City and County of San Francisco
Department of Public Health

Tuberculosis Control Section
L. Masae Kawamura, MD Director

QuantiFERON In-Tube®: Use of Quantitative Information
Provider Information and Guidance

New CDC IGRA Guidelines

Updated CDC guidelines on the use of interferon gamma release assays (IGRA) were published this year *(MMWR,* June 25, 2010/vol.59/RR-5) and call for the reporting of quantitative information in addition to the qualitative result "*to permit a more refined assessment of results and promote understanding of the tests*."

Background

QuantiFERON In-Tube® (QFT-IT) is a blood based TB assay that uses quantitative cut points to determine positive, negative and indeterminate results. These results are based on gamma interferon (IFN-γ) produced by T-cells from whole blood in response to specific *M. tuberculosis* proteins. When antigens are recognized, T-cells release IFN-γ, a chemical messenger or cytokine that is critical for the innate and adaptive immune response against intracellular bacteria.

Quantitative values from the TB antigen containing tubes are compared to negative and positive controls, necessary to determine the validity of the test. If the negative control has levels of IFN-γ that are inappropriately high, the test is considered a "high nil" indeterminate. Likewise, a "low mitogen" indeterminate result can occur due to an inappropriately low IFN-γ mitogen response in the positive control.

IFN-γ responses that are close to the positive cut point of .35 IU/L may represent weak responses to circulating TB antigen. Unlike Tspot-TB, QFT-IT does not have a borderline range or gray zone that can be used when a conservative approach is needed (eg. in particular for immunocompromised patients, contacts with significant exposure to active TB or patients strongly suspected of having active disease).

What is currently known

Multiple studies of serial pre- and post-treatment IGRA testing for active and latent infection have shown that treatment reduces IFN-γ levels. However, many patients on treatment will not lower quantitative values sufficiently to revert their result to negative. Experts conclude that qualitative and quantitative reversion commonly occur with treatment but are not consistent enough to determine treatment efficacy.

Individual variability of quantitative results on different days has been documented by researchers (Detjen 2009, Perry 2008). This may have implications for persons receiving serial testing and the need for review of the quantitative change between tests if no TB exposure has occurred.

In serial testing, higher QFT-IT conversion rates compared to concurrent skin testing have been reported. However, exploratory thresholds using twice the manufacturer's cut-off point (.70 IU/L) improved concordance rates among TST and QFT-IT converters in 2 studies (Pai 2006, Lee 2008).

IFN-γ production may be reduced in immunocompromised persons as documented in a several HIV studies and Japanese rheumatoid arthritis patients with a history of active TB (Maeda 2009).

A risk of progression study observed that very high levels of IFN-γ (≥10IU/L limit) in patients with LTBI preceded disease development (Diel 2008).

QFT-IT Result interpretation

Positive	*Negative*	*Gray Zone*	*Indeterminate*
≥0.35*	<0.35 *	None	*Low mitogen* Mitogen - Nil < 0.50 IU/mL *High Nil* Nil > 8.0 IU/mL

**** (TB Ag - Nil) and assumes appropriate control responses***

When quantitative results may be useful

- Evaluating positive QFT-IT results that are thought to be falsely positive because of lack of exposure
- Evaluating a negative result in immunocompromised persons, contacts with heavy exposure to TB, and foreign-born children who are less than 5 years old with discordant positive TB skin test results
- Evaluating a "converter" on serial testing while understanding that the optimal quantitative threshold for determining new infection from nonspecific variation has not been determined
- Investigating unexpected large proportions of results such as indeterminate rates exceeding 5% or high numbers of converters at a single site

General guidelines

1. Do not use quantitative results if it will not change clinical decisions, particularly when deciding to treat TB suspects based on other clinically relevant information
2. High background nil values may occasionaly elevate an antigen response just over the positive threshold. Reviewing serial results may be helpful when an unexpected positive occurs
3. Handling errors, tube or laboratory problems are usually detected when the proportion of indeterminate results exceed 5% or there is an unexpected increase in positive results. Review of quantitative results of the positive and nil controls is advised to determine a pattern
4. A conservative approach in the interpretation of quantitative QFT-IT results that are just below the positive threshold may be warranted for

    - TB suspects
    - Immunocompromised persons
    - Very young children (under 5)
    - Debilitated elderly and those likely to mount a poor response to the TB skin test

Serial testing - Caution

Quantitative values may vary when an individual receives multiple tests over time. Changes in quantitative results are incompletely characterized and quantitative values that are close to cut points may vary and appear to "wobble" when doing serial testing, sometimes being above or below the positive cut point. It is unclear what clinical implications these low level IFN-γ levels have. However, these minor fluctuations may be misinterpreted as a new infection.

Currently, a quantitative definition of conversion has not yet been determined but is being sought. The CDC has defined QFT-IT and Tspot conversion as a qualitative change from a negative to positive value. Until a new definition emerges, we should always use a conservative approach to patients who are most vulnerable and likely to be infected. In the interim, SF TB Control is evaluating the exploratory threshold of 0.70 IU/L as a definition of conversion for unexposed healthy persons.

Limitations

No diagnostic test can replace clinical judgment.

Normal variation may occur in quantitative results of a single individual but the range of normal, nor its clinical implications has been determined.

Quantitative results cannot determine cure and should be NOT be used to stop treatment for active TB or latent TB infection.

Cautious interpretation of quantitative results is advised until more information is known.

Resources

San Francisco TB Control: 415-206-8524

www.sftbc.org

Francis J. Curry National Tuberculosis Center
Warmline: 415-512-4700

Updated Guidelines for Using Interferon gamma Release Assays to Detect Mycobacterium tuberculosis Infection – United States, 2010

MMWR, Vol. 59/RR-5

www.cdc.gov/tb/publications/guidelines/Testing.htm.