CDPH/CTCA Joint Guidelines

Interferon Gamma Release Assays Clinical Guidelines in California

These guidelines are intended to be used as an educational aid to help clinicians make informed decisions about patient care. The ultimate judgment regarding clinical management should be made by the health care provider in consultation with their patient, in light of clinical data presented by the patient and the diagnostic and treatment options available. Further, these guidelines are not intended to be regulatory and not intended to be used as the basis for any disciplinary action against the health care provider.

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I. Introduction

A. Background

The Centers for Disease Control and Prevention (CDC) issued updated guidelines on the use of Interferon Gamma Release Assays (IGRAs) in diagnosing tuberculosis (TB) infection in 2010. This California TB Controllers Association (CTCA)/California Department of Public Health (CDPH) document is intended to supplement rather than replace the 2010 CDC guideline (http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5905a1.htm?s_cid=rr5905a1_w). This document provides additional, California-specific, guidance in areas where there remains uncertainty in clinical practice.

B. California epidemiology and risk of progression to active disease

Identifying and treating persons with latent tuberculosis infection (LTBI) and thus at risk for progression to active TB disease can be an important measure to prevent TB. A survey of non-institutionalized adults in the United States from 2000 found that 4.2% of the US population has a positive Mantoux tuberculin skin test (TST)[1]. This rate is higher for certain subgroups, including 18.7% among the foreign born. Applying these rates to the California population, which has a higher proportion of foreign born persons, results in an estimated 2.3 million persons latently infected with tuberculosis (6.2%). Among those with LTBI in California, estimated using available data from published registries, surveys and published literature, approximately 130,000 persons have one of the following medical risk factors conferring a higher risk of progression to active TB disease: diabetes, rheumatoid arthritis, HIV, end-stage renal disease on dialysis, Crohn's disease, and organ transplant recipients.

C. Description of IGRAs

Interferon Gamma Release Assays (IGRA) are tests that are conducted on whole blood and were developed in the past decade as an additional test of immunologic response to *Mycobacterium tuberculosis* infection. IGRAs measure release of interferon-gamma from leukocytes in response to exposure to *M. tuberculosis* antigens (i.e., ESAT-6, CFP-10, and TB7.7). IGRAs currently available in the United States include QuantiFERON Gold-In Tube (QFT-GIT), and T-Spot.TB.

D. Operational and clinical similarities and differences from the TST

Both TSTs and IGRAs are aids in the diagnosis of LTBI and active TB disease. Neither test type can definitively rule TB in or out. An IGRA has the advantage over TST of not requiring the patient to return for test result determination (IGRA tests are performed on blood), and current IGRAs do not cross react

[1] Bennett, et.al. Prevalence of TB Infection in the US Population, NHANES survey. Am J Respir Crit Care Med, Vol 177. pp.348-355, 2008

with most non-TB mycobacteria, including BCG vaccine strains. That makes IGRAs more specific for TB infection in some situations and thus better able to identify those most likely to benefit from LTBI treatment. Note that IGRAs do cross react with three species of nontuberculous mycobacteria: *M. marinum, M. szulgai and M.kansasii.*

E. Comparison of different IGRAs and estimates of IGRA sensitivity and specificity

Estimating a test's sensitivity and specificity for LTBI is difficult because there is no gold standard for LTBI diagnosis so the true sensitivity and specificity of a given test is unknown. Studies of sensitivity have generally used populations with diagnoses of active TB disease and compared TST and IGRA results. Studies of specificity generally use populations at very low risk for TB infection and in whom a positive test is likely to be a false positive. However, some positive results considered "false positives" might be true infection from unrecognized risk. Additional areas of caution in interpreting these estimates include: 1) limited data for children; 2) populations evaluated differed across studies and may differ from the population in California; 3) interpretation criteria for IGRAs in these studies differed from current FDA-approved criteria; see CDC guidelines for more information.

TABLE 1: Pooled estimates of sensitivity and specificity for interferon-gamma release assays in studies published before August 2008[2] and compiled by CDC, 2010

IGRA	Specificity	Sensitivity	Sensitivity vs. TST	
			Pooled Estimates	Notes
QFT-GIT	99%	81%	83% vs 89%	Of 11 studies: 6 showed no difference, 3 showed sensitivity greater for TST, 2 showed sensitivity greater for QFT-GIT.
T-Spot.TB[3]	88%	91%	90% vs 89%	Of 12 studies: 9 showed no difference, 3 showed sensitivity greater for T-Spot

Source: Centers for Disease Control and Prevention. Updated Guidelines for Using Interferon Gamma Release Assays to Detect *Mycobacterium tuberculosis* Infection. MMWR 2010; 59(No. RR-5).

