• Capacity of Lab often limits the screening strategy and velocity of the survey

• Characteristics of the TB patients are very different from clinically observed cases i.e. distribution of lower bacteriological load cases
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

**Community Level**

**Microscopy Level**

**SubDistrict Level**

**District Level**

**Regional Labs**

**Reference Labs**

- Integrated NAAT +40% /2h
- LED FM +10%
- LPA Rif / INH 2d
- LPA Rif / INH 2d /16d /30d
- LC / DST 15d /30d
- SC / DST 30d /60d
- ZN 2-3d
- In house DST (MODS, NRA, CRI)
- Special settings and conditions

- Surveillance
- Reference methods
- Network supervision

- Resolution testing (screening-test negative drug resistance)

- Screening
- Passive case finding
- Detect and treat

- Clinical screening
- Primary care
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**

1. Reception, registration and creation of batch of specimens
2. Decontaminate specimens
3. 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-AFB
     - Identification test for MTB
       - Positive
         - MTB
         - DST
       - Negative
         - NTM
         - Contaminants
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
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 – not always necessary for a survey.
- All positive cultures must be speciated to confirm *M. tuberculosis*.

- *The choice of culture method should:*
  - Recommended by WHO (and/or SNRL)
  - Familiar to laboratory staff
  - Common practice

*Direct culture systems are not recommended by WHO Lab Group.*
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 weekly field operation (number of clusters) should be dependant on the lab capacity

Example: If the lab capacity is 250-300 sample per week then 1000 participants per week can be included -> two clusters
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
LESSONS FROM REAL SURVEYS
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\textsuperscript{nd} specimen

Survey specimen: less bacteriological load
- More scanty positive in smear
- $2^+, 3^+ < 1^+, \text{Scanty}$
- $S+C^+ < S-C^+$
- Yield by 2\textsuperscript{nd} specimen seems to be more: 30%?
- And We can't go back field to re-collect

S+ 2C-: might be NTM as culture media is not fit to NTM
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
- False negative due to harsh de-contamination or a poor sample
two culture samples are necessary to have at least one result
Why we need additional exam in Cambodia  FL vs ZN

• Norm- Belief from research setting was different from real world
Smear positive culture negative

• Really TB?
Review Ethiopia Survey Lab + cases
<table>
<thead>
<tr>
<th>sputum</th>
<th>CXR panel Results</th>
<th>Treatment</th>
<th>Sym</th>
<th>final decision</th>
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<tbody>
<tr>
<td>sp1</td>
<td>ma</td>
<td>Normal</td>
<td>on</td>
<td>1</td>
</tr>
<tr>
<td>sp2</td>
<td>TB</td>
<td>Normal</td>
<td>on</td>
<td>1</td>
</tr>
<tr>
<td>c</td>
<td>BE*</td>
<td>Normal</td>
<td>on</td>
<td>1</td>
</tr>
</tbody>
</table>

*BE: Bronchial Echotaxis; **Possible case: excluded from study case
Screening
Why screening

• Decrease work load of Lab
  – Capacity in quantity
  – May have higher quality job
  – Less false positive

• TB is very rare disease (prev. 100-500/100,000 in next survey)

• Quality Lab may have a false positive error per 1000 specimen
  – 12/18 single S+ subjects -> False positive
Challenges

• Gaps due to limitation of screening and diagnostic capacity
  – Lab results
  – Cases along the study case definition
  – Case management: Don't tell that you have TB wrongly – Very strong recommendation by WHO ERC
Symptom screening

- Chronic cough 2-5%
- Any cough or shorter duration 20-35%
- Any TB related symptom: 30-50%
Symptom screening

- Chronic cough 2-5% → 30-60% of S+, 20-50% of C+
- Any cough: 20-35% → Between
- Any TB related symptom: 30-50% → 80-95% of S+, 50-80% of C+

Combination of the symptom such as cough or fever may have value in ACD. However, yields beyond CXR screening seems to be minimum.
CXR

- TB suggestive 1-2% -- 60-80% of S+C+, 50-70% of C+
- Any TB related shadow: 5%: 70-90% of S+C+
- Any Abnormality: 8-15% --- 97-100% of S+C+, 90-95% of C+
Radiography, CXR, X-ray
CXR for screening

• Normal CXR – A normal chest X-ray means clear lung fields and no abnormality detected. Participants with normal CXR have no radiological basis for undergoing bacteriological examination.

