Making the Diagnosis of Tuberculosis

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Testing for TB Infection
Targeted Testing: Key Points

• Test only if plan for ensuring treatment

• De-emphasizes testing of groups that are not at high risk for TB
  – All high risk with positive TB test should be considered for treatment, regardless of age

• Detects persons with LTBI who would benefit from treatment

• Can help reduce the waste of resources and prevent inappropriate treatment
Targeted Tuberculin Testing
Criteria of High Risk Individuals

- Test persons or groups at high risk for recent latent TB infection
  - People recently exposed to person with infectious TB
  - People who have clinical conditions that increase risk of progressing from LTBI to TB disease (e.g., HIV infection)
  - Close contacts of persons with TB disease
  - Individuals who live or work in institutional settings (e.g., hospitals, prisons)
  - Recent immigrants (in US <5 years), from countries with high rates of TB
Testing for \textit{M. tuberculosis} Infection

**Mantoux tuberculin skin test (TST)**

Skin test that produces delayed-type hypersensitivity reaction in persons with \textit{M. tuberculosis} infection

**Interferon-gamma release assay (IGRA)**

Blood test that measures and compares amount of interferon-gamma (IFN-\(\gamma\)) released by blood cells in response to antigens
Mantoux Tuberculin Skin Test

• Preferred method of skin testing for *M. tb* infection

• TST is useful for
  – Determining how many people in a group are infected (e.g., contact investigation)
  – Examining persons who have symptoms of TB

• Multiple puncture tests (e.g., Tine & Heaf tests) are inaccurate and not recommended
Administering the TST

• Inject 0.1 ml of 5 TU PPD tuberculin solution intradermally on volar surface of lower arm using a 27-gauge needle

• Produce a wheal 6 to 10 mm in diameter
Reading the TST (1)

• Measure reaction in 48 to 72 hours

• Measure induration, not erythema

• Record reaction in millimeters, not “negative” or “positive”

• Ensure trained health care professional measures and interprets the TST
• Delayed hypersensitivity reactions to tuberculin usually begin 5-6h after injection, reach a maximum at 48-72h, and subside over a period of a few days, although positive reactions often persist for up to 1 wk*

*MMWR 2000. 49:RR-6
Criteria for TST Positivity by Risk Group

5 mm cut-point

- HIV-positive
- Recent contacts of infectious TB case
- Fibrotic changes on CXR c/w old prior TB
- Patients with organ transplants
- Other immunosuppressed patients ($\geq 15$ mg/day of prednisone for $\geq 1$ mo or TNF-alpha antagonists)
Criteria for TST Positivity by Risk Group

10 mm cut-point

- Recent arrivals (< 5 years) from high TB incidence countries
- Injection drug users
- Residents and employees of high-risk congregate settings: health-care facilities, prisons, shelters, etc.
- Mycobacteriology laboratory personnel
- Persons with “high-risk” clinical conditions
- Children < 4 years of age or infants and children exposed to adults at high-risk
Criteria for TST Positivity by Risk Group

15 mm cut-point *

- Persons with no risk factors for TB

* Although skin testing programs should be conducted only among high-risk groups, certain individuals may require testing for TB infection for employment or school attendance. Diagnosis and treatment of LTBI should always be tied to risk assessment.
### Incidence of TB Disease in Persons With Positive TST

