VIRO_1800-2135

UW Medicine
LABORATORY MEDICINE
VIROLOGY

Laboratory Procedure Manual

Analyte: **TUBERCULOSIS (TB) QUANTIFERON GOLD PLUS (Interferon-gamma)**

Matrix: **Plasma**

Method: **Detection of interferon-gamma by enzyme linked immunosorbent assay (ELISA) is used to identify in vitro responses to a peptide antigens that are associated with Mycobacterium tuberculosis infection.**

As performed by: University of Washington Clinical Virology Laboratory
1616 Eastlake Ave E Suite 320
Seattle, WA 98102
206.685.8037 option 8

Contact: Gregory Pepper
Laboratory Manager

Procedure in service: May 2019

Important Information for Users

The University of Washington Clinical Virology Laboratory periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
TB_K _R	LBXTBN	TB Nil control result
	LBXTBM	TB Mitogen control result
	LBXTB1	TB1 Antigen result
	LBXTB2	TB2 Antigen result
	LBXTBIN	TB coded result

A. Summary of Test Principle and Clinical Relevance

QuantiFERON®-TB Gold Plus (QFT-Plus) is an indirect test for *M. tuberculosis* infection. It is intended for use in conjunction with risk assessment, radiography and other medical and diagnostic evaluations.

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. In the past, 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 QFT-Plus 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.

LTBI must be distinguished from tuberculosis disease, a reportable condition that 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.

The QFT-Plus is an in vitro diagnostic test using a peptide cocktail simulating ESAT-6 and CFP-10 proteins to stimulate cells in heparinized whole blood. Numerous studies have demonstrated that the 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*.

Risk factors for *M. tuberculosis* infection include historical, medical or epidemiological predictors for tuberculosis disease or exposure to tuberculosis. Refer to the most recent CDC guidance (http://www.cdc.gov/nchstp/tb) for detailed recommendations about diagnosing *M. tuberculosis* infection (including disease) and selecting persons for testing.

The QuantiFERON®-TB Gold Plus assay tests 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 non-tuberculous 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 QFT Plus system uses specialized blood collection tubes, which are used to collect whole blood via venipuncture, which include a Nil control tube, two TB Antigen tubes and a Mitogen tube (positive control). The QFT-Plus has two distinct TB antigen tubes: TB Antigen Tube 1 (TB1) and TB Antighen Tube 2 (TB2). Both tubes contain peptide antigens form the MTB-complex-associated antigens, ESAT-6 and CFP-10. Both the TB1 and TB2 tubes contain peptides from ESAT-6 and CFP-10 that are designated to elicit CMI response form 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. The tubes are shaken to mix antigen with the whole blood and incubated at 37°C for 16 to 24 hours. Following the incubation period, plasma is harvested and the amount of IFN-γ that was produced in response to the peptide antigens is measured by ELISA. Results for the test samples are reported in International Units (IU) relative to a standard curve prepared by testing dilutions of a recombinant human IFN-γ standard.
Heterophile antibodies in plasma of certain individuals are known to cause interference with immunoassays. The effect of heterophile antibodies in the QuantiFERON®-TB Gold IT ELISA is minimized by the addition of normal mouse serum to the green diluent and the use of $F(ab')_2$ monoclonal antibody fragments as the IFN-γ capture antibody coated the microplate wells.

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 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 the 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.

B. Safety Precautions

1) Reagent Toxicity or Carcinogenicity
   Green diluent contains 0.01% w/v Thimerosal. Hold hazardous waste for pick up by University of Washington Environmental Health and Safety.
2) Radioactive Hazards
   Not applicable for this procedure.
3) Microbiological Hazards
   Not applicable for this procedure.
4) Mechanical Hazards
   Keep hands away from Mechanical Arm of the DS2 pipette assembly when operating instrument.
5) Protective Equipment
   Personnel protective equipment consists of eye protection, laboratory coat and latex gloves.
6) Training
   All personnel must be trained and proficient in Biosafety Level 2 practices**.** Per the Centers for Disease Control and Prevention/National Institutes of Health, Biosafety Level 2 (BSL2) is suitable for work involving agents of moderate potential hazard to personnel and the environment. It requires that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.
7) Personal Hygiene
   - Wash hands immediately upon contamination, after handling hazardous chemicals and before leaving the laboratory.

- Long hair and loose clothing must be confined when working in the laboratory.
- A soiled or contaminated lab coat should be placed in the soiled hamper and exchanged for a clean one.
- Closed-toe shoes must be worn when working in the laboratory.
- Artificial or long natural fingernails are not permitted in the laboratory. Artificial fingernails includes, but is not limited to , acrylic nails, all overlays, tips, bondings, extensions, tapes, inlays, nail jewelry, and wraps.

8) Disposal of Waste
   a. Collect solid biohazardous waste, collected in biohazard bags, from the bench tops in the main 320 South. Tie or tape bags closed and place into the appropriate biohazard waste shipping container (large, red plastic tub), that is lined with a large red biohazard bag.
   b. Liquid waste is none biohazardous and may be poured directly down the sink with copious amounts of water. The Green diluent contains 0.01% w/v Thimerosal. Hold hazardous waste for pick up by University of Washington Environmental Health and Safety.