[2] FDA approved cut points differ from the cut points used in this table. Increasing from 6 to 8 spots will decrease sensitivity, but increase specificity. As new studies become available, specificity and sensitivities for IGRAs may change.

[3] Results are based on cut points that are lower than FDA approved positive cut points.

II. **Current CDC guidelines**

This section includes a brief summary of the 2010 CDC IGRA guidelines. Please see that guideline for more detail
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5905a1.htm?s_cid=rr5905a1_w

A. Clinical recommendations

1. Who should be screened for TB by IGRAs or TST
   a. Persons likely to benefit from LTBI treatment including persons who are or will be at increased risk of infection or progression to active disease if infected.
   b. Testing persons who have a low risk for both infection and progression to active tuberculosis if infected is not recommended.

2. IGRAs can be used in place of the TST in all situations where TB testing is warranted

3. Test timing and frequency: no different than TST guidelines

4. Test preferences
   a. IGRAs are preferred, but TST acceptable
      i. for testing persons from groups that historically have low rates of returning to have TSTs read (e.g., homeless persons, drug-users)
      ii. for testing persons who have received BCG (as a vaccine or for cancer therapy)
   b. Either IGRA or TST is recommended without preference:
      i. for evaluation of persons with contact to a known or suspected TB case
      ii. for periodic screening of persons who might have occupational exposure to TB (see section IV, B below regarding serial testing)
   c. TST preferred, but IGRA acceptable
      i. for testing children under 5 years of age
   d. Confirmatory IGRA after TST
      i. When additional evidence of infection is required to encourage compliance with LTBI treatment (e.g., foreign-born healthcare workers who believe their positive TST result is attributable to BCG)
      ii. When a healthy person at low risk for infection and progression to active disease has had a positive TST in order to increase the likelihood that the positive test reflects true infection
   e. When to consider using both TST and IGRA: when maximizing the sensitivity of the test for TB infection is important, consideration should be given to using both TST and IGRA result:
      i. When risk for infection, the risk for progression, and the risk for a poor outcome are increased (e.g., children under 5 years of age, persons with HIV infection, or persons receiving immunosuppressive treatment are at risk for infection).

ii. When clinical suspicion exists for active TB and evidence of TB infection is desired.

B. Laboratories are recommended to report quantitative values in addition to standard qualitative test interpretation; see section III for more information on interpretation of test results.

C. Areas of caution

1. Children under 5 years of age: there is additional need to be cautious in the interpretation of test results because of the high rate of progression and higher risk of disseminated disease in this age group.

2. Immunocompromised: IFN-γ production may be reduced in immunocompromised persons as documented in several HIV studies and in Japanese rheumatoid arthritis patients with a history of active TB (Maeda, T. Usefulness and limitations of QuantiFERON-TB Gold in Japanese rheumatoid arthritis patients: proposal to decrease the lower cutoff level for assessing latent tuberculosis infection. Mod Rheumatology; 2010 Feb; 20 (1):18-23.). CD4 counts of less than 200 cells/µl in HIV-infected individuals are associated with lower quantitative values and higher indeterminate results because of low mitogen responses. (Aichelburg MC. Detection and prediction of active TB disease by a whole blood interferon gamma release assay in HIV-1-infected individuals. Clin Infect Dis 2009; 48: 954-62)

III. Interpreting results

A. Test Outcomes (See Appendix A for FDA interpretation criteria for QFT-GIT and T-Spot.TB tests.)

1. Positive Definition: TB antigen response minus nil response at or above cut point, with a nil value below cut point; is interpreted as evidence of TB infection, but further evaluation is needed to determine if TB disease is present.

2. Negative Definition: TB antigen response minus nil below cut point, with adequate control response and nil response below cut point; is interpreted as no evidence of TB infection in healthy person who does not have significant TB risk factors or TB signs or symptoms. However, a negative IGRA does not rule out TB disease in persons with symptoms of TB, or LTBI in a person with a positive TST who is at high risk for progression to TB (i.e., immunocompromised).

3. Indeterminate Definition: either high nil response or low control response. An indeterminate test result indicates test failure due to lack of appropriate responses to the controls. In general, an indeterminate test result does not

provide useful information regarding *M. tuberculosis* infection, however may indicate impairment of the immune system and/or technical errors during the testing process. An indeterminate test result may be due to either low response to mitogen or high nil (control) values. A low mitogen response can be due to a low T-lymphocyte count (in QFT-GIT, lymphocyte numbers controlled in T-Spot.TB), reduced T-lymphocyte activity, or the inability of T-lymphocytes to produce IFN-γ. This can occur in immunocompromised hosts, such as patients with HIV infection, malignancy, or renal dysfunction. High nil values may be due to the presence of heterophile antibodies or to intrinsic IFN-γ production. Other causes of indeterminate test results include improper specimen transport (delayed transport can affect lymphocyte viability) and/or improper specimen handling or storage, as well as other technical factors (e.g., incomplete washing of an enzyme-linked immunosorbent assay plate).

