Diagnosis of Tuberculosis and LTBI

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Objectives

At the completion of this session, the participant will be able to:
• Know how to select the appropriate diagnostic test for patients suspected of tuberculosis as an initial step in the clinical management of the disease.
• Understand that nucleic acid amplification tests (NAAT) are recommended as an adjunct to mycobacterial culture and AFB smear microscopy to enable a rapid diagnosis of TB
• Describe the culture growth-based and molecular methods that can be used to determine drug-susceptibility testing and how to use this data to choose an appropriate TB treatment regimen.
• Identify the appropriate diagnostic testing that is recommended for patients with extra-pulmonary TB
• Joint statement of ATS, IDSA, CDC
• Partners
  - American Academy of Pediatrics
  - Association of Public Health Laboratories
• Harmonization with concurrent and overlapping guidelines
  - AAP Red Book
  - CDC IGRA Guidelines update
  - CDC Nucleic Acid Amplification Testing Guidelines update

GRADE METHODOLOGY
(Grading of Recommendations Assessment, Development, and Evaluation)

• Recommendations based on the certainty in the evidence assessed according to GRADE methodology to address PICO questions, incorporating patient values and costs as well as judgments about tradeoffs between benefits and harms.

PICO = Population, Intervention, Comparison, Outcome

<table>
<thead>
<tr>
<th>Implications</th>
<th>Strong Recommendation</th>
<th>Conditional Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>Most individuals in this situation would want the recommended course of action, and only a small proportion would not.</td>
<td>The majority of individuals in this situation would want the suggested course of action, but many would not.</td>
</tr>
<tr>
<td>Clinicians</td>
<td>Most individuals should receive the intervention. Adherence to this recommendation according to the guideline could be used as a quality criterion or performance indicator. Formal decision aids are not likely to be needed to help individuals make decisions consistent with their values and preferences.</td>
<td>Recognize that different choices will be appropriate for individual patients and that you must help each patient arrive at a management decision consistent with his or her values and preferences. Decision aids may be useful in helping individuals to make decisions consistent with their values and preferences.</td>
</tr>
<tr>
<td>Policy</td>
<td>The recommendation can be adopted as policy in most situations.</td>
<td>Policymaking will require substantial debate and involvement of various stakeholders.</td>
</tr>
</tbody>
</table>

Source: Grading of Recommendations Assessment, Development and Evaluation Working Group [1, 20]
Guideline Contents

• TB: Epidemiology, Transmission, and Pathogenesis
• Diagnostic Tests for LTBI
• Testing for LTBI
• Testing for Pulmonary TB Disease
• Testing for Extrapulmonary TB Disease
• Genotyping of M. TB
• Research Needs

THINK TB....
(and evaluate appropriately)

• Benefits of Diagnostic Testing
  - Prevention of TB
  - Detection of TB
  - Early diagnosis leads to improved morbidity and mortality
Diagnosis of *M. tuberculosis* infection

Person with LTBI (Infected)
- Has a small amount of TB bacteria in his/her body that are alive, but inactive
- **Cannot** spread TB bacteria to others
- Does **not** feel sick, but may become sick if the bacteria become active in his/her body
- Usually has a TB skin test or TB blood test reaction indicating TB infection
- Radiograph is typically normal
- Sputum smears and cultures are negative
- Should consider treatment for LTBI to prevent TB disease
- Does **not** require respiratory isolation
- Not a TB case

Person with TB Disease (Infectious)
- Has a large amount of active TB bacteria in his/her body
- May spread TB bacteria to others
- May feel sick and may have symptoms such as a cough, fever, and/or weight loss
- Usually has a TB skin test or TB blood test reaction indicating TB infection
- Radiograph may be abnormal
- Sputum smears and cultures may be positive
- Needs treatment for TB disease
- May require respiratory isolation
- A TB case

CDC self-study Module 3 – Targeted Testing and the Diagnosis of Latent Tuberculosis Infection and Tuberculosis Disease
The spectrum of TB — from *Mycobacterium tuberculosis* infection to active (pulmonary) TB disease

<table>
<thead>
<tr>
<th></th>
<th>TST</th>
<th>IGRA</th>
<th>Culture</th>
<th>Spinal smear</th>
<th>Infectious</th>
<th>Symptoms</th>
<th>Preferred treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection eliminated with immune response*</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Usually positive</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>Infection eliminated with acquired immune response</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Usually positive</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>Latent TB infection</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive or negative</td>
<td>Mild or none</td>
<td>Preemptive therapy</td>
</tr>
<tr>
<td>Subclinical TB disease</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive or negative</td>
<td>Mild or none</td>
<td>Multi-drug therapy</td>
</tr>
<tr>
<td>Active TB disease</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Severe</td>
<td>Severe</td>
<td>Multidrug therapy</td>
</tr>
</tbody>
</table>

