

## II. Mantoux Tuberculin Skin Testing (TST) and Interferon Gamma Release Assays (IGRAS)

The Mantoux tuberculin skin test is the most accurate skin test for determining TB infection and is the only skin test recommended by TB Control.

- TST should be repeated when interpretations are ambiguous or inconclusive.
- Under nursing law, an RN or LPN may not delegate the administration or reading of a TST to an unlicensed person.
- HCW's responsible for placing and reading TST's should receive classroom training about TB transmission, pathogenesis, testing and treatment of active tuberculosis and latent TB infection. Additionally, the HCW should be observed placing and reading at least 10 positive TST's and 10 negative TST's. A copy of the Quality Control ( QC) procedural observation checklists ( Appendix F ) can be found at the end of this chapter.

### A. Administration

1. Use 0.1 cc of 5TU Purified Protein Derivative (PPD).
2. Use tuberculin syringe with 3/8 inch 26-27 gauge needle.
3. Clean volar or flexor surface of left forearm approximately 2-4 inches below the elbow – allow to dry completely.
4. Give intradermal injection with needle bevel upward; a tense, white wheal of 6-10mm in diameter should be produced when the TST is accurately administered.
5. Repeat injection at another site at least two inches away if part of the antigen is lost or the injection is given too deeply. Specify location of the retest in the record.
6. Follow your agency blood borne pathogens guidelines for standard precautions.
7. Tuberculin solution can be adversely affected by exposure to light or temperature extremes. For storage and handling, **see Chapter VI., C. 1.**
8. Administer TST prior to or simultaneously with live virus vaccines, e.g., measles, mumps, rubella, smallpox and chicken pox. If the TST is not given simultaneously, wait 4-6 weeks before giving the TST.
9. Immediate hypersensitivity reactions to tuberculin (redness, swelling, pruritus, heat) can occur shortly after injection and usually disappear within 24 hours. This has

no clinical significance and is not considered a positive test.

10. There is no contraindication to repeating a TST that was previously positive; a TST should be administered if there is no documentation of a prior mm reading.
11. TST is both safe and reliable throughout the course of pregnancy.
12. TST is safe for infants of any age. A negative reading is considered valid for infant's at least 6 months of age or older (adjust age for premature infants). A positive reading is valid at any age.
13. TST is not contraindicated for individuals who have been vaccinated with BCG.

B. Reading

1. Read TST in 48-72 hours, preferably at 72 hours:
  - a. Instruct individuals to return to the health department if induration occurs after the TST is read;
  - b. Positive TST reactions occurring after 72 hours are considered valid; and
  - c. Negative TST reactions should be repeated when individuals fail to return within 72 hours.
2. Locate induration (not redness) by palpating in a crosswise motion.
3. Measure transversely (crosswise or "east to west") to the long axis of the forearm and record this as a single measurement.
4. Record reaction in mm (example: 0mm, 16mm) and document date of reading and signature of person reading the test.
5. Cold packs or over the counter topical steroid preparations may be used for the relief of pruritus and local discomfort.
6. Evidence of severe scarring at an old TST site denotes a prior positive reaction and a repeat TST may not be indicated.

C. Interpretation

1. A reaction of  $\geq 5$ mm induration is considered positive for:

- a. Close contacts to an individual with known or suspected infectious tuberculosis within the past 2 years;
  - b. Those suspected of having active TB disease based on clinical and/or chest x-ray evidence
  - c. individuals with HIV infection;
  - d. Individuals with fibrotic changes on chest x-ray consistent with prior TB: and
  - e. Individuals with organ transplants and other immunosuppressed patients, including those receiving  $\geq 15$  mg per day of Prednisone for one month or longer or persons taking or considering taking tumor necrosis factor (TNF) inhibitors such as etanercept (Enbrel®), infliximab (Remicade®), adalimumab (Humira®) or anakinra (Kineret™).
2. A reaction of  $\geq 10$ mm induration is considered positive for:
- a. Children younger than 4 years of age;
  - b. Foreign-born individuals from high-prevalence countries, e.g. Asia, Africa, Caribbean, Latin America, Mexico, South America, Pacific Islands or Eastern Europe;  
Low-prevalence countries for TB disease are USA, Canada, Japan, Australia, Western Europe and New Zealand ;
  - c. HIV-negative individuals who inject illicit drugs or use crack cocaine;
  - d. Individuals with medical conditions that have been reported to increase the risk of tuberculosis disease once infected:
    - diabetes mellitus
    - chronic malabsorption syndrome
    - chronic renal failure
    - leukemia, lymphomas, Hodgkin's disease
    - cancer of the head or neck
    - weight loss of  $\geq 10\%$  below ideal body weight
    - silicosis
    - gastrectomy, or jejunioileal bypass
  - e. Residents and staff in long-term care facilities;
  - f. Health care workers;
  - g. Inmates in the Department of Corrections
  - h. staff with direct inmate contact in the Department of Corrections and jails;
  - i. Employees of HIV/AIDS adult daycare centers;
  - j. Homeless shelter residents, employees and volunteers;
  - k. Individuals who increase their mm reading by 10mm or more within two years (converter);
  - l. Mycobacteriology lab personnel;

- m. Children and adolescents exposed to high-risk adults (homeless, substance abuse, incarcerated, HIV positive); and
  - n. Persons who have traveled outside the US and stayed with family and friends who live in high incidence areas, for greater than one month cumulatively.
3. A reaction of  $\geq 15$ mm induration is considered positive for:
- a. Individuals who do not have any of the above risk factors.

D. False Negative TST Reactions

An individual may be infected with M. tuberculosis but have little or no reaction to TST. False negative reactions may be caused by:

- 1. Recent viral infections (rubella, mumps, influenza, measles, chicken pox).
- 2. Overwhelming tuberculosis disease.  
  
**Note:** This means that a negative TST in the work-up of a possible case of TB does NOT rule out TB.
- 3. Immunosuppression due to old age, debility, malnutrition, HIV infection.  
  