4. Borderline: As approved by the FDA, a "borderline" category for T-Spot.TB was established to classify results near the cut point, where small variations can affect interpretation, as neither positive nor negative. There is no borderline result for QFT-GIT. In persons at high risk for TB infection (e.g., recent contacts to TB, known or suspected HIV+, other immunocompromised individuals, TB suspects, persons with CXR changes consistent with tuberculosis) a borderline result can be considered as evidence of TB infection. For others, the significance of a borderline result is unclear and repeating the assay at a later date may be advisable (refer to Appendix A, Table 3). A second borderline result would have to be interpreted on a case by case basis.

B. Interpreting quantitative results

1. The current CDC guidelines on the use of IGRAs *(MMWR,* June 25, 2010;59 (RR-5) recommend 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*." Use of quantitative values in routine clinical practice has not been evaluated and therefore, the following information and guidance should be used with caution.

2. General guidance until research defines how to use quantitative results
Although the clinical usefulness of reviewing quantitative results has not been evaluated, it is advisable to consider quantitative values when making clinical decisions in the following situations/groups.
   a. Immunocompromised/suspects/high risk: since these groups may have lower IFN-γ responses (see section II, C above), review of quantitative values is recommended; a conservative approach (treating the result as positive) in the interpretation of quantitative results that are just below the positive cut point for QFT-GIT, or within the borderline area for T-Spot.TB may be warranted for TB suspects, immunocompromised persons, very young children (under 5) or debilitated elderly and those likely to mount a poor response to the TB skin test.

b. Evaluating potential false positives: high background nil values may occasionally elevate an antigen response just over the positive cutpoint; reviewing serial results of both antigens and control may be helpful when an unexpected positive occurs.
c. Evaluation of conversion/reversion in serial testing: quantitative results may vary by time of day, or day to day, but the magnitude of variance is not well described. Such variations may cause "wobbling" (fluctuations around the cut point, leading to apparent conversion and/or reversion) on serial testing when quantitative values are close to cut point. See section IV, B, 3 and 4 for further discussion of evaluating conversions.
d. High proportion of unexpected results: review of quantitative results of the positive and nil controls in the context of time and location is advised to determine a pattern

3. Quantitative results should **not be used,**
   a. If it will not change clinical decisions, particularly when deciding to treat TB suspects based on other clinically relevant information.
   b. To determine therapeutic response to LTBI or active TB treatment (no "test of cure").

IV. Use in special situations

A. Dual testing (TST and IGRA or IGRA and IGRA): routine testing with both a TST and IGRA is not recommended, however dual testing may be performed in certain situations (see under section II, A, 4 d and e above). Dual testing with two different IGRAs is also not routinely recommended; repeating the same IGRA may be useful if the initial result is indeterminate, borderline, or if the results are unexpected or unusual for the clinical situation (i.e., high nil value). There is little experience with testing using two different IGRAs, but besides using dual testing when outcomes from one assay are repeatedly indeterminate, invalid or borderline, it is unclear in which clinical situations such dual testing would be useful.

1. Interpreting results of dual testing
   a. Concordant results: concordant positive results (+/+) between TST and IGRA or IGRA/IGRA likely indicate TB Infection, especially in a person with known risk factors for TB. On the other hand, concordant negative results (-/-) with TST and IGRA or IGRA and IGRA in a person with low likelihood of TB infection or progression likely indicate that TB infection is not present, but in persons with signs or symptoms of tuberculosis disease, even dual negative tests do not rule out the diagnosis of TB.
   b. Discordant results
      i. Definition: discordance means different results (one positive, one negative or indeterminate) from two different tests (TST/IGRA, or IGRA/IGRA)
      ii. Causes of discordance

- Different sensitivities/specificities: few diagnostic tests are 100% sensitive or 100% specific; thus false positive and false negatives can be expected, and may lead to discordance. Causes of false positive and false negative skin tests have been well-described; causes for false positive and false negative IGRAs have been less-well described, but such results are known to occur. Common situations which can lead to a higher discordance rate include BCG vaccinated individuals or persons with nontuberculous mycobacterial infections, both of whom may have falsely positive skin tests. Review of quantitative values of TB-specific antigen response in IGRAs may be helpful in assessing reason for discordance.
- Subject variability: day to day variation may exist in both TST reactivity and IGRA reactivity; the amount of normal variation in IGRAs is not known, but for TSTs, induration may vary up to several mm from day to day.
- Test variability: assay to assay variability also exists, but the degree of normal variation is not well described.