C. Computerization; Data System Management

Not applicable for this procedure

D. Specimen Collection, Storage, And Handling Procedures

1) Specimen Collection:
   Collect 1mL of blood by venipuncture directly into each of the QuantiFERON®-TB Gold Plus blood collection tubes, which include a Nil Control tube, TB1, TB2 and a Mitogen tube. Tubes should be between 22 ± 5°C at the time of blood draw.
   Immediately after filling tubes, shake them ten times just firmly enough to ensure the entire inner surface of the tube is coated with blood, to solubilize antigens of tube walls. Over energetic shaking, may cause gel disruption and could lead to aberrant results. Hold at room temperature up to 16 hours from draw to incubation. Re-mix tubes by inverting 10 times immediately prior to incubation. Incubate tubes upright at 37 ± 1°C for 16 to 24 hours. Samples are shipped on wet ice by the MECs to the laboratory for testing.
2) Specimen and Reagent Volumes:
   The balck mark on the side of the tubes indicates the validated range of 0.8 to 1.2 mL. If the level of blood in any tube is outside the indicator mark, a new blood sample must be obtained.
3) Sample Minimums:
   See specimen and Reagent Volumes

4) Storage:

Store blood collection tubes at 4°C to 25°C. The blood collection tubes must be at room temperature (17°C to 25°C) at the time of blood draw. Immediately after filling the tubes, shake them ten times just firmly enough to make sure the entire surface of the tube is coated with blood. This dissolves antigens on the tube walls. Following labeling, filing and shaking, the tubes must be transferred to a 37°C ± 1°C incubator within 16 hours collection. If the QFT-Plus blood collection tubes are not incubated directly after collection and shaking, invert the tubes to mix ten times prior to incubation. Incubate the tube upright at 37°C ± 1°C for 16 to 24 hours. After incubation tubes may be held at 4oC to 27°C for up to 3 days prior to centrifugation. Harvest the plasma by centrifuging tubes for 15 minutes at 2000 to 3000g. The gel plug will separate the cells form the plasma. If this does not occur, re-centrifuge the tubes. Plasma samples can be stored for up to 28 day at 2°C to 8°C or below -20°C for extended periods. See Specimen handling for shipment form the Mobile Examination Center (MEC) to the University of Washington Virology Laboratory for analysis.

5) Specimen Handling:

a. NHANES sites will e-mail gregor@uw.edu, paulsf@uw.edu, jh3@uw.edu and uphams@uw.edu the FedEx tracking number, container ID# and excel worksheet of samples to be received in the laboratory.

b. Save attached worksheets in a new folder labeled with the receipt date J:\CVIR\Lab Files\NHANES QFTB4\Pending Worksheets.

c. Once package has arrived in the Virology laboratory. Remove contents and complete Specimen manifest (J:\CVIR\Lab Files\NHANES QFTB4\Specimen Manifest.xlsx). This includes recording:

- Container ID number (use bar code scanner)
- Number of vessels in container
- Shipment conditions (if samples are received frozen on dry ice maintain at -20ºC or colder until ready to run QFTB-Gold assay.
- Receive date
- MLS ID
- Sample storage location at Virology laboratory.

Compare Shipment Manifest Report with actual shipment. Time and date stamp manifest, initial and file in Shipping Manifest Reports Binder. If there is a deviation, report to Gregory Pepper to notify Tolliver, Eric (CDC/DDPHSS/NCHS/DHNES) vys1@cdc.gov and Storandt, Renee (CDC/DDPHSS/NCHS/DHNES) lpq9@cdc.gov

Place shipment containers in storage unit recorded in section C above.

6) Specimen Rejection:
   a. QFT plus assay 4 tubes containing 0.8 – 1.2mL of blood, NIL (gray), TB1 (green), TB2 (yellow) and MITOGEN (purple) for the QFT-Plus assay.
   b. Confirm Blood collection tubes were incubated at 37± 1°C for 16 to 24 hours at originating lab prior to shipping. Following 37 ± 1°C incubation, blood collection tubes may be held between 2°C and 27°C for up to 3 days prior to centrifugation.

E. Procedures For Microscopic Examintations; Criteria For Rejection Of Inadequately Prepared Slides

Not applicable for this procedure

F. Equipment And Instrumentation, Materials, Reagent Prepartion, Calibarators (Standards), And Controls

1) Instrumentation and/or Equipment
   a. DS2 Automated ELISA System DS-Matrix version 1.34.5
   b. Centrifuge capable of centrifuging the blood tubes at least to 3,000 RCF (g)
   c. Calibrated variable-volume pipettes for delivery of 10μL to 1000μL with disposable tips
2) Materials

   QuantiFERON®-TB Gold Plus (QFT®) ELISA kit (cat# 0594-0501-NA) which includes:
   - Microplate strips coated with murine anti-human IFN-γ monoclonal antibody (24 x 8 well strips) ~ 2 x 96 well plates
   - Human IFN-γ Standard, lyophilized (contains recombinant human IFN-γ, bovine casein, 0.01% w/v Thimerosal) ~ 1 x vial (8 IU/mL when reconstituted)
   - Green Diluent (contains bovine casein, normal mouse serum, 0.01% w/v Thimerosal) ~ 1x 30mL
   - Conjugate 100X Concentrate, lyophilized (murine anti-human IFN-γ HRP, contains 0.01% w/v Thimerosal) ~ 1 x 0.3mL (when reconstituted)
   - Wash Buffer 20X Concentrate (pH 7.2) ~ 1 x 100mL
   - Enzyme Substrate solution (contains H_2O_2, 3,3',5,5' Tetramethylbenzidine) ~ 1 x 30mL
   - Enzyme Stopping Solution (contains 0.5M H_2SO_4) ~ 1 x 15mL
3) Reagent Preparation
   a. Reconstitute the human IFN standard with distilled water as indicated on the label. The volume will vary from kit-to-kit. The reconstituted standard is stable for 3 months if stored at

2-8°C. The final concentration is calculated to be 8.0 IU/mL. A 4-point standard curve will be used.

b. Dilute one part Wash Buffer 20X concentrate with 19 parts deionized water. This working strength wash buffer solution is stable for 2 weeks at room temperature.

c. Reconstitute 100X conjugate with 0.3mL of distilled water. Mix gently to minimized frothing and ensure complete solubilization of the conjugate. This solution is stable for 3 months at 2-8°C. **DO NOT** pre-warm the conjugate 100X concentrate.

