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 Intermediate</td>
<td>n/a</td>
<td>n/a</td>
<td>+</td>
<td>+</td>
<td>Minimal</td>
</tr>
<tr>
<td>Solid culture &amp; DST</td>
<td>Central Intermediate</td>
<td>n/a</td>
<td>9 - 12 weeks</td>
<td>+</td>
<td>++</td>
<td>Moderate</td>
</tr>
<tr>
<td>Commercial liquid culture &amp; DST</td>
<td>Central Intermediate</td>
<td>n/a</td>
<td>3 - 5 weeks</td>
<td>+++</td>
<td>+++</td>
<td>Extensive</td>
</tr>
<tr>
<td>Non-commercial culture &amp; DST</td>
<td>Central Intermediate</td>
<td>2 – 21 days 6 – 9 days n/a</td>
<td>3 – 4 weeks 7 – 11 weeks 3 – 5 weeks (liquid culture) 7 – 10 weeks (solid culture)</td>
<td>++</td>
<td>++</td>
<td>Extensive Moderate Extensive</td>
</tr>
<tr>
<td>Line probe assay</td>
<td>Central Intermediate</td>
<td>24-48hrs n/a</td>
<td>3 – 5 weeks</td>
<td>+++</td>
<td>++</td>
<td>Moderate</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

- Clinical screening
  - Primary care

Integrated NAAT +40% /2h

In house DST (MODS, NRA, CRI)
Special settings and conditions

LC / DST d 15d / 30d

LPA / Rif / INH 2d

Integrated NAAT +40% /2h

LED FM +10%

ZN 2-3d

Reference Labs

Regional Labs

District Level

SubDistrict Level

Microscopy Level

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

**In the field**

1. Individual eligible for sputum examination

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.

2. Transport specimens to culture laboratory in cold chain with transportation form

**In culture laboratory**

3. Reception, registration and creation of batch of specimens

4. Decontaminate specimens

5. Concentrated microscopy, culture method, using solid or liquid media

   - Centrifuge
   - Sediment
   - Inoculate 2 culture media
   - Observe growth once a week
   - Growth (primary cultures)
   - ZN staining to confirm AFB

   - AFB
   - Non-ABF

8. Identification test for MTB

   - Positive
     - MTB
   - Negative
     - NTM
     - Contaminants

9. DST
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
Managing the laboratory workload

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
EQA DST

• 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
ADDITIONAL COMMENTS
IKUSHI ONOZAKI
Bottlenecks of the operation

• CXR and Interview → 150-200/day (-300)

• If 10-15% need to submit specimens
  → 15-30 persons/day = 30-60 specimens/day

• If there is a transportation every two days
  → 60-120 specimens/

• If 3 clusters operate at one time
  → Lab will receive 200-300 specimens in a day
Certified lab often failed

• "High contamination rate due to intervals between collection and inoculation" or "low recovery rate due to harsh de-contamination"
• Can't cope with large quantify and poor quality samples

Experiences and practice are essential
continue

Mycobacterium Other than TB

• A few % of S+ and more in S-C+.
• Should be excluded from Study Case

Distinguish "Negative" and "Contamination"

Assess the possibility of Cross-contaminations to avoid false positive diagnosis
Value of 2\textsuperscript{nd} specimen

Survey specimen: less bacteriological load

- More scanty positive in smear
- 2+, 3+ < 1+, Scanty
- S+C+ < S-C+
- Yield by 2\textsuperscript{nd} specimen seems to be more: 30%?
Some more tips

• Assess and confirm the capacity prior to the survey specific training
• Test transportation and examine samples from field