**Note:** Routine anergy testing is not recommended for use in identifying TB infection in immunosuppressed individuals due to the antigen tests' questionable validity and unpredictable variability over time.
- 4. Very recent tuberculosis infection (the individual may not have had time to develop the delayed hypersensitivity reaction, which can take up to eight weeks after exposure).
- 5. Recent (within 4-6 weeks) immunization with certain live virus vaccines (measles, mumps, rubella, chicken pox, smallpox).
- 6. High-dose steroids ( $\geq 15$  mg of Prednisone or its equivalent given daily for one month or longer and other immunosuppressive agents)
- 7. Infants younger than 6 months old (may have false-negative reactions because their immune systems are not fully developed).

8. Improper antigen storage, handling, technique in administration or error in reading.

E. False Positive TST Reactions

An individual may not be infected with M. tuberculosis, but have a false positive reaction. False positive reactions may be caused by:

1. Cross reactions resulting from infection with non-tuberculosis mycobacterium;
2. BCG (bacille Calmette-Guérin) vaccine; and
3. Reading erythema (redness) rather than induration.

F. TST Converters

An increase in reaction size of  $\geq 10$  mm within a period of two years should be considered a converter.

G. Two-Step TST (Booster Phenomenon)

- In some individuals, the ability to react to the TST may gradually diminish over time. If skin tested at this point, these individuals may have a false negative reaction. However, if retested within one week to one year they may then demonstrate a positive reaction (the "booster" phenomenon). The booster phenomenon may occur at any age, but is more common in older persons.
- Two-step testing reduces the likelihood of interpreting a "boosted" reaction as a true conversion or a new infection; it is recommended in situations where there will be repeat testing on a regular basis. Two step testing is required for staff and residents of long term care facilities as well as staff in adult day care centers that provide care for HIV/AIDS clients (see Chapter XI -10A NCAC 41A .0205 (b) 4 and 5). If the individual has had a documented TST within the last 12 months, that TST can be counted as the first step in 2 step testing.
- If the reaction to the first test is positive, consider the individual infected.
- if the reaction to the first test is negative a second test should be given one-to-three weeks later:
  1. If the second test is positive, consider the individual infected; and
  2. If the second test is negative, consider the individual not infected.
- Record reactions in mm and document dates of reading and signature(s) of person(s) reading the tests.

H. Bacille Calmette Guerin (BCG)

BCG was originally derived from *Mycobacterium bovis* and is used in an attenuated or weakened form in high-incidence countries to protect infants and young children against severe forms of TB disease, e.g., miliary, meningeal.

An individual with a positive TST reaction should be considered infected with M. tuberculosis regardless of their BCG history.

1. BCG Vaccination

- a. Evaluate all BCG vaccinated individuals with a positive TST for preventive therapy;
- b. Tuberculin reactivity caused by BCG wanes with time and is unlikely to persist >10 years after vaccination in the absence of M. tuberculosis infection or exposure.

2. Bladder Instillation (Intravesical BCG) For Bladder Cancer Treatment.

Intravesical BCG may cause a reactive TST; a TST prior to treatment is often done for a baseline reading.

M. bovis (a species included in M. tuberculosis complex) can be isolated from individuals who have received intravesical BCG as a treatment for bladder cancer. **(Refer to Chapter IV for diagnosis and treatment of M. bovis.)**

I. Candidates for TST

- TST of individuals and groups should be undertaken only if the diagnostic evaluation and a course of preventive therapy can be completed.
  - **Routine testing of low-risk individuals is not recommended; locally purchased PPD must be used for all low-risk testing, e.g., job-related.**
  - ***State supplied PPD may be used only for persons in categories #1-7 as follows (high-risk for infection or disease).***
1. The following children and adults are legally required (10A NCAC 41A.0205) to receive a TST:
    - a. Household and other close contacts of active cases of pulmonary and laryngeal tuberculosis;

- b. Persons reasonably suspected of having tuberculosis disease;
  - c. Inmates in the custody of, and staff with direct inmate contact, in the Department of Corrections upon incarceration or employment, and annually thereafter;
  - d. Patients and staff in long term care facilities upon admission or employment, using the two-step skin test method;
  - e. Staff in adult day care centers providing care for persons with HIV infection or AIDS upon; and employment, using the two-step skin test method
  - f. Persons with HIV infection or AIDS.
2. The following children and adults should receive a baseline TST when they initially present for health care:
- a. Foreign-born individuals from high incidence areas, such as, Asia, Africa, Caribbean, Latin America, Mexico, South America, Pacific Islands or Eastern Europe;  
Low-prevalence countries for TB disease are USA, Canada, Japan, Australia, Western Europe and New Zealand:
  - b. Individuals who inject illicit drugs or use crack cocaine;
  - c. Migrants, seasonal farm workers, and the homeless (if unable to ensure completion of evaluation and TLTBI, screen for disease);
  - d. Persons who have traveled outside the US and stayed with family and friends who live in high incidence areas, for greater than one month cumulatively;
  - e. Children and adolescents exposed to high-risk adults (homeless, substance abuse, incarcerated, HIV positive); and
  - f. Persons with conditions that increase the risk of progression to disease once infected
    - diabetes mellitus,
    - chronic renal failure,
    - chronic malabsorption syndrome,
    - leukemia, lymphomas, Hodgkin's disease,
    - cancer of the head or neck,
    - silicosis,
    - weight loss of > 10% ideal body weight, and
    - gastrectomy or intestinal bypass.

A subsequent TST is not necessary unless there is a continuing risk of exposure to persons with tuberculosis disease.