Methods for Detecting *M. tb* Infection

- Mantoux tuberculin skin test (TST)
- Interferon Gamma Release Assays (IGRAs):
  - QuantiFERON-TB Gold In-Tube (QFT-GIT)*, and
  - T-Spot.*
- These tests do not exclude LTBI or TB disease
- Decisions about medical/public health management should not rely only on TST/IGRA results, but consider TB risk, setting, patient and source case factors
William Osler

“Medicine is a science of uncertainty and an art of probability.”

“It is much more important to know what sort of a patient has a disease than what sort of a disease a patient has.”

Mantoux Tuberculin Skin Test

• Tuberculin made from proteins derived from inactive tubercle bacilli
• 0.1 ml of 5 tuberculin units of liquid tuberculin injected intradermally
• Most people who have TB infection will have a reaction at injection site
  - Health care worker reads reaction within 48 to 72 hours
  - Measure induration in mm (not redness)
### Factors that May Affect the TST Reaction

<table>
<thead>
<tr>
<th>Type of Reaction</th>
<th>Possible Cause</th>
</tr>
</thead>
</table>
| False-positive  | • Nontuberculous mycobacteria  
• BCG vaccination  
• Using wrong solution (ie tDap, dTap)  
• Measurement or interpretation |
| False-negative  | • Anergy  
• Viral, bacterial, fungal co-infection  
• Recent TB infection  
• Very young age; advanced age  
• Live-virus vaccination  
• Overwhelming TB disease  
• Renal failure/disease  
• Lymphoid disease  
• Low protein states  
• Immunosuppressive drugs  
• Problems with TST administration |

### The booster phenomenon with the TST

Person becomes infected with *M. tuberculosis*  

Person is skin tested years later  

Person has negative reaction due to lessened ability to react to tuberculin  

However, this skin test “jogs the memory” of the immune system to recognize and react to tuberculin  

Person is skin tested again, up to 1 year later. *(for this example, assume that the person was NOT exposed to TB during this time)*  

Person has a positive reaction  

**Boosted reaction** due to TB infection that occurred a long time ago, not during the time between the two skin tests
Two-Step Testing
(only for TST, not IGRA)

Baseline skin test

- Reaction
  - Negative
    - Retest 1-3 weeks later
  - Positive
    - Person probably has TB infection
      (Unless BCG history—Get IGRA)

- Reactivity
  - Negative
    - Person probably does NOT have TB infection
  - Positive
    - Reaction is considered a boosted reaction (due to TB infection that occurred a long time ago)
      - Repeat at regular intervals; a subsequent positive reaction will probably be due to a recent TB infection
    - Retesting not necessary,
      Future screening of risk and symptoms

Interferon Gamma Release Assays

- Whole blood test using purified antigens from MTB to stimulate peripheral-blood lymphocytes to produce interferon-gamma (IFN-γ)
  - QuantiFERON tests (QFT) measures level of IFN-γ in supernatant of the cell suspension
  - TSPOT measures # of cells producing IFN-γ (ELISpot assay)
QFT vs T-SPOT Results

<table>
<thead>
<tr>
<th>QFT-GIT</th>
<th>T.SPOT.TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (&gt; 0.35 IU/mL)</td>
<td>Positive (&gt; 8 spots)</td>
</tr>
<tr>
<td>Negative (&lt; 0.35 IU/mL)</td>
<td>Negative (&lt; 4 spots)</td>
</tr>
<tr>
<td>Borderline (5-7 spots)</td>
<td></td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Invalid</td>
</tr>
<tr>
<td>- Low mitogen</td>
<td>- Low mitogen</td>
</tr>
<tr>
<td>- High nil</td>
<td>- High nil</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Invalid</td>
</tr>
<tr>
<td>- Low mitogen</td>
<td>- Low mitogen</td>
</tr>
<tr>
<td>- High nil</td>
<td>- High nil</td>
</tr>
<tr>
<td>Failed</td>
<td>Failed</td>
</tr>
<tr>
<td>- Inadequate blood volume</td>
<td>- Inadequate blood volume</td>
</tr>
<tr>
<td>- Broken tube</td>
<td>- Broken tube</td>
</tr>
<tr>
<td>- Delayed incubation</td>
<td>- Delayed incubation</td>
</tr>
</tbody>
</table>