2. Management of discordant results: management decisions should be individualized on the basis of risk of infection and progression, and review of quantitative values of IGRAs and the size of induration of TSTs.
   a. For patients who are at low risk for both infection and progression, a positive result in one test can be discounted as a false positive if the other test is negative. For patients who are BCG-vaccinated and do NOT have an increased risk of progression if infected, a TST reaction of less than 15 mm induration may be discounted as false positive **if** IGRA is clearly negative (very low or no reactivity to TB specific antigens).
   b. A positive result in either test should be considered as evidence of infection in the following:
      i. Patients who are at high risk for infection (i.e., contacts),
      ii. Patients with high risk for disease progression if infected,
      iii. Patients for whom poor outcomes are more likely with TB disease,
      iv. Patients for whom TB disease is suspected (signs, symptoms, radiographic evidence),
   c. For other situations not described above, inadequate evidence exists on which to base recommendations. In general, clinicians should make a careful assessment of the patient's underlying risk factors for TB, determine the patient's motivation for treatment, and have an individualized discussion with the patient to determine course of action.

B. Serial Testing

1. Indications: serial testing is indicated in two main situations: evaluation of contacts to TB cases (contact investigations, or CIs) and screening of individuals at risk for TB infection because of occupation, travel, or other activities. Use of IGRAs in CIs may be beneficial if the population tested has a high prevalence of prior BCG vaccination, and studies suggest that a

positive IGRA is more strongly associated with greater recent exposure than is a positive TST. IGRAs are more specific for TB infection, which is useful for the serial testing of employees and others at risk, especially if the pre-test probability of infection is low. Finally, IGRAs are advantageous in serial testing because they do not cause the "boosting" phenomenon. Serial testing is not indicated during the treatment of either active disease or LTBI, and should not be used for a "test of cure" (see section III, B 3 above).

2. Frequency of serial testing. Serial testing of contacts to TB cases should be done at the interval recommended by the CDC, which is at baseline (once contact is identified) and again 8-10 weeks after the end of the exposure period if baseline testing is negative. For occupational screening, frequency depends on the underlying risk of the occupation or the institution in which the individual works, but for most healthcare institutions in California, testing would be done on an annual basis. Also, the recent Cal OSHA Aerosol Transmissible Diseases (ATD) Standard advises testing of all employees covered by the standard annually. There are no recommendations for the frequency of testing for others at risk.

3. Definition of conversion and reversion.
   a. Conversion: the change from a negative result (value below the cut point) to a positive result (value at or above the positive cut point) in two serial samples.
   b. Reversion: the change from a positive result to a negative result in two serial samples.
   c. Note: the T-Spot. TB has a "borderline" result, so the cut points for positive and negative are different ($\geq$ 8 for positive, $\leq$ 4 for negative). There is no borderline result for QFT -GIT, which has a single cut point ($\geq$ 0.35 is positive, < 0.35 is negative). Therefore, a conversion or reversion of a T-Spot.TB would necessitate at least a 4 spot change, whereas a conversion/reversion of a QFT-GIT could happen with a change as little as 0.01 IU/ml in the TB response.

4. Evaluation of possible conversion. Evaluation of a possible conversion should include a review of the patient's risk factors for TB, the details of the possible exposure (if known) and review of the quantitative values of the IGRA response if the conversion is in doubt.
   a. Defined TB exposure: a conversion in a close contact to an infectious case of TB is unlikely to be due to an error, and should be accepted as a true result
   b. No defined TB exposure/low risk: conversions can occur due to true infection, or apparent conversion can occur due to specimen handling or laboratory error, or due to the normal variation in responses to TB antigens (see "wobbling", section III, B, 2, c); review of current and prior quantitative results can help determine the cause
      i. Prior results reveal moderate degree of reactivity to TB antigens (i.e., near to but below the cut point for positive): may be a "wobbler", (especially if there is a history of prior positive skin test or distant

exposure to a TB case), or may indicate a true conversion; consider doing another IGRA to clarify. Wobblers should not be considered converters, but evaluation for TB disease should be done with consideration given to treatment for LTBI if none has been given in the past; further serial testing with IGRAs should not take place on wobblers, but continued screening for TB symptoms should take place.

ii. Prior results reveal little or no reactivity to TB antigens: can be due to lab/specimen error or true conversion. Repeat the assay, and if the reactivity to TB antigens is the same or higher, likely true conversion, whereas if it is low or negative, the positive result was likely a false positive.

V. Troubleshooting

A. Managing high levels of indeterminate results: having high numbers of indeterminates usually indicates a problem with specimen collection or processing, or faulty tube lots; review specimen collection technique and all steps of specimen handling and processing (contact laboratory) to determine problem, review quantitative values (see section III, B, 2, d).

