Quantiferon–TB Gold Plus

Effective 05/22/2018 our facility will be upgrading our interferon–gamma release assay (IGRA) from the QIAGEN Quantiferon–TB Gold to the QIAGEN Quantiferon–TB Gold Plus.

Both of these assays are used for the indirect detection of infection with *Mycobacterium tuberculosis*. In brief, the peripheral blood of individuals infected with TB contain high levels of T-cells which can be stimulated to release interferon–gamma when exposed *in vitro* to TB–specific peptides. This interferon–gamma can be detected and quantified using immunochemical techniques such as enzyme–linked Immunosorbent Assay (ELISA). [1]

The previous assay, QIAGEN Quantiferon–TB Gold, used three peptides specific to *M. tuberculosis*: TB7.7, ESAT-6 and CFP-10. These long–chain peptides stimulated the MHC class II binding sites on CD4+ T cells, causing them to secrete interferon. These proteins are absent from all BCG (*Bacillus Calmette–Guerin*) strains and from most non–TB mycobacteria, with the exception of *M. kansasi*, *M. szulgai*, and *M. marinum*. [1]

QIAGEN has updated the Quantiferon–TB Gold Plus assay by removing the TB7.7 peptide and including a second antigen tube that contains both long and short chain peptides for ESAT-6 and CFP-10. The short chain peptides stimulate MHC class I binding sites on CD8+ T cells, causing them to secrete interferon in much the same way as the long chain peptides stimulated the MHC class II binding sites on the CD4+ T cells. According to the manufacturer, CD8+ T cells as well as CD4+ T cells regulate the immune response to *M. tuberculosis* and, in the case of CD8+ T cells, they are directly involved with the destruction of cells infected with TB. Recent research has also discovered that CD8+ T cells are more frequently detected in patients with active tuberculosis and in patients who are recovering from a recent infection. According to the manufacturer, by stimulating CD8+ T–cells in addition to CD4+ T cells in the reformulated IGRA, the sensitivity of the assay is increased while maintaining similar specificity. To date, any increases in clinical sensitivity and/or specificity have been negligible: In a study performed by the manufacturer in the United States comparing the Quantiferon–TB Gold test to the Quantiferon–TB Gold Plus test, sensitivity was 88.7% and 88.7% for QFT and QFT–Plus respectively and specificity was 99.06% and 98.11% for QFT and QFT–Plus respectively. Similar studies (Yi, L et. al.) have also shown little difference in sensitivity and specificity between the methods. [1,2,3]

Comparison studies performed by the manufacturer and other independent laboratories have shown a high (>95%) degree of qualitative agreement between the new and old methods. Quantitatively, results obtained using the old method also showed a high degree of correlation with the new method, with Pearson’s correlation coefficient [R] values of 0.74 and 0.75 for TB1 and TB2 respectively. In the study performed by Moon et al, most discordant results (84.8% for QFT TB versus QFT–Plus TB1 and 88.6% for QFT TB versus QFT–Plus TB2) lie in a range of 0.2 – 0.7 IU/mL. In house, our comparison studies have also yielded a high degree (100%) of qualitative agreement between the two methods. [1,2,3]

Regarding the clinical interpretation of positive results at or near the manufacturer recommended cut–off, QIAGEN used population based statistics to derive the positive cutoff value of 0.35 IU/mL. Studies performed in house and by other independent laboratories found that individual patients can demonstrate clinically significant
variability in initially positive results at or near the manufacturer recommended cutoff. The reasons for this variability are still speculative, but are inherent to the IGRA test. For this reason, we will continue to report positive values that are ≥0.35 IU/mL but less than 1.50 IU/mL as Weak Positive and recommend repeating these samples 1–3 months after the initial positive test. [2,4]

This test is intended for use in conjunction with risk assessment, radiography and other diagnostic medical evaluations. As with the previous assay, QuantiFERON–TB Gold Plus results should be interpreted with caution in individuals who are immunosuppressed. It is also recommended to consult the latest CDC and IDSA guidelines for the diagnosis of TB disease and latent tuberculosis infection. [5]

For specimen collection, the only change is the addition of a second antigen tube, and the color of the antigen tubes will change from red to green and yellow. For result reporting, qualitative results for the QuantiFERON–TB Gold plus test will be reported as positive when either TB antigen tube is positive.

