MODULE FOUR

TB Diagnostics

Treatment Action Group

TB/HIV Advocacy Toolkit
Topics to be covered

• Basic TB diagnostic concepts and vocabulary.
• Commonly used TB diagnostic tools.
• New TB diagnostic tools, and what more is needed.
• Advocacy priorities to improve access to current diagnostics and potential to develop the new TB diagnostics.
Section 1: Basic TB diagnostic concepts and vocabulary
## Commonly used diagnostics

<table>
<thead>
<tr>
<th>Tool</th>
<th>What condition does it detect?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermometer</td>
<td>Fever</td>
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<tr>
<td>Pap smear</td>
<td>Abnormal development or growth of cells</td>
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<tr>
<td>X-ray</td>
<td>Broken bones</td>
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<tr>
<td>ELISA</td>
<td>HIV antibodies</td>
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<tr>
<td>Checking for yellowing of the eyes</td>
<td>Jaundice</td>
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<tr>
<td>HIV viral load</td>
<td>Amount of HIV RNA per milliliter of blood</td>
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</table>
What is a diagnostic test?

• A medical diagnostic test is any method or tool used to measure, identify, or analyze conditions of the body. Diagnostic tests detect physiological or biochemical changes in our bodies that indicate an unhealthy state or look for the source of disease or ill health.

• TB diagnostics are used to identify latent TB infection or active TB disease, as well as to provide information about drug resistance.
TB diagnostic vocabulary

1. Sensitivity
   a. Is caused by low sensitivity. People with the disease are wrongly identified as not having the disease. These wrongly classified people will not be treated for their condition and might be at risk for transmitting the infection to others and for becoming sick and dying.

2. Specificity
   b. Is able to diagnose resistant strains of TB.

3. False Positive
   c. The process of growing TB bacteria on a substance that provides nutrients.
   d. Measures the proportion of people without a disease that are correctly identified by a diagnostic tool as not having the condition.
   e. Measures the proportion of people with a disease that are correctly identified by a diagnostic tool as having the condition.

4. False Negative
   f. Is caused by low specificity. People without a condition may be wrongly diagnosed as having it. These people run the risk of being wrongly treated for a condition they do not have.

5. Media
   g. Substances (solid or liquid) containing nutrients on which TB bacteria are grown in a laboratory.

6. Culture

7. DST
Section 2: Commonly used TB diagnostic tools
Tests commonly used to confirm TB disease

- Sputum smear microscopy
- Culture test
Sputum Smear Microscopy

**Procedure**
1. Collect sputum
2. Smear small volume of sputum on glass slide
3. Stain with acid fast stain (Ziehl-Neelsen)
4. Flood slide with methylene blue counterstain
5. Decolorize with acid-alcohol solution
6. Scan entire sample under 40x magnification.
7. Confirm the presence or absence of MTB

**Advantages:**
+ Conclusive results may be obtained the same day.

**Disadvantages**
- Very low sensitivity (50%) among all TB cases.
- Only useful for pulmonary TB.
- Difficult to obtain sample from children.
- Up to 61% of HIV-positive individuals have sputum smear-negative TB, so test incorrectly diagnoses as not having TB.
Bacteria Culture on Solid Media

Bacteria culture is the growth of bacteria in vitro (or in a controlled environment outside of the body).

**Procedure**

1. Collect sputum or tissue sample
2. Sputum is processed to make it more liquid (usually by NaOH solution) to reduce the potential of contamination and non-interpretable results
3. Smear sputum on culture plates
4. Incubate at body temperature (37°C) in sterile incubator
   1. Incubation time can vary depending on presence of CFUs.
   2. A negative result is usually confirmed after 42 days
5. Visually confirm MTB CFUs based on cell shape and color.
6. DST can be done by adding TB drugs into the media and looking for growth

**Advantages**
+ high sensitivity
+ high specificity

**Disadvantages**
- 3 to 6 weeks for results
- DST takes nearly 2 months
- Requires laboratories with advanced equipment and trained staff
Drug susceptibility testing (DST)

• DST is done using solid or liquid culture
• DST can provide information about:
  – Which TB drugs the bacteria is susceptible to
  – The appropriate treatment regimens
  – Whether a person has multidrug-resistant TB (MDR-TB) or extensively drug-resistant TB (XDR-TB)
Other commonly used TB diagnostic tests