B. Managing sporadic indeterminate results: the optimal follow-up of persons with an indeterminate test result has not been determined; however, the following strategies can be used depending on the patient's preference and program resources.

1. Patients should be informed that an indeterminate result means test failure and can result from either technical or host factors. Available alternative strategies should be discussed and offered to the patient unless the program decides it will systematically adopt a single follow-up strategy

2. Possible follow up strategies include:
   a. Retesting using the same IGRA: factors causing indeterminate results are more likely to resolve the longer you wait to re-test; an interval of at least one week is recommended,
   b. Retesting using a different IGRA immediately
   c. Retesting with a TST immediately
   d. Using prior positive TST results, if available
   e. Determine likelihood of infection by review of risk factors and symptoms and obtaining a CXR if risk factors or symptoms are present. Granuloma, typical upper lobe scarring or active appearing infiltrates may be indicative of TB infection or disease and may require further work-up

C. IGRA studies that have assessed the risk factors that influence indeterminate results, retesting strategies and the timing of retesting are very limited. A study from Japan suggests that QFT- Gold mitogen control failure is associated with elderly and immunocompromised patients,

lymphocytopenia and hypoalbuminemia (Kobashi et al, Eur Respir J. 2009 Apr;33(4):812-5. Epub 2009 Jan 7). Retesting 40 patients with mitogen failure immediately with T-spot yielded valid results in 65% of the patients. Managing discordant results: see section IV, A, 2 above.

VI. Laboratory Considerations

A. Clinicians should notify the laboratory if a high proportion of unusual results are being seen (high levels of indeterminates, positives in low-risk individuals) to help determine what the potential cause may be.

B. CDC guidelines recommend that laboratories report quantitative values in addition to the qualitative interpretation; if a laboratory does not routinely report quantitative values, the clinician can contact them for those values when they are needed to assist in test interpretation.

C. Clinicians should NOT call laboratories to help to determine the clinical significance of a result; if the clinicians need assistance either in interpreting results or in making clinical decisions based on the results, he/she should consult the local TB Control Program, the Curry International TB Center Medical Consultation Service (see section VII, D below), or the California Department of Public Health TB Control Branch.

VII. Resources

A. IGRA Frequently Asked Questions (Appendix B)

B. Provider IGRA flyer (Appendix C)

C. CDC. MMWR 2010; 59 (RR-5) Updated Guidelines for Using Interferon Gamma Release Assays to Detect *Mycobacterium tuberculosis* Infection – United States.
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5905a1.htm?s_cid=rr5905a1_w

D. Curry International Tuberculosis Center medical consultation service: http://www.currytbcenter.ucsf.edu/medconsult/index.cfm or by phone at 877-390-6682 or 415-502-4700

E. 2009 National TB Controllers Association Conference; Managing Indeterminate QuantiFERON®-TB Gold and QuantiFERON®-TB Gold In-Tube Results in Program Practice. Grinsdale, J. & Kawamura, M. San Francisco Department of Public Health, Tuberculosis Control Section.

F. California Department of Public Health, Tuberculosis Control Branch at 510-620-3000.
http://www.cdph.ca.gov/programs/tb/Pages/ResourcesLHDsTBCB.aspx