Sample handling, stability, incubation and test performance such as reference intervals, cutoff values, result interpretation and methodology will be unchanged. However, due to the cancellation of many samples due to over-filled tubes, we will reiterate the sample collection and handling information below:

**Specimen Requirements:**

The QuantiFERON–TB Gold Plus collection procedure uses FOUR distinct heparinized tubes for each blood collection: A grey Nil tube, a green TB1 tube, a yellow TB2 tube and a purple Mitogen tube. ALL FOUR of these tubes MUST be collected at the same time. Although there is no stated requirement for order of collection, a common collection order is: Nil, TB1, TB2, and Mitogen.

**Patient Preparation:**

None

**Specimen Preparation:**

Obtain tubes by calling the clinical lab at 916–734–0500 prior to anticipated collection. Tubes will be supplied with additional collection and handling instructions. These instructions are critical and must be followed to adequately preserve the sample. Tubes must be at room temperature (17–27°C) at the time of collection.

**Storage/Transport:**

Immediately invert tubes 10 times after collection and deliver at room temperature (17–27°C) to the laboratory for immediate processing. If sample cannot be delivered to the laboratory for immediate processing it may be stored at room temperature (17–27°C) but must be delivered to and processed by the lab within 16 hours.

**Stability:**

Room temperature (17–27°C) for up to 16 hours.

**Minimum volume:**

Collect 1mL of blood in each tube. Each tube must contain ONLY 1mL of blood. **Do not overfill tubes.** The black mark on the side of the tube indicates a validated range of 0.8–1.2mL. **If the level of blood is below or above this mark, the specimen must be redrawn**

**Unacceptable Conditions:**

Refrigerated, Frozen, specimens not delivered to the lab within 16 hours. Samples with volume outside the validated range of 0.8–1.2mL.

**Routine Testing:**

Monday, Tuesday, Thursday and Friday Day Shift.

**Methodology:**

IGRA (interferon–gamma release assay)
Reference Interval: Negative

References:

1. Package insert: QuantiFERON–TB Gold Plus (QFT–Plus), Qiagen, Germantown, MD, August 2017

If you have questions or need additional information please contact Laboratory Client Services at 734-7373 or email patholclientserv@ucdavis.edu

www.testmenu.com/ucdavis
Blood Collection

1. Collect 1 mL of blood into each of the QFT-Plus blood collection tubes.
   a. **All four tubes must be collected together.**
   b. **The tubes fill slowly.** Keep the tube on the needle for 2-3 seconds once the tube appears to have completed filling to ensure the full volume is drawn.
   c. Blood collection tubes must be at room temperature (17-27°C) at the time of blood collection. (see Figure 1)
   d. Although there is no stated requirement for order of collection, a common collection order is: Nil, TB1, TB2, and Mitogen.
   e. **Do not overfill tubes.** The black mark on the side of the tubes indicates the validated range of 0.8 – 1.2 mL. **If the level of blood is below or above this mark, the specimen must be redrawn.** (see Figure 2)
   f. A lithium heparin purge tube should be used in situations where a butterfly needle is used to collect blood.

2. Immediately shake or invert all four tubes ten (10) times
   a. Thorough mixing is required to dissolve antigens on the tube walls. Ensure the entire inner surface of the tube is coated with blood. (see Figure 3)
   b. Do not shake the tube so hard as to disrupt the gel layer.

3. Label all four tubes appropriately.
   a. Do not completely cover the black mark or back window.
   b. Incorrect labeling will cause delays in results.

Specimen Stability and Transport

4. Immediately transport specimens at room temperature (17-27°C) to the laboratory.
   a. If sample cannot be delivered to the laboratory for immediate processing it may be stored at room temperature but must be delivered to and processed by the lab within 16 hours.
   b. **Samples should never be refrigerated.**

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**Blood collection tubes catalog no. 622536**
- QuantiFERON Nil Tube (gray cap, white ring)
- QuantiFERON TB1 Tube (green cap, white ring)
- QuantiFERON TB2 Tube (yellow cap, white ring)
- QuantiFERON Mitogen (purple cap, white ring)

For questions please call the clinical laboratory at: 916-734-0500

An excellent collection training video can be found on QIAGEN's website: