Objectives

• At the completion of this TB webinar, participants will:
  – Be familiar with the tests to diagnose latent tuberculosis and active tuberculosis
  – Recognize the tests available to detect Mycobacterium tuberculosis in clinical specimens
  – Understand the value of molecular tests to detect TB

History of TB Diagnostics

• Robert Koch announced in 1882 that he had found a microbe, Mycobacterium tuberculosis, that was the cause of "White Death", a disease responsible for one-seventh of all deaths in Europe in the late part of the 1800's.
**Timeline of TB Infection**

- **Exposure**
- **4-6 wks**
- **Adaptive T cell response**
- **Latent TB (LTBI)**
- **Active TB**

*Prevention efforts focus on detecting LTBI. Most LTBI do not advance to active disease but those patients are at high risk particularly if they become immunocompromised.

---

**TB Infection vs. TB Disease**

<table>
<thead>
<tr>
<th>TB In the body</th>
<th>TB In the body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest X-ray normal</td>
<td>Chest X-ray abnormal</td>
</tr>
<tr>
<td>Sputum not done</td>
<td>Sputum smear and culture positive</td>
</tr>
<tr>
<td>No symptoms</td>
<td>Symptoms: cough, fever, weight loss</td>
</tr>
<tr>
<td>Not infectious</td>
<td>Infectious</td>
</tr>
<tr>
<td>Not a case of TB</td>
<td>Case of TB</td>
</tr>
</tbody>
</table>

---

**TB Algorithm**

- Collect sputum specimens at 3 different times and 8 hours apart (at least one must be a first morning specimen) for AFB smear and mycobacterial culture.
- Perform MTD or NAAT test on the first smear positive sputum specimen
Diagnosis of TB

- Clinical picture
  - History and symptoms
- Chest XRay
- Antigen Test
  - Skin test (TST)
  - Blood Test (IGRA)
- AFB (Acid Fast Bacilli) microscopy of sputum
- NAAT Testing
- Culture (up to 6 weeks)
  - Solid medium
  - Liquid (MGIT)
- Nucleic Acid Amplification Testing (NAAT)
  - Molecular probes
  - PCR
- Sensitivity Testing
- Genotyping

Requirements to get a high quality specimen to the laboratory

- Collect specimens before therapy started
- Even after a few days of therapy, AFB may be killed or numbers decreased to longer be detectable
- Specimens must be handled properly to guarantee successful cultures
- Promptly transport specimens

Specimen Type: Varies with symptoms

- Pulmonary
  - Sputum (spontaneous, induced)
  - Bronchoalveolar Lavage
- Gastric Lavage (children)
- Tissue and Body fluids (CSF, pleural, blood)
- Wounds, skin lesions (exudates)
Specimen Collection and Processing: special considerations

• Biohazard
  – Aerosol transmission
• Prevent contamination of specimen
  – Slow growth rate of TB
• Evaluate at least 3 specimens per patient

Sputum collection considerations

• Instruct patients that nasopharyngeal discharge and saliva are not sputum
• Sputum = thick, yellowish (sometimes blood-tinged) exudative material brought up from the lungs after a deep, productive cough
• First rinse mouth with mouthwash to decrease bacterial contamination
• Collect specimen into appropriate container

Sputum cont...

• About 10 ml of sputum is sufficient
• If patient cannot provide an adequate specimen then sputum induction is acceptable
  – Warm, aerosolized hypertonic salt solution
  – Be certain to label the specimen as “induced sputum”
Specimen Transport

• From the time of collection until the specimen is processed, the other bacteria present will over grow (contaminate) the slower growing *Mycobacteria* sp.
  – Speed is important
    • Courier
    • Ship cold when possible
    • Shipping cold slows bacterial growth

Specimen Processing

• If collected from a non-sterile site (sputum), then digest and decontaminate before culture
  – Kill off other microbes
  – Liquefy mucin
  – Remove organic debris
  – Homogenize tissue
• N-acetyl-L-cysteine (NALC)-NaOH method
• Concentrate specimen

Summary of Standard Diagnostic Techniques

• Direct from specimen
  – AFB Smear – cold kinyoun and fluorescent
  – Culture in broth and on solid media
  – Direct detection by NAAT
• From growth of organism
  – Probe (accuprobe)
  – Biochemicals
  – 16S ribosomal RNA
  – Sensitivity Testing
**TB Specimen Processing**

- Specimen
- Culture
- Smear Positive
  - Probe
  - Biochemical
  - Sensitivity
  - Genotyping
  - NAAT

**Laboratory Tests: Non-specific**

- AFB smear
  - Semi-quantitative as a measure of patient infectiousness
- Culture
  - Liquid and solid media (up to 6 weeks)
  - Automated commercial systems widely used
  - Semi-quantitative

**Diagnosis of TB: AFB Smear Microscopy**

- Make a “smear” on a slide
- Stain for acid-fast bacteria
  - Cold Kinyoun
  - Ziehl Neelsen
  - Fluorochrome (Auramine-Rhodamine)
Diagnosis of TB: AFB Smear Microscopy

- **Strengths**
  - Easy, fast, cheap (ZN)

- **Weakness**
  - 50-60% of TB patients are smear negative
    - Need at least 10,000 CFU/ml sputum for positive result
  - Cannot differentiate *Mycobacteria* species

Importance of acid-fast bacilli smear microscopy as a primary diagnostic tool

- Initial diagnosis
- Monitoring treatment
- Determination of time to release from isolation

How sensitive is the smear?

- Peterson et. al. JCM 1999 vol. 37:3564-68.

<table>
<thead>
<tr>
<th>Number of specimens</th>
<th>Direct AFB smear sensitivity</th>
<th>Concentrated AFB smear sensitivity</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>353 culture positive for Mycobacteria</td>
<td>34%</td>
<td>58%</td>
<td>Direct smear cannot be relied on</td>
</tr>
<tr>
<td>208 cultures positive for M. tuberculosis</td>
<td>42%</td>
<td>74%</td>
<td>Concentrated smear most reliable</td>
</tr>
<tr>
<td>Analysis of 3 specimens per patient</td>
<td>81%</td>
<td>91%</td>
<td>Concentrated smear is still the most reliable</td>
</tr>
</tbody>
</table>
Direct detection of TB in the specimen
- MTD test – Genprobe – transcription mediated amplification
- In house developed Nucleic Acid Amplification test (NAAT)
- GeneXpert Cepheid NAAT

Interpretation

<table>
<thead>
<tr>
<th>Smear</th>
<th>NAAT</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>Presumed Positive TB, No Additional Testing</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>If first sputum specimen: smear positive and NAAT-negative, repeat on one additional specimens, if negative then presume negative for TB.</td>
</tr>
<tr>
<td>--</td>
<td>+</td>
<td>Additional specimens (limit 2). Presumptive positive for TB if the subsequent specimen positive</td>
</tr>
<tr>
<td>--</td>
<td>-</td>
<td>Presumptive negative for TB. Two specimens recommended.</td>
</tr>
</tbody>
</table>

TB NAAT Algorithm Respiratory Specimen
Diagnosis of TB: Culture

• Solid Media Culture
  – Agar Middlebrook 7H10/7H11
  – Egg based Lowenstein-Jensen
• Liquid – Broth Culture
  – 7H9
  – Commercial broth and monitoring systems
    • Becton Dickinson MGIT
    • ThermoScientific, TREK Diagnostic Systems, Versa TREK Myco
• Use a solid and a liquid media

Laboratory Tests: Specific

• Biochemical tests
  – Require sub-culture
  – Ex. Niacin, Nitrate, Tween 80 Hydrolysis, 68 Catalase
• High performance liquid chromatography (HPLC) of cell wall mycolic acids
• Molecular probes
  – Culture confirmation
  – Direct from growth in broth or on slant
• DNA sequence analysis
  – 16S rRNA gene