IGRA: General Points

- **Highly specific** (~95% in low TB incidence areas)
  - Both QFT and T-SPOT are substantially more specific than PPD since they contain antigens not found in BCG
  - Distinguish most NTM
    - Except M. Kansasii, M. marinum, M. szulgai, M. flavescens
    - PPD contains large number of mycobacterial proteins not specific to M. tuberculosis
- **Sensitivity:** T-SPOT.TB assay appears higher than QFT or TST (90%, 80%, and 80%, respectively)
  - Sensitivity diminished by HIV infection, immunosuppression, in children

Pai et al. Clinical Microbiology Reviews, 2014
What are 5 advantages for using an IGRA as compared to the TST?

IGRA Limitations

• Within-subject variability
• Serial testing of healthcare workers (HCW) using IGRA in low risk settings (U.S. and Canada):
  - Unusually high IGRA conversion rates (4-7%) compared to historical or concurrent TST conversions rates (0.0-0.9%)
  - 60-75% with IGRA conversion reverted to (-) on repeat testing
  - TST before IGRA may cause boosting (9% QFT, 11% TSPOT)
• Test agreement fair; discordance between tests more common than concordance among those with + tests
• Delay in IGRA conversion compared to TST may account for some discordant TST/IGRA results in recently exposed contacts

Dorman, Am J Respir Crit Care Med. 2014;189(1):77-87
Candidates for testing and treatment of LTBI

1. Persons with risk for recent infection with *M. tuberculosis* (exposure risk)
2. Persons with risk of progression to active TB if infected with *M. tuberculosis* (Medical risk)
Table 1: Criteria for Classifying Positive TST Reactions

<table>
<thead>
<tr>
<th>Positive IGRA result or a TST reaction of 5 or more millimeters of induration is considered positive in</th>
<th>Positive IGRA result or a TST reaction of 10 or more millimeters of induration is considered positive in</th>
</tr>
</thead>
<tbody>
<tr>
<td>- HIV-infected persons</td>
<td>- Recent immigrants (≥ 5 years) from high-prevalence countries</td>
</tr>
<tr>
<td>- Recent contacts of a TB case</td>
<td>- Injection drug users</td>
</tr>
<tr>
<td>- Persons with fibrocavitory changes on chest radiograph consistent with old TB</td>
<td>- Residents and employees of high-risk congregate settings (e.g., correctional facilities, nursing homes, homeless shelters, hospitals, and other health care facilities)</td>
</tr>
<tr>
<td>- Organ transplant recipients</td>
<td>- Microbiological laboratory personnel</td>
</tr>
<tr>
<td>- Persons who are immunosuppressed for other reasons (e.g., taking the equivalent of ≥ 15 mg/day of prednisone for 1 month or longer, taking TNF-α antagonists)</td>
<td>- Children under 5 years of age, or children and adolescents exposed to adults in high-risk categories</td>
</tr>
</tbody>
</table>

Positive IGRA result or a TST Reaction of 15 or more millimeters of induration is considered positive in

- Persons with no known risk factors for TB*

*Although skin testing programs should be conducted only among high-risk groups, certain individuals may require TST for employment or school attendance. An approach independent of risk assessment is not recommended by CDC or the American Thoracic Society.

Paradigm for evaluation of those with LTBI based on risk of infection, risk of progression to TB, and benefit of therapy

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2017 ATS/CDC/IDSA TB Diagnosis Guidelines
Testing for LTBI

Step 1. Determine Risk of Infection: Likely or Unlikely
Step 2. Determine Risk of Disease Progression: Low, Intermediate, High

Diagnostic Guidelines: LTBI Testing #1a

- We recommend performing an IGRA rather than a TST in individuals 5 years or older who meet the following criteria:
  
  (1) are likely to be infected with \textit{Mtb},
  (2) have a low or intermediate risk of disease progression,
  (3) it has been decided that testing for LTBI is warranted, and
  (4) either have a history of BCG vaccination or are unlikely to return to have their TST read

\textit{Strong recommendation, moderate-quality evidence}

Remarks: A TST is an acceptable alternative, especially in situations where an IGRA is not available, too costly, or too burdensome.
Diagnostic Guidelines: LTBI Testing #1b

• We recommend performing an interferon-γ release assay (IGRA) rather than a tuberculin skin test (TST) in individuals 5 years or older who meet the following criteria:
  
  (1) are likely to be infected with *Mtb*,
  (2) have a low or intermediate risk of disease progression,
  (3) it has been decided that testing for LTBI is warranted

  *Conditional recommendation, moderate-quality evidence*

  *Remarks:* A TST is an acceptable alternative, especially in situations where an IGRA is not available, too costly, or too burdensome.