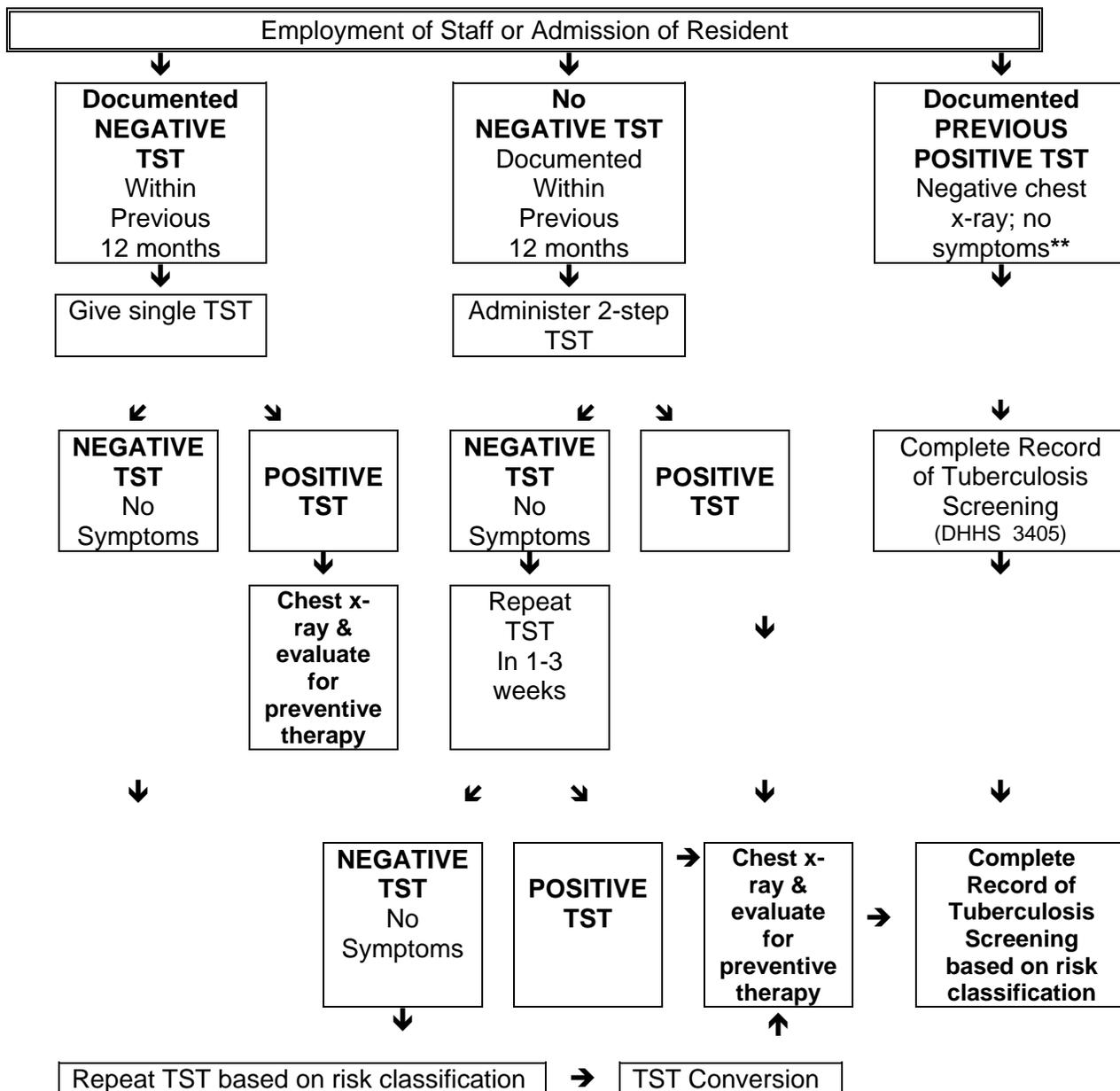
3. **Persons taking or considering taking tumor necrosis factor (TNF) inhibitors (e.g., etanercept, infliximab, adalimumab or anakinra) which can suppress the immune system are at high risk for TB disease if infected. Before starting these drugs, a TST should be done (preferably a two-step test) and treatment for LTBI started if the reading is 5 mm or greater. If there is a credible history of exposure to TB, LTBI should be initiated regardless of TST result.**
4. Clinically assess all household members in the immediate environment of a child  $\leq 2$  years of age with a newly identified positive TST to rule out an undiagnosed case of tuberculosis. An assessment should include an evaluation of symptoms for TB disease and may include a TST, bacteriological examination and chest x-ray, if indicated.
5. Clinically assess all household members in the immediate environment of a pregnant woman with a newly identified positive TST to rule out TB exposure in the immediate environment that the newborn infant will be entering. An assessment should include an evaluation of symptoms for TB disease and may include a TST, bacteriological examination and chest x-ray, if indicated.
6. Homeless shelters
  - a. Staff and volunteers should be educated regarding the symptoms of tuberculosis disease. Those clients with symptoms suggesting TB disease should be promptly evaluated for active disease.
  - b. Staff and volunteers should receive a two-step TST on employment.
  - c. Routine TST of clients should be undertaken only if the diagnostic evaluation and course of preventive therapy can be initiated and completed.
7. Local jails and detox units
  - a. All jail facilities must conduct a facility specific risk assessment; for additional guidance see: prevention and Control of Tuberculosis in Correctional and Detention Facilities: Recommendations from CDC. MMWR 2006;55 (No RR-9).
  - b. TST staff [upon employment] using the two-step method. Annual TST is based on the facility's current risk level.
  - c. Verbally screen all inmates for symptoms of TB on admission. Inmates with symptoms suggesting TB disease should be evaluated for active disease.

8. Staff and residents in occupational settings where TST is required by regulatory and/or agency policy:
  - a. Two-step TST [upon admission or employment] individuals who cannot provide a documented negative TST within the preceding 12 months:
    - Individuals who can provide a documented negative TST within the preceding 12 months should receive a single TST and use this result as the second part of the two-step test; and
    - Individuals who can provide a documented positive TST should have a Record of Tuberculosis Screening (DHHS 3405) completed using the most recent chest x-ray report.
  - b. Individuals with a previously documented positive TST should be re-x-rayed only when symptoms for tuberculosis disease are present.

J. Chest x-rays

1. A posterior-anterior view of the chest should be obtained on all adults:
  - a. With a newly identified positive TST;
  - b. With symptoms suggestive of TB disease regardless of TST results;
  - c. With suspected extrapulmonary TB disease; and
  - d. With negative TST and starting treatment for LTBI, i.e. close contact.
2. A posterior-anterior and lateral view of the chest should be obtained on children under 5 years of age:
  - a. With a newly identified positive TST;
  - b. With symptoms suggestive of TB disease regardless of TST results;
  - c. With suspected extrapulmonary TB disease; and
  - d. With negative TST and starting INH, i.e. close contact.
3. For chest x-rays during pregnancy, see Chapter III, G.
4. Individuals with a previously documented positive TST and a negative chest x-ray should have a repeat x-ray only when symptoms for tuberculosis disease are present (see Chapter III for chest x-ray recommendations when starting treatment for LTBI for someone with a remote or prior positive TST).

