Guidelines for Using the QuantiFERON®-TB Test for Diagnosing Latent *Mycobacterium tuberculosis* Infection

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Summary

Until 2001, the only test used to diagnose latent tuberculosis infection (LTBI) was the tuberculin skin test (TST). However, in 2001, a new test (QuantiFERON®-TB or QFT; manufactured by Cellestis Limited, Carnegie, Victoria, Australia) that measures the release of interferon-gamma in whole blood in response to stimulation by purified protein derivative was approved by the Food and Drug Administration. This statement provides interim recommendations for using and interpreting QFT. As with TST, interpretation and indicated applications of QFT differ for persons according to their risk for LTBI and for developing tuberculosis (TB). This report provides guidance for public health officials, health-care providers, and laboratorians with responsibility for TB control activities in the United States in their efforts to incorporate QFT testing for detecting and treating LTBI. Regardless of the test used to identify LTBI, testing should be primarily targeted at diagnosing infected patients who will benefit from treatment.

Introduction

In 2001, the QuantiFERON®-TB test (QFT) (manufactured by Cellestis Limited, Carnegie, Victoria, Australia) was approved by the Food and Drug Administration (FDA) as an aid for detecting latent *Mycobacterium tuberculosis* infection (*1*). This test is an in vitro diagnostic aid that measures a component of cell-mediated immune reactivity to *M. tuberculosis*. The test is based on the quantification of interferon-gamma (IFN-γ) released from sensitized lymphocytes in whole blood incubated overnight with purified protein derivative (PPD) from *M. tuberculosis* and control antigens.

Tuberculin skin testing (TST) has been used for years as an aid in diagnosing latent tuberculosis infection (LTBI) and includes measurement of the delayed type hypersensitivity response 48–72 hours after intradermal injection of PPD. TST and QFT do not measure the same components of the immunologic response and are not interchangeable. Assessment of the accuracy of these tests is limited by lack of a standard for confirming LTBI.

As a diagnostic test, QFT 1) requires phlebotomy, 2) can be accomplished after a single patient visit, 3) assesses responses to multiple antigens simultaneously, and 4) does not boost anamnestic immune responses. Compared with TST, QFT results are less subject to reader bias and error. In a CDC-sponsored multicenter trial, QFT and TST results were moderately concordant (overall kappa value = 0.60). The level of concordance was adversely affected by prior bacille Calmette-Guérin (BCG) vaccination, immune reactivity to nontuberculous mycobacteria (NTM), and a prior positive TST (*2*). In addition to the multicenter study, two other published studies have demonstrated moderate concordance between TST and QFT (*3,4*). However, one of the five sites involved in the CDC study reported less agreement (*5*).

Limitations of QFT include the need to draw blood and process it within 12 hours after collection and limited laboratory and clinical experience with the assay. The utility of QFT in predicting the progression to active tuberculosis has not been evaluated.

This report provides interim recommendations for using and interpreting QFT results based on available data. As with TST, interpretation and indicated applications of QFT differ between those persons at low risk and those at increased risk

for LTBI. This report should assist public health officials, health-care providers, and laboratorians who are responsible for TB control activities in the United States in their efforts to incorporate QFT testing for detecting and treating LTBI.

QFT Performance, Interpretation, and Use

Tuberculin testing is performed for persons who are 1) suspected as having active TB; 2) at increased risk for progression to active TB; 3) at increased risk for LTBI; or 4) at low risk for LTBI, but are tested for other reasons (Table 1).

QFT Performance

Aliquots of heparinized whole blood are incubated with the test antigens for 16–24 hours.* The antigens included in the test kits are PPD from *M. tuberculosis* (tuberculin)[†] and PPD from *Mycobacterium avium* (avian sensitin). The kits also include phytohemaglutinin (a mitogen used as a positive assay control), and saline (negative control or nil). After incubation, the concentration of IFN-γ in the separated plasma is determined by enzyme-linked immunosorbent assay (ELISA).

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* Additional technical information is available from the manufacturer at http:// www.cellestis.com.
[†] PPD from *M. tuberculosis* is referred to by the manufacturer and in FDA documents as human PPD.

TABLE 1. Interim recommendations for applying and interpreting QuantiFERON®-TB (QFT) (Cellestis Limited, Carnegie, Victoria, Australia)