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>TB cases/1,000 person-years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent TB infection</td>
<td></td>
</tr>
<tr>
<td>Infection &lt;1 yr past</td>
<td>12.9 (6)*</td>
</tr>
<tr>
<td>Infection 1–7 yr past</td>
<td>1.6</td>
</tr>
<tr>
<td>Human immunodeficiency virus (HIV) infection</td>
<td>35.0–162 (28)</td>
</tr>
<tr>
<td>Injection drug use</td>
<td></td>
</tr>
<tr>
<td>HIV seropositive</td>
<td>76.0 (31)</td>
</tr>
<tr>
<td>HIV seronegative or unknown</td>
<td>10.0 (31)</td>
</tr>
<tr>
<td>Silicosis</td>
<td>68 (36)</td>
</tr>
<tr>
<td>Radiographic findings consistent with prior TB</td>
<td>2.0–13.6 (32–34)</td>
</tr>
<tr>
<td>Weight deviation from standard</td>
<td></td>
</tr>
<tr>
<td>Underweight by ≥15%</td>
<td>2.6 (35)</td>
</tr>
<tr>
<td>Underweight by 10–14%</td>
<td>2.0</td>
</tr>
<tr>
<td>Underweight by 5–9%</td>
<td>2.2</td>
</tr>
<tr>
<td>Weight within 5% of standard</td>
<td>1.1</td>
</tr>
<tr>
<td>Overweight by ≥5%</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are reference numbers.
TST Shortcomings

• Patients must return to a health-care provider for test reading

• Reading the test is subject to inaccuracies and bias

• False-positive and false-negative results
Factors That May Cause False-Positive TST Reactions

- **Nontuberculous mycobacteria**
  - Reactions caused by nontuberculous mycobacteria are usually $\leq 10$ mm of induration

- **BCG vaccination**
  - Reactivity in BCG vaccine recipients generally wanes over time; positive TST result is likely due to TB infection if risk factors are present
Role of BCG

• BCG was developed to prevent the most serious forms of TB in young children

• Effectiveness in young children (50-80%)
  – Clinical history (contact) and risk factor for TB disease is more important than BCG history

• Disregard in adults
  – Sensitivity highly variable, effectiveness wanes with time
  – Risk factors most important in predicting TB disease

• TST not contraindicated with BCG
Factors That May Cause False-Negative TST Reactions (1)

• Anergy
  – Inability to react to a TST because of a weakened immune system
  – Usefulness of anergy testing in TST-negative persons who are HIV infected has not been demonstrated

• Recent TB infection
  – 2 to 10 weeks after exposure

• Very young age
  – Newborns
Factors That May Cause False-Negative TST Reactions (2)

• Live-virus vaccination close to TST administration
  – For example, measles or smallpox
  – Can temporarily suppress TST reactivity

• Overwhelming TB disease

• Poor TST administration technique
  – For example, TST injection too shallow or too deep, or wheal is too small
Some people with LTBI may have a negative skin test reaction when tested years after infection because of a waning response.

An initial skin test may stimulate (boost) the ability to react to tuberculin.

Positive reactions to subsequent tests may be misinterpreted as new infections rather than “boosted” reactions.
Two-Step Testing (1)

• A strategy to determine the difference between boosted reactions and reactions due to recent infection
  – If first TST is positive, consider the person infected
  – If first TST is negative, give second TST 1–3 weeks later
  – If second TST is positive, consider the person infected
  – If second TST is negative, consider the person uninfected at baseline

• Use two-step tests for initial baseline skin testing of adults who will be retested periodically (e.g., health care workers)
Two-Step Testing (2)

True Infection vs. Booster Effect

(mm induration)

<table>
<thead>
<tr>
<th>Situation</th>
<th>Time</th>
<th>0</th>
<th>1 week</th>
<th>1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>14</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>2</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>12</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>
Interferon-gamma release assay (IGRA)

• QuantiFERON®-Gold TB Test (QFT-G)
  – FDA approved May 2005

• QuantiFERON®-Gold In-tube (QFT-GIT)
  – FDA approved Oct 2007

• T-Spot®. TB
  – FDA approved July 2008
How Do IGRAs Work?