K. Two-Step Tuberculin Skin Testing (TST) \*



\* Initial two-step TST is required for employees and residents of long-term care facilities and facilities providing adult day care for HIV positive individuals. Other agency/institution requirements may vary.

\*\* Repeat x-ray ONLY if signs and symptoms of tuberculosis disease are present. Obtain sputums and x-ray individuals with symptoms regardless of TST result.

L. Quality Control Procedural Observation Checklist from MMWR2  
2005;54 (NoRR-17) Appendix F

Appendix F. Quality control (QC) procedural observation checklists

Quality Control (QC) Procedural Observation Checklist for Placing Tuberculin Skin Tests (TSTs) — Mantoux Method		
Date _____	Trainer (QC by) _____	Trainee (TST placed by) _____
<div style="border: 1px solid black; padding: 2px; display: inline-block;">                     Scoring: ✓ or Y = Yes    X or N = No    NA = Not Applicable                 </div>		
<p><b>1. Preliminary</b></p> <p>_____ Uses appropriate hand hygiene methods before starting.</p> <p>_____ Screens patient for contraindications (severe adverse reactions to previous TST).*</p> <p>_____ Uses well-lit area.</p> <p><b>2. Syringe<sup>†</sup> filled with exactly 0.1 mL of 5 tuberculin units (TU) purified protein derivative (PPD) antigen<sup>§</sup></b></p> <p>_____ Removes antigen vial from refrigeration and confirms that it is 5 TU PPD antigen.<sup>¶</sup></p> <p>_____ Checks label and expiration date on vial.</p> <p>_____ Marks opening date on multidose vial.</p> <p>_____ Fills immediately after vial removed from refrigeration.</p> <p>_____ Cleans vial stopper with antiseptic swab.</p> <p>_____ Twists needle onto syringe to ensure tight fit.</p> <p>_____ Removes needle guard.</p> <p>_____ Inserts needle into the vial.</p> <p>_____ Draws slightly over 0.1 mL of 5 TU PPD into syringe.</p> <p>_____ Removes excess volume or air bubbles to exactly 0.1 mL of 5 TU PPD while needle remains in vial to avoid wasting of antigen.</p> <p>_____ Removes needle from vial.</p> <p>_____ Returns antigen vial to the refrigerator immediately after filling.</p> <p><b>3. TST administration site selected and cleaned</b></p> <p>_____ Selects upper third of forearm with palm up ≥2 inches from elbow, wrist, or other injection site.**</p> <p>_____ Selects site free from veins, lesions, heavy hair, bruises, scars, and muscle ridge.</p> <p>_____ Cleans arm site with antiseptic swab using circular motion from center to outside.</p> <p>_____ Allows site to dry thoroughly before administering antigen.</p> <p><b>4. Needle inserted properly to administer antigen</b></p> <p>_____ Rests arm on firm, well-lit surface.</p> <p>_____ Stretches skin slightly.<sup>††</sup></p>	<p>_____ Holds needle bevel-up and tip at 5°–15° angle to skin.</p> <p>_____ Inserts needle in first layer of skin with tip visible beneath skin.</p> <p>_____ Advances needle until entire bevel is under the first layer of skin.</p> <p>_____ Releases stretched skin.</p> <p>_____ Injects entire dose slowly.</p> <p>_____ Forms wheal, as liquid is injected.</p> <p>_____ Removes needle without pressing area.</p> <p>_____ Activates safety feature of device per manufacturer's recommendations, if applicable.</p> <p>_____ Places used needle and syringe immediately in puncture-resistant container without recapping needle.</p> <p>_____ Immediately measures wheal to ensure 6–10 mm in diameter (Actual wheal measurement _____mm).</p> <p>_____ If blood or fluid is present, blots site lightly with gauze or cotton ball.</p> <p>_____ Discards used gauze or cotton ball according to local standard precautions.</p> <p>_____ If the TST is administered incorrectly (too deeply or too shallow) and the wheal is inadequate (&lt;6 mm), a new TST should be placed immediately. Applying the second TST on the other arm or in a different area of the same arm (at least 2 inches from the first site) is preferable so that the TST result will be easier to read.</p> <p>_____ Documents all information required by the setting (e.g., date and time of TST placement, person who placed TST, location of injection site and lot number of tuberculin).</p> <p>_____ Uses appropriate hand hygiene methods after placing TST.</p> <p><b>5. Explanation to the client regarding care instructions for the injection site</b></p> <p>_____ The wheal (bump) is normal and will remain about 10 minutes.</p> <p>_____ Do not touch wheal; avoid scratching.</p> <p>_____ Avoid pressure or bandage on injection site.</p> <p>_____ Rare local discomfort and irritation does not require treatment.</p> <p>_____ May wash with soap and water (without pressure) after 1 hour.</p> <p>_____ No lotions or liquids on site, except for light washing, as above.</p> <p>_____ Keeps appointment for reading.</p>	

\* Severe adverse reactions to the TST are rare but include ulceration, necrosis, vesiculation, or bullae at the test site, or anaphylactic shock, which is substantially rare. These reactions are the only contraindications to having a TST administered.

† Use a ¼–½-inch 27-gauge needle or finer, disposable tuberculin (preferably a safety-type) syringe.

§ Prefilling syringes is not recommended. Tuberculin is absorbed in varying amounts by glass and plastics. To minimize reduction in potency, tuberculin should be administered as soon after the syringe has been filled as possible. Following these procedures will also help avoid contamination. Test doses should always be removed from the vial under strictly aseptic conditions, and the remaining solution should remain refrigerated (not frozen). Tuberculin should be stored in the dark as much as possible and exposure to strong light should be avoided. SOURCE: American Thoracic Society, CDC, Infectious Disease Society of America. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med* 2000;161:1376–95.

¶ Preventing tuberculin antigen and vaccine (e.g., Td toxoid) misadministration is important. Measures should include physical separation of refrigerated products, careful visual inspection and reading of labels, preparation of PPD for patient use only at time of testing, and improved record keeping of lot numbers of antigens, vaccines, and other injectable products. SOURCE: CDC. Inadvertent intradermal administration of tetanus toxoid-containing vaccines instead of tuberculosis skin tests. *MMWR* 2004;53:662–4.

\*\* If neither arm is available or acceptable for testing, the back of the shoulder is a good alternate TST administration site. SOURCE: National Tuberculosis Controllers Association, National Tuberculosis Nurse Consultant Coalition. Tuberculosis nursing: a comprehensive guide to patient care. Smyrna, GA: National Tuberculosis Controllers Association; 1997.

†† Stretch skin by placing nondominant hand of health-care worker (HCW) on patient's forearm below the needle insertion point and then applying traction in the opposite direction of the needle insertion. Be careful not to place the nondominant hand of the HCW opposite the administration needle if the patient is likely to move during the procedure, which might cause an accidental needle-stick injury. In children and others who are likely to move during the procedure, certain trainers prefer stretching the skin in the opposite direction of the needle insertion by placing the nondominant hand of the HCW under the patient's forearm. This method should not be used for persons with poor skin turgor.

**Appendix F. (Continued) Quality control (QC) procedural observation checklists**

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**Quality Control (QC) Procedural Observation Checklist for Reading Tuberculin Skin Test (TST) Results — Palpation Method**

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Date \_\_\_\_\_ Trainer (QC by) \_\_\_\_\_ Trainee (TST placed by) \_\_\_\_\_

Scoring: ✓ or Y = Yes    X or N = No    NA = Not Applicable

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<p><b>1. Preliminary</b></p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Uses appropriate hand hygiene methods before starting.</li> <li><input type="checkbox"/> Keeps fingernails shorter than fingertips to avoid misreading TST result.</li> <li><input type="checkbox"/> Keeps TST reading materials at hand (eyeliner pencil or ballpoint pen,* and ruler).</li> <li><input type="checkbox"/> Uses well-lit area.</li> <li><input type="checkbox"/> Inspects for the site of the injection.</li> </ul> <p><b>2. Palpate — finding margin ridges (if any)</b></p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Palpates with arm bent at elbow at a 90° angle.</li> <li><input type="checkbox"/> Lightly sweeps 2-inch diameter from injection site in four directions.</li> <li><input type="checkbox"/> Uses zigzag featherlike touch.</li> <li><input type="checkbox"/> Repeats palpation with arm bent at elbow at a 45° angle to determine presence or absence of induration.</li> </ul> <p><b>If induration is present, continue with these steps†:</b></p> <p><b>3. Placing marks</b></p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Holds palm over injection site.</li> <li><input type="checkbox"/> Uses fingertips to find margins of the induration.</li> <li><input type="checkbox"/> Marks the induration by placing small dots on both sides of the induration.</li> <li><input type="checkbox"/> Inspects dots, repeats finger movements toward indurated margin, and adjusts dots if needed.</li> <li><input type="checkbox"/> Marks dots transverse (perpendicular) to long axis of forearm.</li> </ul>	<p><b>4. Placing and reading ruler</b></p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Places the "0" ruler line inside the edge of the left dot. Reads the ruler line inside right dot edge (uses lower measurement if between two gradations on millimeter scale) (see Figure 1).</li> <li><input type="checkbox"/> Uses appropriate hand hygiene methods after reading TST.</li> </ul> <p><b>5. Documenting results</b></p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Records all TST results in millimeters, even those classified as negative. Does not record only as "positive" or "negative." Records the absence of induration as "0 mm."</li> <li><input type="checkbox"/> Correctly records results in mm; only a single measured induration in mm should be recorded.             <ul style="list-style-type: none"> <li>Trainee's measurement _____ mm.</li> <li>Trainer's (gold standard) measurement _____ mm.</li> <li>Trainee's result within 2 mm of gold standard reading?§</li> <li>Yes _____ No _____</li> </ul> </li> </ul> <p><b>NOTE:</b> In rare instances, the reaction might be severe (vesiculation, ulceration, or necrosis of the skin). Report severe adverse events to the FDA MedWatch Adverse Events Reporting System (AERS), telephone: 800-FDA-1088; fax: 800-FDA-0178; <a href="http://www.fda.gov/medwatch">http://www.fda.gov/medwatch</a> report form 3500, Physicians' Desk Reference.</p>
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\* A fine-tipped eyeliner pencil or ballpoint pen can be used as a marker. An eyeliner pencil is useful for TST training and for blinded independent duplicate readings (BIDRs) because the dots are easy to remove with a dot of lubricant (e.g., baby oil). Alternative TST result reading methods have been described, including the pen method.

† If induration is not present, record the TST result as 0 mm and go to the end of this form (Documenting results).

§ For example, if the TST trainer reads the TST result (the gold standard reading) as 11 mm, the trainee's TST reading should be between 9–13 mm to be considered correct. Only a single measured induration in millimeters should be recorded.

M. North Carolina Guidelines For the Use of Interferon Gamma Release Assays (IGRAS) in Tuberculosis Diagnosis

NORTH CAROLINA GUIDELINES FOR THE USE OF INTERFERON GAMMA RELEASE ASSAYS (IGRAS) IN TUBERCULOSIS DIAGNOSIS

Background

Interferon gamma release assays (IGRAs) are relatively new tests for tuberculosis (TB) infection. These tests measure the patient's immune response (interferon gamma release) after stimulation of white blood cells in a test tube with two-to-three relatively TB-specific antigens. In contrast to the tuberculin skin test, which requires two separate visits for placement and reading, IGRAs offer the possibility of testing for TB infection with a single blood draw at a single visit. In addition to the logistical advantage of requiring a single visit, IGRAs may have other advantages over the tuberculin skin test. The antigens used for the IGRA tests are not present in the Bacille Calmette-Guerin vaccine (BCG), so false positive tests due to BCG are unlikely to occur. The IGRA antigens are also not present in most nontuberculous mycobacteria, so false positive tests due to nontuberculous mycobacterial exposure or infection are less likely to occur with IGRAs than with tuberculin skin testing.