- Symptom screen
- Tuberculin Skin Test (TST), also referred to the Mantoux or purified protein derivative (PPD)
- Chest X-ray
Clinical symptoms

- Current cough
- Fever and night sweats
- Chest pain
- Weight loss
- Hemoptysis (blood-stained sputum)
- If extrapulmonary TB, symptoms can vary based on site of infection

**Advantages**
+ Often triggers health-seeking behavior and providers start the diagnostic process.

**Disadvantages**
- Not conclusive as many infections might have similar symptoms.
- Can be missed by many until disease is quite developed.
- More challenging for extrapulmonary cases as symptoms vary depending on site of disease.
Tuberculin Skin Test (TST)

- Tuberculin Skin Test (TST), also referred to as the Mantoux or purified protein derivative (PPD) test, detects TB exposure through the skin.

**Advantages**
+ Simple and easy to administer.
+ Can detect TB infection and disease

**Disadvantages**
- The test needs to be refrigerated
- Because the test it is injected, administration requires staff training.
- False negatives: the failure of a bump to develop can be the result of a compromised immune system.
- False positives: the bacterial particles in the BCG vaccine can stimulate the production of TB antibodies, causing a false positive.
- Sensitivity varies widely across populations and is inconclusive for children, HIV patients with low CD4 counts (especially under 200), and BCG-vaccinated individuals.
- The test cannot distinguish infection from disease, and needs to be followed by other conclusive tests to ensure appropriate treatment.
Chest X-ray

**Advantages**
- Fast
- Can rule out active infection after a positive TST

**Disadvantages**
- Misses latent TB infection
- Only used to detect pulmonary TB
- HIV-positive patients with active TB commonly have abnormal x-rays not consistent with TB

Abnormal Chest X-Ray
Decision Algorithms for TB Diagnosis

Decision algorithms help guide clinicians in determining the most accurate and time-saving method of diagnosis.

Algorithms have been designed to help medical providers identify smear-negative and extrapulmonary TB in HIV prevalence settings.
Section 3: New TB diagnostic tools, and what more is needed
So, what would you want in a TB diagnostic?

Any ideas??
## Minimum specifications for a point-of-care TB diagnostic test

<table>
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<tr>
<th>Minimum Specifications Required</th>
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### Medical Decision

**Treatment Initiation**

### Sensitivity—adults (regardless of HIV status)

- **Pulmonary TB:**
  - Smear-positive, culture-positive: 95%
  - Smear-negative, culture-positive: 60–80%*

  (Detection of extrapulmonary TB being a preferred but not a minimal requirement)

### Sensitivity—children (including extrapulmonary TB regardless of HIV status)

- 80% compared to culture of any specimen and
- 60% of probable TB (noting the problem of lack of a gold standard)

### Specificity

- **Adults:**
  - 95% compared to culture
- **Children:**
  - 95% compared to culture
  - 90% for culture negative, probable TB (noting the problem of lack of a gold standard)

### Time to results

- Maximum three hours (patient must get result the same day, desirable would be <15 minutes)

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*The group could not decide on a definite minimal value*
New TB diagnostic tools

• Between 2007 and 2010 the WHO has recommended several new diagnostic tests to improve TB diagnosis:
  – Fluorescent microscopy using LED technology—2009
  – Liquid culture: MGIT (mycobacterium growth indicator tubes) and MODS (microscopic observation drug susceptibility assay)—2007/2009
  – Nucleic acid amplification test (NAAT): Hain MDR TB Plus (also known as the Hain Line Probe Assay)—2007
  – Nucleic Acid Amplification Test (NAAT): Xpert MTB/RIF—2010
  – WHO-recommended symptom screen algorithm to rule out active TB in people with HIV—2010
Fluorescent microscopy using light emitting diode (LED) technology

**Advantages**
- Can be done in same laboratory where microscopy is being done and provides improvements over conventional microscopy.
- The bacteria are easier to detect.
- Offers about 10% increase in sensitivity over conventional light-source microscopy.
- Reduces time it takes to detect bacteria by nearly 25-50%.