Appendix A

	QFT-GIT	T-SPOT
What laboratories currently run the test in California?	Please visit www.quantiferon.com for the most up-to-date list of labs currently offering QFT testing as a service in California. Note this listing is sortable by state. There are over 50 labs in California running QFT, with many additional Quest and Lab Corp Patient Service Centers collecting QFT samples.	RDL Reference Laboratory, Los Angeles HIBM Reference Laboratory, Los Angeles Solano County Public Health Laboratory, Fairfield Oxford Immunotec, Inc. has created access to the T-SPOT.*TB* test for all healthcare providers and facilities in the state of California through its California licensed laboratory, Oxford Diagnostic Laboratories®.
Explain the steps involved from blood draw to receiving IGRA results	1. 1mL of blood is collected into three QFT tubes and incubated at 37°C for 16-24 hours. 2. The sample is centrifuged to isolate the plasma above the gel. 3. Plasma is tested in a laboratory using a standard ELISA method for the presence of IFN-gamma.	1. Collect the blood specimen in a standard green top collection tube (either sodium or lithium heparin). No special collection tubes or incubation required. 2. Package the specimen and ship to the laboratory. 3. Test results will be reported thirty-six hours after receipt of the specimen. Customers have the option to receive test results either through a secure web portal or by fax.
As a provider, what are some of the issues that I need to consider, before switching over to IGRA?	1. Reallocate budget to the laboratory. 2. Become familiar with guidelines recommending the use of IGRAs, such as QFT.	Logistics and cost. Oxford Diagnostic Laboratories has addressed most, if not all of the logistical challenges historically associated with implementation of an IGRA. In addition, tools are available that will allow the customer to easily and systematically calculate the cost of a TST vs. an IGRA.
Getting samples tested (including, identifying a lab, blood collection, storage, shipping, etc)?	3. Identify a convenient QFT testing laboratory. 4. Define logistics of getting QFT blood tubes into the 37°C incubator within 16 hours of collection and to a QFT testing lab.	Cut-off time for specimen collection. Blood specimens being sent to Oxford Diagnostic Laboratories can be drawn all day, every day. (Note: certain holidays may be excluded. Weekend courier charge may apply. Contact the laboratory at 877-59 TBLAB for further details.) Specimen stability. T-SPOT.*TB* specimens are stable at room temperature up to 30 hours post collection.
Interpreting IGRA results?	1. Should be used as an aid in the diagnosis of TB infection. 2. The sample is identified as negative, positive, or indeterminate – there is no grey zone for QFT. 3. Positive result – MTB infection is likely. Negative result – MTB infection is unlikely. Indeterminate result – TB antigen responsiveness cannot be determined. In the presence of a negative TB result, an indeterminate result may indicate a poorly functioning immune system. 4. QFT eliminates the risk of false positives due to BCG vaccination. 5. QFT is highly specific for TB and does not cross-react with most strains of non-tuberculosis mycobacteria.	Both the qualitative and quantitative results are reported per the recommendations of the CDC.[1] [1]MMWR 2010; 59(No.RR-5):1-25.

This information was collected from the two companies in 2011

Appendix B

IGRA Frequently Asked Questions

Decision to use IGRAs in California

1. **Which test for latent TB infection (TST, T-SPOT or QFT-GIT) is most appropriate to use in California?**
   Both TSTs and IGRAs are aids to diagnosis of LTBI and active TB disease and neither test can definitively rule TB in or out. However, IGRAs have several advantages over the TST because of its higher specificity (99% for QFT-GIT and 88% for T-Spot compared to 85% for TST) and not requiring a second clinic visit for test results. For these reasons, the current CDC IGRA guidelines state that IGRAs are preferred over TST when testing individuals who have been BCG vaccinated or those who are unlikely to return for their skin test. In pediatrics however, due to the paucity of data on the use of IGRAs in children under 5 years, the TST is preferred over IGRAs, although it is not contraindicated. Therefore, the appropriate TB test will depend on what population is being served, and may include patient or parent preferences if both TST and IGRA tests are available.

Considerations when implementing IGRAs in your practice/hospital/TB clinic

2. **I want to switch over to using IGRA testing. What is the best way to make this transition in my practice?**
   Prior to transitioning from TST to an IGRA within any patient screening program, administrators should ensure that they address the following;

   - Logistics – Ensure blood samples are collected in the appropriate tubes and can get to the appropriate laboratory for processing.
   - Communication with laboratory– Liaisons between IGRA testing site and laboratory should be strengthened to ensure timely delivery of specimens and reporting of qualitative and quantitative IGRA results.
   - Staff training – Proper techniques of drawing and collecting blood samples should be demonstrated and reviewed.
   - Documentation - Written protocols are in place to appropriately document both qualitative and quantitative IGRA results as well as outline how the results should be interpreted. Guidance should be provided for indeterminate or borderline results.
   - Education - Provide adequate patient education on new test and possible results.
   - Quality assurance – Ensure that there is a mechanism to review test results on a daily/monthly/quarterly basis to identify problems with blood collection/laboratory processing.

3. **I've recently switched over to IGRA testing. How can I assess if testing and processing of specimens is happening correctly?**
   On-going communication with your laboratory and clear understanding of how to report irregular patterns is always important. Regular review of indeterminate rates can help you assess specimen handling, occasional bad IGRA lots, and other

laboratory issues. When indeterminate rates go above 5% or there is an excess of unexpected positive results investigation may be warranted to determine if there is a laboratory or clinical issue. Looking at quantitative interferon gamma release assay values and clustering of positives by location, dates or batched runs can help you.

Cost of IGRA

4. Why switch to IGRA if using TST is more cost effective?

Current cost-effectiveness studies often show that although the TST may be less costly per test, IGRAs alone or as a confirmatory test is more cost effective than the TST alone. Cost per test will vary based on the volume of testing (lower cost when bought in larger quantities) lab/personnel costs, and access to and follow up of population tested.