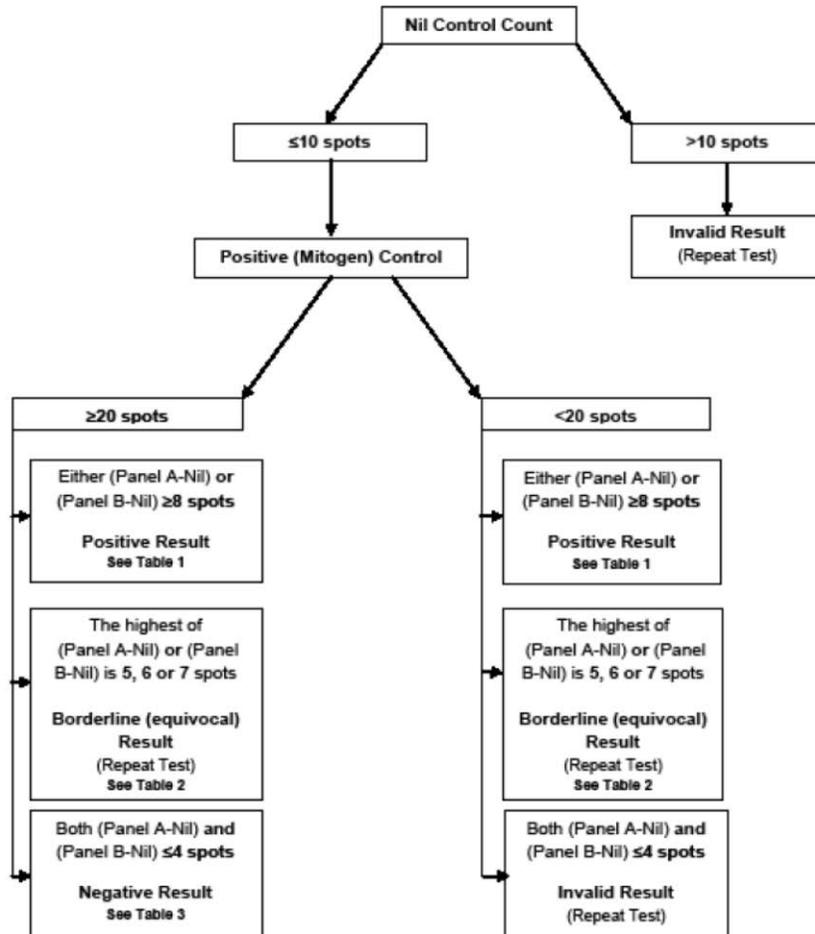
Despite these potential advantages, the IGRAs are imperfect tests for TB diagnosis. There is a large body of data demonstrating the association between a positive tuberculin skin test and the risk to develop active tuberculosis, but the data demonstrating a similar association between a positive IGRA and risk for active tuberculosis are much more limited. Additionally, every study comparing the two available IGRA tests has demonstrated a significant proportion of discordant results, the significance of which is unknown. This discordance means that the IGRAs are not interchangeable; switching among the IGRAs (or for that matter, between an IGRA and tuberculin skin testing) in the setting of serial testing may result in false "conversions" caused by discordance between the different tests used (as opposed to new TB infection). Furthermore, the cost of an IGRA is significantly greater than the cost of a tuberculin skin test.

Currently two IGRAs are approved for use in the United States by the Food and Drug Administration. The Quantiferon Gold in-tube® is an enzyme-linked immunosorbent assay-based test. The test measures the concentration of interferon gamma in whole blood in 3 separate tubes: a nil tube (negative control), a tube containing 3 TB antigens (ESAT-6, CFP-10, and TB7.7), and a tube containing phytohemagglutinin (a mitogen used as a positive control). Blood is drawn from the patient directly into each tube (about 1 ml of blood each), and the tubes must then be shaken vigorously. The tubes must be placed into an incubator at 37 C within 14 hours of the blood draw, and are then incubated for 16-24 hours. After incubation, a machine is used to measure the concentration of interferon gamma in each tube. The criteria for test interpretation are listed below (from the package insert, available at [www.cellestis.com](http://www.cellestis.com)).

Nil [IU/mL]	TB Antigen minus Nil [IU/mL]	Mitogen minus Nil [IU/mL] <sup>1</sup>	QuantiferON®-TB Gold IT Result	Report / Interpretation
≤ 8.0	≥ 0.35 and ≥ 25% of Nil value	Any	<b>Positive<sup>2</sup></b>	<i>M. tuberculosis</i> infection likely
	< 0.35 OR ≥ 0.35 and < 25% of Nil value	≥ 0.5	<b>Negative</b>	<i>M. tuberculosis</i> infection NOT likely
> 8.0 <sup>4</sup>	Any	< 0.5	<b>Indeterminate<sup>3</sup></b>	Results are indeterminate for TB Antigen responsiveness
		Any		

The T-SPOT.TB® is an enzyme-linked immunospot test. The test measures the number of spots on a plate containing four different antigens: nil (negative control), two TB antigens (ESAT-6 and CFP-10, also called Panel A and Panel B), and phytohemagglutinin (positive control). Each spot theoretically represents a white blood cell that is secreting interferon gamma. Blood is drawn from the patient (8 mL for adults, 4 mL for children 2-9 years old, and 2 mL for children under 2 years) and then must be processed in the laboratory within eight hours. The white blood cells are separated from the rest of the blood, and a standard number of white cells is placed into each plate. The cells are incubated with the antigens for 16-20 hours, and then further steps are used to develop the spots for each plate. The criteria for test interpretation are outlined in the diagram below (from the package insert at [http://www.oxfordimmunotec.com/Technical\\_Documents\\_North\\_America](http://www.oxfordimmunotec.com/Technical_Documents_North_America)).

Figure 3: Algorithm Flow Diagram



A brief comparison of the tuberculin skin test and the two IGRAs follows in the Table.

	Tuberculin skin test	Quantiferon Gold in-tube	T-SPOT.TB
Number of visits required	2*	1	1
Time frame to get blood to lab	N/A	<14 hours (can be incubated in portable incubator)	<8 hours
Need for additional processing of blood before incubation	N/A	No	Yes
Result format	mm of induration	Concentration of interferon gamma (IU/mL)	Number of spots on a plate

Reliability among observers	+/-	++	++
Potential as a “send out” test to distant labs	N/A	Yes	Not at present
Available in NC as of 5/2009	Yes	Yes	No
Cost to health department or healthcare facility	Reagent inexpensive, labor somewhat more	Moderately expensive	Unknown, probably will be similar to Quantiferon
Cross-reacts with BCG, nontuberculous mycobacteria	Yes (mostly an issue in foreign-born populations)	No	No

\* 4 visits may be necessary if 2-step testing is performed

#### Guidelines for use of IGRAs in North Carolina

Medicine is constantly changing, and IGRAs are a very active area of research. **Clinical judgment based on the latest scientific evidence, with emphasis on how a given test will affect patient management, should always be used in deciding to order any diagnostic test and in interpreting the results.** The guidelines that follow are a consensus statement by the North Carolina Tuberculosis Control Program staff and the North Carolina Tuberculosis Medical Advisory Committee, designed to assist providers in determining when IGRAs may be useful in clinical practice. At the time of this writing, only Quantiferon Gold in-tube® is available to clinicians in North Carolina, but this may change with time.

