

Laboratory Procedure Manual

Analyte: **QuantiFERON[®]-TB Gold**

Matrix: **Whole Blood**

Method: **QuantiFERON[®]-TB Gold IT kit**

Method No.:

Revised: *September, 2013*

as performed by: **University of Washington**

Contact: **Dr. Kovel**

Important Information for Users

The University of Washington 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.

This document details the Lab Protocol for testing. The file name is TB_QFT_G. The variable name is LBXTBA with SAS label (TB antigen result), LBXTBN with SAS label (TB numeric result), LBXTBIN with SAS label (TB Nil Number Result) and LBXTBM with SAS label (TB mitogen control result).

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Clinical Significance:

QuantiFERON[®]-TB Gold IT 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.

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. 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 QuantiFERON[®]-TB Gold IT 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 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.

Numerous studies have demonstrated that the peptide antigens (ESAT-6, CFP-10 and TB7.7) used in QuantiFERON[®]-TB Gold IT 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, CFP-10 and TB7.7 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.

Principle:

The QuantiFERON[®]-TB Gold IT test 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 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 QuantiFERON[®]-TB Gold IT system uses specialized blood collection tubes, which are used to collect whole blood via venipuncture, which include a Nil control tube, TB Antigen tube and a Mitogen tube

(positive control). The tubes are shaken to mix antigen with the whole blood and incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 16 to 24 hours. Following the incubation period, plasma is harvested and the amount of IFN- γ 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.

2. SAFETY PRECAUTIONS

- A. **Follow all procedures and policies in the Laboratory Safety Manual, including the Universal Blood and Body Substance Technique (UBBST). Consider all specimens received for analysis potentially positive for infectious agents.**
- B. **Wear gloves, lab coat, and safety glasses while handling all specimens. Dispose analyzed specimens and contaminated supplies in autoclave/biohazard bags; seal and autoclave. Wipe all work surfaces with disinfectant solution.**
- C. **Recommend to laboratory personnel performing the assay that they receive the HBV vaccine. Maintain records of vaccination or signed declination forms.**
- D. **Label all reagents indicating the preparation date, expiration date, formula, lot number if applicable, hazards of the reagent, antidote of contact with hazard, and the initials of the technician.**

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

- A. Microsoft Excel software on a PC and our Laboratory Information Systems (L.I.S.) are used to manage the data.
- B. Results are entered into spreadsheet and double-checked.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

Collect 1 ml of blood by venipuncture directly into each of the QuantiFERON[®]-TB Gold IT blood collection tubes, which include a Nil Control tube, TB Antigen tube and a Mitogen tube. Tubes should be between $22^{\circ}\text{C} \pm 5^{\circ}\text{C}$ 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. Ship tubes to laboratory at $22^{\circ}\text{C} \pm 5^{\circ}\text{C}$ as soon as possible and within 16hrs of collection. Do not refrigerate or freeze the blood samples.

The assay is set up at least once a week or more frequently depending on work load. Samples are stored for a minimum 7 days at $2^{\circ} - 8^{\circ}\text{C}$ after final results have been posted.

5. **PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES**

Not applicable for this procedure.

6. **EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS**

Materials:

QuantiFERON®-TB Gold IT kit 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) ~ 1 x 30 ml
- Conjugate 100X Concentrate, lyophilized (murine anti-human IFN- γ HRP, contains 0.01% w/v Thimerosal) ~ 1 x 0.3 ml (when reconstituted)
- Wash Buffer 20X Concentrate (pH 7.2, contains 0.01% w/v Thimerosal) ~ 1 x 100 ml
- Enzyme Substrate solution (contains H₂O₂, 3,3',5,5' Tetramethylbenzidine) ~ 1 x 30 ml
- Enzyme Stopping Solution (contains 0.5M H₂SO₄) ~ 1 x 15 ml

37° C \pm 1° C Incubator (with or without CO₂)

Calibrated variable-volume pipettes for delivery of 10 μ l to 1000 μ l with disposable tips.

Calibrated multichannel pipette capable of delivering 50 μ l and 100 μ l with disposable tips.

1 ml microtubes with caps in 96 well format racks or uncoated microtitre plates with plastic seals for plasma storage (26 patients / rack or plate) – not essential.

Centrifuge capable of centrifuging the blood tubes at least to 3,000 RCF (g).

Microplate shaker capable of speeds between 500 and 1,000 rpm.

Deionised or distilled water – 2 L.

Microplate washer (for safety in handling plasma samples, an automated washer is recommended).

Microplate reader fitted with 450 nm filter and 620 nm to 650 nm reference filter.

Variable speed vortex.

Timer

Measuring cylinder – 1 L or 2 L.

Reagent reservoirs.

7. **CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES**

There are no calibration procedures for this assay.

