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

The Mantoux tuberculin skin test (TST) and interferon gamma release assays (IGRAs) are tests for the presence of infection with *Mycobacterium tuberculosis*. These tests should be performed in persons who are at risk of infection with *M. tuberculosis* and/or at high risk for progression to active TB if infected. These tests have a number of limitations:

- They do not distinguish between latent tuberculosis infection (LTBI) and active TB disease
- Both false-negative (negative results in persons truly infected with *M. tuberculosis*) and false-positive (positive results in persons not infected with *M. tuberculosis*) results may occur
- They have issues with test-retest reliability, so if the same person is tested on more than one occasion, a different result may be obtained despite no change in TB infection. These issues arise from multiple sources, including reader variability (TST), biologic variability, specimen handling (IGRAs), and laboratory variability (IGRAs)
- Testing of low-risk populations is likely to yield many false-positive results, resulting in unnecessary treatment

At the time of this writing, two IGRAs are commercially available in North Carolina: the Quantiferon Gold in-tube® and T-SPOT.TB®. For most purposes these tests are roughly equivalent, and either can be used when an IGRA is indicated.

While either a TST or an IGRA may be used to screen for latent TB, in certain situations one test may be preferred. The decision to perform a TST or an IGRA will depend on a number of factors, including cost, logistics, and local test availability. The table below provides general guidance as to which type of test would be preferred in different clinical situations.

<table>
<thead>
<tr>
<th>Situation</th>
<th>Preferred test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person 5 years or older born outside the U.S.</td>
<td>IGRA</td>
</tr>
<tr>
<td>Person known to have received BCG (vaccine or bladder cancer treatment)</td>
<td>IGRA</td>
</tr>
<tr>
<td>Person unlikely to return for TST reading</td>
<td>IGRA</td>
</tr>
<tr>
<td>Child under 5 years old</td>
<td>TST*</td>
</tr>
<tr>
<td>Person at low risk for TB infection (e.g. administrative screening)</td>
<td>IGRA</td>
</tr>
<tr>
<td>Testing in settings where the TST is infrequently performed</td>
<td>IGRA</td>
</tr>
</tbody>
</table>

* Emerging evidence suggests that IGRAs may reduce false-positive tests among BCG-vaccinated children, so an IGRA is a reasonable alternative for such children
Mantoux skin testing

The Mantoux tuberculin skin test is the only skin test recommended for latent TB screening by NC TB Control.

- The TST should be repeated when interpretations are ambiguous or inconclusive.
- Under nursing regulations, an RN or LPN may not delegate the administration or reading of a TST to an unlicensed person.
- Healthcare workers responsible for placing and reading TSTs should receive classroom training about TB transmission, pathogenesis, testing and treatment of active tuberculosis and latent TB infection. Additionally, the healthcare worker 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 a 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 2 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 after these vaccines 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 infants 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, but as noted above IGRA's are preferred for most such individuals.

B. Reading

1. Read TST 48-72 hours after placement, 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
   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 (e.g. 1% hydrocortisone) 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 ≥5mm 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

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™), or other similar biologic immunosuppressive agents

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 jejunoileal 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 2 years (converter)

l. mycobacteriology lab personnel

m. children and adolescents exposed to high-risk adults (homeless, substance abuse, incarcerated, HIV positive)

n. persons who have traveled outside the US and stayed with family and friends who live in high incidence areas, for greater than 1 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 occur in any patient, but are more common in the following situations:

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 associated with old age, debility, malnutrition, HIV infection, medications, and malignancy.

**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 8 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 (>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 nontuberculous mycobacteria
2. BCG (bacille Calmette-Guérin) vaccine
3. Reading erythema (redness) rather than induration

F. TST Converters
An increase in reaction size of ≥ 10 mm within a period of 2 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 1 week to 1 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 (c)1-4. 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 1 to 3 weeks later:
  1. if the second test is positive, consider the individual infected
  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 is an attenuated or weakened form of tuberculosis, originally derived from Mycobacterium bovis, and is used in high-incidence countries to protect infants and young children against severe forms of TB disease (miliary and meningeal disease). BCG is also used as an intravesicular therapy (instilled into the bladder) for treatment of bladder cancer. BCG (identified as M. bovis) can frequently be isolated from the urine of persons who have recently received intravesicular therapy, but this does not always require treatment.

Both BCG vaccination and intravesical BCG can cause a reactive TST; this effect seems to wane over time but can persist for years. IGRAs are therefore preferred for most persons who have received the BCG vaccine.