• Entails mixing blood samples with TB specific antigens from *M. tuberculosis* and controls and incubating for 16 to 24 hours

• Cells that recognize the antigen release interferon-γ

• Amount of interferon released in response to tuberculin is compared to amount released in response to other antigens
Interferon gamma (IFN-γ)

- Component of cell mediated immune response
- Antigen specific secretion
- Stable & measurable

### Species Specificity of ESAT-6 and CFP-10

<table>
<thead>
<tr>
<th>Tuberculosis complex</th>
<th>Antigens</th>
<th>Environmental strains</th>
<th>Antigens</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ESAT</td>
<td>CFP</td>
<td>ESAT</td>
</tr>
<tr>
<td><strong>M tuberculosis</strong></td>
<td>+</td>
<td>+</td>
<td>M abcessus</td>
</tr>
<tr>
<td><strong>M africanum</strong></td>
<td>+</td>
<td>+</td>
<td>M avium</td>
</tr>
<tr>
<td><strong>M bovis</strong></td>
<td>+</td>
<td>+</td>
<td>M branderi</td>
</tr>
<tr>
<td><strong>BCG substrain</strong></td>
<td></td>
<td></td>
<td><strong>ESAT</strong></td>
</tr>
<tr>
<td>gothenburg</td>
<td>-</td>
<td>-</td>
<td>M celatum</td>
</tr>
<tr>
<td>moreau</td>
<td>-</td>
<td>-</td>
<td>M chelonea</td>
</tr>
<tr>
<td>tice</td>
<td>-</td>
<td>-</td>
<td>M fortuitum</td>
</tr>
<tr>
<td>tokyo</td>
<td>-</td>
<td>-</td>
<td>M gordonii</td>
</tr>
<tr>
<td>danish</td>
<td>-</td>
<td>-</td>
<td>M intracellulare</td>
</tr>
<tr>
<td>glaxo</td>
<td>-</td>
<td>-</td>
<td>M kansasii</td>
</tr>
<tr>
<td>montreal</td>
<td>-</td>
<td>-</td>
<td>M malmoense</td>
</tr>
<tr>
<td>pasteur</td>
<td>-</td>
<td>-</td>
<td>M marinum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M oenavense</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M scrofulaceum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M smegmatis</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>M szulgai</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M terrae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M vaccae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M xenopi</td>
</tr>
</tbody>
</table>
QuantiFERON®-TB Gold (QFT-G)

Stage 1 Whole Blood Culture in Tissue Culture Plate

- Draw blood with heparin
- Make 1 ml aliquots & add antigen
- Incubate overnight.
- γ γ γ from sensitized lymphs

Stage 2: Measure [IFN-\(\gamma\)] & Interpret

- Harvest plasma from above settled cells
- Measure [IFN-\(\gamma\)] in ‘Sandwich’ ELISA
- Computerized interpretation
Stage 1: Whole Blood Culture in special blood collection tubes

- Collect 1mL of blood in 3 tubes
- Incubate at 37°C for 16-24 hours
- Centrifuge 5 minutes to separate plasma above gel

Stage 2: Measure [IFN-γ] & Interpret

- Collect 50 µL of plasma for ELISA
- Measure [IFN-γ] in ‘Sandwich’ ELISA

*Mtb = ESAT-6 + CFP-10 & TB 7.7
T-Spot. TB® (T-Spot)

• Collect blood in CPT tube
• Recover, wash, & count blood cells (peripheral blood mononuclear cells - PBMCs)
• Aliquot 250,000 PBMCs to 4 wells with anti-IFN-γ
• Add saline, + control (mitogen), ESAT-6 or CFP-10 & incubate
• Wash away cells
• Develop & count spots where cells produced IFN-γ

Saline Nil  ESAT-6  CFP-10  Positive Control
## Differences in Currently Available IGRAs