8. **PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS**

A. Procedure

1. When QFTB tubes are received, check to make sure:
 - a) There are 3 tubes containing 0.8 – 1.2 ml of blood, NIL (gray), ANTIGEN (red) and MITOGEN (purple)
 - b) All tubes should be transported at room temperature (22° C \pm 5° C):

Blood collection was done <16 hours from the current time, Re-mix tubes by inverting 10 times immediately prior to incubation. Incubate tubes upright at 37° C \pm 1° for 16 to 24 hours **or**

Blood collection tubes were incubated at 37° C \pm 1° for 16 to 24 hours at originating lab prior to shipping. Following 37° C \pm 1° incubation, blood collection tubes may be held between 2° C and 27° C for up to 3 days prior to centrifugation

2. If all criteria are met, fill out spreadsheet that is stored in the QFTB folder in log in. Place a post-it note on the request form that states the time that the tubes should be removed from the incubator. (If blood collection tubes were incubated at originating lab proceed to step 3).
3. After incubation of the tubes at 37° C \pm 1° for 16 to 24 hours (following incubation blood collection tubes may be held between 2° C and 27° C for up to 3 days prior to centrifugation), centrifuge tubes at 2000 – 3000g (RCF) for

15 minutes. The gel plug will separate the cells from the plasma. If this does not occur, the tubes need to be re-centrifuged at a higher speed. Centrifuged tubes should be handled with care to avoid mixing of plasma.

4. Samples are to be stored in the refrigerator in W-8814 (log-in) in the QFTB test to be done box. Complete the spreadsheet with the date and time of centrifugation and refrigeration of the samples. Plasma in centrifuged tubes is stable for up to 28 days when stored at 2° - 8° C (for extended periods, harvest the plasma and hold below -20° C).
5. Pull up QFTB worksheet including sample CID's. All plasma samples and reagents must be brought to room temperature (22°C +/- 5° C) prior to use. **DO NOT** pre-warm the conjugate 100X concentrate. Allow at least 60 minutes for equilibration.

B. Automated Procedure:

1. On the automated Dynex instrument, run the assay using Revelation DSX version 6.12, assay protocol DSX QFT 4pt ver4.12 output file. See DSX procedure version 1200-215-05. Rename plate accordingly (QFTB mmddyy - initials)/(NHANES_mm.dd.yyyy_run#). See manual procedure if Dynex is not available.
 - 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. **NOTE: Wash buffer contains 0.01% w/v Thimerosal. Hold hazardous waste for pick up by University of Washington Environmental Health and Safety.**
 - C. Reconstitute 100X conjugate with 0.3 ml 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.
 - 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:
 - Mix thoroughly but gently to avoid frothing.
 - Working strength conjugate should be used within 6 hours of preparation.
 - **Return and unused Conjugate 100X concentrate to 2° C and 8° C immediately after use.**

C. Manual Procedure:

1. Pull out the number of strips needed for the assay. You will need 8 wells for a 4-point standard curve plus 3 wells for each patient. There is a plate layout template in the Appendix A. Fill out the plate template for your run. Refer to DYNEX for automated set-up procedure.
2. 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°C - 8° C. The final concentration is calculated to be 8.0 IU/ml. A 4 point standard curve will be used. Prepare standard dilutions as follows:
 - a) "Standard 1" add 150 µl of reconstituted kit standard and 150 µl of green diluent (final concentration 4.0 IU/ml).
 - b) "Standard 2" add 300 µl of green diluent and 100 µl of standard 1 (final concentration 1.0 IU/ml).
 - c) "Standard 3" add 300 µl of green diluent and 100 µl of standard 2 (final concentration 0.25 IU/ml).
 - d) "Standard 4" green diluent (concentration 0.0 IU/ml).
3. 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. **NOTE: Wash buffer contains 0.1% Thimerosal. Hold hazardous waste for pick up by University of Washington Environmental Health and Safety.**
4. Reconstitute 100X conjugate with 0.3 ml of distilled water. Mix gently to minimized frothing and ensure complete solubilization of the conjugate. This solution is stable for 3 months at 4° C.
 - 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:
 - Mix thoroughly but gently to avoid frothing.

- Working strength conjugate should be used within 6 hours of preparation.
 - **Return and unused Conjugate 100X concentrate to 2° C and 8° C immediately after use.**
5. Add 50 µl of freshly prepared working strength conjugate to each well.
 6. Add 50 µl of test plasma samples to appropriate wells following the plate template. (See Appendix A for layout).
 7. Add 50 µl of standards 1 – 4. The standards are assayed in duplicate (see plate map).
 8. Mix the plate on a microplate shaker for 1 minute at 500 – 1000 RPM.
 9. Cover and incubate the plate for at room temperature (22° C ± 5°C) for 120 ± 5 minutes.
 10. Wash wells with approximately 200µl of working strength wash buffer 6 times. Tap out excess fluid.
 11. Add 100 µl of enzyme substrate solution and mix the plate on a microplate shaker for 1 minute at 500 – 1000 RPM.
 12. Cover and incubate the plate for 30 minutes at room temperature (22° C ± 5°C). Plate should not be exposed to direct sunlight during incubation.
 13. Add 50 µl of enzyme stop solution to wells in the same order and at approximately the same speed as the substrate in step 18.
 14. Measure the OD of each well at 450 nm/630 nm within 5 minutes of adding the stop solution.

D. Calculations:

Revelation DSX version 6.12 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.