Diagnostic Guidelines: LTBI Testing #2

• There are insufficient data to recommend a preference for either a TST or IGRA in individuals 5 years or older who meet the following criteria:
  
  (1) are likely to be infected with *Mtb*,
  (2) have a high risk of disease progression,
  (3) it has been decided that testing for LTBI is warranted
Diagnostic Guidelines: LTBI Testing #3

“Guidelines recommend that persons at low risk for Mtb infection and disease progression NOT be tested for Mtb infection.

We concur with this recommendation.”

However, we also recognize that such testing may be obliged by law or credentialing bodies. If diagnostic testing for LTBI is performed in individuals who are unlikely to be infected with Mtb despite guidelines to the contrary:

**Recommendation 3a:**
We suggest performing an IGRA instead of a TST test in individuals 5 years or older.

*Conditional recommendation, low quality evidence*

**Remarks:** A TST is an acceptable alternative in settings where an IGRA is unavailable, too costly, or too burdensome.
**Recommendation 3b:**
We suggest a second diagnostic test if the initial test is positive in individuals 5 years or older.

**Conditional recommendation, very low quality evidence**

Remarks: The confirmatory test may be either an IGRA or a TST. When such testing is performed, the person is considered infected only if both tests are positive.

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**Summary of Recommendations for Testing for LTBI**

<table>
<thead>
<tr>
<th>Group</th>
<th>Testing Strategy</th>
<th>Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Likely to be Infected</td>
<td><strong>Preferred</strong>: IGRA where available</td>
<td>Prevalence of BCG vaccination, Expertise of staff and/or laboratory infrastructure, Test availability, Patient perceptions, Staff perceptions, Programmatic concerns</td>
</tr>
<tr>
<td>High Risk of Progression (TST ≥ 10mm)</td>
<td><strong>Accepted</strong>: IGRA or TST</td>
<td></td>
</tr>
<tr>
<td>Children 5–5 years of age</td>
<td>Consider dual testing where a positive result from either test would be considered positive</td>
<td></td>
</tr>
<tr>
<td>Preferred: TST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accepted: IGRA or TST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Likely to be Infected</td>
<td><strong>Preferred</strong>: IGRA where available</td>
<td>Prevalence of BCG vaccination, Expertise of staff and/or laboratory infrastructure, Test availability, Patient perceptions, Staff perceptions, Programmatic concerns</td>
</tr>
<tr>
<td>Low to Intermediate Risk of Progression (TST &lt; 10mm)</td>
<td><strong>Accepted</strong>: IGRA or TST</td>
<td></td>
</tr>
<tr>
<td>Unlikely to be Infected</td>
<td>Testing for LTBI is not recommended if necessary: <strong>Preferred</strong>: IGRA where available, <strong>Accepted</strong>: Either IGRA OR TST for serial testing, <strong>Accepted</strong>: Either IGRA OR TST</td>
<td>Prevalence of BCG vaccination, Expertise of staff and/or laboratory infrastructure, Test availability, Patient perceptions, Staff perceptions, Programmatic concerns</td>
</tr>
<tr>
<td>(TST &gt; 15mm)</td>
<td>Consider repeat or dual testing where a negative result from either would be considered negative</td>
<td></td>
</tr>
</tbody>
</table>
Mr. G is a 25 year old medical student. He was born in Haiti, and moved to the U.S. with his family after the earthquake. His medical school is requiring that he have a test for TB infection before he starts his clinical rotations.

**WHY?**

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**TB Testing Programs**

• TB testing programs in health care settings, corrections, residential facilities, etc. aim to:

1. Identify people who have LTBI or TB disease so they can be given treatment as needed
   • Individual benefit
   • Community benefit

2. Determine whether TB is being transmitted in facility
   • Staff or residents convert from negative to positive TST/IGRA
1. Which test for TB infection should Mr. G have at baseline?

2. Mr. G’s initial QFT-GIT is indeterminate. What should you do now?

3. Mr. G’s repeated baseline QFT-GIT is negative. When he starts the second year of his clinical rotations, his QFT-GIT is read:
   - Mitogen: >10.0  TB nil: 0.08  TB Ag: 0.50
   - TB  Ag-nil: 0.42

4. He has no knowledge of contact to TB, denies traveling home or contact to known TB. Now what should you do?
Serial IGRAs for Healthcare Workers

• IGRAS are not a solution to false-positive results associated with serial testing in low-risk individuals

• “At present there is insufficient information available to guide the establishment of definitive criteria for the conversion and possible reversion of IGRAS.....[especially] in the context of serial testing.”