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 and #9 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 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 1 month cumulatively
   e. children and adolescents exposed to high-risk adults (homeless, substance abuse, incarcerated, HIV positive)
   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  
- gastrectomy or intestinal bypass  
- current or planned use of immunosuppressive medications, particularly biologic agents (e.g. infliximab, adalimumab, etanercept)

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 or IGRA should be done (preferably a 2-step test if the TST is used) and treatment for LTBI started if the TST reading is 5 mm or greater or the IGRA is positive. If there is a credible history of exposure to TB, TLTBI should be initiated regardless of TST result.

4. Clinically assess all household members in the immediate environment of a child ≤ 2 years of age with a newly identified positive TST or IGRA to rule out an undiagnosed case of tuberculosis. An assessment should include an evaluation of symptoms for TB disease and may include a TST or IGRA, 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 or IGRA, 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 or IGRA on employment  
   c. routine TST or IGRA 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. Test staff with a TST (using the two-step method) or IGRA upon employment. Annual TST or IGRA 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
   • 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

9. Refugee Notification of Arrival: Class A and B Conditions (also see chapter IX -18 for reporting in NCEDSS)

a. prior to entering this country, federal regulations require newly arrived refugees ≥ 15 year of age* to have a medical exam consisting of a chest x-ray. Sputum smears are only done if the chest x-ray indicates possible TB disease. The overseas exam may not include a TST or sputum for culture. Furthermore the required chest x-ray may have been done up to 2 years prior to arrival in the US. Because of these uncertainties it is recommended that each refugee be fully evaluated for TB disease, including a TST and chest x-ray if not done overseas. If a chest x-ray was taken overseas, repeat chest x-ray if the original is of poor quality or x-ray is > 6 months old. Sputum should be obtained for smear and culture, if indicated. When TB disease is ruled out, consider treating for LTBI if indicated
b. refugees arriving in this country without an A or B classification status (normal chest film) who are determined to have a positive TST or IGRA during their initial evaluation should have a new chest film taken before starting treatment for LTBI

c. Class A TB with waiver
   • Applicants who have tuberculosis disease and have been granted a waiver

d. Class B1 TB, Pulmonary, No Treatment
   • Applicants who have medical history, physical exam, or chest x-ray findings suggestive of pulmonary tuberculosis but have negative AFB sputum smears and cultures and are not diagnosed with tuberculosis or can wait to have tuberculosis treatment started after immigration

e. Class B1 TB, Pulmonary, Completed Treatment
   • Applicants who were diagnosed with pulmonary tuberculosis and successfully completed directly observed therapy prior to immigration. The cover sheet should indicate if the initial sputum smears and cultures were positive and if drug susceptibility testing results are available

f. Class B1 TB, Extrapulmonary
   • Applicants with evidence of extrapulmonary tuberculosis.

g. Class B2 TB LTBI Evaluation
   • Applicants who have a tuberculin skin test > 10 mm or positive IGRA but otherwise have a negative evaluation for tuberculosis. The size of the TST reaction or IGRA result, the applicant’s status with respect to LTBI treatment, and the medication(s) should be documented on the overseas evaluation form. Contacts with a TST ≥ 5 mm or positive IGRA should receive this classification if they are not already classified as a Class B1 TB, Pulmonary. For

h. Class B3 TB, Contact Evaluation
   • Applicants who are a recent contact of a known tuberculosis case.

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 or IGRA
   b. with symptoms suggestive of TB disease regardless of TST/IGRA results
   c. with suspected extrapulmonary TB disease
   d. with negative TST/IGRA 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/IGRA
   b. with symptoms suggestive of TB disease regardless of TST/IGRA results
   c. with suspected extrapulmonary TB disease
   d. with negative TST/IGRA and starting latent TB treatment, i.e. close contact

3. For chest x-rays during pregnancy, see Chapter III, page 5

4. Individuals with a previously documented positive TST/IGRA 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/IGRA)
K. **Two-Step Tuberculin Skin Testing (TST)** *

Employment of Staff or Admission of Resident

- **Documented NEGATIVE TST**
  - Within Previous 12 months
  - Give single TST

- **No NEGATIVE TST**
  - Documented Within Previous 12 months
  - Administer 2-step TST

- **Documented PREVIOUS POSITIVE TST**
  - Negative chest x-ray; no symptoms**

**NEGATIVE TST**
- No Symptoms
  - Chest x-ray & evaluate for preventive therapy

**POSITIVE TST**
- Repeat TST In 1-3 weeks

**NEGATIVE TST**
- No Symptoms
  - Repeat TST based on risk classification

**POSITIVE TST**
- Chest x-ray & evaluate for preventive therapy
  - Complete Record of Tuberculosis Screening based on risk classification

**NEGATIVE TST**
- No Symptoms
  - TST Conversion

---

* 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. Interferon Gamma Release Assays (IGRAs)

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 2-3 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).
The T-SPOT.TB® is an enzyme-linked immunospot test. The test measures the number of spots on a plate containing 4 different antigens: nil (negative control), 2 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 8 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)).