**Disadvantages**
- Needs a specialized microscope.
- Needs trained microscopist.
- Needs electricity.
- Only detects pulmonary TB.
Liquid culture: MGIT (mycobacterium growth indicator tubes) and MODS (microscopic observation drug susceptibility assay).

**Advantages**

+ Both are a lot faster than solid media (2 wks vs. 3-6 wks).
+ MGIT had 81.5% sensitivity and 99.6% specificity in detecting MTB.
+ MGIT results had high concordance with solid media: 97% for rifampin and 96% for isoniazid resistance.
+ MODS is close to 98% sensitive and nearly 99% specific in detecting rifampin resistance, and 98% sensitive and 96% specific for isoniazid resistance.
+ MODS is inexpensive.

**Disadvantages**

- Both MGIT and MODS require trained technicians, sample processing, and biosafety levels appropriate only for reference laboratory settings.
- MGIT is costly (@$30,000 per machine and $5 for a tube).
- MGIT also requires another test to do rapid speciation to distinguish between MTB and non-TB mycobacteria.
- MODS may have standardization issues that can hamper its accuracy.
Nucleic acid amplification test (NAAT): Hain MDR TB Plus

**Advantages**

+ Sensitivity and specificity for detecting rifampin resistance alone are 99% and specificity for isoniazid alone are 89% and 99%.
+ Can be done on sputum-smear positive samples, dried sputum, or culture.
+ Can provide a result in a matter of hours.

**Disadvantages**

- Test requires manual processing to extract and amplify DNA.
- Cannot be decentralized, as it requires high-biosafety-level laboratories and trained laboratory staff.
Nucleic acid amplification test (NAAT): Xpert MTB/RIF

**Advantages**

+ MTB detection is 98% sensitivity in smear-positive samples and 73% for smear-negative samples; specificity is 99%.
+ Sensitivity and specificity for rifampin resistance is nearly 98%.
+ Gives results within 2 hours.
+ Test is automated and is conducted in a cartridge.
+ Requires minimal sample preparation.
+ Does not require high biosafety.

**Disadvantages**

- Requires expensive machinery: currently about $17,000 for the machine and $17 per cartridge.
- Machine requires electric supply and annual calibration.
Limitations of new tests

• Not appropriate for use as point-of-care tests in local settings where most TB patients are seen.
• MODS, MGIT, and Hain MDR-TB Plus require high biosafety levels to protect the workers and get accurate results.
• All these tests require electricity, equipment, and some degree of infrastructure.
What to ask when assessing a new diagnostic tool

- Is the test sensitive and specific?
- What indications (e.g., smear-negative TB, drug resistance) and/or communities (e.g., infants, people with HIV) is it useful for?
- How long does the test take?
- What does the test tell us (e.g., infection, disease)?
- Does it need a lot of technology and training?
- What type of specimen does it need (e.g., sputum, blood, urine)?
- Does it need a high level of biosafety? (e.g., does it involve culturing the bacteria)?
- What does it cost?
Why don’t we have better diagnostics?

• TB was discovered more than 125 years ago but we still do not have good biomarkers that predict TB immunity, disease, or cure.

• Antibodies and antigens that can be used as biomarkers are yet to be discovered and validated.

• Technologies that can be used to detect these biomarkers also need to be developed and evaluated.
2010 funding for TB diagnostics research - $48,410,889

Global Plan target - $340,000,000
What needs to happen?

- Increased funding for TB diagnostics research.
- Greater investment in basic science for TB.
- Funders must coordinate to ensure that resources are being used efficiently and fund a comprehensive research agenda.
- TB diagnostics must become an advocacy priority for all TB, HIV, and social justice advocates addressing the impact of poverty.
- The WHO’s recommended tools must be taken up by national programs and budgeted for in funding proposals.
- Laboratory capacity needs to be strengthened.
Module review

• What are sensitivity and specificity?
• What is DST used for?
• What is culture?
• Name two tools commonly used to diagnose TB. Name one advantage and one disadvantage of each
• Name three gaps in smear microscopy and/or culture.
• Name a tool used to diagnose latent TB infection. Name two benefits and two disadvantages of this test.
• Name two new diagnostic tools that the WHO has recommended since 2007. Name one advantage and one disadvantage of each.
• Name three advocacy priorities that can improve potential to develop the needed TB diagnostics.