4) Calibrators

Not applicable for this procedure

5) Controls

Not applicable for this procedure

G. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Not applicable for this procedure

H. OPERATING PROCEDURE INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

1) Sample Preparation

a. Prepare pending worksheets using excel files J:\CVIR\Lab Files\NHANES QFTB4\Pending Worksheets

- Sort file by Analyte type (order Z to A).
- Sort file by Slot No (order smallest to Largest).
- Divide into runs of a maximum 22 samples in descending order of container ID followed by sample ID. Note specimen ID 9 digits long, first 6 identify the patient and last 3 indentify the vessel (233-nil, 224- TB1 antigen, 225 TB2 Antigen, 226-mitogen)

b. Include QC repeat samples. 2% of samples will be repeated using a rolling and random basis. The samples to be repeated will be selected from the previous run using http://www.mathgoodies.com/calculators/random_no_custom.html. Include a QFTB QC cover sheet. Place the worksheets in the NHANES to be run folder.

c. Bring all plasma samples and reagents to room temperature (22 ± 5°C) prior to use. Allow at least 60 minutes for equilibration. **DO NOT** pre-warm the conjugate 100X concentrate. Centrifuge tubes at 2000 – 3000g (RCF) for 5 minutes prior to set up to insure material on the surface of the gel plug are not disturbed.

2) Sample Analysis

On the automated DS2 instrument, run the assay using Matrix DS2 version 1.34.5, assay protocol DS2 DS2 QFT-Plus_v2.6 for QFT Plus. See 1200-1020 DS2 automated ELISA system guideline. Save as NHANES _mm.dd.yyyy_initials_runx.

a. Reconstitute the human IFN standard with distilled water as indicated on the label. The volume will vary from kit-to-kit. The reconstituted standard is stable for 3 months if stored at 2-8°C. The final concentration is calculated to be 8.0 IU/mL. A 4-point standard curve will be used.

b. Dilute one part Wash Buffer 20X concentrate with 19 parts deionized water. This working strength wash buffer solution is stable for 2 weeks at room temperature.

c. Reconstitute 100X conjugate with 0.3mL of distilled water. Mix gently to minimized frothing and ensure complete solubilization of the conjugate. This solution is stable for 3 months at 2-8°C. **DO NOT** pre-warm the conjugate 100X concentrate.

d. Working strength conjugate is prepared by diluting the required amount of reconstituted 100X concentrate in green diluent. **NOTE:** Green diluent contains 0.01% w/v Thimerosal. Hold hazardous waste for pick up by University of Washington Environmental Health and Safety. Use the following table:

NUMBER OF STRIPS	VOLUME OF CONJUGATE 100X CONCENTRATE	VOLUME OF GREEN DILUENT
3	15µL	1.5mL
4	20µL	2.0mL
5	25µL	2.5mL
6	30µL	3.0mL
7	35µL	3.5mL
8	40µL	4.0mL
9	45µL	4.5mL
10	50µL	5.0mL
11	55µL	5.5mL
12	60µL	6.0mL

- Mix thoroughly but gently to avoid frothing.
- Working strength conjugate must be used within 6 hours of preparation.
- **Return and unused Conjugate 100X concentrate to 2 - 8°C immediately after use.**

3) Operation

Not applicable for this procedure

4) Data Recording

Not applicable for this procedure

5) Calculations

The DS2 Matrix version 1.34.5 software will automatically analyze the raw data and calculate the 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.

6) Data Analysis and Interpretation

QuantiFERON®-TB Gold Plus results are interpreted using the following criteria:

NOTE: Diagnosing or excluding tuberculosis disease, and assessing the probability of LTBI, requires a combination of epidemiological, historical, medical, and diagnostic findings that should be taken into account when interpreting QuantiFERON®-TB Gold-Plus results. See general guidance on the diagnosis and treatment of TB disease and LTBI (http://www.cdc.gov/nchstp/tb/).

Nil [IU/mL]	TB1 Antigen minus Nil [IU/mL]	TB2 Antigen minus Nil [IU/mL]	Mitogen minus Nil [IU/mL][1]	QuantiFERON®-TB Plus Result	Reporting
≤ 8.0	≥ 0.35 and ≥ 25% of Nil value	Any	Any	Positive[2,3]	POS
	Any	≥ 0.35 and ≥ 25% of Nil value			POS
	< 0.35 OR ≥ 0.35 and < 25% of Nil value	< 0.35 OR ≥ 0.35 and < 25% of Nil value	≥ 0.5	Negative	NRN
			< 0.5	Indeterminate[d]	INDET
> 8.0	Any	Any	Any	Indeterminate[e]	INDET

a. Responses to the Mitogen positive control and 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 QuantiFERON®-TB Gold IT software as > 10 IU/mL.

b. Where *M. tuberculosis* infection is not suspected, initially positive results can be confirmed by retesting the original plasma samples in duplicate in the QuantiFERON®-TB Gold IT ELISA. If repeat testing of one or both replicates is positive, the test result is considered positive.