<table>
<thead>
<tr>
<th>Nil [IU/mL]</th>
<th>TB Antigen minus Nil [IU/mL]</th>
<th>Mitogen minus Nil [IU/mL]</th>
<th>QuantiFERON®-TB Gold IT Result</th>
<th>Report / Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 8.0</td>
<td>≥ 0.35 and ≥ 25% of Nil value</td>
<td>Any</td>
<td>Positive²</td>
<td>M. tuberculosis infection likely</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.35 OR ≥ 0.35 and &lt; 25% of Nil value</td>
<td>≥ 0.5</td>
<td>Negative</td>
<td>M. tuberculosis infection NOT likely</td>
</tr>
<tr>
<td>&gt; 8.0⁴</td>
<td>Any</td>
<td>Any</td>
<td>Indeterminate³</td>
<td>Results are indeterminate for TB Antigen responsiveness</td>
</tr>
</tbody>
</table>

The T-SPOT.TB® is an enzyme-linked immunospot test. The test measures the number of spots on a plate containing 4 different antigens: nil (negative control), 2 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 8 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.

<table>
<thead>
<tr>
<th></th>
<th>Tuberculin skin test</th>
<th>Quantiferon Gold in-tube</th>
<th>T-SPOT.TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of visits required</td>
<td>2*</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Time frame to get blood to lab</td>
<td>N/A</td>
<td>&lt;14 hours (can be incubated in portable incubator)</td>
<td>&lt;8 hours</td>
</tr>
<tr>
<td>Need for additional processing of blood before incubation</td>
<td>N/A</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Result format</td>
<td>mm of induration</td>
<td>Concentration of interferon gamma (IU/mL)</td>
<td>Number of spots on a plate</td>
</tr>
<tr>
<td>Reliability among observers</td>
<td>+/-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Test-retest variability</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Potential as a “send out” test to distant labs</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes (always done this way)</td>
</tr>
<tr>
<td>Cost to health department or healthcare facility</td>
<td>Reagent inexpensive, labor somewhat more</td>
<td>Moderately expensive</td>
<td>Moderately expensive</td>
</tr>
<tr>
<td>Cross-reacts with BCG, nontuberculosis mycobacteria</td>
<td>Yes (mostly an issue in foreign-born populations)</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

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

**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.

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 ≥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 ≥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.

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%, the sensitivity of the Quantiferon Gold in-tube® was 70%, 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.
Appendix F
Quality Control Procedural Observation Checklist (from MMWR2 2005;54 (RR-17))

Appendix F. Quality control (QC) procedural observation checklists

| Quality Control (QC) Procedural Observation Checklist for Placing Tuberculin Skin Tests (TSTs) — Mantoux Method |
|---|---|---|---|---|
| Date (QC by) | Trainee (TST placed by) | Scoring: X or Y = Yes | X or N = No | NA = Not Applicable |

1. Preliminary
   - Use appropriate hand hygiene methods before starting.
   - Screen patient for contraindications (severe adverse reactions to previous TST). *
   - Use well-lit area.

2. Syringe * filled with exactly 0.1 mL of purified protein derivative (PPD) antigen
   - Injects antigen via intradermal injection and confirms that it is 5 TST PPD antigen.
   - Checks label and expiration date on vial.
   - Marks opening date on multidose vial.
   - Fills immediately after vial removed from refrigeration.
   - Closes vial stopper with antiseptic swab.
   - Twists needle onto syringe to ensure tight fit.
   - Removes needle guard.
   - Injects needle into the vial.
   - Delays slightly over 0.1 mL of 5 TST PPD into syringe.
   - Injects excess volume or air bubbles to exactly 0.1 mL of 5 TST PPD while needle remains in vial to avoid wasting of antigen.
   - Removes needle from vial.
   - Returns antigen vial to the refrigerator immediately after filling.

3. TST administration site selected and cleansed
   - Selects upper third of forearm with palm up ±2 inches from elbow, wrist, or other injection site. **
   - Selects site free from veins, lesions, hairy hair, bruises, scars, and muscle lumps.
   - Cleans site with antiseptic swab using circular motion from center to outside.
   - Allows site to dry thoroughly before administering antigen.