Molecular Probes for Mycobacteria identification

• MYCOBACTERIUM TUBERCULOSIS Complex Culture Identification Test – For identification of M. tuberculosis, M. bovis, M. bovis (BCG), M. africanum, M. canetti, M. microti etc. isolated from culture.
• MYCOBACTERIUM AVIUM Culture Identification Test - For the identification of Mycobacterium avium isolated from culture.
• MYCOBACTERIUM INTRACELLULARE Culture Identification Test - For the identification of Mycobacterium intracellulare isolated from culture.
• MYCOBACTERIUM AVIUM Complex Culture Identification Test - For the identification of Mycobacterium avium complex (M. avium, M. intracellulare, and other members) isolated from culture.
• MYCOBACTERIUM GORDONAE Culture Identification Test - For the identification of Mycobacterium gordonae isolated from culture.
• MYCOBACTERIUM KANSASII Culture Identification Test - For the identification of Mycobacterium kansasii isolated from culture.
### Molecular Probe Performance Characteristics

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. avium</td>
<td>99.3%</td>
<td>100%</td>
</tr>
<tr>
<td>M. intracellulare</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>M. avium complex</td>
<td>99.0%</td>
<td>100%</td>
</tr>
<tr>
<td>M. gordonae</td>
<td>98.8%</td>
<td>99.7%</td>
</tr>
<tr>
<td>M. kansas</td>
<td>93.4%</td>
<td>100%</td>
</tr>
<tr>
<td>M. tuberculosis complex</td>
<td>99.2%</td>
<td>99.0%</td>
</tr>
</tbody>
</table>

Biochemical tests for *M. tuberculosis* complex
- 8 species make up the complex
  - Mycobacterium tuberculosis
  - Mycobacterium africanum
  - Mycobacterium bovis
  - Mycobacterium bovis (BCG)
  - Mycobacterium microti
  - Mycobacterium canettii
  - Mycobacterium pinnipedii
  - Mycobacterium mungi
- Differentiate by biochemical testing
  - Niacin
  - Nitrate
  - Others

Antimicrobial susceptibility testing
- Required for all MTB complex patients
  - Absolute concentration
  - Resistance ratio
  - Proportion
- Recommended for some NTM species
Drug susceptibility testing of *M. tuberculosis*

- Culture based DST remains the gold std
  - Reliable for INH & Rif, inconsistent for Ethambutol resistance
- Genotypic methods
  - Sequencing
  - Line probe hybridization assays (commercial)
  - Molecular beacons (GeneXpert)
  - Loop mediated isothermal amplification

Genotyping

- MMWR Controlling TB in the US Nov. 2005
- Refers to procedures to identify *M. tuberculosis* isolates that are identical in specific parts of the genome
- Along with epi investigation, genotype used to confirm transmission
CDC program for genotyping *M. tuberculosis* isolates

- DNA Fingerprinting – Restriction Fragment Length Polymorphism (RFLP)

![Graph showing DNA fingerprinting results.](image)

http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5412a1.htm

**TABLE 2. Essential laboratory tests for tuberculosis control.**

<table>
<thead>
<tr>
<th>Test</th>
<th>Maximum turnaround time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy for acid-fast bacilli</td>
<td>≤24 hours from specimen collection or, if test is performed offsite, ≤24 hours from receipt in laboratory; if latter, time from specimen collection to laboratory receipt should be ≤24 hours</td>
</tr>
<tr>
<td>Nucleic acid amplification assay</td>
<td>≤48 hours from date of specimen collection</td>
</tr>
<tr>
<td>Mycobacterial growth detection by culture</td>
<td>≤14 days from date of specimen collection</td>
</tr>
<tr>
<td>Identification of cultured mycobacteria</td>
<td>≤21 days from date of specimen collection</td>
</tr>
<tr>
<td>Drug susceptibility testing</td>
<td>≤30 days from date of specimen collection</td>
</tr>
<tr>
<td>Drug susceptibility testing of second-line drugs</td>
<td>≤4 weeks from date of request</td>
</tr>
</tbody>
</table>