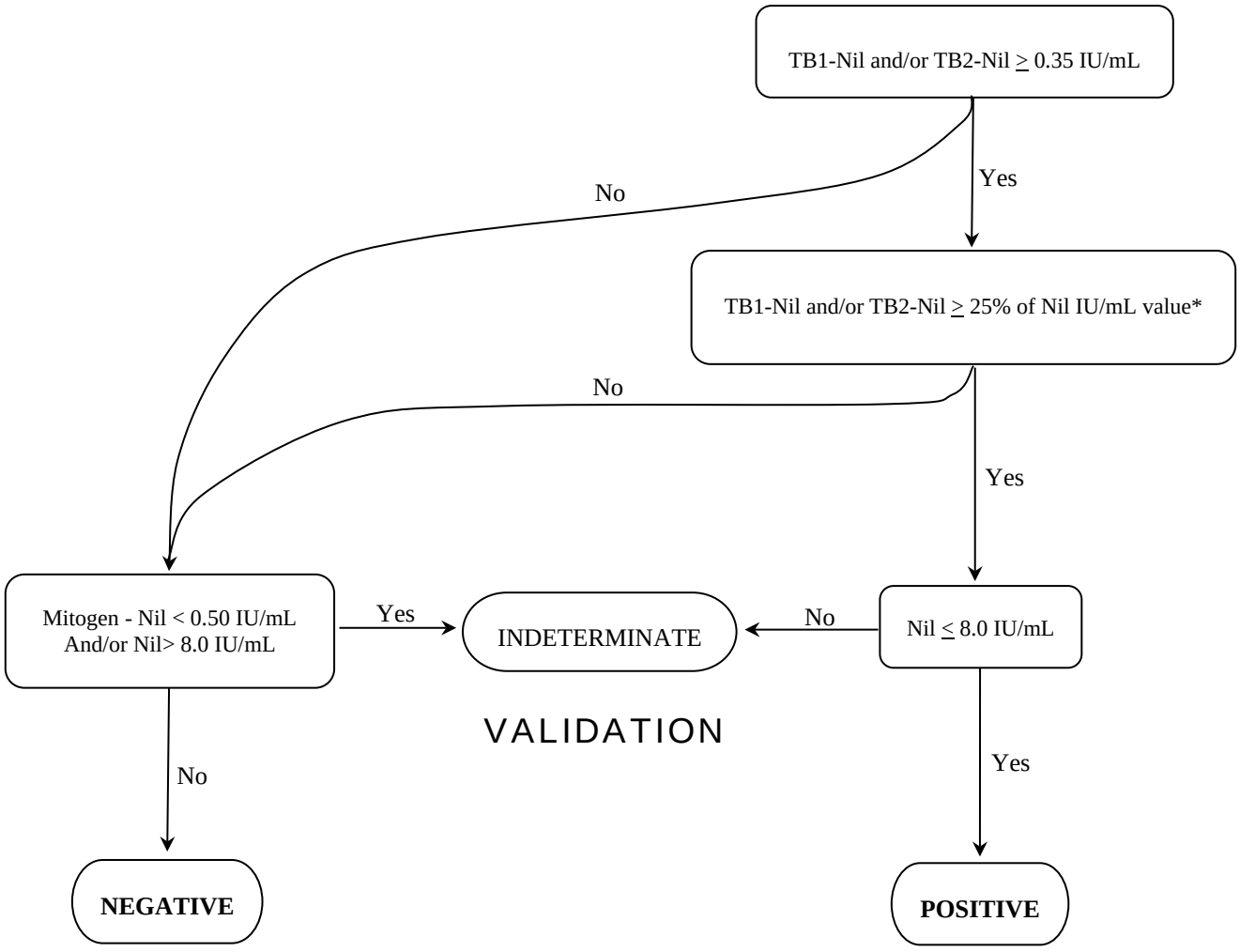
c. A positive TB response to persons who are negative to mitogen is rare, but has been seen in patients with TB disease (Mitogen minus Nil <0.5 IU/mL). This indicates the IFN-γ response to TB antigen is greater than that to mitogen, which is possible, as the level of mitogen does not maximally stimulate IFN-γ production by lymphocytes.

d. Indeterminate results may be related to the immune status of the individual being tested or be related to technical factors:
   - Excessive levels of circulating IFN-γ or presence of heterophile antibodies.
   - Incorrect transport/handling of blood specimens.
     - Insufficient mixing of blood collection tubes
     - Storage of filled blood collection tubes outside the recommended temperature range prior to incubation
     - Longer than 16 hours from blood draw to incubation
     - Incomplete washing of the ELISA plate
     - Deviations from the procedure

e. In clinical studies, less than 0.25% of subjects had IFN-γ levels of > 8.0 IU/mL for the Nil control.

NOTE: 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.

Interpretation Flow Diagram



QFT-Plus test interpretation * 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.

7) Transfer to network drive
   a. Upon completion of the QFTB assay. Print the NHANES_mm.dd.yyyy_initials_runx run report.
   b. Export the OD values (raw test data) in the .cvs file to a memory stick. Transfer raw data run folders J:\CVIR\Lab Files\NHANES QFTB4\Raw Data
   c. Transpose raw data from vertical format to match DS2 plate map. Open in Macro Raw Data 1 J:\CVIR\Lab Files\NHANES QFTB4\Macro Raw Data 1.xlsm. Run Macro 1 and Macro 2. The data is now in a format that can be copies and paste into the QuantiFERON®-TB Gold IT analysis software (version 2.17) see Appendix
   d. Complete excel file located Z:\Lab Files\NHANES QFTB\Pending worksheets\receipt date. This includes:
      - Analysis date
      - Run number
      - Analyte result
        - Nil value (11 coded value for >10)
        - Antigen value (11 coded value for >10)
        - Interpretation coded values
          - 1 – positive
          - 2 – negative
          - 3 – indeterminate low mitogen
          - 4 – indeterminate high nil
        - Mitogen value (11 coded value for >10)
      - Result comment coded values
        - 0 – Ok (required for all valid results)
        - 103 – above the limit of detection (use with interpretation code 11)
        - 126 - overincubated at site
        - 127 - improper specimen handling (ie. gel plug not separated from plasma at site)
        - 62 - frozen sample (use for all sample received on dry ice)
      - QC repeat (if required)
      - LOD (limit of detection) coded value
        - 10 – above limit of detection (use for all analyte result >10 IU/mL)

e. Initial and date NHANES Quantiferon worksheet results enter section.

f. Copy QC repeat sample data when applicable to Z:\Lab Files\NHANES QFTB\QC Repeats.xls file. Enter the following data:

- QC repeat date
- QC repeat run number
- Tech ID
- Container ID

g. Review all resulted data. Initial and date NHANES Quantiferon worksheet result QC check section. Post results on securetransfer2.westat.com

User name: lab

Password: Rta$6171

- Upload To /Distribution/LAB/Lab115

h. Move resulted folders:

- From Z:\Lab Files\NHANES QFTB\Pending worksheets\receipt date to Z:\Lab Files\NHANES QFTB\Pending worksheets
- To Z:\Lab Files\NHANES QFTB\Pending worksheets\reported results\result posted

I. Reportable Range

Not applicable for this procedure

J. Quality Control

1) In-house positive, negative and indeterminate controls are included with each new kit lot or a minimum of every 6 months.
   - Record controls in the Serology EIA_IFA Quality Control (QC) chart J:\CVIR\Lab Files\Serology QC\Serology EIA_IFA QC chart - current.xlsx tab QFTB plus. %CV ≤ 25% for TB1 and TB2 antigen compared to the mean.
2) Proficiency testing is done using CAP series QF-B (QF)
3) Print the report and worklist generated by the Matrix version 1.34.5software. Compare Sample ID printout to worksheet CID printout to ensure all vessels were loaded in the correct location.
4) The accuracy of test results is dependent on the generation of an accurate standard curve. Therefore, results derived from the standards are examined before test sample results can be interpreted.

