TB Prevalence Surveys
Laboratory Requirements

Christopher Gilpin PhD MPH

TB Diagnostics and Laboratory Strengthening Unit
StopTB Department
World Health Organization, Geneva

25 May 2011
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>Microscopy</td>
<td>Peripheral Intermediate Central</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>9 - 12 weeks</td>
<td>+</td>
<td>++</td>
<td>Moderate</td>
<td>++</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 MODS NRA CLI</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

- **SubDistrict Level**
  - ZN 2-3d

- **District Level**
  - Integrated NAAT +40% /2h

- **Regional Labs**
  - LC
  - DST
  - Rif / INH 2d
  - LPA

- **Reference Labs**
  - In house DST (MODS, NRA, CRI)
  - Special settings and conditions

- **Community Level**
  - Clinical screening
  - Primary care

- **Microscopy Level**
  - LED FM +10%

- **Surveillance**
  - Reference methods
  - Network supervision

- **Resolution testing**
  - (screening-test negative drug resistance)

- **Screening**
  - Passive case finding
  - Detect and treat

- **Combined**
  - Combined methods
Figure 8.1
Diagram of the recommended protocol for specimen collection and processing

**In the field**
- 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.

- Transport specimens to culture laboratory in cold chain with transportation form

**In culture laboratory**
- Reception, registration and creation of batch of specimens
- Decontaminate specimens

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
  - Identification test for MTB
    - Positive
      - MTB
      - DST
    - Negative
      - NTM
      - Contaminants
  - Non-AFB
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
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
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 $2^{nd}$ specimen

Survey specimen: less bacteriological load

- More scanty positive in smear
- $2+, 3+ < 1+, \text{ Scanty}$
- $S+C+ < S-C+$
- Yield by $2^{nd}$ 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