#### *Reporting of IGRA results*

Reporting of IGRA results as “Positive,” “Negative,” or “Indeterminate” is clinically useful, but may be suboptimal in certain circumstances. Both clinically available IGRAs have a certain amount of test-retest variability (“wobble” — for example see Pai M et al., American Journal of Respiratory and Critical Care Medicine 2006 174: 349). Particularly when results are near the threshold for a positive test, repeat testing has a significant probability of producing a result on the other side of the threshold. This problem is of particular concern in the setting of repeat testing, when a patient may test negative at just below the threshold on one occasion, and then test positive at just above the threshold on another occasion solely due to inter-test variability.

Recommendations: To assist with test interpretation, the NC Tuberculosis Control Program recommends that laboratories report the following information:

#### Quantiferon Gold in-tube®

- Nil tube interferon gamma concentration (IU/mL)
- TB antigen tube interferon gamma concentration (IU/mL)
- TB-Nil value (difference, IU/mL)
- Criteria for a positive value (i.e. difference of  $\geq 35$  IU/mL and  $>25\%$  of nil)
- Interpretation (“Positive,” “Negative,” “Indeterminate”)

#### T-SPOT.TB®

- Nil plate spots (number)

- TB antigen plate spots (number, both plates)
- Highest difference (TB antigen-nil)
- Criteria for a positive value (i.e. difference of  $\geq 8$  spots)
- Interpretation (“Positive,” “Negative,” “Indeterminate”)

#### *Management of indeterminate results*

Both IGRAs may yield indeterminate results, either due to high Nil background or an inadequate interferon gamma response to mitogen. Indeterminate results are more common in the setting of immunosuppression, but can also occur in apparently immunocompetent hosts. However, a significant proportion of persons with indeterminate results on one occasion will have a non-indeterminate result on repeat testing. Similarly, while an indeterminate result is associated with an anergic response to the tuberculin skin test, not all persons with an indeterminate response from an IGRA will have anergy in response to the tuberculin skin test. In most studies, the T-SPOT.TB® produces a smaller proportion of indeterminate results than the Quantiferon Gold in-tube®.

#### Recommendations:

- If an indeterminate result is obtained from IGRA testing, repeating the same IGRA test should be considered.
- If an indeterminate result is obtained from one IGRA test (outside the setting of serial testing) performing a different IGRA test (if available) or a tuberculin skin test can be considered

#### *Diagnosis of active tuberculosis*

Like the tuberculin skin test, IGRAs are at best imperfect tools in the diagnosis of active TB. A recent meta-analysis estimated that the sensitivity of the tuberculin skin test among persons with active tuberculosis was 77 percent, the sensitivity of the Quantiferon Gold in-tube® was 70 percent, and the sensitivity of the T-SPOT.TB® was 90% (Pai M et al., Annals of Internal Medicine 2008; 149: 177). IGRAs will therefore be falsely negative in a significant proportion of persons with active TB. Also, IGRAs cannot discriminate between latent TB infection and active TB disease in a given patient. However, one study did demonstrate that IGRAs were useful in distinguishing children with TB cervical lymphadenitis from children with nontuberculous mycobacterial lymphadenitis (Clin Infect Dis. 2007; 45(3):322 ) in a low-incidence area.

#### Recommendations:

- Like the tuberculin skin test, IGRAs should not be relied upon to make or disprove the diagnosis of active TB in adults.
- IGRAs may be used in children as part of diagnostic algorithms for TB diagnosis, keeping in mind their imperfect test characteristics.
- Microbiologic diagnosis (culture) is the gold standard and should be aggressively pursued in both adults and children.

#### *Targeted testing for latent TB infection in immunocompetent adults and older children*

A large body of epidemiologic evidence associates an elevated risk to develop TB disease with a positive tuberculin skin test. No such evidence exists for any IGRA test at this time outside of a few studies of persons who were contacts to infectious TB cases. While IGRAs are clearly more specific for TB infection than the tuberculin skin test, some investigators believe that the tuberculin skin test may be more sensitive for remote TB

infection. The use of IGRAs for targeted TB testing may therefore result in fewer positive tests (and thus a lower number of persons offered latent TB treatment), with the possibility of missing some persons who truly have latent TB. The present costs of either IGRA from a public health department perspective are significantly greater than the cost of a tuberculin skin test, and routine use of IGRAs for targeted testing would divert scarce resources away from higher-priority activities.

Recommendations:

- The tuberculin skin test is preferred for routine targeted testing for latent TB infection in immunocompetent adults and children 5 years and older in the public health setting.
- IGRAs are acceptable alternatives for targeted testing for latent TB infection in immunocompetent adults and children 5 years and older, and may be preferred in some settings (see below “*Screening for latent TB infection at sites where tuberculin skin testing is not frequently performed*”).
- If serial testing (healthcare workers, residents of long-term care facilities) is planned, see specific recommendations below.

*Contact investigations*

IGRAs have been studied in a number of contact investigation settings, and a positive IGRA result generally correlates more closely with the extent of TB exposure than a positive tuberculin skin test. Limited data suggest that IGRAs predict subsequent progression to active TB disease after TB exposure at least as well as tuberculin skin testing (Diel R. et al., American Journal of Respiratory and Critical Care Medicine 2006; 174: 349 and Bakir M et al, Annals of Internal Medicine 2008 149: 777). The primary advantages of IGRAs over the tuberculin skin test are the availability of a result with one patient encounter (as opposed to two with the tuberculin skin test) and the lack of IGRA cross-reactivity with BCG and most nontuberculous mycobacteria. The cost of IGRAs, however, limits their general use in the public health setting.