   For the ELISA to be valid:
   - The mean OD for Standard 1 must be ≥ 0.600.
   - The % coefficient of variation (%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 units from their mean.
- The correlation coefficient (r) calculated from the mean absorbance values of the standards must be $\geq$ 0.98.

K. Remedial Action If Calibration Or QC Systems Fail To Meet Acceptable Criteria

If the above criteria are not met, the run is invalid and must be repeated/reviewed by Supervisory personnel prior to reporting.

- Refer to QuantiFERON-TB GOLD package insert ELISA trouble-shooting.
- The mean OD value for Standard 4 (green diluent) should be $\leq$ 0.150. If the mean OD is > 0.150, the plate washing procedure should be investigated.

L. Limitations Of Method; Interfering Substances And Conditions

1) Limitations of Method
   a. 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.
   b. The predictive value of a positive QuantiFERON®-TB Gold Plus result in diagnosing *M. tuberculosis* infection depends on the probability of infection, which is assessed by historical, epidemiological, diagnostic and other findings.
   c. A diagnosis of LTBI requires that tuberculosis disease must be excluded by medical evaluation.
   d. 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.
2) Interfering Substances and Conditions
   a. The effect of lymphocytes count on the reliability of the QFT-Plus results is unknown. The minimum number of lymphocytes required for a reliable test result has not been established and also may be variable.
   b. A false-negative QFT-Plus result can be caused by incorrect blood sample collection or improper handling of specimen affectint lymphocyte function.
   c. Delay in incubation may cause false or indeterminate results.

M. Reference Ranges (Normal Values)

Not applicable for this procedure

N. Critical Call Results ("Panic Values")

Not applicable for this procedure

O. Specimen Storage And Handling During Testing

Bring all plasma samples to room temperature (22 ± 5°C) prior to use. Allow at least 60 minutes for equilibration. During testing the samples remain on the automated DS2 instrument. Samples are removed from the instrument and frozen -10 to -25°C for long term storage after testing is completed.

P. Alternate Methods For Performing Test Or Storing Specimens If Test System Fails

A backup automated DS2 instrument is available to run the procedure. In-house control material is available for comparison prior to use.

Q. Test Result Reporting System; Protocol For Reporting Critical Calls

Not applicable for this procedure

R. Transfer Or Referral Of Specimens; Procedures For Specimen Accountability And Tracking

A specimen manifest is maintained (J:\CVIR\Lab Files\NHANES QFTB4\Specimen Manifest.xlsx)
This includes recording:

1. Container ID number (use bar code scanner)
2. Number of vessels in container
3. Shipment conditions (if samples are received frozen on dry ice maintain at -20°C or colder until ready to run QFTB-Gold assay.
4. Receive date
5. Tech ID
6. Sample storage location at Virology laboratory.

S. Summary Statistics and QC Graphs

Not applicable for this procedure

REFERENCES

1. QuantiFERON®-TB Gold Plus (QFT®) ELISA Package Insert measuring responses to ESAT-6, CFP10 peptide Antigens. (Rev. Date August 2017).

2. Ferrara, G., *et al*. Routine hospital use of commercial whole blood interferon- gamma assay for tuberculosis infection. *Am J Respir Crit Care Med*, 2005. 172:631-5.

APPENDIX

Generation of Standard Curve and Sample Values using QuantiFERON®-TB Gold IT analysis software *(If matrix version 1.34.5 analysis software is not used)*

1) Locate the QFTB (READ ONLY) file and double click on it (J:\CVIR\Lab Files\QFTB (READ ONLY).
    - For QFT select QFTB_gold_v2.17.3_setup (READ ONLY) and follow direction below starting with Figure 1.
    - For QFT-Plus select QFT_TBGoldPlus_2.71.2_Build06_SOW-58-0-00 and run program set up for the QFT-Plus 4 tube/4 point standard curve (follow directions per QFTv2.17.3 program but remember to select the correct format either QFTB PLUS 4 point standard full plate or QFTB PLUS 4 point standard partial plate).