• In serial LTBI testing of U.S. healthcare workers, IGRA conversions noted be 6-8%, 6-9x higher than for TST and thought to be false conversions¹

• For further guidance on healthcare worker LTBI testing, refer to 2005 CDC Guidelines for Preventing the Transmission of *M. TB* in Health-Care Settings²

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Diagnosis of TB Disease
Case: Mrs. H

• 72 year old F from the Philippines; she has been living with her daughter in the U.S. for 3 months.
• She has lost about 18 pounds over the past 4 months. She also reports night sweats, fevers and a cough for several months, and has recently noticed a reddish tinge to her sputum.
• She is a known contact to her father who was treated for TB when she was a child, but she was never evaluated
• She has insulin dependent diabetes

What parts of Mrs. H’s medical history should lead a clinician to suspect TB?
Mr. S is from Pakistan. He has been complaining of cough, intermittent fevers and sweating profusely at night. He has lost 15 pounds and is very weak. He is admitted to the hospital for evaluation. His physician suspects TB and orders an IGRA.

Q. For patients with symptoms of TB disease, should clinicians wait for TST or IGRA results before starting other diagnostic tests?

A.

What would you like to do next to evaluate Mr. S for TB disease?

• Components of medical evaluation:

  1.
  2.
  3.
  4.
  5.
Medical History

• Clinicians should ask patients if they have:
  - Symptoms of TB disease
    • General: fever, chills, weight loss, malaise, anorexia, night sweats
    • Pulmonary: cough, chest pain, shortness of breath, hemoptysis
    • Extrapulmonary: any site
  - Been exposed to a person with infectious TB or have risk factors for exposure to TB
  - Any risk factors for developing TB disease
  - Had LTBI or TB disease before
  - Had treatment for LTBI/TB disease before

Physical Examination

A physical examination cannot confirm or rule out TB disease, but can provide valuable information
Chest X-Ray

• When a person has TB disease in the lungs, the CXR usually appears abnormal. It may show:
  - Infiltrates (collections of fluid and cells in lung tissue)
  - Cavities (hollow spaces within the lung)


Can the results of a chest x-ray confirm that a person has TB disease? Why or why not?
Bacteriologic Examination of Clinical Specimens

Bacteriologic examination has 5 parts

1. Specimen collection
2. AFB smear classification
3. Nucleic acid amplification testing (NAA, NAAT, PCR etc., DNA probe)
4. Culture and identification
5. Drug-susceptibility testing
Mr. L has a cough and other symptoms of TB disease, and his CXR strongly suggests TB disease. However, he is unable to cough up any sputum on his own for the bacteriologic examination.

What should be done?

Sputum Sample Specimen Collection

• Easiest and least expensive method is to have patient cough into sterile container
• HCWs should coach and instruct patient
• Should have at least 3 sputum specimens examined
  - Collected in 8 to 24 hour intervals
  - At least one early morning specimen

• See instructions in multiple languages:
Recommendation #10: Use sputum induction rather than flexible bronchoscopic sampling as the initial respiratory sampling method for individuals with suspected pulmonary TB who are either unable to expectorate sputum or whose expectorated sputum is AFB smear microscopy negative.

*Conditional recommendation, low-quality evidence*

- Induced sputum has equal or greater diagnostic yield than bronchoscopic sampling, has fewer risks, and is less expensive.

**Induced Sputum Collection**

- Creates extra moisture in the airways to loosen and thin out secretions so they may be coughed up more easily.
- Patient inhales hypertonic saline mist, irritates airways, stimulates secretion of thick watery mucous, causes deep coughing
- Performed in a negative pressure room or booth, or the practitioner performing induction must wear a N-95 mask
- Specimen often clear and watery, should be labeled “induced specimen”
Recommendation #11: Use flexible bronchoscopic sampling, rather than no bronchoscopic sampling, in individuals with suspected pulmonary TB from whom a respiratory sample cannot be obtained via induced sputum

*Conditional recommendation, very low-quality evidence*

- Bronchoscopy in a patient with possible pulmonary TB can:
  - Differentiate TB disease from alternative diseases
  - Obtain specimens for cultures that provide isolates for drug susceptibility testing

Recommendation #12: Postbronchoscopy sputum specimens should be collected from all individuals with suspected pulmonary TB who undergo bronchoscopy

*Conditional recommendation, low-quality evidence*

- Postbronchoscopy AFB smears have a diagnostic yield of 9%–73% and postbronchoscopy mycobacterial cultures have a yield of 35%–71% (multiple studies)
- In HIV infected patients, the yield of postbronchoscopy sputum cultures was 80% in a single study
- Specimens obtained via bronchoscopy can undergo AFB smear microscopy, mycobacterial culture, NAAT, and histopathological analysis.
Ms. T is a 65 year old white female. She complains of a worsening cough for 2 months, pleuritic chest pain and dyspnea.