4. Needle inserted properly to administer antigen
   - Points arm on firm, well-lit surface.
   - Stretches skin slightly. 11
   - Holds needle bevel-up and tip at 5°–15° angle to skin.
   - Inserts needle in first layer of skin with tip visible beneath skin.
   - Advances needle until entire bevel is under the first layer of skin.
   - Places syringe against skin.
   - Injects syringe slowly.
   - Forces needle, as liquid is injected.
   - Removes needle without pressing area.
   - Activates safety feature of device per manufacturer’s recommendations, if applicable.
   - Places used needle and syringe immediately in puncture-resistant container without recapping needle.
   - Immediately measures wheal to ensure 6–10 mm in diameter (Actual wheal measurement ______ mm).
   - If blood or fluid is present,blots site lightly with gauze or cotton ball.
   - Documents used gauze or cotton ball according to local standard procedures.
   - If the TST is administered incorrectly (too deeply or too shallow) and the wheal is inadequate (≤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.
   - Documents all information required by the setting (e.g., date and time of TST placement, person who placed TST, location of injection site, lot number of tuberculin).
   - Uses appropriate hand hygiene methods after placing TST.

5. Explanation to the client regarding care instructions for the injection site
   - The wheel (tump) is normal and will remain about 10 minutes.
   - Do not touch wheel, avoid scratching.
   - Avoid pressure or bandage on injection site.
   - Raw local discomfort and irritation does not require treatment.
   - May wash with soap and water (without pressure) after 1 hour.
   - No lotions or liquids on site, except for light washing, as above.
   - Keeps appointment for reading.

* Severe adverse reactions to the TST are rare but include urticaria, necrotizing, vascular blister, or bullae at the site, or anaphylactic shock, which is extremely rare. These reactions are the only contraindications to having a TST administered.

1 Use a 0.5-inch 25-gauge needle or first, disposable tuberculin (preferably a safety-type) syringe.

2 Prefilling syringes is not recommended. Tuberculin is absorbed in varying amounts by glass and plastic. To minimize reduction in potency, tuberculin should be administered as soon after the syringe is filled as possible. Following these procedures will also help avoid contamination. Each dose should always be removed from the vial under sterile 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 tuberculois in adults and children. Am J Resp Cit. Care. 2000;160:1278-85.

3 Preventing tuberculin antigen and vaccine (e.g., Td booster) 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 tuberculin skin tests. MMWR 2004;53:662-4.

** If neither arm is available or acceptable for testing, the belt of the shoulder is a good alternate TST administration site.

SOURCE: National Tuberculosis Controllers Association, National Tuberculosis Nurses Consultant Coalition. Tuberculosis nursing: a comprehensive guide to patient care, spring, Ga, National Tuberculosis Controllers Association; 1997.

11 Stretch skin by placing nondominant hand of health-care worker (HCW) on patients forearm below the needle insertion point and then applying traction in the opposite direction of the needle insertion. Care 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 and 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

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

<table>
<thead>
<tr>
<th>Date</th>
<th>Trainer (QC by)</th>
<th>Trainers (TST placed by)</th>
</tr>
</thead>
<tbody>
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Scoring: ✓ or Y = Yes  X or N = No  NA = Not Applicable

1. Preliminary
   - Uses appropriate hand hygiene methods before starting.
   - Keeps implements shorter than fingertips to avoid misreading TST result.
   - Keeps TST reading materials at hand (eyeliner pencil or ballpoint pen, and ruler).
   - Uses well-lit area.
   - Inspects the site of the injection.

2. Palpate — finding margin ridges (if any)
   - Palpates with arm bent at elbow at a 90° angle.
   - Lightly sweeps 2-inch diameter from injection site in four directions.
   - Uses rigid featherlike touch.
   - Reposition palpation with arm bent at elbow at a 45° angle to determine presence or absence of induration.

If induration is present, continue with these steps:

3. Placing marks
   - Holds palm over injection site.
   - Uses fingertips to find margins of the induration.
   - Marks the induration by placing small dots on both sides of the induration.
   - Inspects dots, repeats finger movements toward indurated margin, and adjusts dots if needed.
   - Marks dots transverse (perpendicular) to long axis of forearm.

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.

4. Placing and reading ruler
   - Place the 0° ruler line inside the edge of the left dot. Read the ruler line inside right dot edge (smallest lower measurement if between two graduations on millimeter scale — see Figure 1).
   - Use appropriate hand hygiene methods after reading TST.

5. Documenting results
   - 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."
   - Correctly records results in mm; only a single measured induration in mm should be recorded.
   - Trainer’s measurement ________ mm.
   - Trainer’s (gold standard) measurement ________ mm.
   - Trainer's result within 2 mm of gold standard reading? Yes __ No __