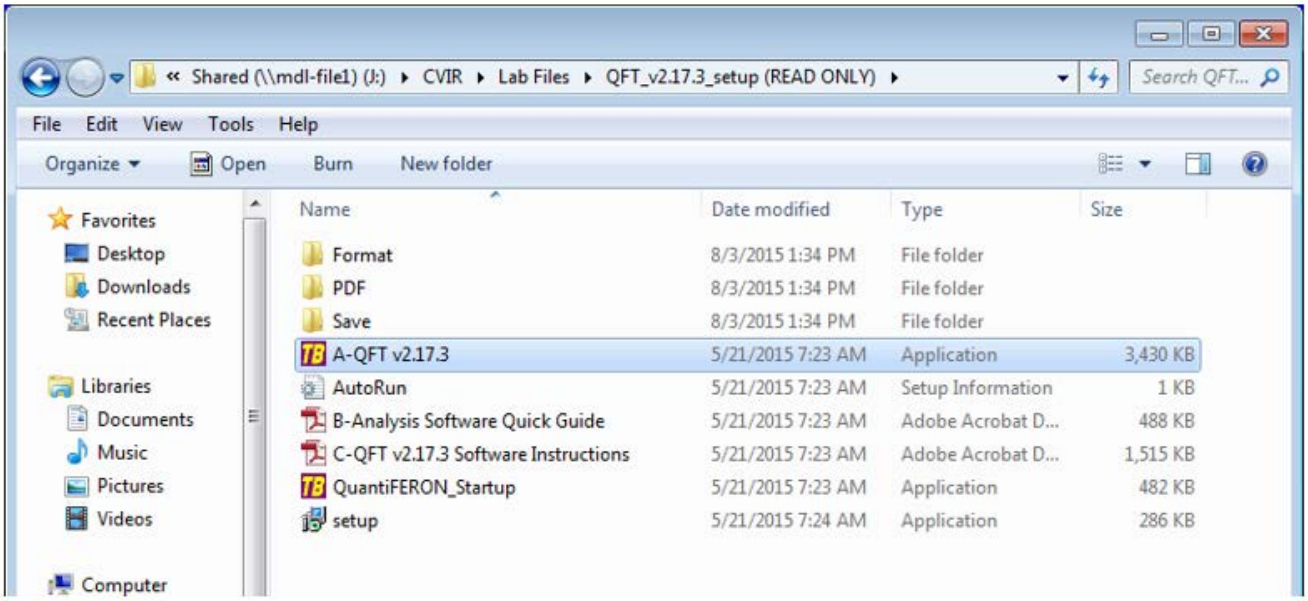


Figure 1

2) Double click TB A-QFT v2.17.3 and RUN.

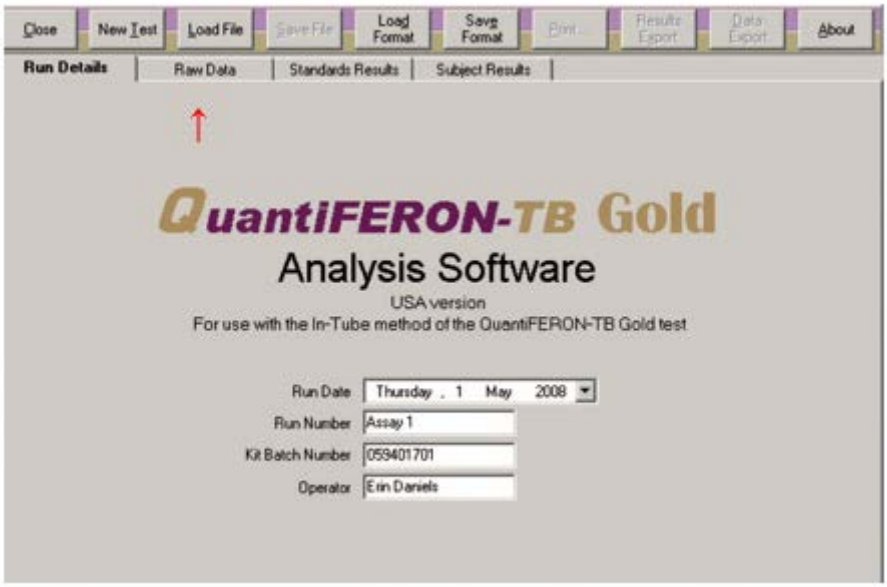


Figure 2

- Enter the following information in the fields provided
  - Run date (drop-down calendar)
  - Run Number
  - Kit Batch Number
  - Operator

3) Select the Raw Data tab to advance to the next screen (see Figure 2 ↑).

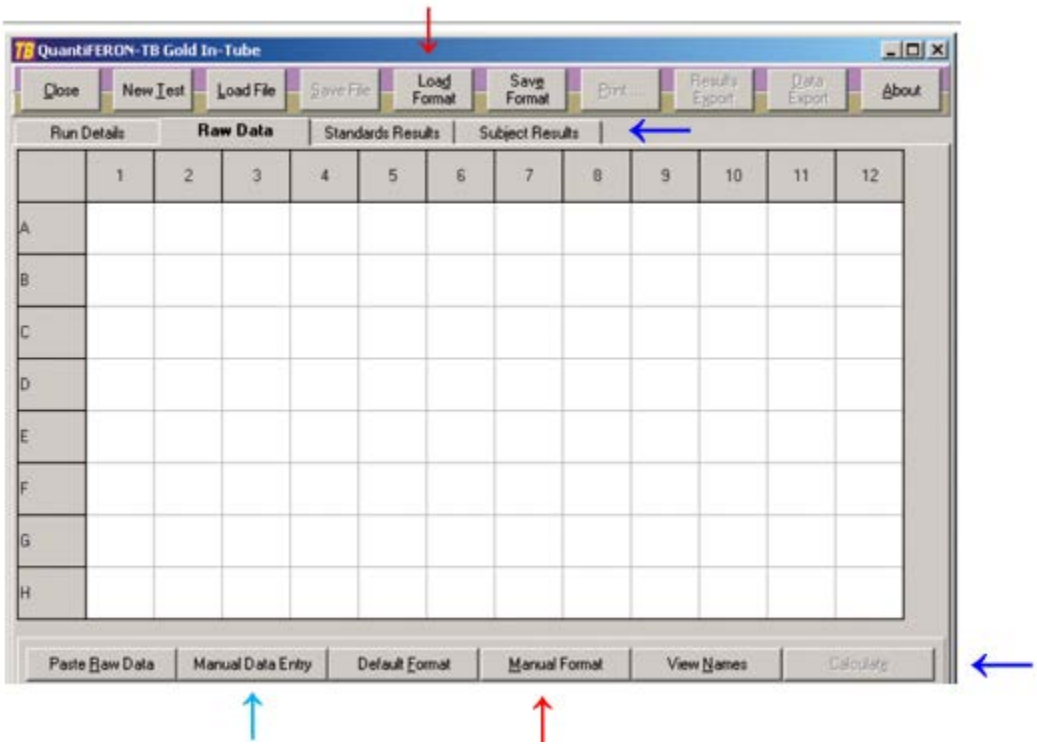


Figure 3

4) Select the Load Format Button (Figure 3 ↓) followed by the QFTB 8 point standard partial plate.qff to automatically assign the testing layout for the standards. Set up the standards in the same configuration as outlined in Figure 4. If a full plate was run select the QFTB 8 point full plate.qff.