QuantiFERON®-TB Gold IT analysis software (version 2.17.2) may also be used to analyze raw data and calculate results. See QuantiFERON®-TB Gold IT analysis software Appendix C

Interpretation of Results

QuantiFERON®-TB Gold IT 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 IT results. See general guidance on the diagnosis and treatment of TB disease and LTBI (<http://www.cdc.gov/nchstp/tb/>).

1. Responses to the Mitogen positive control (and occasionally TB antigen) can be outside the range of the microplate reader. This has no impact on test results. Values > 10 IU/ml are reported by the QuantiFERON®-TB Gold IT software as > 10 IU/ml.
2. 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.
3. Indeterminate results are uncommon and may be related to the immune status of the individual being tested, but may also be related to technical factors:
 - Longer than 16 hours from blood draw to incubation at 37° C +/- 1° C
 - Storage of filled blood collection tubes outside the recommended temperature range (22° C +/- 5° C) prior to the incubation at 37° C +/- 1° C
 - Insufficient mixing of blood collection tubes
 - Incomplete washing of the ELISA plate

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

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

E. Reporting:

Enter the IU/ml results for each of 3 tubes (nil, antigen, and mitogen).

Enter interpretation as:

- QFPOS2: **Positive** - Evidence of *Mycobacterium tuberculosis* specific memory T cell response. This test cannot differentiate between latent tuberculosis infection (LTBI) and active *M. tuberculosis* infection. Clinical correlation advised.
- QFPOS3: (¶) **Positive** - Evidence of *Mycobacterium tuberculosis* specific memory T cell response. **A positive TB response to persons who are negative to mitogen is rare, but has been seen in patients with TB disease.** This test cannot differentiate between latent tuberculosis infection (LTBI) and active *M. tuberculosis* infection. Clinical correlation advised.
- QFNEG: **Negative** - *M. tuberculosis* infection NOT likely
- QFILM: **Indeterminate** - Quantiferon test not interpretable due to insufficient reaction in the mitogen control. This could be due to underlying immune suppressive condition or improper specimen collection or handling. If latent TB suspected and no immune suppression present, repeat sample is recommended.
- QFIHN: **Indeterminate** - Quantiferon test not interpretable due to elevated reaction in the Nil control tube, repeat sample is recommended.

Print completed worksheet to double check the computer entries

9. **REPORTABLE RANGE OF RESULTS**

Results are reported as positive, negative or indeterminate.

10. **QUALITY CONTROL (QC) PROCEDURES**

Print dat file and .sid file. Compare SID printout to DAT printout to ensure all vessels were loaded in the correct location. Initial and date SID printout.

The accuracy of test results is dependent on the generation of an accurate standard curve. Therefore, results derived from the standards must be examined before test sample results can be interpreted.

For the ELISA to be valid:

- The % coefficient of variation (%CV) between replicates for standards 1 and 2 must be $\leq 15\%$.
- Replicate OD values for standards 3 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 QuantiFERON[®]-TB Gold IT analysis software calculates and reports these quality control parameters.

If the above criteria are not met, the run is invalid and must be repeated.

- Refer to QuantiFERON-TB GOLD package insert ELISA trouble shooting.

If test results are delayed, contact Laboratory Reference Services at 206-685-6066 to inform the clients.

Proficiency testing is done using CAP series QF-B (QF)

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

Remedial action for out of control conditions includes examination of the pipetting and detection equipment and examination of reagent materials.

12. LIMITATIONS OF THE METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- 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 QuantiFERON[®]-TB Gold IT 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.
- 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.

Indeterminate results may occur due to:

- Deviations from the procedure.
- Incorrect transport/handling of blood specimens
- Excessive levels of circulating IFN- γ or presence of heterophile antibodies
- Longer than 16 hours from blood specimen collection to incubation at 37° C \pm 1°

13. REFERENCE RANGES (NORMAL VALUES)

A negative value is normal for this type of testing.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable for this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Store specimens at 2-8° until analysis. Specimens reach room temperature during analysis.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Samples will remain in refrigerator until instrument is back in operation. If the system is inoperable >36 hours, Specimens in the original NHANES tubes may be refrozen until analysis is possible.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Test results that are not abnormal are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an Excel file, either through Internet FTP transfer of files or electronic mail or other electronic means.

All data are reported electronically to Westat within 21 days of receipt of specimens.
Internet FTP transfer of files is available and is preferred for data transfer.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Shipments of frozen specimens are logged in upon receipt by the Laboratory. All notebooks disks and files containing raw data, final data, QC information, communications, etc. are saved. These serve as documentation for specimen accountability and tracking.

Microsoft Excel spreadsheets are used to keep records and track specimens with the data taken from the Laboratory Information System. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study.

19. SUMMARY STATISTICS AND QC GRAPHS

Qualitative assays are assays with a positive, negative or borderline/indeterminate result. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.

REFERENCES

1. QuantiFERON-TB GOLD (In-Tube Method) measuring responses to ESAT-6, CFP1- and TB & .7 peptide Antigens Package Insert.
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.

TB_QFT in blood
NHANES 2011-2012