



**MOBILE CLINIC
POLICY AND PROCEDURE**

DIVISION: LABORATORY

POLICY NUMBER: CP-2.4.3

SUBJECT: QUANTIFERON N TB GOLD PLUS
(QFT-PLUS) SEND-OUT
TEST PROTOCOL

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SECTION: PHLEBOTOMY & CENTRAL
RECEIVING

ORIGINAL ISSUE DATE: 06/24/2022

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APPROVED BY: ABSALON GALAT MD.
MEDICAL DIRECTOR *Agalatt*

REVISION DATE:
APPROVED DATE: 06/24/2022

TO BE PERFORMED BY:
ALL APPLICABLE EMPLOYEES

SUPERSEDES DATE:

PRINCIPLE:

The QuantiFERON®-TB Gold PLUS (QFT-PLUS) assay is a type of interferon-gamma release assay whole blood test used as an aid in diagnosing Mycobacterium tuberculosis infection, including latent tuberculosis infection (LTBI) and tuberculosis (TB) disease.

Blood samples are mixed with antigens (substances that can produce an immune response) and controls. The antigens include a mixture of synthetic peptides, representing M. tuberculosis proteins. The M. tuberculosis specific antigens are ESAT- 6, CFP-10 and TB 7.7(p4). After incubation of the blood with antigens for 16-24 hours, the amount of interferon-gamma (IFN-gamma) is measured. If the patient is infected with M. tuberculosis, their white blood cells will release IFN-gamma in response to contact with the TB antigens. The QFT-PLUS results are based on the amount of IFN gamma that is released in response to the antigens.

POLICY:

All personnel must follow the protocol.

PURPOSE:

This protocol provides instructions to ensure the proper collection and processing of the QFT-PLUS blood assay. QFT-PLUS is an interferon gamma release assay. To initiate interferon gamma release by T cells, vigorous mixing (at the time of specimen collection and at the time of specimen receipt by the laboratory) is required to ensure adequate exposure of T cells within the sample to TB antigen coating the tube. The reaction is temperature dependent, thus, QuantiFERON specimens must be incubated at 37C within 16 hours of specimen collection to ensure accurate results. Hence, manufacturer guidelines and specifications as summarized in this protocol must be followed.

The QuantiFERON-TB Gold Plus Assay is considered positive when either TB1, TB2 or both tubes are significantly above the Nil value. The mitogen tube serves as a positive control for each specimen tested. Low mitogen response indicates an indeterminate result when the sample also has a negative response to the TB antigens.

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Preferred Specimen(s):

1 mL whole blood collected in four tubes (Nil (gray), TB Antigen Tube 1-TB1 (Green), TB Antigen Tube 2-TB2 (Yellow), and Mitogen (purple) — (Specimen must be drawn as close to black line on specimen tube as possible).

(NOTE: DO NOT EXCEED 1ml)

Rejection Criteria:

- a. Received >16 hours after specimen collection
- b. Received refrigerated or frozen
- c. Specimens not collected in Quantiferon®-TB Gold PLUS blood collection tubes.

PROCEDURE:

1. Specimen Collection

- a. Order “Quantiferon TB GOLD PLUS-PHL” to obtain labels (4 accession labels should print for the tubes). Print an additional label for the Quantiferon- TB Specimen Tracking Log. Verify patient identification against labels that will be placed on specimen.
- b. Although there is no set order for collecting the tubes, it is recommended that tubes be collected in the following order:
 - 1st Grey Cap – Nil tube
 - 2nd Green Cap – TB Antigen 1 tube
 - 3rd Yellow Cap- TB Antigen 2 tube
 - 4th Purple Cap – Mitogen tube