Cost analyses done via mathematical modeling in the literature offer variable results. A recent analysis in healthcare workers (de Perio, Arch Intern Med Jan 2009) suggests either QFT-G or QFT-GIT would be more effective and less costly compared with TST regardless of prior history of BCG and prevalence of LTBI in the community. Other studies suggest that the most cost-effective use of QFT is in BCG-vaccinated individuals (contact screening; Marra, Int J Tuberc Lung Dis, Dec 2008) or as a confirmatory IGRA test after a positive TST result (Oxlade, Int J Tuberc Lung Dis, Jan 2007; Diel, CHEST, May 2007; and Diel R, et al. ERJ 2007 30: 321-332).

5. In California, is there an IGRA code for MediCal and Medicare reimbursement?

Recently, MediCal updated its reimbursement policy regarding IGRAs based on the CDC 2010 updated IGRA guidelines. IGRAs are reimbursable with CPT-4 code 86480 by either MediCal or Medicare for the diagnosis of TB infection. As of September 2011, rates of reimbursement are $69.27 and $87.22, respectively.

IGRA use in specific situations/screenings

6. We have been told previously that the recommendations are not to do both TST and IGRA due to the possibility of conflicting results. Can you please clarify?

In general, using both tests is unnecessary, more time consuming for patients and expensive. Conflicting results add another layer of confusion, especially when there is a lack of expertise in using IGRA results. However, when maximum sensitivity is needed to make critical clinical decisions in TB suspects, very young children or immunocompromised individuals, serial testing with the TST and IGRA can be helpful. In these situations, any test result that is positive should be interpreted as evidence of TB infection. On the contrary, negative results would not rule out infection or disease.

7. We get inundated at the beginning of each school year with children needing TB screening for school entry. Can I use IGRA in this population?

IGRAs can be used in children although TST is preferred in children under 5. Blood draw in the very young can sometimes be challenging. The amount of blood necessary to process any of the IGRAs is the same for adults and children (QFT-GIT

requires 3 cc, T-Spot.*TB* requires 6cc). Due to the lack of data on interpreting IGRA in children <5 years old, negative results should be interpreted with caution. There is additional need to be cautious because of the high rate of progression and higher risk of disseminated disease in this age group. However, for children who recently received BCG, an IGRA may be beneficial in that the results are more specific for TB infection than a TST.

8. **Many of my patients have HIV and we have a poor TST reading rate. What do I need to consider in deciding to use IGRA in my practice?**
   Unread TSTs increase TST indeterminate rates and therefore significantly lower its sensitivity. IGRAs, on the other hand give you results almost every time, and may improve surveillance, clinical care and prevention by increasing test result rates. However, as with the case of very young children, a negative IGRA result in immunocompromised persons should be interpreted with caution. There are limited studies regarding sensitivity of IGRAs among immunocompromised persons. There is some evidence that patients with low CD4 counts (less than 200 cells/µl) might have lower quantitative values and higher rate of indeterminate results. Therefore, review of quantitative results and consultation with an expert in TB may be beneficial; some advocate use of lower cut points in immunocompromised individuals. Clinically, you may experience more wobblers (quantitative results that hover above and below the cut point) in HIV infected individuals receiving serial tests because of immunosuppression and fluctuating immune status.

9. **Can IGRA be used in TB screening of staff and clients in homeless shelters, where incidence in the area is low?**
   Although the TB incidence of an area may be low, homeless populations are notoriously mobile and constantly changing. IGRAs can be useful in screening shelter clients and staff because a result can be obtained with a single visit.
   In high incidence areas, IGRA can also be used as part of the annual TB screening process.

10. **How would you implement IGRAs into a high-risk hospital setting? Should we use it for both contact investigations and employee health screenings (many of our employees are foreign-born)?** Serial testing is indicated in two main situations: evaluation of contacts to TB cases (contact investigations) and screening of individuals at risk for TB infection due to occupation, travel, or other activities. Use of IGRAs in contact investigations may be beneficial if the population tested has a high prevalence of prior BCG vaccination, and studies suggest that a positive IGRA is more strongly associated with greater recent exposure than a positive TST. IGRAs are more specific for TB infection, which is useful for the serial testing of employees and others at risk, especially if the pre-test probability of infection is low. Contact your local health department for assistance in targeting the appropriate use of IGRAs in contact investigation situations.

    Finally, IGRAs are advantageous in serial testing because they do not cause the "boosting" phenomenon. However, a quantitative definition for an IGRA conversion is currently being sought. Preliminary reports on serial testing among healthcare workers have noted unexpected higher rates of conversions and reversions

compared to the TST, which may be due to fluctuating gamma interferon levels upon serial testing. Therefore, regular review of quantitative values of interferon responses may be beneficial.