http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5412a1.htm

**What are the expected TAT?**

<table>
<thead>
<tr>
<th>Test</th>
<th>Standard</th>
<th>SHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB smear</td>
<td>&lt;24 h</td>
<td>7 h</td>
</tr>
<tr>
<td>NAAT</td>
<td>&lt;48 h</td>
<td>&lt;24 h</td>
</tr>
<tr>
<td>Growth in culture</td>
<td>&lt;14 d</td>
<td>NA</td>
</tr>
<tr>
<td>ID of culture</td>
<td>&lt;21 d</td>
<td>14 d</td>
</tr>
<tr>
<td>Sensitivity Testing</td>
<td>&lt;30 d</td>
<td>21 d</td>
</tr>
</tbody>
</table>
Tuberculin Skin Test (TST)

- In routine use since 1910
- TST is the most used test for \textit{M. tuberculosis} infection in U.S.
- Delayed type hypersensitivity reaction to PPD, a polyvalent mycobacterial antigen mixture

TST Pro’s Con’s

**Advantages**
- Inexpensive
- Good performance
- No special equipment
- Long history of experience

**Limitations**
- Reader variability
- “Boost” response
- Low specificity
  - Cross reaction with BCG and NTM
- Low sensitivity
- Need for 2 visits

Interferon Gamma Release Assays:

**Principle:**
- Persons exposed to \textit{M. tuberculosis} develop T-cells (lymphocytes that recognize and respond to TB-specific antigens

\[ \text{Dendritic cell processes antigen and presents antigen to T Cell} \]
IGRA (continued)

• When stimulated with TB-specific antigens, these primed T-cells release the cytokine, interferon-gamma (IFN-γ).

![Diagram of T cell releasing IFN-γ](Image)

Antigens: ESAT 6 & CFP 10

T cell releases IFN-γ

IGRA (continued)

• The released IFN-γ can then be detected and serves as an indirect indicator of exposure to TB.

T-SPOT
FDA approved August 2008

http://www.oxfordimmunotec.com/na/healthcare/howitworks.html
QuantiFERON TB- Gold In-Tube

• Blood test that measures and compares amount of interferon-gamma (IFN-γ) released by blood cells in response to antigens
• FDA approved in May 2005 – Cellestis, Carnegie, Australia

QFT Procedure – Clinic and Lab

• Procedures in Clinic
  – Blood Collection
  – Shaking of Tubes
  – Blood Incubation
  – Plasma Separation

• Procedures in Lab
  – ELISA
  – Data Analysis
Data Analysis and Results

- Results are reported as:
  - Positive
  - Negative
  - Indeterminate
- Indeterminate
  - Low mitogen
  - CMI response
  - High Nil
  - live vaccines
  - secondary infection

Technology Comparison

<table>
<thead>
<tr>
<th></th>
<th>T-SPOT</th>
<th>QFTB In-Tube</th>
<th>TST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigens</td>
<td>ESAT-6 &amp; CFP10</td>
<td>ESAT-6, CFP10, TB7.7</td>
<td>PPD</td>
</tr>
<tr>
<td>Boosting effect with repeat tests</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>TAT</td>
<td>16-20 h</td>
<td>16-24 h</td>
<td>48-72 h</td>
</tr>
<tr>
<td>Readout units</td>
<td>IFN-Gamma spot forming cells</td>
<td>International units of IFN-Gamma</td>
<td>Millimeters of induration</td>
</tr>
<tr>
<td>Technology</td>
<td>ELISpot</td>
<td>ELISA</td>
<td>NA</td>
</tr>
<tr>
<td>Readout system</td>
<td>Count of spots</td>
<td>Measurement of optical density values using an automated reader</td>
<td>Palpable induration</td>
</tr>
<tr>
<td>Subjective Reading</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
CDC advises that IGRA's can be used in all circumstances in which the TST is used, including…

