on "Local Disk (C:)".
     - Double click on "Flex-E" folder.
     - Double click on "TBG" folder.
     - Click on the exported file (.txt).
   - Click "Open"
   - Click "Accept". This will place the ODS into the QFT's "Raw Data" screen.

7. Click on "Run Detail" tab and verify data are imported for "Run Number", "Kit Batch Number", and "Operator". "Run Date" will automatically default to current date. Change date if necessary.

8. Click "Raw Data" tab and select "Manual Format".

9. Check box that says "Standard", then "Vertical".

10. Click mouse in the A1 box on the screen. S1-S8 will appear in the first column. Repeat for box A2. These are the O.D. results for the 8-point Standard curve.

11. Now click the box “Subject”, then “Vertical”. In the subject ID line, type in the accession number for the first patient. HINT: slate readout printed with the Flex-E results. Click the mouse in A3. The first 3 wells of that column will populate with 1N, 1A, and IM. Move onto the next box (A6) and repeat for the next patient. Do this until all the samples have been assigned.
    - When running Positive patients is duplicate:
        - Click “Random”. The “N”(Nil) button is selected.
        - In the subject ID line, type in patients’ accession number.
        - Click the mouse in the first box corresponding to the Nil tube of the patient.
        - The “A” (Antigen) button is now selected.
        - In the subject ID line, type in the patients’ accession number.
        - Click the mouse in the first box corresponding to the Antigen tube of the patient.
        - The “M” (Mitogen) button is now selected.
        - In the subject ID line, type in the patients’ accession number.
        - Click the mouse in the first box corresponding to the Mitogen tube of the patient.
    - For the duplicate results, repeat these steps. When typing in the patients accession number, put an “a” after the number. This will differentiate between the patients duplicate results. Then click the mouse in the second boxes for the N, A, and M tubes.

12. Click “Complete”, then “Calculate”. Verify that the standard curve ‘passes” and the run is valid.

13. Click on “Subject Results”. Verify that the accession numbers are now listed with the interpretation.

14. Click on “Save File”.

15. Click on “Results Export”.
    - Export Type: “Export to File”.
    - OK
    - Save

16. Click on “Print”.
    - Save as PDF
    - Print
    - Close print window.

17. Close out of “TB GOLD: QFT v.2.17.3”.

18. Exit out of “Flex-E results to TB Gold v.4.8”.

19. Open “TB Gold to ASTM v.4.8”
    - A disclaimer will pop up. Click OK.
    - De-select all “Result Types” except “Qualitative Result”.
    - Click “Browse”.
    - Find the exported file (DDmonthYY_ResultExpa.txt) in folder (My Documents/QuantiFeron/Save).
    - Click “Open”. The “Results Table” is filled in with the patients’ accession numbers and correlating results.
    - Click “Start Export”
    - Yes
    - OK
    - Exit

20. Specimens testing Positive and Indeterminate need to be run again in duplicate on another run.
    - Retesting in Duplicate.

- Load patients.
- Select test
- Close
- Open “Folder”
- Drop down “Look in:” and click on “Result”.
- Drop down “Files of type:” and click on “Result Files(*.res)”.
- Choose file that contains patient to be run in duplicate.
- Click “Open”
- Click “Edit”
- Click “Retest’
- Highlight patients’ accession numbers (3 per patient).
- OK
- Click “Utilities”
- Click “Patient details”
- Find patient, click on ;the “+” to open and verify that 2 tests are requested.
- OK
- Restore Down file.
- Exit file.
- Export: No.
  - If at least one result of the duplicate run is positive, report QFT Positive.
  - if both results of the duplicate run are negative, report QFT NEGATIVE.

21. “CLIP” the specimens and store in the walk in refrigerator.

REPORTING RESULTS

The TB tool software converts the OD values into concentration of IU/ml, which in turn produces a result of Positive, Negative, or Indeterminate. Report these values in Cerner, with an additional comment, if necessary.

A. POSITIVE: M. tuberculosis infection LIKELY.
Persons with a positive QFT-G result, regardless of symptoms or signs, should be evaluated for TB disease before latent TB infection is diagnosed. *(qftpos*)

B. NEGATIVE: M. tuberculosis infection NOT likely
The majority of healthy adults who have negative QFT-G results are unlikely to have M. tuberculosis infection and do not require further evaluation. However, if recent contact or exposure (with persons who have infectious TB) is suspected, negative QFT-G results should be confirmed with a repeat test performed 8-10 weeks after the exposure. (*qftneg*)

C. INDETERMINATE: Results are Indeterminate.
For persons who are unlikely to have M. tuberculosis infection, no further tests are necessary. For persons with an increased likelihood of M. tuberculosis infection who have an indeterminate QFT-G result, repeat testing may be indicated. (*qftind*)

LIMITATIONS

Unreliable or Indeterminate results may occur due to:

Incorrect incubation times or temperatures
Incorrect transport of blood specimens
Excessive levels of circulating IFN-γ or presence of heterophile antibodies

>16 hours between blood draw and incubation at 37°C

Performance of this test has not been extensively evaluated for the following groups of individuals:

Those with impaired or altered immune function
Pregnant women
Individuals <17 years of age

REFERENCES

A. Morbidity and Mortality Weekly Report, December 16, 2005. *Guidelines for Using the Quantiferon-TB Gold Test for Detecting Mycobacterium tuberculosis Infection, United States.* Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

B. Cellestis, Inc., 2007. *Quantiferon-TB Gold (In-Tube Method)*, Package insert.

C. Biorad, Inc. 2007*. Evolis Microplate Automation Operator's Manual*

DOCUMENT APPROVAL Purpose of Document / Reason for Change:			
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