APPENDIX A: Quantiferon Testing

APPENDIX A QUANTIFERON TB GOLD COLLECTION

Test Name: Quantiferon In-Tube

CPT Code: 86480

Test Includes: Quantiferon TB reported as negative, positive, or indeterminate

Method: Enzyme linked Immunosorbant Assay (ELISA)

Reporting: Samples are tested on Fridays and reported Friday afternoon.

Critical values: n/a

Specimen requirements:

Specimen type: Whole Blood. Special collection kits are required. Follow instructions from the kit. The tubes fill slowly. If using a butterfly needle a discard tube must be drawn before collecting the quantiferon tubes.

Special processing required: Sample collectors must read Quantiferon TB Gold In-Tube Procedure, become familiar with instructions for sample collection and receive training before collecting Quantiferon samples.

COLLECT BLOOD USING GREY, RED, and PURPLE TUBES
(special tubes provided by PHLET)

[Image of three Quantiferon TB Gold Blood Collection Tubes]

3 tubes required

Quantiferon TB Gold Blood Collection Tubes

Proper training in specimen collection is necessary to ensure accurate results. New employees should be trained in the proper technique to collect a quantiferon sample and demonstrate proper sample collection and handling 5 times before collecting the specimens without supervision. Training materials are available from PHLET.
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Sample rejection: Mislabeled specimens, insufficient or excess volume in the QFT tubes, specimens not processed according to Quantiferon TB Gold In Tube Procedure including: Specimens not incubated for the time specified by the Quantiferon TB gold in Tube procedure (16-24 hours at 37°C) and specimens not received by the laboratory within 72 hours of incubation.

Limitations:

1. A negative QFT result does not preclude the possibility of Mycobacterium tuberculosis infection or tuberculosis disease: false negative results can be due to stage of infection (e.g. specimen obtained prior to the development of cellular immune response), co-morbid conditions which affect immune function, or other immunological factors.
2. A false negative QFT result can be caused by incorrect blood sample collection or improper handling of the antigens affecting lymphocyte function. Blood tubes must be incubated at 37°C +/- 1°C with stimulation antigens within 16 hours of collection: delay in incubation may cause false negative or indeterminate results (refer to Package insert, Technical information section) and other technical parameters may affect ability to detect a significant IFN-γ response.
3. Incorrect performance of the assay may cause false negative results.
4. Incorrect performance of the assay may cause false positive QFT responses. A positive Quantiferon test should not be the sole or definitive basis for determining infection with M. tuberculosis.
5. The effect of lymphocyte count on reliability of QFT results is unknown. Lymphocyte counts may vary over time for an individual person, and from person to person. The minimum number of lymphocytes required for a reliable test result has not been established and may also be variable.
6. A positive QFT result should be followed by further medical evaluation for active tuberculosis disease (e.g. Acid fast bacilli (AFB) smear and culture, chest x ray).
7. A positive QFT result can support and the diagnosis tuberculosis disease. ESAT-6, CFP-10, AND TB 7.7(p4) are present in Mycobacterium tuberculosis, but infection by other mycobacteria, including M. kansasii, M szulgai, and M. marinum may also cause positive results. Other diagnostic evaluations (e.g. AFB smear and culture, chest x-ray) besides QFT are needed to confirm tuberculosis disease.
8. The predictive value of a negative QFT result in immunosuppressed persons has not been determined.
9. The performance of the QFT test has not been extensively evaluated with specimens from the following groups:
   • Individuals who have impaired or altered immune function such as those who have HIV infection or AIDS, those who have transplant managed with immunosuppressive treatment or others who receive immunosuppressive drugs (e.g. corticosteroids, methotrexate, azathioprine, chemotherapy) and those who have other clinical conditions: diabetes, silicosis, chronic renal failure, hematological disorders (e.g. leukemia and lymphomas), and other specific malignancies (e.g. carcinoma of the head or neck or kung)
   • Individuals younger than 17 years of age
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Quantiferon TB Gold In-Tube Procedure (revised February 21, 2013)
For Quantiferon Blood Collection tubes T05090-0301, page 1 of 2

Quantiferon®-TB Gold IT uses the following collection tubes:
1. Nil Control (GREY cap with white ring).
2. TB Antigen (RED cap with white ring).
3. Mitogen Control (PURPLE cap with white ring).
Tubes should be maintained at room temperature before collecting the sample.