- Mrs. T’s sputum smear results were reported as 4+, 3+, and 4+ acid fast bacilli (AFB)
- What do these results tell you about Ms. Thompson’s diagnosis and her infectiousness?

- 70% overall sensitivity in culture confirmed TB testing 3 sputum smears for AFB
  - 1st 54%; 2nd + 11%; 3rd + 2-5%
### Examination of AFB Smears

<table>
<thead>
<tr>
<th>Classification of Smear</th>
<th>Smear Result</th>
<th>Infectiousness of Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>Strongly positive</td>
<td>Probably very infectious</td>
</tr>
<tr>
<td>3+</td>
<td>Strongly positive</td>
<td>Probably very infectious</td>
</tr>
<tr>
<td>2+</td>
<td>Moderately positive</td>
<td>Probably infectious</td>
</tr>
<tr>
<td>1+</td>
<td>Moderately positive</td>
<td>Probably infectious</td>
</tr>
<tr>
<td>Actual number of AFB seen (no plus sign)</td>
<td>Weakly positive</td>
<td>Probably infectious</td>
</tr>
<tr>
<td>No AFB seen</td>
<td>Negative</td>
<td>May not be infectious</td>
</tr>
</tbody>
</table>

### Recommendations #5: AFB smear microscopy should be performed, rather than no AFB smear microscopy, in all patients suspected of having pulmonary TB

*Strong recommendation, moderate quality evidence*

**Remarks:**
- A negative AFB smear result does not exclude pulmonary TB.
- A positive AFB smear result does not confirm pulmonary TB.
- At least 2 respiratory specimens should be tested, at least one of which should be a first morning specimen.
- Providers should request a sputum volume of at least 3 mL, but the optimal volume is 5 to 10 mL.
- Concentrated respiratory specimens and fluorescence microscopy are preferred.
How do you know if Mrs. T has TB or another mycobacterial infection?

1.
2.

Always consider:
- Patient’s risk of TB exposure
- Medical conditions associated with progression from LTBI to TB disease
- Clinical presentation

Mycobacterial Culture

- Laboratory gold standard

- Step 1: Detect growth of a mycobacteria
  - Solid media: 3 to 6 weeks
  - Liquid media: 4 to 14 days

- Step 2: Identify organism that has grown (isolate)
  - Nucleic acid probes can detect *M. tuberculosis complex*: 2 to 4 hrs
Recommendation #6: Both liquid and solid mycobacterial cultures should be performed for every specimen obtained from an individual with suspected TB disease

*Conditional recommendation, low quality evidence*

- **Liquid cultures** alone are reasonably sensitive and highly specific, but limited by bacterial contamination
- **Solid cultures** alone are not sufficiently sensitive to reliably diagnose TB and generally take longer to yield results; however, some Mtb isolates are detected only on solid medium.

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Culture and Response to TB Therapy

- **Negative culture does not rule out TB disease**
  - Some patients with negative cultures are diagnosed with TB based on signs and symptoms ("culture-negative" or "clinical case of TB")
- **Bacteriological examinations are important for assessing infectiousness and response to treatment**
  - Specimens should be obtained monthly until 2 consecutive cultures are negative
  - Culture conversion is the most important objective measure of response to TB treatment
Nucleic Acid Amplification Tests (NAAT)

- Directly identify *M. tb* from sputum specimens by amplifying (copying) DNA and RNA segments in hours
- Highly accurate in distinguishing TB from other NTM
- Can help guide clinician’s decision for patient therapy and isolation
  - 2 negative geneXPERT → unlikely infectious and can be cleared from airborne infection isolation in hospital
- **NAAT do not replace AFB culture or clinical judgment; a negative test does not rule out TB**
  - Not sensitive enough
  - Need isolate for drug susceptibility testing

Recommendation #7: A diagnostic **NAAT should be performed on the initial respiratory specimen** from patients suspected of having pulmonary TB

*Conditional recommendation, low quality evidence*

**Evidence Basis for NAAT (detect M. Tb complex)**

- **AFB Smear Positive**\(^1\)
  - Sensitivity 96%, Specificity 85%
- **AFB Smear Negative**\(^1\)
  - Sensitivity 66%, Specificity 98%
- Not stratified by AFB smear microscopy\(^2\)
  - Sensitivity 85%, Specificity 97%

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\(^1\) Greco S. Thorax 2006; 61(9): 783-90.