<table>
<thead>
<tr>
<th></th>
<th>QFT-G</th>
<th>QFT-GIT</th>
<th>T-Spot</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Format</strong></td>
<td>Stimulate whole blood by 12 hrs</td>
<td>Stimulate whole blood, 37ºC incubation by 16 hrs</td>
<td>Stimulate PBMCs by 8 hrs</td>
</tr>
<tr>
<td><strong>TB Antigen</strong></td>
<td>ESAT-6 &amp; CFP-10</td>
<td>ESAT-6, CFP-10, &amp; TB 7.7</td>
<td>ESAT-6 &amp; CFP-10</td>
</tr>
<tr>
<td></td>
<td>Separate wells</td>
<td>Incubation in single blood drawing tube</td>
<td>Separate wells</td>
</tr>
<tr>
<td><strong>Measurement</strong></td>
<td>Total IFN-γ</td>
<td>Total IFN-γ</td>
<td>Number of IFN-γ producing cells (spots)</td>
</tr>
<tr>
<td><strong>Possible results</strong></td>
<td>Positive, negative, indeterminate</td>
<td>Positive, negative, indeterminate</td>
<td>Positive, negative, borderline</td>
</tr>
</tbody>
</table>
Interpretation of the QuantiFERON®-Gold test (QFT-G)

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Nil*</th>
<th>TB Response†</th>
<th>Mitogen Response§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive††</td>
<td>Any</td>
<td>≥0.35 IU/ml and ≥50% of Nil</td>
<td>Any</td>
</tr>
<tr>
<td>Negative**</td>
<td>≤0.7</td>
<td>&lt;0.35 IU/ml</td>
<td>≥0.5</td>
</tr>
<tr>
<td>Indeterminate†††</td>
<td>≤0.7</td>
<td>&lt;0.35 IU/ml</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td></td>
<td>&gt;0.7</td>
<td>&lt;50% of Nil</td>
<td>Any</td>
</tr>
</tbody>
</table>

## Interpretation of the T-Spot.

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Nil*</th>
<th>TB Response†</th>
<th>Mitogen§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive†</td>
<td>≤10 spots</td>
<td>≥8 spots</td>
<td>Any</td>
</tr>
<tr>
<td>Borderline**</td>
<td>≤10 spots</td>
<td>5, 6, or 7 spots</td>
<td>Any</td>
</tr>
<tr>
<td>Negative‡‡</td>
<td>≤10 spots</td>
<td>≤4 spots</td>
<td>Any</td>
</tr>
<tr>
<td>Indeterminate**</td>
<td>&gt;10 spots</td>
<td>Any</td>
<td>Any</td>
</tr>
<tr>
<td></td>
<td>≤10 spots</td>
<td>&lt;5 spots</td>
<td>&lt;20 spots</td>
</tr>
</tbody>
</table>

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**Note:**

- **Nil**: No visible granulomas.
- **Positive**: Moderate to high levels of IFN-γ release.
- **Borderline**: Intermediate levels of IFN-γ release.
- **Negative**: Low levels of IFN-γ release.
- **Indeterminate**: Inadequate control.
Interpreting IGRA Results (1)

• **Negative:** Same interpretation as negative TST
  – No further TB evaluation unless indicated by clinical judgment

• **Positive:** Same interpretation as positive TST
  – Medical evaluation and chest x-ray are still needed to exclude TB disease and confirm LTBI
Interpreting IGRA Results (2)

- **Indeterminate:** Test failure
  - Repeat IGRA or administer TST as diagnostic aide for TB or LTBI. IGRA results may be indeterminate due to laboratory error or patient anergy. If two different specimens from a patient yield indeterminate results, do not repeat IGRA for that person.

- **Borderline:** (T-Spot only) Falls within borderline zone close to negative/positive cut point

*Like the TST, IGRA*s are a useful but imperfect diagnostic aide. It should not replace clinical judgment.*
Advantages of IGRA

IGRA vs. TST

**in vitro**, controlled lab test with minimal inter-reader variability
- TB specific antigens used
- No boosting; 2 step testing not needed
- 1 patient visit
- Unaffected by BCG or most environmental mycobacteria
- Simple yes/no result
- Exposure characteristics associated with increased risk of infection correlate better with IGRAs, especially among BCG vaccinated contacts