Antigens have been dried onto the inner wall of the blood collection tubes so it is essential that the contents of the tubes be thoroughly mixed with the blood. The tubes must be transferred to a 37°C ± 1°C incubator as soon as possible and within 16 hours of collection.

A. For each subject collect 1mL of blood by venipuncture directly into each of the QuantiFERON®-TB Gold IT blood collection tubes. Tubes should be drawn in the following order: GREY, RED, PURPLE

- As 1mL tubes draw blood relatively slowly, keep the tube on the needle for 2-3 seconds once the tube appears to have completed filling, to ensure that the correct volume is drawn.

The black mark on the side of the tubes indicates the 1mL fill volume. QuantiFERON®-TB Gold IT blood collection tubes are manufactured to draw 1mL ± 10% and perform optimally within the range of 0.8 to 1.2mL. If the level of blood in any tube is not close to the indicator line, it is recommended to obtain another blood sample. Under or over-filling of the tubes outside of the 0.8 to 1.2mL range may lead to erroneous results. QuantiFERON®-TB Gold IT blood tubes have been validated to draw between 0.8mL and 1.2mL at altitudes from sea-level to 3,000 feet. Above this altitude users should ensure that blood is drawn into each tube within these limits. If low blood draw volume does occur, blood can be collected using a syringe and 1mL transferred to each of the three tubes. For safety reasons, this is best performed by removing the syringe needle, ensuring appropriate safety procedures, removing the caps for the three QuantiFERON®-TB Gold IT tubes and adding 1mL of blood to each (to the black mark on the side of the tube label). Replace the tube caps securely and mix as described below.

- If a “butterfly needle” is being used to collect blood, a “purge” tube should be used to ensure that the tubing is filled with blood prior to the QuantiFERON®-TB Gold IT tubes being used.

B. Mix the tubes by shaking them 10 times just firmly enough to coat the entire inner surface of the tube with the blood. Place tubes upright in a test tube rack.

- Thorough mixing is required to ensure complete integration of the tube’s contents into the blood.

C. Label tubes appropriately.

- Ensure each tube (Nil, TB Antigen, Mitogen) is identifiable by its label or other means once the cap is removed.

D. The tubes must be transferred to a 37°C ± 1°C incubator as soon as possible, and within 16 hours of collection. Prior to incubation, maintain tubes at room temperature (22°C ± 5°C). Do not refrigerate or freeze the blood samples. If incubation on site is not available send specimen to PHLET within 16 hours of collection.

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Quantiferon TB Gold In-Tube Procedure (revised February 21, 2013)
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INCUBATION Notes:

OPTION 1: Incubate tubes at Collection site
2.1 If blood is not incubated immediately after collection, remix the tubes by inverting 10 times prior to incubation.
2.2 Incubate the tubes upright at 37°C +/- 1°C for 16 to 24 hours. The incubator does not require CO2 or humidification.
2.3 Preferred method: Ship incubated tubes to the testing laboratory (samples must be received within 3 days) Maintain tubes at 4-27°C. Label tubes “Incubated.”
2.4 Alternatively after incubation samples can be centrifuged for 15 minutes at 2000 to 3000 RCF (G).
2.5 Once centrifuged samples can be store at 2 to 8°C (refrigerated) for up to 28 days.

OPTION 2 Incubate tubes at Laboratory
2.6 Blood must be received by laboratory as soon as possible and within 16 hours of collection Ship tubes to laboratory at 17 to 27°C (room temperature) do not refrigerate or freeze sample.
2.7 Label tubes “Not Incubated”

Shipping instructions:

Only certified shippers may package and ship blood specimens.

Call the lab prior to shipping samples.

Ship specimens to:

Public Health Laboratory of East Texas
University of Texas Health Science Center at Tyler
11949 US Highway 271
Tyler, Texas 75708
Attention: Janine Yost
Phone: 903-877-5071