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NAAT and AFB Smear Results

• If NAAT and AFB smears are both positive:
  - Patient presumed to have TB and should begin treatment

• If NAAT is positive and AFB smear is negative:
  - If clinical suspicion for TB is intermediate to high:
    • Positive NAAT result can be used as presumptive evidence of TB (few false-positive results); start therapy
    • Negative NAAT cannot exclude pulmonary TB because false-negative results are common

• If NAAT is negative and AFB smears are positive:
  - NAAT false-negative results 4%, so negative NAAT with AFB+ smear makes TB disease unlikely
  - Patient may have NTM

Growth-based Drug Susceptibility Testing

• Determines which drugs kill TB bacilli
  - Tubercle bacilli killed by a particular drug are susceptible to that drug
  - Tubercle bacilli that grow in presence of a particular drug are resistant to that drug

• DST results obtained within 7 - 14 days for liquid medium method and up to 21 days for solid medium method

• Should be repeated if cultures still positive at 2 months despite therapy
Ms. J is a 49 yo F from Uzbekistan. She was noted to have an abnormal CXR overseas but her cultures were negative and she was allowed to immigrate. She reports to the local health department for B-1 follow up, and was noted to have an abnormal CXR, symptoms, and 3 positive AFB smears (3+, 2+ and 3+). She admits that she had TB treatment 10 years ago in Uzbekistan.

What do you want to know?

Molecular tests for Drug Susceptibility

• Drug resistance is caused by mutations in specific *M. tb* genes, can be detected by molecular assays
  - Xpert MTB/RIF assay: non-sequencing; detects MTBC and resistance to rifampin in <2h
  - Line-probe assays (LPAs): rapid identification of INH and RIF (MTBDRplus, Hain Lifesciences)
  - Sequencing-based assays: reports actual mutations (e.g., Sanger sequencing, pyrosequencing, CDC Molecular Detection of Drug Resistance [MDDR] service)
• Conventional DST should be done in conjunction with molecular tests
Recommendation #8: Perform **rapid molecular DST for rifampin with or without isoniazid** using the respiratory specimens of persons who are either AFB smear positive or Hologic Amplified MTD positive and who meet one of the following criteria:

1. have been treated for TB in the past,
2. were born in or have lived for at least 1 year in a foreign country with at least a moderate TB incidence ($\geq 20$ per 100,000) or a high primary MDR-TB prevalence ($\geq 2\%$),
3. are contacts of patients with MDR-TB, or
4. are HIV infected

*Strong recommendation, moderate-quality evidence*

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**DIAGNOSTIC APPROACH:**
**TESTING FOR SUSPECTED EXTRAPULMONARY TB**
Extrapulmonary TB

- Specimens other than sputum may be obtained
- Depends on part of body affected

<table>
<thead>
<tr>
<th>Site</th>
<th>Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>The first-voided midstream urine specimen is preferred, and if possible, the patient should not be receiving broad-spectrum antibiotics at the time of collection because the antibiotics may inhibit mycobacterial growth. Three specimens are recommended to demonstrate the presence of mycobacteria.</td>
</tr>
<tr>
<td>Blood and bone marrow</td>
<td>Blood or bone marrow for mycobacterial culture may be collected in a commercially available lytic centrifugation system. Alternatively, blood may be inoculated directly into a commercially available broth medium designed specifically for culture of mycobacteria from blood.</td>
</tr>
<tr>
<td>Cerebrospinal and other sterile body fluids</td>
<td>Sterile body fluid (cerebrospinal, pleural, peritoneal, and pericardial) findings typical of tuberculous disease are elevated protein concentration, lymphocytosis, and low glucose; however, this is not diagnostic. Culture must be performed. For diagnosis of tuberculous meningitis, a minimum of 5 ml of cerebrospinal fluid should be collected in a sterile container for AFB smear, although rarely positive, and mycobacterial culture. For cerebrospinal fluid, obtaining larger volumes may increase the diagnostic yield. Similarly, at least 5 ml of pleural, peritoneal, or pericardial fluid should be submitted for AFB smear and mycobacterial culture. Due to the generally low inoculum of organisms in extrapulmonary TB, the more fluid that is sent to the laboratory, the greater the likelihood of a positive culture.</td>
</tr>
<tr>
<td>Tissue biopsy</td>
<td>Tissue specimens for histological analysis and mycobacterial culture from the lung, pleura, pericardium, lymph nodes, bones and joints, brain, liver, bowel, sputum, epididymis, or other sites of disease should be considered when noninvasive techniques do not provide a diagnosis. The specimen should be divided, and very importantly, the portion sent for culture should not be placed in formalin or other fixatives.</td>
</tr>
</tbody>
</table>
Recommendation #13: If induced sputum is AFB smear negative or a respiratory sample cannot be obtained via induced sputum, use flexible bronchoscopic sampling in individuals with suspected miliary TB and no alternative lesions that are accessible for sampling.