Reason for testing	Population	Initial screening	Positive results	Evaluation
Tuberculosis (TB) suspect	Persons with symptoms of active TB	Tuberculin skin testing (TST) might be useful; QFT not recommended	Induration ≥5 mm	Chest radiograph, smears, and cultures, regardless of test results
Increased risk for progression to active TB, if infected	Persons with recent contact with TB, changes on chest radiograph consistent with prior TB, organ transplants, or human immunodeficiency virus infection, and those receiving imumnosuppressing drugs equivalent of ≥15 mg/ day of prednisone for ≥1 month*	TST; QFT not recommended	Induration ≥5 mm	Chest radiograph if TST is positive; treat for latent TB infection (LTBI) after active TB disease is ruled out
	Persons with diabetes, silicosis, chronic renal failure, leukemia, lymphoma, carcinoma of the head, neck, or lung, and persons with weight loss of ≥10% of ideal body weight, gastrectomy, or jejunoileal bypass*	TST; QFT not recommended	Induration ≥10 mm	
Increased risk for LTBI	Recent immigrants, injection-drug users, and residents and employees of high-risk congregate settings (e.g., prisons, jails, homeless shelters, and certain health-care facilities)[†]	TST or QFT	Induration ≥10 mm; percentage tuberculin response ≥15[§]	Chest radiograph if either test is positive; confirmatory TST is optional if QFT is positive; treat for LTBI after active TB disease is ruled out; LTBI treatment when only QFT is positive should be based on clinical judgment and estimated risk
Other reasons for testing among persons at low risk for LTBI	Military personnel, hospital staff, and health-care workers whose risk of prior exposure to TB patients is low, and U.S.-born students at certain colleges and universities[†]	TST or QFT	Induration ≥15 mm; percentage tuberculin response ≥30[§]	Chest radiograph if either test is positive; confirmatory TST if QFT is positive; treatment for LTBI (if QFT and TST are positive and after active TB disease is ruled out) on the basis of assessment of risk for drug toxicity, TB transmission, and patient preference

*QFT has not been adequately evaluated among persons with these conditions; it is not recommended for such populations.
[†]QFT has not been adequately evaluated among persons aged <17 years, or among pregnant women; it is not recommended for such populations.
[§]The following additional conditions are required for QFT to indicate *Mycobacterium tuberculosis* infection: 1) mitogen – nil and tuberculin – nil are both >1.5 IU, and 2) percentage avian difference is ≤10.

QFT results are based on the proportion of IFN-γ released in response to tuberculin as compared with mitogen, or (tuberculin – nil) / (mitogen – nil) × 100 = percentage tuberculin response.§ The difference in the amount of IFN-γ released in response to tuberculin as compared with avian sensitin is expressed as (avian – nil) – (tuberculin – nil) / (tuberculin – nil) × 100 = percentage avian difference. A computer program is available from the test manufacturer that performs these calculations and interprets the test results.¶

QFT Interpretation

Interpretation of QFT results (Table 2) is stratified by estimated risk for infection with *M. tuberculosis*, in a manner similar to that used for interpreting TST with different cut-off values. QFT results indicative of *M. tuberculosis* infection include the following three criteria:

1. (mitogen – nil) and (tuberculin – nil) are both ≥1.5 IU; and
2. percentage avian difference ≤ 10; and
3. percentage tuberculin response ≥ 15 (increased risk for LTBI) or ≥ 30 (low risk for LTBI).

Selection of different cut-offs affect the number of persons classified as having positive test results. Using 15 as the percentage tuberculin response cut-off for interpreting a QFT test as positive identifies approximately the same number of persons compared with using a TST induration cut-off of 10

mm. Using 30 as the percentage tuberculin response cut-off for interpreting a QFT test as positive identifies approximately the same number of persons compared with using a TST induration cut-off of 15 mm. The test is considered negative if (mitogen – nil) ≥ 1.5 IU but (tuberculin – nil) < 15% (mitogen – nil). Results are considered indeterminate if (mitogen – nil) < 1.5 IU, which might be observed among anergic persons.

Using QFT for Persons at Increased Risk for LTBI

QFT can aid in detecting *M. tuberculosis* infections among certain populations who are at increased risk for LTBI (6). These populations include recent immigrants (i.e., immigrated within the previous 5 years) from high-prevalence countries where tuberculosis case rates are ≥30/100,000, injection-drug users, residents and employees of prisons and jails, and health-care workers who, after their preemployment assessment, are considered at increased risk for exposure to tuberculosis. For these populations, a percentage tuberculin response of ≥15 should be considered a positive QFT result.

Using QFT for Persons at Low Risk for LTBI

CDC discourages use of diagnostic tests for LTBI among populations at low risk for infection with *M. tuberculosis* (6). However, initial testing is occasionally performed among certain population groups for surveillance purposes or where cases of active, infectious tuberculosis might result in extensive trans-

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§ Percentage tuberculin response is referred to by the manufacturer and in FDA documents as percentage human response.
¶ Available at http://www.cellestis.com.

