TB Prevalence Surveys
Laboratory Requirements

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Laboratory planning -

- Core elements of laboratory services
  - Laboratory infrastructure and maintenance;
  - Equipment validation and maintenance;
  - Specimen transport and referral mechanisms;
  - Testing protocols
  - Management of laboratory commodities and supplies;
  - Laboratory information and data management systems;
  - Laboratory quality management systems;
  - Appropriate, adequate strategies and funding for laboratory human resource development.
## Summary: Characteristics and laboratory requirements of WHO-approved technologies

<table>
<thead>
<tr>
<th>Diagnostic tool or method</th>
<th>Laboratory service level</th>
<th>Time to detection of MDR</th>
<th>Equipment</th>
<th>Consumables</th>
<th>Training needs</th>
<th>Infrastructure (Risk category)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct</td>
<td>Indirect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopy</td>
<td>Peripheral</td>
<td>n/a</td>
<td>n/a</td>
<td>+</td>
<td>+</td>
<td>Minimal</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Central</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid culture &amp; DST</td>
<td>Central</td>
<td>n/a</td>
<td>9 - 12 weeks</td>
<td>+</td>
<td>++</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial liquid culture &amp; DST</td>
<td>Central</td>
<td>n/a</td>
<td>3 - 5 weeks</td>
<td>+++</td>
<td>+++</td>
<td>Extensive</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-commercial culture &amp; DST</td>
<td>MODS</td>
<td>2 – 21 days</td>
<td>3 – 4 weeks</td>
<td>++</td>
<td>++</td>
<td>Extensive</td>
</tr>
<tr>
<td></td>
<td>NRA</td>
<td>6 – 9 days</td>
<td>7 – 11 weeks</td>
<td>+</td>
<td>++</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>CLI</td>
<td>n/a</td>
<td>3 – 5 weeks (liquid culture)</td>
<td>+</td>
<td>++</td>
<td>Extensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 – 10 weeks (solid culture)</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Line probe assay</td>
<td>Central</td>
<td>24-48hrs</td>
<td>3 – 5 weeks</td>
<td>+++</td>
<td>++</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>SM pos</td>
<td>n/a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SM neg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Positioning in tiered health system

- Surveillance
- Reference methods
- Network supervision

Resolution testing (screening-test negative drug resistance)

- Screening
- Passive case finding
- Detect and treat

In house DST (MODS, NRA, CRI)

Special settings and conditions

LPA Rif / INH 2d

Integrated NAAT +40% /2h

Community Level

Microscopy Level

SubDistrict Level

ZN 2-3d

LED FM +10%

District Level

Regional Labs

Reference Labs

LQ 15d

DST DST d 16d 30d

4
Figure 8.1
Diagram of the recommended protocol for specimen collection and processing

<table>
<thead>
<tr>
<th>In the field</th>
<th>Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual eligible for sputum examination¹</td>
<td></td>
</tr>
</tbody>
</table>

Two specimens are required. The timing of specimen collection can be either (i) the same day for both specimens, with an interval of 1 hour between collection of each specimen, or (ii) one specimen collected on-the-spot and the second collected the following morning (with the morning specimen used for culture examination). The choice between the two methods depends on operational considerations.

<table>
<thead>
<tr>
<th>In culture laboratory</th>
<th>Reception, registration and creation of batch of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Decontaminate specimens</td>
</tr>
</tbody>
</table>

Concentrated microscopy, culture method, using solid or liquid media²

<table>
<thead>
<tr>
<th>Concentrated microscopy, culture method, using solid or liquid media²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuge</td>
</tr>
<tr>
<td>Sediment</td>
</tr>
<tr>
<td>Inoculate 2 culture media</td>
</tr>
<tr>
<td>Observe growth once a week</td>
</tr>
<tr>
<td>Growth (primary cultures)</td>
</tr>
<tr>
<td>ZN staining to confirm AFB</td>
</tr>
<tr>
<td>AFB</td>
</tr>
<tr>
<td>Non-AFB</td>
</tr>
</tbody>
</table>

Identification test for MTB

<table>
<thead>
<tr>
<th>Identification test for MTB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive MTB</td>
</tr>
<tr>
<td>Negative NTM</td>
</tr>
<tr>
<td>Contaminants</td>
</tr>
</tbody>
</table>

