Considerations in the Use of QuantiFERON-TB Gold In-Tube (QFT-GIT) Assay

The diagnosis of latent tuberculosis infection (LTBI) is both an important clinical screening test and is often a requirement for any newly hired employees. In that arena the Interferon Gamma (IFN-γ) Release Assays (IGRA’s) have become the “gold standard” and fueled an increase in their utilization as an alternative to the tuberculin skin test (TST).

IGRAs are blood tests that measure a T-cell response after stimulation with antigens that are specific to Mycobacterium tuberculosis. Although IGRAs are thought to offer improved specificity over the TST in certain populations that receive Mycobacterium bovis BCG vaccinations, this specificity may not be as high when testing in low-risk North-American healthcare workers and college students. It is important that variability due to preanalytical, analytical, postanalytical, manufacturing and immunological factors be minimized. A recent article in the Journal of Clinical Microbiology (1) deals in detail with these issues and is summarized below.

PREANALYTICAL:

There are multiple preanalytical steps that can cause variability.

1. Disinfection of the skin is important: inadequate disinfection of the skin and rubber septums of the collection tubes can introduce microbial contaminants that may have an immunomodulatory effect potentially causing both false positives or false negatives.
2. The order of the collection tubes is also important (starting with the nil tube and finishing with the mitogen tube) since contamination of the antigen tube with mitogen can cause a false positive and contamination of the nil tube with mitogen can cause a false negative.
3. The collected volume is also important: studies have shown that blood volume inversely correlates with TB response in infected individuals and can result in false negatives in some individuals.
4. Excessive shaking of QFT-GIT tubes can non-specifically increase IFN-γ response in nil or antigen tubes and lead to either a false-positive or false negative result depending on which tube is shaken excessively.
5. Delay in processing between collection and incubation can be an important source of variability with a decrease in TB response. Incubation delay has also been shown to increase the indeterminate rate.
**ANALYTICAL:**
As with any laboratory test, analytical variability even in a stable assay can lead to dichotomous results when samples have values that are close to the assay cutoff.

Postanalytical: Manual entry can lead to clerical error resulting in false positive or negative results.

Manufacturing defects: False positive QFT-GIT results related to faulty antigen tubes have been documented a number of times. Periodic review of the positivity rate at UVMMC has been introduced to assess this issue. Indeterminate QFT-GIT results due to faulty mitogen tubes have also been reported, though not all users were affected, perhaps indicating the importance of uniform collection techniques.

**IMMUNOLOGICAL:**
A significant increase in TB response has been seen when IGRA assays follow a TST by more than 3 days. It is not clear how long this increase persists.

Important steps to minimize the variability of IGRAs:
1. Uniform disinfection of skin and tubes similar to that of blood culture.
2. Standardized tube order of draw per package insert.
3. Standardize blood volume to 1 mL.
4. Standardize gentle shaking of QFT-GIT tubes per package insert.
5. Minimize delay in processing of cells.
6. Institute a QA program to monitor positivity and indeterminate rates.
7. Draw blood samples for IGRA within 72 hrs of TST if a two-step testing system is used.

**REFERENCE**