The California Department of Public Health (CDPH) Licensing and Certification (L&C) Program revised their procedures for requesting program flexibility to use IGRAs for healthcare employee testing. The All Facilities Letter (AFL-10-23): Procedures for Requesting Program Flexibility for the Use of IGRA to Identify TB Infection in California Health care Workers" was released September 2010. A full copy of the letter and the requirements for submission can be found on their website at, http://www.cdph.ca.gov/certlic/facilities/Pages/LnCAFL10.aspx. Consult with the CDPH L&C district office in your region for questions about current requirements.

11. **What would you recommend in a situation where an employee had a prior positive TST and now has a negative IGRA? Are these individuals considered to have LTBI? Will they need an annual symptom review or an annual IGRA?**
Discordant results are always difficult and confusing, especially when you don't know which test to believe. Remember, false positives and negatives occur with both the TST and IGRAs. No test is perfect and IGRAs are certainly not panaceas. If you are using an IGRA result in a serial testing setting, experts have advised to disregard prior TST results and make clinical decisions based on the result you have at hand. Both quantitative and qualitative results should be requested on IGRA results and documented. You should manage an IGRA result as you would a TST result; that is, if the TST or IGRA is positive, evaluate for TB disease and offer treatment for LTBI if no active disease is identified. If the TST or IGRA is negative, repeat annually. Further testing with an IGRA after a positive result is not warranted, unless a false positive is suspected. Quantitative IGRA results may change with treatment of LTBI, but it is not recommended to use these tests to monitor response to treatment. Again, until more research is done, the most prudent approach is to treat an IGRA result like a TST result.

Interpreting results

12. **How long should you wait to retest someone with either a TST or IGRA after an initial indeterminate IGRA results?**
The optimal follow-up of persons with indeterminate test results has not been determined, however any follow-up strategy should include education to the patient as to what the indeterminate result means (either technical or host factor). When an indeterminate result is obtained, the medical provider should reassess the client for dates of live virus vaccinations. As per CDC recommendations, IGRAs should have a 4-6 week window after the administration of any live virus vaccine.

Recommended follow-up on an indeterminate could include one of the following;

Retesting Options	Suggested Interval
Retesting using the same IGRA	Waiting 4 weeks after the initial test may increase valid results although NYC and SF TB programs report >70% valid QFT-IT results when retesting within 1 week. Discuss urgency of results with patient
Retesting using a different IGRA	Immediately
Retesting with a TST	Immediately

13. How should an IGRA conversion (initial IGRA test negative with subsequent IGRA test positive) be evaluated when there is no documented TB exposure?

Before using any test for TB infection, patients should be assessed for their individual risk of TB exposure and disease progression. Low risk patients should be discouraged from being tested in order to reduce false positive results. Reviewing the quantitative results can be helpful in determining false positives from high background nil results or wobblers (see main IGRA guidelines for further clarification) around the cut point. In other situations a 3rd test is needed to further evaluate true conversion or the possibility of a false positive. Clinical decision making should always be done with full disclosure to the patient.

14. How should we use quantitative data in interpreting IGRA results?

Generally, quantitative results should not be used if they will not change clinical decisions and should not be used to determine therapeutic response to LTBI or active TB treatment. Although CDC recommends that laboratories report both qualitative and quantitative IGRA results, the use of quantitative values in routine clinical practice has not been established or evaluated.

Situations where providers may benefit from reviewing quantitative results, include:

a. Low risk of exposure and evaluating potential false positive IGRA results.
b. Evaluating high-risk persons with negative IGRA results (e.g., immunocompromised persons, contacts with significant exposure to TB, and foreign-born children who are less than 5 years old with discordant positive TB skin test results)
c. Evaluating a test conversion in an individual receiving serial testing, particularly in low risk transmission settings and when there is no history of TB contact
d. The evaluation of unexpected large proportions of results such as indeterminate rates exceeding 5% or high numbers of converters at a single location

15. Are there any programs around the state that have experience using IGRAs and have familiarity with interpreting the results?

More TB programs are now using IGRAs across the state and the US. However, help for interpreting IGRA results for specific cases can be obtained from a regional training and medical consultation center (RTMCC). For the western region, go to the website www.nationaltbcenter.ucsf.edu for more information on how to contact the "Consult Warmline" at the Curry International TB Center in San Francisco or contact the California Department of Public Health TB Control Branch medical officer at 510-620-3000.

Other resources:

- CDC Division of TB Elimination has an IGRA Fact Sheet and has posted the new IGRA guidelines at www.cdc.gov/tb/publications/factsheets/default.htm
- More IGRA educational material may be found at the www.findtbresources.org - a site maintained by the CDC.
- Cellestis (makers of QFT) also has a FAQ for healthcare providers located on their website www.cellestis.com
- Oxford Immunotec (makers of T-SPOT.TB) provide an overview about their product at their website http://www.oxfordimmunotec.com/How_It_Works_North_America

APPENDIX C

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).

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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 occasionally 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.

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