**in vivo**, subject to errors during administration and interpretation
- Less specific PPD used
- Boosting, with repeated testing
- 2 patient visits
- False-positive test common after BCG and environmental mycobacteria exposure
- Interpretation based on patient’s relative risk for TB exposure or development of disease
Limitations of IGRAs

- Limited data in many groups, such as those with impaired immune function and children (<5 yrs of age)
- Increase in indeterminate results shown in studies
- The ability of IGRAs to predict risk of LTBI progression to TB disease has not been determined. The risk may be different than in those with a positive TST
Sensitivity

• No gold standard for latent TB infection

• TB disease used as a surrogate
  – Problematic

• Overall, tests are comparable
  – Trends for increased sensitivity with T-Spot, but limited head-to-head comparison
Specificity

• No gold standard
• Measured in persons with low or no identifiable risk for *M. tuberculosis* infection
• Variation in population from study to study
• Trend toward increased specificity with QFT-GIT, but head-to-head comparison data lacking
• Limited published specificity data on T-Spot in general
Current Guidelines for Use of IGRAs

• Populations/situations in which IGRAs are preferred
  – Testing persons who have received BCG vaccination (vaccines or cancer therapy)
  – Testing persons unlikely to return for TST reading

• Population/situation in which TST is preferred
  – Testing children younger than 5 years old

• Populations/situations in which there is no preference between TST or IGRA
  – Testing of contacts and periodic screening for occupational exposure

MMWR 2010; Vol. 59(No RR-15)
What do you do about a positive test result?

• Persons with positive TST or IGRA should receive the following to rule out TB disease
  – Physical exam, including symptom assessment
  – Chest x-ray

• If chest x-ray is normal, treatment for LTBI should be offered

• If chest x-ray is abnormal, patient should be evaluated for TB disease
  – Sputum smear exam and culture
**LTBI vs. Pulmonary TB Disease**

**Latent TB Infection**
- TST or IGRA positive
- Negative chest radiograph
- No symptoms or physical findings suggestive of TB disease

**Pulmonary TB Disease**
- TST or IGRA usually positive
- Chest radiograph may be abnormal
- Symptoms *may* include one or more of the following: fever, cough, night sweats, weight loss, fatigue, hemoptysis, decreased appetite
- Respiratory specimens *may* be smear or culture positive
Diagnosis of TB Disease

See lectures on Chest Radiographs & Laboratory Techniques
Diagnostic Evaluation

• Medical history
• Physical examination
• Testing for TB (previously covered)
• Radiologic examination (*X-Ray or CT scan of chest*)
• Bacteriologic examination
Medical History

• Symptoms of disease and duration

• History of TB exposure, infection or disease

• Previous treatment for TB (completion status)

• Demographic risk factors for TB (age, country of origin, etc.)

• Medical risk factors for TB (HIV infection, diabetes, etc.)
Symptoms of Pulmonary TB

- Cough: productive, prolonged
- Chest pain
- Hemoptysis
- Systemic symptoms:
  - Fever, chills, night sweats, loss of appetite, weight loss

Specific symptoms of extrapulmonary TB depend on site of disease.
Clinical Features of Pulmonary TB

- 188 adults with pulmonary TB, Los Angeles, prospective evaluation

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>78</td>
</tr>
<tr>
<td>Weight loss</td>
<td>74</td>
</tr>
<tr>
<td>Fatigue</td>
<td>68</td>
</tr>
<tr>
<td>Fever</td>
<td>60</td>
</tr>
<tr>
<td>Night sweats</td>
<td>55</td>
</tr>
<tr>
<td>Chills</td>
<td>51</td>
</tr>
<tr>
<td>Anorexia</td>
<td>46</td>
</tr>
<tr>
<td>Chest pain</td>
<td>40</td>
</tr>
<tr>
<td>SOB</td>
<td>37</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>28</td>
</tr>
</tbody>
</table>

When to Suspect TB Disease

- **Cough illness ≥2-3 weeks +**
  - Fever, night sweats, weight loss, and/or hemoptysis