Recommendations:

- IGRAs can be used for adults and children 5 years and older in contact investigations in place of the tuberculin skin test.
- The tuberculin skin is preferred for children <5 years old who are contacts to a case of active TB. T-SPOT.TB (currently not available in NC) can be used for contacts <5 years in place of the tuberculin skin test. **Note: Neither the tuberculin skin test nor IGRAs are particularly sensitive for TB infection in children under 6 months of age. Per NC guidelines, children under 6 months old who are close contacts to an infectious TB case should be given presumptive treatment for latent TB infections regardless of skin test/IGRA results.**
- The use of IGRAs should be particularly considered in populations with suboptimal healthcare access/utilization (e.g. homeless, substance abusers, migrant workers) who are unlikely to return for tuberculin skin test reading.
- Where possible, IGRA use in contact investigations should be paired with opt-out testing for the human immunodeficiency virus to identify close contacts who would be candidates for latent TB treatment regardless of IGRA result.
- The same test (IGRA or TST) should be used for initial and repeat (8-week post-exposure) testing of contacts.

*Serial testing in healthcare workers*

The use of IGRAs in serial testing of healthcare workers has not been extensively studied. However, the problem of test “wobble” has become apparent in early studies (Pai M et al., American Journal of Respiratory and Critical Care Medicine 2006 174: 349); that is, persons whose results are close to the cutoff for a positive result on one occasion have a high likelihood to have a different test interpretation if retested. The cost of serial testing of healthcare workers with IGRAs may be a significant additional burden on infection control programs that should be assessed in the context of the entire program. However, IGRAs may increase acceptance of latent TB treatment among healthcare workers who test positive, especially in those workers who have received BCG vaccine (Sahni R et al., Infection Control and Hospital Epidemiology 2009; 30: 197).

**Recommendations:**

- IGRAs should not generally be used for serial testing of healthcare workers until more data become available
- If serial testing of healthcare workers (or other persons at risk of TB infection) is performed, the same test should be consistently used in the same person over time. Switching among the tuberculin skin test, Quantiferon Gold in-tube®, and T-SPOT.TB® may result in false conversions due solely to test discordance.
- IGRAs may be considered as confirmatory tests for healthcare workers at low risk for progression to TB disease as a tool to increase acceptance of latent TB treatment (particularly among BCG-vaccinated healthcare workers)

*Screening of young children (<5 years) for latent TB infection*

Limited data exist demonstrating the utility of IGRAs in young children. At present, the T-SPOT.TB® has more extensive data justifying use in young children (e.g. Bakir M et al, Annals of Internal Medicine 2008 149: 777), and reported rates of indeterminate results for T-SPOT.TB® are generally lower in young children than for Quantiferon Gold in-tube® (e.g. Bergamini BM et al., Pediatrics 2009; 123: e419). However, not all studies support the superiority of T-SPOT.TB® in children (e.g. Kampmann B et al., European Respiratory Journal 2009; epub).

**Recommendations:**

- IGRAs should not generally be used for screening children <5 years old for latent TB infection.
- IGRAs should not generally be used as confirmatory tests after a positive tuberculin skin test among children <5 years old.

*Screening for latent TB infection at sites where tuberculin skin testing is not frequently performed*

One of the chief deficiencies of the tuberculin skin test is the poor inter-reader reliability when performed by inexperienced healthcare workers. In settings where tuberculin skin tests are not frequently performed, IGRAs may provide a more reliable test result than the tuberculin skin test.

**Recommendation:**

- IGRAs should be considered instead of the tuberculin skin test for latent TB screening (adults and children 5 years and older) at sites where tuberculin skin testing is not frequently performed.

- If latent TB screening of children <5 years old is indicated, referral to a site where tuberculin skin testing is frequently performed (e.g. public health clinic) may be appropriate if the referring site does not frequently perform tuberculin skin testing.

#### *Screening for latent TB infection in immunocompromised populations*

An increasing body of data has evaluated the performance of IGRAs in immunocompromised populations, including persons infected with the human immunodeficiency virus and persons on treatment for rheumatic disease. Similar to the tuberculin skin test, IGRAs are less sensitive for active tuberculosis in immunocompromised persons than in immunocompetent persons (e.g. Raby E et al. PLoS ONE 2008 3: e2489). Immunocompromised persons may have both false-negative IGRA results and indeterminate IGRA results in the setting of TB disease. The T-SPOT.TB® seems less likely to give an indeterminate result in some immunocompromised populations than the Quantiferon Gold in-tube® (e.g. Ferrara G et al, Lancet 2006 367: 1328), but the clinical importance of this finding is unclear.

#### Recommendations:

- The tuberculin skin test is still the first-line test for screening immunocompromised populations for latent TB infection.
- If the patient is unlikely or unwilling to return for reading of the tuberculin skin test, an IGRA may be considered.
- A negative IGRA in an immunocompromised individual does not exclude either latent infection or active disease.

#### Conclusions

IGRAs represent a significant new development in TB diagnostics, but also a challenge for a healthcare system with limited resources. Targeted use of IGRAs in the public health setting may provide significant benefits. These guidelines should be interpreted in the context of local expertise and resources, and will evolve as scientific knowledge of IGRAs continues to evolve. As always, clinical judgment, accompanied by expert consultation where appropriate, is vital in the use and interpretation of any new test.

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Quick reference table for recommended use of IGRAs. “+” indicates that the test is recommended for use in a given setting.

<b>Population</b>	<b>TB skin test</b>	<b>IGRA</b>	<b>Comment</b>
Suspect adult active TB			Culture diagnosis best
Suspect child active TB		May be helpful	Culture diagnosis best
Targeted testing for latent TB infection	+	Acceptable, not preferred	TB skin test preferred in most settings
Contact investigation	+	+	IGRA best in high-risk individuals unlikely to return for read, especially paired with HIV testing
Serial testing (healthcare workers, others)	+		May be helpful as “confirmation” of a positive skin test in otherwise low-risk, BCG-vaccinated persons unlikely to take latent TB treatment otherwise
Children < 5 years old	+		May be helpful in BCG vaccinated child < 5 who has not lived in endemic country and who has no documented TB exposure
Immunocompromised patients (latent TB screening)	+		
Screening of low-risk adults and older children (5+) at sites where tuberculin skin testing is not commonly performed		+	