DST³
Estimating Laboratory capacity

- number of microscopes;
- number and size of sinks to prepare slides;
- number of biosafety class I or II cabinets;
- facility with unidirectional airflow and a minimum of 6-12 air changes per hour;
- number and size of centrifuges;
- incubator space and how many tubes can be incubated at a time, taking into account that solid media tubes need to be incubated for 8 weeks and liquid cultures for 6 weeks before being reported as negative;
- distilled water machines, and their throughput time per litre, to prepare buffer, media and for autoclaving; and
- waste disposal equipment such as autoclaves and incinerators
Sample management

• Collection of specimens
  – Instructions for collection (Where and How)
  – Assessment of sample quality
• Timing of collection
  – Which sampling strategy
• Where are samples to be tested?
  – What is the transportation delay
  – Maintaining a cold chain
  – What is the transportation mechanism
• Quantify or estimate diagnostic need to identify cases
  – Number of participants to be screened and the anticipated number of TB suspects
  – Needs good planning so as not to over-burden the laboratory services
Microscopy

• LED fluorescence microscopy has approx. 10% increase in sensitivity over bright field microscopy and ZN staining
• Microscopy is suitable for peripheral and higher level laboratories
• Microscopy can be done safely with minimal bio-safety precautions
• Microscopy has limited sensitivity, which is further reduced in HIV-positive individuals
• Microscopy identifies AFB and not *M. tuberculosis*;
• Microscopy will not differentiate between viable and non-viable organisms
• Sensitivity and specificity of microscopy will vary with HIV prevalence
• One technician could read 25-30 ZN smears or up to 100 FM smears per day
Culture

- Culture is suitable for national or regional level laboratories
- Both solid and liquid culture are recommended by WHO but require a high level of bio-safety precautions
- Liquid culture, is more expensive than solid culture, but results are available more rapidly
- All positive cultures must be speciated to confirm *M. tuberculosis*

- The choice of culture method should:
  - Recommended by WHO
  - Familiar to laboratory staff
  - Common practice

*Direct culture systems are not recommended*
Decontamination methods for Culture

• Decontamination is the critical step for MTB culture

  • Balance between killing normal respiratory flora and protecting MTB
    • Too harsh decontamination results in poor sensitivity of culture
    • Too gentle decontamination results in bacterial overgrowth
  • NaOH NALC recommended for liquid culture

  • Incubation needs: solid media (8 weeks) and liquid cultures (6 weeks)
How to choose between solid or liquid culture?

• Liquid Culture (manual or automated) is preferred over Solid Culture

• Advantages
  • Higher sensitivity
  • Shorter time to detection
  • Requires highly functional laboratories
  • Samples must be maintained in cold chain

• Disadvantages
  • More prone to contamination
  • Higher cost
  • Automated MGIT has limited capacity (960 tubes)
Managing the laboratory workload

• In one working day a technician
  • Can decontaminate and inoculate approx 20-30 specimen
  • Read 500 solid media cultures
  • Read 500 manual MGIT cultures

• Automated liquid culture reduces the need to read but may restrict the number of samples which can be tested.

• One MGIT instrument has an maximum annual capacity of approx. 6000 tests
EXAMPLE

- Target sample 40,000
- 90% eligible individual participate
- 10-15% eligible for sputum examination
- Approx. 3,600-5,400 participants are required to submit sputum
- Determining cluster size should be dependant on the lab capacity

If the lab capacity is 100-150 sample per week than 500 participants per week can be included
Performance Indicators are essential to determine the laboratory quality

- The AFB smear positivity rate among new TB suspects
- The AFB positivity rate among follow-up specimens from persons on treatment
- The proportion of AFB smear negative culture positive specimens among total positive cultures
- The proportion of new smear positive cases that are culture positive
- Contamination rates in both solid and liquid media need to be determined separately and fall within acceptable limits.
  - 2-5% contamination on solid media
  - 8-10% in liquid media
- Overall bacterial contamination rates
- The proportion of NTM isolated should remain constant in different epidemiological settings
- Consistency within a case series.
- Isolated positive results need to be investigated
• It is essential that laboratories performing drug susceptibility testing participate in a quality assurance programme to ensure proficiency.

• This should be coordinated with the National TB Reference Laboratory in each setting or with the Supranational Reference Laboratory in the region.

• A panel on strains of known susceptibility patterns should be tested at least annually. The sensitivity and specificity of DST testing for isoniazid and rifampicin should exceed 95%
Key messages

✔ The laboratory is critical to the success of a prevalence survey

✔ Ensure that the additional workload does not overburden the laboratory

✔ Plan to ensure the quality of laboratory testing