CPAL
Central Pennsylvania Alliance Laboratory

# Technical Bulletin
**No. 172**

**January 10, 2019**

## QuantiFERON®-TB Gold Plus Assay

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**Effective Date:** January 28, 2019

**Ordering Information:** There are two options for ordering the QFT Gold Plus Assay.

**Option 1: QFTB Plus (4 tube collection method)**

| | | |
|---|---|---|
| **Test Name** | QFT Gold Plus | |
| **Test Code** | 3520020 | |
| **Specimen** | Collect 1 mL of blood into each of the QFT Gold Plus tubes (4 tubes). | Collection tubes supplied through CPAL. |
| **Specimen Handling** | After collection, shake tubes 10 times. Tubes should remain at Room Temperature prior to incubation(up to 16 hours). Remix tubes by inverting 10 times prior to incubation. Incubate at 37ºC for 16 to 24 hours. Once incubated, specimens are stable for 3 days before needing to be centrifuged. | |

**Option 2: QFTB Plus (1 tube collection method)**
***This method requires collection time restrictions.***

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|---|---|
| **Test Name** | QFT Gold Plus-1 |
| **Test Code** | 3520030 |
| **Specimen** | Draw 5 mL of blood into a green Lithium Heparin tube. Mix well. **Actual collection time must be recorded on the tube**. |
| **Specimen Handling** | Allow specimen to sit at Room temperature for at least 15 minutes but no longer than 3 hours. Then refrigerate for 16 to 48 hours. Send tubes to CPAL at 2-8º C as soon as possible. **Specimens must be received at CPAL within 48 hours of collection** to allow for inoculation and incubation of tubes within the specified time limits set by the manufacturer. Additionally, **specimens must be received at CPAL no later than the Friday evening courier run**. Specimens should not be collected on weekends. |

***Proper collection, incubation, and processing is required for accurate results!!***
See separate collection and processing instructions QFT PLUS BLOOD COLLECTION INSTRUCTION FLYER (Available at www.cpallab.com under the Technical Notes section).

**Testing Performed:**
Monday-Saturday (days)

**Summary:**
QuantiFERON®-TB Gold Plus (QFT-Plus) test is an *in vitro* diagnostic test. It is the fourth generation in QuantiFERON®-TB testing technology, assessing cell-mediated immune response to peptide antigens that simulate mycobacterial proteins. These proteins, ESAT-6 and CFP-10, are absent from all BCG strains and from most non-tuberculosis mycobacteria with the exception of *M. kansasii*, *M. szulgai*, and *M. marinum*. Individuals infected with M. tuberculosis complex organisms 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 by enzyme-linked immunosorbent assay (ELISA) forms the basis of this test. As with all Interferon-Gamma Release Assays (IGRA), QFT-Plus is an indirect test for *M. tuberculosis* (MTB) infection and is intended for use in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations.

In MTB infection, CD4+ T cells play a critical role in immunological control through their secretion of the cytokine IFN-γ. Evidence now supports a role for CD8+ T cells participating in the host defense to MTB by producing IFN-γ and other soluble factors, which activate macrophages to suppress growth of MTB, kill infected cells, or directly lyse intracellular MTB. IFN-γ producing MTB-specific CD8+ cells have been detected in subjects with latent *M. tuberculosis* infection (LTBI) and with active TB. Moreover, ESAT-6 and CFP-10 specific CD8+ T lymphocytes are described as being more frequently detected in subjects with active TB disease versus LTBI and may be associated with a recent MTB exposure. In addition, MTB-specific CD8+ T cells producing IFN-γ have also been detected in active TB subjects with HIV co-infection and in young children with TB disease.

QFT-Plus has two distinct TB antigen tubes: TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2). Both tubes contain peptide antigens from the MTB-complex-associated antigens, ESAT-6 and CFP-10. Both the TB1 tube and TB2 tubes contain peptides from ESAT-6 and CFP-10 that are designed to elicit CMI responses from 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. Each set of QFT-Plus tubes also includes a NIL tube as a baseline of response, and a Mitogen tube that serves as an IFN-γ positive control for each specimen.

A QFT-Plus assay is considered positive if the IFN-γ response to either TB antigen tube is significantly above that of the Nil tube. A low response in the Mitogen tube (<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 improper specimen handling or filling/mixing of the Mitogen tube, 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 with intrinsic IFN-γ secretion. The Nil tube adjusts for background (e.g., elevated levels of circulating IFN-γ or presence of heterophile antibodies). The IFN-γ level of the Nil tube is subtracted from the IFN-γ level for the TB antigen tubes and the Mitogen tube.

**CDC Guidelines for Interpreting IGRA Tests for *Mycobacterium tuberculosis*:**

Diagnoses of *M. tuberculosis* infection and decisions about medical or public health management should not be based on IGRA or tuberculin skin test (TST) results alone; but should include consideration of epidemiologic and medical history as well as other clinical information. In healthy persons who have a low likelihood both of *M. tuberculosis* infection and of progression to active tuberculosis if infected, a single positive IGRA or TST result should not be taken as reliable evidence of *M. tuberculosis* infection. *Because of the low probability of infection, a false-positive result is more likely.* In such situations, the likelihood of *M. tuberculosis* infection and of disease progression should be reassessed, and the initial test results should be confirmed. Repeat testing, with either the initial test or a different test, should be considered.