Quantiferon Gold-Plus plate map

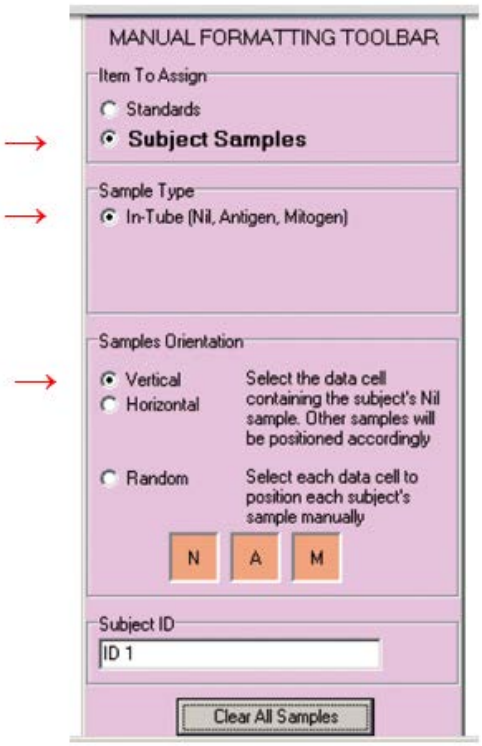
	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	1N	3N	5N	7N	9N	11N	13N	15N	17N	19N	21N
B	S1	1TB1	3TB1	5TB1	7TB1	9TB1	11TB1	13TB1	15TB1	17TB1	19TB1	21TB1
C	S2	1TB2	3TB2	5TB2	7TB2	9TB2	11TB2	13TB2	15TB2	17TB2	19TB2	21TB2
D	S2	1M	3M	5M	7M	9M	11M	13M	15M	17M	19M	21M
E	S3	2N	4N	6N	8N	10N	11N	13N	16N	18N	20N	22N
F	S3	2TB1	4TB1	6TB1	8TB1	10TB1	12TB1	14TB1	16TB1	18TB1	20TB1	22TB1
G	S4	2TB2	4TB2	6TB2	8TB2	10TB2	12TB2	14TB2	16TB2	18TB2	20TB2	22TB2
H	S4	2M	4M	6M	8M	10M	12M	14M	16M	18M	20M	22M

Figure 4

- S1 (standard 1), S2 (standard 2), S3 (standard 3), S4 (standard4),
- 1N (sample1. Nil control plasma):1TB1 (sample1. TB1 Antigen plasma): 1TB2 (sample1. TB2 Antigen plasma):1M (sample1.Mitogen Control plasma).

5) Select the Manual Format button to open the manual formatting toolbar (Figure 3 ↑). This toolbar is used to manually assign plate format and sample ID's. Alternatively, subject

identification can be added by left-clicking on the colored block for each subject and typing (scanning barcode) the identification in the pop-up box.



- In order to assign sample information to the data select the Subject sample radio button
- Assign sample information using the vertical radio button. Click the cell containing the Nil sample. Antigen and mitogen will be positioned accordingly. The sample information will be set out in the same configuration as outlined in figure 4 Plate Layout.
- Prior to assigning the sample data the subjects name/ID are to be entered in the Subject ID field. Subject names can be changed at any stage by left clicking on the colored block for each subject and typing the new name in the pop up box.
- Finish by selecting the Complete button.

6) Select the Manual Data Entry button. Click on the cell to enter the data manually. Press **Enter** or click on another cell to store the value. (Figure 3 ↑).

7) Once the format has been generated and data entered, select the Calculate button which will now be active (Figure 3 ←), the standard curve for the assay will be automatically analyzed and the standard results screen displayed.

8) The results of the Quality Control acceptance criteria will be shown as PASS or FAIL. If any of the QC criteria are not met, the ELISA test is invalid and must be repeated (see package insert for further details of the acceptance criteria). Select the Save File button (Fgure 5 ↑) to Save File as .qdf file. Use the following nomenclature “mmddyy QFTB- initials”.