- Contact investigations
- Evaluation of recent immigrants who have had BCG vaccination
- TB screening of health care workers and other individuals in high risk settings
- IGRA is in place of (not an addition to) TST

General Benefits of IGRA over TST

- Requires only one patient visit
- Assesses responsiveness to *M. tuberculosis* antigens
- Does not boost previous responses
- Interpretation less subjective than for TST
Limitations of IGRAs

- Cross-reactivity is possible with some atypical Mycobacteria infections:
  - *M. kansasii*, *M. szulgai*, and *M. marinum*
- Testing logistics:
  - Specimen transport time
  - Requirement for specialized testing equipment
- Additional data needed in certain patient populations
  - Children, Immunocompromised, Pregnancy

BCG Vaccinated Patients

- IGRA benefit the BCG vaccinated patient
- Many false positive TST due to vaccination status
- Treatment is costly, carries risk of significant side effects
- Treatment is not always needed since most do not have LTBI

Performance of IGRAs and the TST:

*An up-to-date TB Test Meta-Analysis*

R Diel, R Loddenkemper and A Nienhaus
Evidence based comparison of commercial interferon-gamma release assays for detecting active tuberculosis – a meta-analysis.

*Chest*, 2009, Published on Dec 18, 2009 in electronic format;

*Chest* April 2010 137:952-968; doi:10.1378/chest.09-2350
Contact Investigations

- For persons with recent TB exposure, negative IGRA results should be confirmed with a repeat test 8-10 weeks after exposure (end of window period) per CDC. This is the same as for a negative TST.
  - “3 months interval from the diagnosis of the index case will be enough for the final decision of the infection of contacts.”
  - N=25, 8 positive QFTB-G

For high risk contacts...

- When “window prophylaxis” has been started for high-risk contacts exposed to an infectious TB patient, a negative IGRA result at the end of the window period should be interpreted in light of all other clinical and epi data
  - A full course of LTBI TX should be considered even with a negative result when the rate of TB transmission to other contacts is high or when a false-negative is suspected because of immune status.

Use of IGRA
Baseline and Serial Testing

- Baseline testing with IGRA
  - Establish baseline with single negative IGRA
  - HCWs with positive IGRA result should be referred for diagnostic evaluation
- Serial testing for infection control
  - A conversion is a change from negative to positive
Cost Barrier?

– Cost-effective alternative to TST
  • Reduction in false positive test results
  • No second visit needed to complete testing
  • Two-step testing not needed
  • Reduction in rates of CXR (due to higher specificity for *M. tuberculosis*)

Are IGRAs cost effective?

• DePerio et al: Arch Intern Med. 2009
  – Use of IGRA “leads to superior clinical outcomes and lower costs than the TST and should be considered in screening non-BCG-vaccinated and BCG vaccinated new HCWs for LTBI.”
  – “Selected use of QFT-G appears to be cost effective if used in targeted fashion.”

IGRA Summary

• IGRAs are more specific than TST and are not confounded by previous BCG vaccination
  – Less unnecessary preventive treatment
• IGRAs are more sensitive than TST
TB antibody tests

- Tests that detect IgG antibody to TB
- Highly variable results for sensitivity and specificity
- Do not have a roll in the diagnosis of TB
- Not FDA approved
- Recently confused with IGRA in the news.

Take Home Message

- Culture of TB remains the gold standard
- AFB smears are the most cost effective
- NAAT are sensitive and rapid but cannot differentiate between dead and viable TB
- IGRA do not differentiate between active and latent TB
- There are new tests on the horizon

Let’s not forget…
• Requirements to get a high quality specimen to the laboratory

• Standard diagnostic techniques for the detection and identification of Mycobacteria

• Improvement of acid-fast bacilli smear microscopy as a primary diagnostic tool

• Drug susceptibility testing of M. tuberculosis

• Rapid methods for the identification and drug susceptibility testing of M. tuberculosis as they compare to the traditional methods

• CDC program for genotyping M. tuberculosis isolates

• Interferon Gamma Assays (IGRA) – Pros and Cons