Persons with a positive TST or IGRA result should be evaluated for the likelihood of *M. tuberculosis* infection, for risks for progression to active tuberculosis if infected, and for symptoms and signs of active tuberculosis. In persons who have symptoms, signs, or radiographic evidence of active tuberculosis or who are at increased risk for progression to active tuberculosis if infected, a positive result with either an IGRA or TST should be taken as evidence of *M. tuberculosis* infection. However, negative IGRA or TST results are not sufficient to exclude infection in these persons.

If risks, symptoms, or signs are present, additional evaluation is indicated to determine if the person has LTBI or active tuberculosis. A diagnosis of LTBI requires that active tuberculosis be excluded by medical evaluation, which should include a medical history and a physical examination to check for suggestive symptoms and signs, a chest radiograph, and, when indicated, testing of sputum or other clinical samples for the presence of *M. tuberculosis*. Neither an IGRA nor TST can distinguish LTBI from active tuberculosis.

In persons with discordant test results (i.e., one positive and the other negative), decisions about medical or public health management require individualized judgment in assessing the quality and magnitude of each test result, the probability of infection, the risk for disease if infected, and the risk for a poor outcome if disease occurs.

**Notes:**

1. Heterophile (e.g., human anti-mouse) antibodies in serum or plasma of certain individuals are known to cause interference with immunoassays. The effect of heterophile antibodies in the QFT-Plus 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 to the microplate wells.

2. The effect of lymphocyte count on reliability of QuantiFERON®-TB Gold Plus IT results is unknown. Lymphocyte counts may vary over time for any individual 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.

3. The predictive value of a negative result in immunosuppressed individuals has not been determined.

4. While ESAT-6 and CFP-10 are absent from all BCG strains and from most known nontuberculous mycobacteria, it is possible that a positive QFT-Plus result may be due to

infection by *M. kansasii, M. szulgai,* or *M. marinum*. If such infections are suspected, alternative tests should be performed.

5. The performance of the USA format of the QFT-Plus test has not been extensively evaluated with specimens from the following groups of individuals:
   a. Individuals who have impaired or altered immune functions, such as those who have HIV infection or AIDS, those who have transplantation managed with immunosuppressive treatment or others who receive immunosuppressive drugs (e.g., corticosteroids, methotrexate, azathioprine, cancer chemotherapy), those who have other clinical conditions, such as diabetes, silicosis, chronic renal failure, and hematological disorders (e.g., leukemia and lymphomas), or those with other specific malignancies (e.g., carcinoma of the head or neck and lung);
   b. Individuals younger than age 17 years;
   c. Pregnant women.

**Validation Studies:**

***Method Comparison:***

Results of studies that compare the QFT-Plus to the QFT Gold assay can be found in the scientific literature. These studies show equivalent performance of the assays. After discussions with CPAL members as well as colleagues who had completed the comparison studies at academic institutions, CPAL determined that it was not plausible to complete such studies in our lab due to the different sample requirements of the two assays, volume of sample required to validate across two instruments and a manual method, and coordination needed to obtain such samples from patients at member facilities. Therefore, since appropriate comparative data is available in the literature, CPAL performed comparison studies of QFT-Plus utilizing samples with known results for the QFT-Plus assay. These samples consisted of a 44-member panel supplied by the manufacturer (Qiagen) as well as 25 samples from an outside laboratory that had completed validation and migration to the QFT-Plus assay. A total of 69 samples (34 positive and 35 negative) were tested utilizing the manual method as well as two GSD ThunderBolt systems that automate the steps of the ELISA portion of the test (resulting in 3 replicates for each of the 69 samples). CPAL obtained results that agreed with the expected result for 100% of the 207 tests performed.

***Reproducibility:***

Once comparison studies were completed, negative and positive pools were made for each of the four tube types utilizing the remaining known positive and negative samples. These pools were used for within-run and between-run reproducibility studies. Studies were performed utilizing the manual method as well as the two GSD ThunderBolt systems (resulting in 60 replicates for each of the two pools). All replicates produced the expected result.

**Suggested coding:** These are suggested codes, which must be verified by each member.

| CPAL PDM Number | | Test Name | CPT Code | LOINC Code |
|---|---|---|---|---|
| | 3520020 | QFT Gold Plus | 86480 | 45323-3 |
| Components: | | | | |
| | 3520022 | TB1(CD4+)-NIL | | 64084-7 |
| | 3520024 | TB2(CD4/8+)-NIL | | 88517-8 |
| | 3520026 | QFT Interp | | 45323-3 |

| CPAL PDM Number | | Test Name | CPT Code | LOINC Code |
|---|---|---|---|---|
| | 3520030 | QFT Gold Plus-1 | 86480 | 45323-3 |
| Components: | | | | |
| | 3520022 | TB1(CD4+)-NIL | | 64084-7 |
| | 3520024 | TB2(CD4/8+)-NIL | | 88517-8 |
| | 3520026 | QFT Interp | | 45323-3 |

**References:**

QuantiFERON®-TB Gold Plus Package Insert, 08/2017.

Morbidity and Mortality Weekly Report; Recommendations and Reports; June 25, 2010 Vol. 59/No. RR-5. Updated Guidelines for Using Interferon Gamma Release Assays to Detect *Mycobacterium tuberculosis* Infection — United States, 2010.

H Hoffman et al. Equal sensitivity of the new generation QuantiFERON-TB Gold plus in direct comparison with the previous test version QuantiFERON-TB Gold IT. Clinical Microbiology and Infection, 22 (2016) 701e703.