*Conditional recommendation, very low-quality evidence*

- Sampling should include bronchial brushings and/or TBB, as the yield from washings is substantially less and the yield from BAL unknown
- Can get a rapid presumptive diagnosis by identifying histopathologic findings consistent with TB

Recommendation 16: AFB smear microscopy should be performed on specimens collected from sites of suspected extrapulmonary TB

*Conditional recommendation, very low-quality evidence*

Recommendation 17: Mycobacterial cultures should be performed on specimens collected from sites of suspected extrapulmonary TB

*Strong recommendation, low-quality evidence*
Recommendation 18: NAAT should be performed on specimens collected from sites of suspected extrapulmonary TB

*Conditional recommendation, very low-quality evidence*

- Positive test suggests TB, negative doesn’t exclude TB
- NAAT testing on specimens other than sputum is an off-label use of the test

Recommendation #19: Histological examination be performed on specimens collected from sites of suspected extrapulmonary TB

*Conditional recommendation, very low-quality evidence*

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**Rapid Molecular Tests for Drug Susceptibilities in other samples**

- Rapid molecular tests may be performed on some non-respiratory specimens in laboratories capable of validating the assay.
- PCR can be used on fixed (paraffin) specimens
  - Send to CDC Pathology Lab through State Lab and if positive they will send to CDC TB Lab for MDDR
- Specialized reference laboratories have the capacity to attempt molecular diagnosis through DNA extraction of formalin-fixed tissue (need for safety hood/precautions and effect formalin has on DNA)
- Not FDA approved
Recommendation #14: **Cell counts and chemistries** should be performed on amenable fluid specimens collected from sites of suspected extrapulmonary TB

*Conditional recommendation, very low-quality evidence*

- Pleural, cerebrospinal, ascitic, and joint fluids.
- TB often associated with high protein, high % lymphocytes and low glucose.

Recommendation #15a: **ADA levels** should be measured on fluid collected from patients with suspected pleural TB, TB meningitis, peritoneal TB, or pericardial TB

*Conditional recommendation, low-quality evidence*

Recommendation #15b: **Free IFN-γ levels** should be measured on fluid collected from patients with suspected pleural TB or peritoneal TB

*Conditional recommendation, low-quality evidence*
Recommendation #20. One culture isolate from each mycobacterial culture-positive patient should be submitted to a regional genotyping laboratory for genotyping

*Strong recommendation, very low-quality evidence*

**Benefits of Genotyping:**
- Detect (and interrupt) recent transmission
- Identify unsuspected relationships
- Enhance contact and outbreak investigations
  - Identify previously unidentified source cases, locations
- Distinguish TB disease from new infection vs. recurrence or relapse of previous TB
- Recognize false positives

1-800-4-TB-INFO!!
Resources

• Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children
  Official-American-Thoracic-Society-Infectious

• Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis

• Guidelines for the Diagnosis for LTBI in the 21st Century

• Guide to the Application of Genotyping to Tuberculosis Prevention and Control,


Resources

Fact sheets

• Testing for TB,

• A New Tool to Diagnose Tuberculosis: The Xpert MTB/RIF Assay,

• Recommendations for Human Immunodeficiency Virus (HIV) Screening in Tuberculosis (TB) Clinics,
  http://www.cdc.gov/tb/publications/factsheets/testing/hivscreening.htm

• Interferon-Gamma Release Assays (IGRAs) - Blood Tests for TB Infection,
  http://www.cdc.gov/tb/publications/factsheets/testing/igra.htm

• Tuberculin Skin Testing
  http://www.cdc.gov/tb/publications/factsheets/testing/skintesting.htm

• Diagnosis of Tuberculosis Disease
  http://www.cdc.gov/tb/publications/factsheets/testing/diagnosis.htm

• Targeted Tuberculosis Testing and Interpreting Tuberculin Skin Test Results,
  http://www.cdc.gov/tb/publications/factsheets/testing/skintestresults.htm