• Abnormal CXR (Eligible for sputum exam)- An abnormal chest X-ray means any lung (including pleura) abnormality detected on interpretation by the medical officer (e.g. opacities, cavitation, fibrosis, pleural effusion, calcification(s), any unexplained or suspicious shadow, etc.).

• Other Abnormal CXR: Congenital abnormalities, normal variants, and bony abnormalities like fractures are excluded by definition as are findings like increased heart size and other heart-related abnormalities.
• A more detailed interpretation (audited reading) can be performed at the central level
• The central team should classify x-rays based on a classification decided upon earlier (as mentioned in the x-ray reference manual)
• May help identify quality issues with lab
CXR Selection

- ? Technology
- ? Number of units
- ? Value additions (e.g. CAD, Teleradiology)
X-ray technologies

CONVENTIONAL
• Conventional radiography
• Conventional with autoprocessor

DIGITAL
• Computed radiography (CR)
• Direct radiography (DR, DDR)
Conventional radiography
Autoprocessor
Computed Radiography (CR)

1. X-ray Exposure
   Patient

2. Image Reader

3. Image Scaling

4. Image Record

5. Computed Radiograph

X-ray system

Phosphor plate
Direct Radiography
DDR

- Flat panel
- CCD
- CMOS
- Slot-scan
Post processing – Digital only
Digital image

- 2nd reading, central reading (Audit reading) will be easier – to increase sensitivity, avoiding human errors

- Disadvantage – Staff are not familiar with image reading on screen
Value additions

- Teleradiology
- CRRS
- Computer-Aided-Detection (CAD)
- Computed-Aided-Diagnosis (CADx)
- Temporal subtraction imaging
## Comparison chart

<table>
<thead>
<tr>
<th>No.</th>
<th>Feature</th>
<th>Conventional</th>
<th>CR</th>
<th>Digital</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Electronic data collection, reporting and storage, data management &amp; privacy, back-up data</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
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<tr>
<td>2</td>
<td>High Image readability and quality</td>
<td>NO</td>
<td>YES/NO</td>
<td>YES</td>
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<tr>
<td>3</td>
<td>Value additions (CAD, Teleradiology)</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>4</td>
<td>Use of films and chemicals (potential environmental issues)</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>5</td>
<td>Radiation safety</td>
<td>NO*</td>
<td>NO*</td>
<td>YES*</td>
</tr>
<tr>
<td>6</td>
<td>Cost*</td>
<td>Cheap initially</td>
<td>Intermediate</td>
<td>Cheap in long run</td>
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<tr>
<td>7</td>
<td>Faster throughput</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>8</td>
<td>Immediate image reproducibility</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
</tr>
</tbody>
</table>
Costs

• Conventional: 10-25,000 USD
• Autoprocessor: 7-12,000 USD
• CR: 50-70,000 USD
• DR with imaging panel: 100-120,000 USD
• DDR: 150,000 USD and above
CXR Requirements

- Planning
- Procurement
- Teamwork
- Allied equipment
- Radiation safety
- Legal and regulatory requirements
- Logistic requirements
- Technical assistance
Planning & Procurement

• Local technical expertise with TA
• Frequent bottleneck and time-consuming step
• Initiate early
• Attention to minute details
  – Accessories
  – Software/hardware
• Legal/regulatory issues
Radiation exposure

- MBUR Referral guidelines, Royal College of Radiologists London: ‘typical effective dose = 0.02 mSv = 3 days app. Equivalent period of natural background radiation
- HPA – RCR: CXR associated risk of childhood cancer is very low and acceptable when compared with natural risk. Radiation doses resulting from Dx procedures present a negligible risk of induced hereditary disease in descendants of the unborn child
- ACR: Some procedures (incl. CXR in 1st & 2nd trimester) render so low exposures that pregnancy status need not be considered for a “medically indicated” exam, as long as good radiation practice is ensured
- At 1 meter, occupational exposure (if no apron is worn) is 0.1% of that which enters the patient.
Regulatory

- No ‘safe’ radiation, use regulated
- Radiation regulatory authority/body clearance
- Ethics committee clearance
- Consent, voluntary participation
- Exclude children, pregnant participants
- Good comprehensive protocol
- Timely engagement
Logistics
Fieldwork
Thank you!