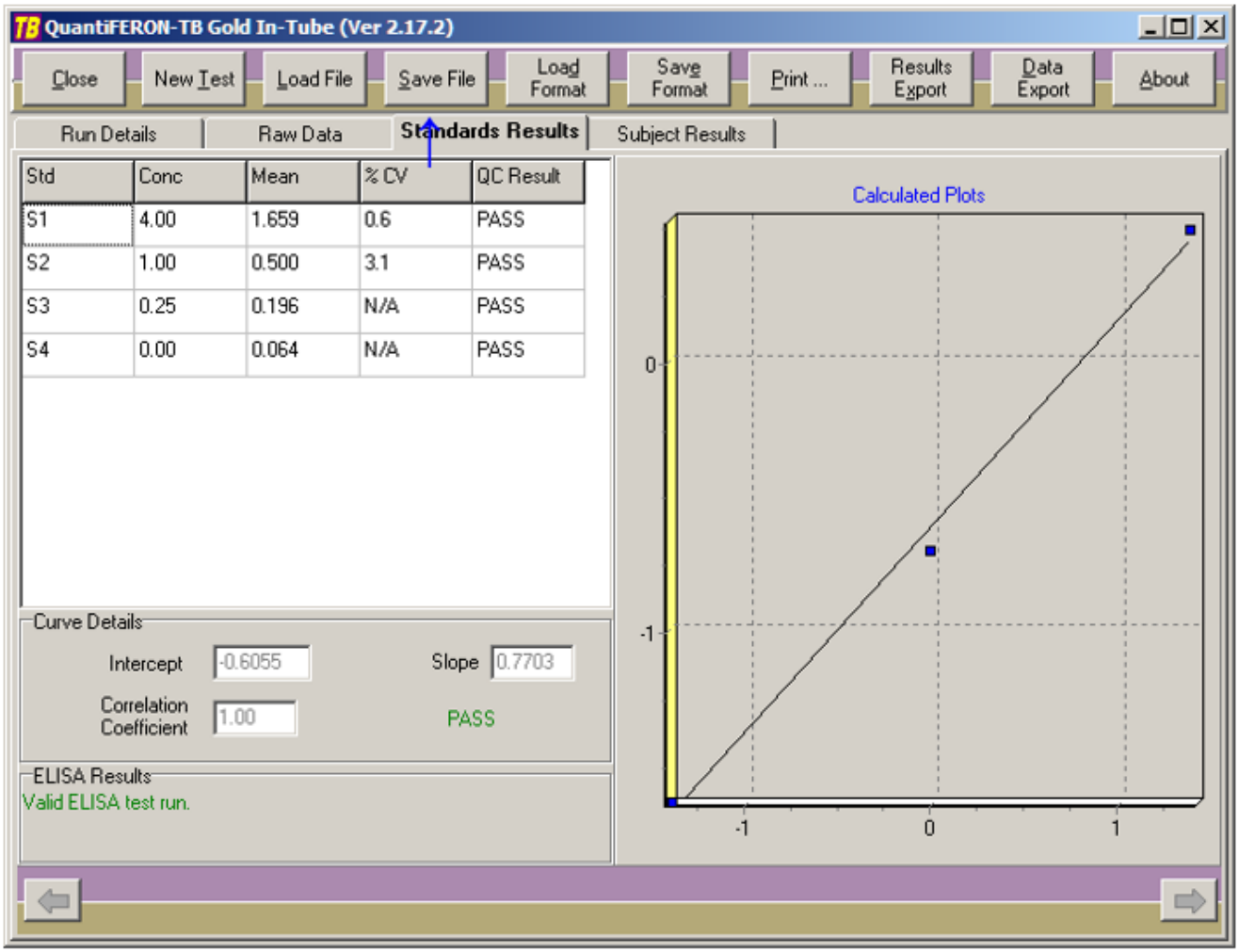
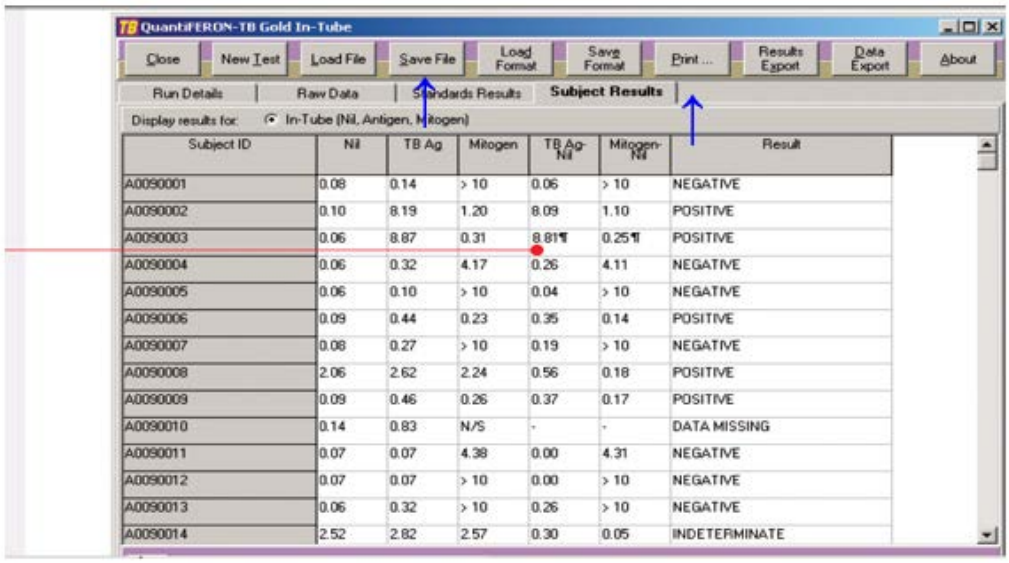


Figure 5

9) Select the Subject Results tab to proceed to next screen (figure 3 ←)

If the mitogen minus Nil esults is less than 0.35IU/mL the result is flagged as a possible mix up. This warning helps to limit the possibility of a false positive result due to a mix up of the TB antigen and Mitogen samples.



Subject ID	Nil	TB Ag	Mitogen	TB Ag-Nil	Mitogen-Nil	Result
A0090001	0.08	0.14	> 10	0.06	> 10	NEGATIVE
A0090002	0.10	8.19	1.20	8.09	1.10	POSITIVE
A0090003	0.06	8.87	0.31	8.81¶	0.25¶	POSITIVE
A0090004	0.06	0.32	4.17	0.26	4.11	NEGATIVE
A0090005	0.06	0.10	> 10	0.04	> 10	NEGATIVE
A0090006	0.09	0.44	0.23	0.35	0.14	POSITIVE
A0090007	0.08	0.27	> 10	0.19	> 10	NEGATIVE
A0090008	2.06	2.62	2.24	0.56	0.18	POSITIVE
A0090009	0.09	0.46	0.26	0.37	0.17	POSITIVE
A0090010	0.14	0.83	N/S	-	-	DATA MISSING
A0090011	0.07	0.07	4.38	0.00	4.31	NEGATIVE
A0090012	0.07	0.07	> 10	0.00	> 10	NEGATIVE
A0090013	0.06	0.32	> 10	0.26	> 10	NEGATIVE
A0090014	2.52	2.82	2.57	0.30	0.05	INDETERMINATE

Figure 6

Missing Data is reported if any of a subject's plasma samples display the value N/S (no sample)

10) Select the print button (Figure 6 ↑) to generate a report hard copy, then select Save File (Figure 5 ↑) (save as .pdf file). Use the following nomenclature "mmddyy QFTB- initials".