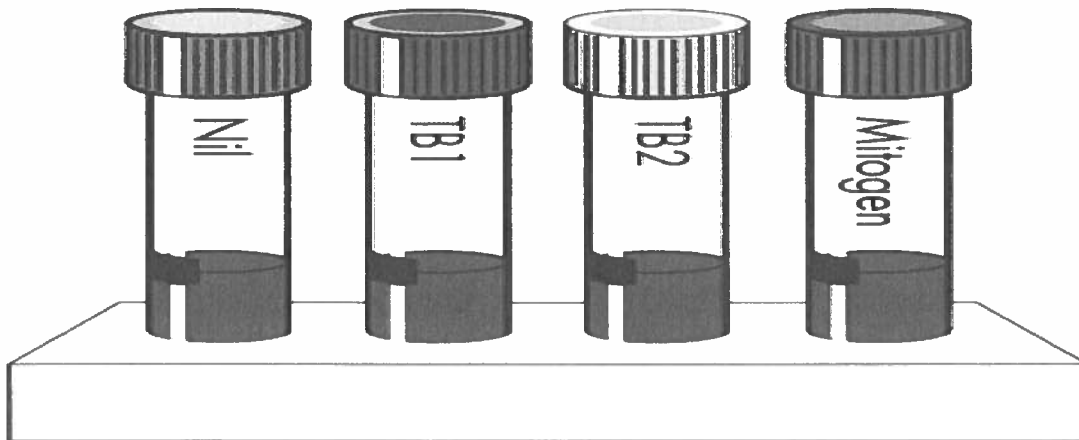


Figure 1. Four QFT-GIT tubes with properly attached labels.

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- c. Draw blood from the patient and collect 1 mL into each QFT-PLUS tube (4 tubes total) and label.
- As 1 mL tubes draw blood relatively slowly, keep the tube on the needle for 2-3 seconds once the tube appears to have completed filling.
 - The black 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 of the indicator mark, a new blood sample should be obtained.
 - 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 QFT-Plus Blood Collection Tubes being used.
 - Immediately after filling the tubes, simultaneously shake the four tubes 10 times, just firmly enough to make sure the entire inner surface of the tube is coated with blood. This will dissolve antigens on tube walls. Foaming is to be expected during the shaking process and should not be a concern.

Important: Overly vigorous shaking may cause gel disruption and could lead to aberrant results.

2. Storage and incubation in Central processing and shipping to LA County PHL

- a. After accessioning the QFT-PLUS tubes, re-mix all four tubes 10 times, just firmly enough to make sure the entire inner surface of the tube is coated with blood. And place **upright** into a rack with loose rubber band and store at room temperature.
- b. Manually print a label to place on PHL Form “Quantiferon-TB Specimen Tracking Log”.
- c. QFT-PLUS tubes must be transferred to a 37C+/-1C incubator at collection site within 16 hours of collection.
- d. Re-mix the specimens by inverting tubes 10 times and place Quantiferon specimen rack in incubator with tubes placed UPRIGHT.
- e. Document time specimens are placed in the 37C incubator (preferably 6 pm; the latest time for start of incubation should be before the end of evening shift).
- f. Remove specimens from incubator after 16-24 hours of incubation and log time out of incubator on the QFT form.
- g. Centrifuge specimens at 2000-3000 x G for 15 minutes.
- h. Package specimens for pickup by the courier in refrigerator (2-8C) for transport on cold pack as soon as possible.

References:

Quantiferon TB GOLD PLUS-Tube Package Insert (www.quantiferon.com) Public Health Laboratory Update (October 17, 2018)

Forms: Quantiferon- TB Specimen Tracking Log (Attachment 1)



PUBLIC HEALTH LABORATORY UPDATE

October 17, 2018

ANNOUNCING A NEW 4-TUBE QUANTIFERON TB-PLUS ASSAY

On November 1, 2018, the Los Angeles County Public Health Laboratory will be replacing the QuantiFERON test (QFT-Gold In-Tube) with an updated version called QuantiFERON-TB Gold Plus or QFT-Plus. This change is required because the vendor is no longer supporting the previous test.

QuantiFERON is a type of interferon-gamma release assay that detects latent infection with *Mycobacterium tuberculosis*. Test interpretation is qualitative (Positive, Negative, or Indeterminant) and numerical values are shown for informational purposes only.

QuantiFERON-TB Gold Plus blood collection tubes will be distributed to all sites prior to the launch date of the new test. Starting November 1, the new 4 tube QuantiFERON-TB Gold Plus collection tubes must be used.

Specimens collected using the previous 3 tube collection kit after the new test start date will be rejected. The 3 tube QuantiFERON-Gold In-Tube collection kits expire 10/31/18. Please return all unused tubes to the Public Health Laboratory.

