Prevalence Survey Preparatory Workshop

Part III: Bacteriological Examination

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WHO Stop TB Department
Outline

• Sputum collection
• Microscopy and culture techniques
• Minimum requirements
• Transport of sputum specimens
• Biosafety concerns
• Implementation of laboratory services
Why a laboratory diagnosis?

- TB signs and symptoms non-specific
- Radiological manifestations suggestive but non-specific
- Definitive diagnosis by demonstrating acid-fast bacilli (AFB) on microscopy and/or *Mycobacterium tuberculosis* on culture of sputum specimens
Sputum collection

- Aerosol infection risk
- Supervised collection recommended
- Good quality essential
- 3ml - 5ml recommended
- Induced sputum a possibility
- Transport to laboratory ASAP
Sputum quality
Sputum characteristics and microscopy yield
SAMRC prevalence surveys 1972 – 1984 (paired specimens, n=17 941)

<table>
<thead>
<tr>
<th>Sputum characteristics*</th>
<th>SM+ (%)</th>
<th>M-H odds ratio (significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality sufficient</td>
<td>1.48</td>
<td></td>
</tr>
<tr>
<td>Quality insufficient</td>
<td>0.51</td>
<td>2.89 (p&lt;0.05)</td>
</tr>
<tr>
<td>Quantity sufficient</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>Quantity insufficient</td>
<td>0.67</td>
<td>1.80 (p&gt;0.05)</td>
</tr>
</tbody>
</table>

*Sufficient quality: Macroscopic presence of mucopurulent material
*Sufficient quantity: Volume > 1ml
Sputum characteristics and culture yield
SAMRC prevalence surveys 1972 – 1984 (paired specimens, n=17 941)

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<thead>
<tr>
<th>Sputum characteristics*</th>
<th>C+ (%)</th>
<th>M-H odds ratio (significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality sufficient</td>
<td>2.85</td>
<td></td>
</tr>
<tr>
<td>Quality insufficient</td>
<td>1.15</td>
<td>2.49 (p &lt; 0.05)</td>
</tr>
<tr>
<td>Quantity sufficient</td>
<td>2.10</td>
<td></td>
</tr>
<tr>
<td>Quantity insufficient</td>
<td>1.48</td>
<td>1.41 (p &gt; 0.05)</td>
</tr>
</tbody>
</table>

*Sufficient quality: Macroscopic presence of mucopurulent material
*Sufficient quantity: Volume > 1ml
Microscopy

- Rapid, robust, inexpensive
- Identifies ~80% of infectious TB cases
- Cannot distinguish viable from non-viable organisms
- Cannot differentiate mycobacterial species
- Sensitivity reduced in advanced HIV
- Sensitivity low in extra-pulmonary TB
- Turnaround time <24 hours
- Two options: Light vs Fluorescence
Acid-fast bacilli on smear

**Light microscopy**
(Ziehl-Neelsen staining)

**Fluorescence microscopy**
(Auramine-O staining)
Quantification

<table>
<thead>
<tr>
<th>Number of AFB seen</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AFB per 100 fields</td>
<td>Negative</td>
</tr>
<tr>
<td>1 - 9 AFB per 100 fields*</td>
<td>Exact number</td>
</tr>
<tr>
<td>10 - 99 AFB per 100 fields</td>
<td>1+</td>
</tr>
<tr>
<td>1 - 10 AFB per field</td>
<td>2+</td>
</tr>
<tr>
<td>&gt;10 AFB per field</td>
<td>3+</td>
</tr>
</tbody>
</table>

*(‘Scanty’) not well correlated with culture, could reflect non-viable organisms or laboratory contamination; repeat microscopy investigation or preferably culture should be done

- AFB highly predictive (>95%) for *M. tuberculosis*
The chance of finding AFB in a smear increases with the concentration of bacilli in the specimen

<table>
<thead>
<tr>
<th># of bacilli observed</th>
<th>Estimated concentration of bacilli/ml sputum</th>
<th>Probability of a positive result</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 in 100 or more fields</td>
<td>&lt; 1 000</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>1 – 2 in 300 fields</td>
<td>5 000 – 10 000</td>
<td>50%</td>
</tr>
<tr>
<td>1 – 9 in 100 fields</td>
<td>~ 30 000</td>
<td>80%</td>
</tr>
<tr>
<td>1 – 9 in 10 fields</td>
<td>~ 50 000</td>
<td>90%</td>
</tr>
<tr>
<td>1 – 9 per field</td>
<td>~ 100 000</td>
<td>96.2%</td>
</tr>
<tr>
<td>10 or more per field (3+)</td>
<td>~ 500 000</td>
<td>99.95%</td>
</tr>
</tbody>
</table>

Incremental yield in microscopy with multiple specimens

- Systematic review of 37 eligible studies
- Incremental diagnostic yield of 3rd specimen

<table>
<thead>
<tr>
<th>1st specimen</th>
<th>2nd specimen</th>
<th>3rd specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>85.8%</td>
<td>11.9%</td>
<td>3.1%</td>
</tr>
</tbody>
</table>


Light vs Fluorescence?