TABLE 2. QuantiFERON®-TB (Cellestis Limited, Carnegie, Victoria, Australia) results and interpretation

M – N* (IU/mL)	T – N† (IU/mL)	Avian difference (%)	Tuberculin response (%)§	Report and interpretation	Interpretation
≤1.5		All other response profiles		Interferon-gamma (IFN-γ) response to mitogen is inadequate	Indeterminate
≥1.5		All other response profiles	<15	Percentage tuberculin response is <15 or not significant	Negative: *Mycobacterium tuberculosis* infection unlikely
≥1.5	≥1.5	≤10	≥15 but <30	Percentage tuberculin response is 15–30	Conditionally positive: *M. tuberculosis* infection likely if risk is identified, but unlikely for persons who are at low risk
≥1.5	≥1.5	≤10	≥30	Percentage tuberculin response is ≥30	Positive: *M. tuberculosis* infection likely

*M – N is the IFN-γ response to mitogen minus the IFN-γ response to nil antigen.
†T – N is the IFN-γ response to purified protein derivative from *M. tuberculosis* minus the IFN-γ response to nil antigen; this must be ≥1.5 IU/mL for a patient to be considered QuantiFERON-TB–positive for *M. tuberculosis* infection. If T – N < 1.5 IU/mL, the persons are deemed negative for *M. tuberculosis* infection, regardless of their percentage tuberculin response and percentage avian difference results.
§A percentage tuberculin response cut-off of 15% is used for persons with identified risk for tuberculosis infection, whereas a cut-off of 30% is used for persons with no identified risk factors.

mission to highly susceptible populations. These populations include military personnel, hospital staff and health-care workers whose risk of prior exposure to TB was low, and U.S.-born students at higher education institutions (e.g., as a requirement for admission to U.S. colleges and universities). For these populations, a percentage tuberculin response of ≥30 should be considered a positive QFT result.

Recommendations

The highest priority of targeted tuberculin testing programs remains one that identifies persons at increased risk for TB who will benefit from treatment for LTBI. Following that principle, targeted tuberculin testing should be conducted among groups at risk for recent infection with *M. tuberculosis* and those who, regardless of duration of infection, are at increased risk for progression to active TB.

The role of QFT in targeted testing has not yet been defined, but QFT can be considered for LTBI screening as follows:

- initial and serial testing of persons with an increased risk for LTBI (e.g., recent immigrants, injection-drug users, and residents and employees of prisons and jails);
- initial and serial testing of persons who are, by history, at low risk for LTBI but whose future activity might place them at increased risk for exposure, and others eligible for LTBI surveillance programs (e.g., health-care workers and military personnel); or
- testing of persons for whom LTBI screening is performed but who are not considered to have an increased probability of infection (e.g., entrance requirements for certain schools and workplaces).

Before QFT testing is contemplated, arrangements should be made with a qualified laboratory. Those arrangements should include quality assurance and collection and transport of blood within the required 12 hours.

Confirmation of QFT results with TST is possible because performance of QFT does not affect subsequent QFT or TST results. The probability of LTBI is greatest when both the QFT and TST are positive. Considerations for confirmation are as follows:

- When the probability of LTBI is low, confirmation of a positive QFT result with TST is recommended before initiation of LTBI treatment. LTBI therapy is not recommended for persons at low risk who are QFT-negative or who are QFT-positive but TST-negative.
- TST can also be used to confirm a positive QFT for persons at increased risk for LTBI. However, the need for LTBI treatment when QFT is positive and the subsequent

TST is negative should be based on clinical judgment and perceived risk.
- Negative QFT results do not require confirmation, but results can be confirmed with either a repeat QFT or TST if the accuracy of the initial test is in question.

Contraindications

Because of insufficient data on which to base recommendations, QFT is not recommended for

- evaluation of persons with suspected tuberculosis. Active tuberculosis is associated with suppressed IFN-γ responses, and in prior studies, fewer persons with active TB had positive QFT results than TST results. The degree of suppression appears to be related to the severity of disease and the duration of therapy. Studies are under way that compare the sensitivity of QFT and TST among persons with untreated active TB.
- assessment of contacts of persons with infectious tuberculosis, because rates of conversion of QFT and TST after a known exposure to *M. tuberculosis* have not been compared, and concordance of QFT and TST after exposure and with serial LTBI screening have not been studied.
- screening of children aged <17 years, pregnant women, or for persons with clinical conditions that increase the risk for progression of LTBI to active TB (e.g., human immunodeficiency virus infection). Further studies are needed to define the appropriate use of QFT among these persons.
- detection of LTBI after suspected exposure (i.e., contact investigation after a resident or employee is diagnosed with active TB or a laboratory spill of *M. tuberculosis*) of persons participating in longitudinal LTBI surveillance programs. The approach of using QFT for initial screening, followed by QFT and TST 3 months after the end of the suspected exposure, has not been evaluated.
- confirmation of TST results because injection of PPD for TST might affect subsequent QFT results. Although QFT is not recommended for confirmation of TST results, QFT can be used for surveillance <12 months after a negative TST, if the initial QFT is negative.
- diagnosis of *M. avium* complex disease.

Conclusions

These interim recommendations are intended to achieve a high rate of acceptance and completion of testing for LTBI among groups who have been identified for targeted testing.

Testing programs using TST or QFT should only be implemented if plans are also in place for the necessary follow-up medical evaluation and treatment (e.g., chest radiograph or LTBI treatment) of persons who are diagnosed with LTBI and quality laboratory services are ensured.

References

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