- **High risk for TB, unexplained illness, including respiratory symptoms of ≥ 2-3 weeks duration**
  - Recent exposure, known (+) TST, HIV, drug use, immigrant ≤5 years from high-risk region, high-risk congregate setting, homeless, immunosuppressed, advanced CKD, silicosis, others

- **HIV (+), unexplained cough, fever**

- **High risk and unresponsive CAP after 7 days**

- **High risk and worrisome CXR**

*MMWR 54:1 (2005)*
Physical Examination

• An essential part of the evaluation of any patient
• Cannot be used to confirm or rule out TB
• Can affect how TB is treated
Diagnosis of TB Disease

• Consideration of these factors leads to high, medium, or low “index of suspicion”
  – Epidemiologic information
  – Clinical, pathological, chest x-ray findings

• Know global and local TB epidemiology
  – Demographics of cases
  – Locations where potential transmission has occurred
  – Endogenous and exogenous risk factors
Radiologic Examination

• PA & lateral views
  – Other views or additional studies may be necessary (e.g., apical lordotic or oblique views, CT/PET scans)

• Abnormalities often seen in apical or posterior segments of upper lobe or superior segments of lower lobe

• May have unusual appearance in HIV-positive

• Abnormalities on chest x-rays may be suggestive of, but cannot confirm, a diagnosis of TB
Bacteriologic Examination

- Sputum specimens (= 3 specimens obtained 8-24 hrs apart, one being an early morning specimen) for AFB microscopy and culture

- Shift from previous guideline of 3 morning specimens
## Bacteriology

<table>
<thead>
<tr>
<th>Specimen</th>
<th>AFB Smear</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum day 1</td>
<td>AFB smear positive 2+</td>
<td>Pending</td>
</tr>
<tr>
<td>Sputum day 2</td>
<td>AFB smear positive 3+</td>
<td>Pending</td>
</tr>
</tbody>
</table>

24 hours | 1–3 wks | 3 – 8 wks

ZN X 1,125 X 1440
Is there another test you can order to help you make a diagnosis sooner?

Specimen collection and processing

- AFB Smear
- Liquid Culture (Liquid medium, automated system)
- Agar Plate M H 711

NAAT: Nucleic acid amplification test
*Mycobacterium tuberculosis* Direct Test (MTD) or Amplicor
Nucleic Acid Amplification (NAA) Tests

- Direct, rapid, detection of *Mycobacterium tuberculosis* complex (rRNA)
  - Patients suspected of TB
  - Takes about 4 to 5 hours
  - Approved for respiratory specimens only

- Smear positive and smear negative
  - Non-respiratory specimen (validated by labs)
  - Can detect fewer than 10 organisms
  - Does not distinguish live vs dead organism

MMWR July 7, 2000
## CDC Guidelines - 2009

### Nucleic Acid Amplification Test

- Collect specimen for AFB, culture, & NAA
- Interpret results with AFB smear

<table>
<thead>
<tr>
<th>NAA</th>
<th>AFB</th>
<th>Recommend</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td><strong>Start treatment. PPV &gt;95% NAA in AFB+ cases</strong></td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td><strong>Repeat NAA test. Presume TB if ≥2 NAA (+)</strong></td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td><strong>Repeat NAA. Test for inhibitors, if none detected. Presume nontuberculous mycobacteria (NTM)</strong></td>
</tr>
</tbody>
</table>
| −   | −   | • **Use clinical judgment**  
• NAA sensitivity 50-80% in detection AFB (-)  
• Culture (+) pulmonary TB |

MMWR 2009
Other Techniques

• May need bronchosscopic lavage & biopsy

• Video-assisted thorascopic surgery (VATS)

• Open procedure
• A decision to administer a TST or IGRA is a decision to treat latent TB infection

• Diagnosis of TB disease is a cumulative pathway that includes:
  – Clinical picture
  – Epidemiological factors
  – Radiologic findings
  – Smear microscopy, + NAAT
  – Culture