- Existing infrastructure
- Skills capacity and training need
- Anticipated workload
- Specimen and process flow
- Quality assurance
- New technologies (LED)
- Cost
Culture

- High sensitivity, even with HIV co-infection
- Single positive result confirms diagnosis
- Up to 40% increased case detection
- Decontamination of sputum always required, with adverse effect on mycobacteria
- Diagnosis always delayed due to slow growth rate of *M. tuberculosis*
Conventional culture methods

• Solid medium
  – Inexpensive
  – Not technically sophisticated
  – Slow
  – Requires adequate biosafety
  – Egg-based
  – Agar-based

• Liquid medium
  – Standard in developed countries
  – Rapid
  – Technically sophisticated
  – Expensive
  – Requires adequate biosafety
  – Commercial systems (BACTEC, MGIT - Becton Dickinson; MBBacT - BioMérieux; Bact-Alert - Organon Technika)
Mycobacterium tuberculosis

Löwenstein-Jensen egg-based medium
- Cream
- Rough
- Waxy
- Non-pigmented
Implementing liquid culture: Lessons learnt (1)

- Laboratory infrastructure
  - Space constraints
  - Biosafety
  - Emergency power supply
  - Air conditioning
- General infrastructure (power supply)
- Reliable, regular specimen transport
Implementing liquid culture: Lessons learnt (2)

- Staff training
- Standard operating procedures
- Contamination
- Internal QC and external QA
- Retention of solid culture
- Increased work load!
  - Smear confirmation
  - Culture purity checks
  - Species identification
  - Strict requirements for sub-culturing for DST
Implementing liquid culture: Lessons learnt (3)

- Supply shortages
- Stock rotation

- Rapid, effective communication of results
- Integration of information systems
- Improved patient management?
Molecular methods

- Good performance characteristics
  - Rapid
  - Sensitive (approaching culture)
  - Specific
- Expensive
- Requires sophisticated technology
- Core technology and platform suitable for developing country use under development
- Not yet available
Other diagnostic options

• PCR
  – False-positive and false-negative results
• Nucleic acid probes
  – Useful for species identification
• Rapid serological tests
  – Poor sensitivity in HIV+ patients
  – Poor specificity for active TB vs infection, BCG vaccination, previous TB
• Adenosine deaminase (ADA)
  – Useful as adjunct to diagnosis of TB peritonitis and pleural effusion
  – Useless in diagnosis of pulmonary TB
  – Increased levels in many infectious and haematological diseases
• ESR, WBC, C-reactive protein, etc.
  – Results highly variable
  – Not useful in TB diagnosis
FIND product deliverables 2006-2013

Reference Lab
- Speciation test
- Liquid culture MTB & DST
- Automated NAAT

Peripheral Lab
- Phage based resistance test
- LED Fluor Microscopy
- POC NAAT
- Urinary NAAT

Clinic Health post
- Urinary AG detection
- Reader based LAT Flow
- Improved AG/AB strip test

% Access after 5 years
- Reference Lab: 10-40%
- Peripheral Lab: 70%
- Clinic Health post: 95%
## HBC laboratory capacity 2006
(Source: WHO Global Report 2008)

<table>
<thead>
<tr>
<th></th>
<th>National Reference Laboratory</th>
<th># culture laboratories per 5 million population</th>
<th># DST laboratories per 10 million population</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>Yes</td>
<td>0.03</td>
</tr>
<tr>
<td>2</td>
<td>China</td>
<td>Yes</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>Indonesia</td>
<td>No</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>South Africa</td>
<td>Yes</td>
<td>1.3</td>
</tr>
<tr>
<td>5</td>
<td>Nigeria</td>
<td>No</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>Bangladesh</td>
<td>Yes</td>
<td>0.1</td>
</tr>
<tr>
<td>7</td>
<td>Ethiopia</td>
<td>Yes</td>
<td>0.1</td>
</tr>
<tr>
<td>8</td>
<td>Pakistan</td>
<td>No</td>
<td>0.1</td>
</tr>
<tr>
<td>9</td>
<td>Phillipines</td>
<td>Yes</td>
<td>0.2</td>
</tr>
<tr>
<td>10</td>
<td>DR Congo</td>
<td>Yes</td>
<td>0.1</td>
</tr>
<tr>
<td>11</td>
<td>Russian Federation</td>
<td>No</td>
<td>34</td>
</tr>
<tr>
<td>12</td>
<td>Viet Nam</td>
<td>Yes</td>
<td>1.0</td>
</tr>
<tr>
<td>13</td>
<td>Kenya</td>
<td>Yes</td>
<td>0.3</td>
</tr>
<tr>
<td>14</td>
<td>UR Tanzania</td>
<td>Yes</td>
<td>0.4</td>
</tr>
<tr>
<td>15</td>
<td>Uganda</td>
<td>Yes</td>
<td>0.5</td>
</tr>
<tr>
<td>16</td>
<td>Brazil</td>
<td>Yes</td>
<td>5.1</td>
</tr>
<tr>
<td>17</td>
<td>Mozambique</td>
<td>Yes</td>
<td>0.2</td>
</tr>
<tr>
<td>18</td>
<td>Thailand</td>
<td>Yes</td>
<td>5.1</td>
</tr>
<tr>
<td>19</td>
<td>Myanmar</td>
<td>Yes</td>
<td>0.2</td>
</tr>
<tr>
<td>20</td>
<td>Zimbabwe</td>
<td>Yes</td>
<td>0.4</td>
</tr>
<tr>
<td>21</td>
<td>Cambodia</td>
<td>Yes</td>
<td>1.1</td>
</tr>
<tr>
<td>22</td>
<td>Afghanistan</td>
<td>No</td>
<td>0.2</td>
</tr>
</tbody>
</table>
TB diagnostic services