The QuantiFERON-TB Gold Plus test differs from the previous generations of assays because it has two distinct TB antigen tubes: TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2). A total of 4 QFT tubes are collected (Nil, TB1, TB2, and Mitogen). The tube caps are color coded to differentiate tube type.

Collection tube storage, specimen draw volume, order of draw, shaking, incubation, storage, and transport procedures are like the previous QuantiFERON test. The order of draw is Nil (gray), TB1 (green), TB2 (yellow), then Mitogen (purple). Refer to the attached QuantiFERON-TB Gold Plus Blood Collection Quick Guide for details.

The QuantiFERON-TB Gold Plus assay is considered positive when either TB1, TB2, or both tubes are significantly above the Nil value. The mitogen tube serves as a positive control for each specimen tested. Low mitogen response indicates an indeterminate result when the sample also has a negative response to the TB antigens.

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A table showing QuantiFERON-TB Gold Plus tube values and interpretations is shown below.

Interpretation of QuantiFERON-TB Gold Plus test results

Nil IU/mL	TB1 – Nil IU/mL	TB2 – Nil IU/mL	Mitogen – Nil IU/mL	QFT-Plus Result	Interpretation
≤ 8.0	≥ 0.35 and ≥ 25% Nil	Any	Any	Positive	<i>M. tuberculosis</i> infection likely
	Any	≥ 0.35 and ≥ 25% Nil			
	< 0.35 or ≥ 0.35 and <25% Nil	< 0.35 or ≥ 0.35 and <25% Nil	≥ 0.5	Negative	<i>M. tuberculosis</i> infection NOT likely
> 8.0	< 0.35 or ≥ 0.35 and <25% Nil	< 0.35 or ≥ 0.35 and <25% Nil	< 0.5	Indeterminate	Likelihood of <i>M. tuberculosis</i> infection cannot be determined
	Any	Any	Any		

Specific questions regarding this change may be directed to the Public Health Laboratory staff listed below. The main laboratory phone number is 562-658-1330.

For training or clinical consultation:

Dr. Nicole Green, Laboratory Director
nrcgreen@ph.lacounty.gov

For technical consultation:

Dr. Peera Hemarajata, Assistant Laboratory Director
pemarajata@ph.lacounty.gov

Dr. Lee Borenstein, Public Health Microbiology Supervisor II
lborenstein@ph.lacounty.gov

For specimen issues and results:

Michael Brown, Public Health Microbiology Supervisor I
mibrown@ph.lacounty.gov

For collection tubes and courier issues:

William Chen, Supervising Toxicologist
wchen@ph.lacounty.gov

QuantiFERON®-TB Gold Plus (QFT-Plus) — Blood Collection

Blood collection

1. Label QFT-Plus Blood Collection Tubes. There are four tubes per collection kit.
 - **Important:** QFT-Plus Blood Collection Tubes should be at room temperature (17–25°C) at the time of blood collection.
2. Collect 1 mL of blood by venipuncture directly into each of the QFT-Plus Blood Collection Tubes.
 - As 1 mL tubes draw blood relatively slowly, keep the tube on the needle for 2–3 seconds once the tube appears to have completed filling.
 - The black 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 of the indicator mark, a new blood sample should be obtained.
 - 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 QFT-Plus Blood Collection Tubes being used.



Tube shaking

1. Immediately after filling the tubes, shake them ten (10) times just firmly enough to make sure the entire inner surface of the tube is coated with blood. This will dissolve antigens on tube walls.
 - **Important:** Overly vigorous shaking may cause gel disruption and could lead to aberrant results.



Incubation and shipping to LA County PHL

1. QFT-Plus tubes must be transferred to a 37°C ±1°C incubator at collection site within 16 hours of collection.
 - Tubes should be transferred to an incubator at collection site as soon as possible after collection. If tubes are not incubated immediately after blood collection, remix tubes by inverting 10 times immediately prior to incubation.
 - Incubate tubes **UPRIGHT** at 37°C ±1°C for 16–24 hours.
 - After incubation is completed, centrifuge specimens at 2000–3000 x G for 15 minutes. Store specimens at 2–8 °C and send on cold pack as soon as possible
 - o If unable to centrifuge, specimens must be sent on cold pack as soon as possible to the laboratory. Specimens must be centrifuged within 72 hours of incubation.

Have questions? Call LA County Public Health Laboratories at 562-658-1330.