Unsatisfactory performance attributed to

- Inadequate human resources
- Lack of recognition of the importance of TB laboratory services
- Weak communication between NTP and laboratory services
- Insufficient financial resources
- Problems related to availability and accessibility of TB diagnostic services
- Delay in technology transfer from industrialized to resource-limited countries
- No or minimal interaction with private-sector laboratories
- Biosafety concerns (M/XDR, HIV infection in laboratory staff)
Excess occupational risk

<table>
<thead>
<tr>
<th>Work location</th>
<th>TB incidence rate ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(relative to general population TB incidence rate)</td>
</tr>
<tr>
<td>Outpatient facilities</td>
<td>4.2 – 11.6</td>
</tr>
<tr>
<td>General medical wards</td>
<td>3.9 – 36.6</td>
</tr>
<tr>
<td>Inpatient facilities</td>
<td>14.6 – 99.0</td>
</tr>
<tr>
<td>Emergency rooms</td>
<td>26.6 – 31.9</td>
</tr>
<tr>
<td>Laboratories</td>
<td>78.9</td>
</tr>
</tbody>
</table>


Classification of infective organisms by risk group


• Risk Group 1
  ‘A microorganism that is unlikely to cause human or animal disease’

• Risk Group 2
  ‘A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited’

• Risk Group 3
  ‘A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available’

• Risk Group 4
  ‘A pathogen that usually causes serious human or animal disease and that can be readily spread from one individual to another, directly or indirectly. Effective treatment and preventive measures are usually not available’
1. Minimum biosafety levels

- **American Institute of Architects (IAI) Guidelines for the Design and Construction of Health Care Facilities, 2006**
  - Minimum of 6ACH and 100% exhaust of air

- **CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 5th ed. 2007**
  - BSL2 (P2): ‘No specific requirement on ventilation systems. However, planning of new facilities **should consider mechanical ventilation systems** that provide and inward flow of air **without recirculation** to spaces outside of the laboratory.’
  - BSL3 (P3): ‘A **ducted air ventilation system** is required. This system must provide sustained directional airflow by drawing air into the laboratory from ‘clean’ areas toward ‘potentially contaminated areas’. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.’

  - BSL2: ‘Planning of new facilities **should consider mechanical ventilation systems** that provide an inward flow of air **without recirculation**. If there is no mechanical ventilation, windows should be able to be opened. ’… a **standby generator** is desirable…’
  - BSL3: ‘There must be a **controlled ventilation system** that maintains a directional airflow into the laboratory. **Air may be HEPA filtered, reconditioned and re-circulated** within the laboratory.’ (In addition to separate anteroom to maintain pressure differential, as well as on-site autoclave for waste disposal)
Laboratory preparedness for introduction of new technologies requires addressing:

- Infrastructure and equipment
- Staffing and training
- Biosafety aspects
- Development of procedures and SOPs
- Quality assurance systems

based on:

- Product requirements
- Local needs and capacity
- Level of health care systems where technology will be used
- Adaptation to the national context
Laboratory preparedness for introduction of new technologies requires recognition of differences

<table>
<thead>
<tr>
<th>Emerging Economies</th>
<th>Low-income/Asia</th>
<th>Low-income/Africa</th>
</tr>
</thead>
<tbody>
<tr>
<td>eg. China, India, S. Africa, Russia, Brazil</td>
<td>eg. Indonesia, Bangladesh, Pakistan, Philippines, Afghanistan, Cambodia, Myanmar, Vietnam</td>
<td>eg. DR Congo, Ethiopia, Kenya, Mozambique, Nigeria, Uganda, Tanzania</td>
</tr>
</tbody>
</table>

- Less dependent on international donors, funding mostly from national budgets
- Human resource capacity stronger
- Current laboratory services more developed
- Private for-profit sector role often considerable

- More dependent on international donors for funding
- Moderate human resource capacity
- Current laboratory services weak to moderate
- Private for-profit role significant

- Very dependent on international donors
- Generally weak human resource capacity
- Current laboratory services weak
- Private for-profit role present, but variable
More than technology transfer