Minimal MDR-TB laboratory requirements

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State Agency of Tuberculosis and Lung Diseases
Does laboratory needed to diagnose MDR-TB?
At this point of time MDR-TB is still laboratory diagnosis.
Options

Laboratory (-ies) performing sputum smear microscopy only.
Finding and treating most infectious TB cases.
If treatment failure for 1\textsuperscript{st} category – probability to have MDR-TB case (~ 5% of failures)
If treatment failure for 2\textsuperscript{nd} category – probability to have MDR-TB case (~80% % of failures)
Drug Resistance Survey results needed.
Laboratory design

- Separated lab
- Double entry airlock or anteroom
- Controlled ventilation system (HEPA-filtered air)
- Air condition
- Water resistant walls, floors and ceilings
- Lab sealable for gaseous decontamination
- Backflow-precaution devices to the water supply
- Autoclav available in the containment lab
- Closed windows
Good quality laboratory work can not compensate for a poor quality specimen.
Microscopy vs. culture

- 6000 to 10000 AFB/ml for microscopy positive
- 10 to 100 AFB/ml for culture positive
- Microscopy positive patient is most infectious
Value of acid-fast microscopy

- Rapid, first bacteriologic evidence of tuberculosis
- Identifies infectious patients
- Indicates patient status during therapy
Significance of culture

• Basic method: inoculation of 2 LJ slants
• For selected patients: inoculation of 1 BACTEC MGIT tube and 2 LJ slants
Problems with culture

• Specimens from “non-sterile” body sites have to be decontaminated prior to inoculation
• Slow growth = delayed diagnosis, spread of infection
• More resources needed to perform
Growth time

- The mycobacteria are aerobic, non-spore-forming, non-motile bacilli.
- Growth for mycobacteria is slow to very slow, with generation times ranging by species from 2 to more than 20 hours.
- The *M. tuberculosis* DNA-dependent RNA-polymerase exerts properties which result in a rate of RNA chain growth only approximately one-tenth seen in *E. coli*.
- *M. tuberculosis* 2-8 weeks
- *NTB* 1 week to 2 months
Precondition to perform culture isolation

A well equipped laboratory
(including safety cabinets)
<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Biosafety Level</th>
<th>Laboratory Type</th>
<th>Laboratory Practices</th>
<th>Safety Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Basic - Biosafety Level 1</td>
<td>Basic teaching research</td>
<td>GMT</td>
<td>None; open bench work</td>
</tr>
<tr>
<td>2</td>
<td>Basic - Bisafety Level 2</td>
<td>Primary health services; diagnostic services; research</td>
<td>GMT plus protective clothing, biohazard sign</td>
<td>Open bench plus BSC for potential aerosols</td>
</tr>
<tr>
<td>3</td>
<td>Containment – Biosafety Level 3</td>
<td>Special diagnostic services, research</td>
<td>As Level 2 plus special clothing, controlled access, directional airflow</td>
<td>BSC and /or other primary devices for all activities</td>
</tr>
<tr>
<td>4</td>
<td>Containment – Biosafety Level 4</td>
<td>Dangerous pathogen units</td>
<td>As Level 3 plus airlock entry, shower exit, special waste disposal</td>
<td>Class III BSC, or positive pressure suits in conjunctions with Class II BSCs, double-ended autoclave, filtered air</td>
</tr>
</tbody>
</table>

BSC = biosafety cabinet; GMT= good microbiological techniques
## Summary of biosafety level requirements

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Biosafety Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation(^a) of laboratory</td>
<td>1   2   3   4</td>
</tr>
<tr>
<td>Room sealable for decontamination</td>
<td>No  No  Yes  Yes</td>
</tr>
<tr>
<td><strong>Ventilation:</strong></td>
<td></td>
</tr>
<tr>
<td>Inward airflow</td>
<td>No  Desirable  Yes  Yes</td>
</tr>
<tr>
<td>Controlled ventilating system</td>
<td>No  Desirable  Yes  Yes</td>
</tr>
<tr>
<td>HEPA-filtered air exhaust</td>
<td>No  No  Yes/No(^b)  Yes</td>
</tr>
<tr>
<td>Double-door entry</td>
<td>No  No  Yes  Yes</td>
</tr>
<tr>
<td>Airlock</td>
<td>No  No  No  Yes</td>
</tr>
<tr>
<td>Airlock with shower</td>
<td>No  No  No  Yes</td>
</tr>
<tr>
<td>Anteroom</td>
<td>No  No  Yes</td>
</tr>
<tr>
<td>Anteroom with shower</td>
<td>No  No  Yes/No(^c)  No</td>
</tr>
<tr>
<td>Effluent treatment</td>
<td>No  No  Yes/No(^c)  Yes</td>
</tr>
<tr>
<td><strong>Autoclave:</strong></td>
<td></td>
</tr>
<tr>
<td>On site</td>
<td>No  Desirable  Yes  Yes</td>
</tr>
<tr>
<td>In laboratory room</td>
<td>No  No  Desirable  Yes</td>
</tr>
<tr>
<td>Double-ended</td>
<td>No  No  Desirable  Yes</td>
</tr>
<tr>
<td>Biological safety cabinets</td>
<td>No  Desirable  Yes  Yes</td>
</tr>
<tr>
<td>Personnel safety monitoring capability</td>
<td>No  No  Desirable  Yes</td>
</tr>
</tbody>
</table>

\(^a\) Environmental and functional isolation from general traffic

\(^b\) Dependent on location of exhaust

\(^c\) Dependent on agent(s) used in the laboratory

\(^d\) For example, window, closed-circuit television, two-way communication
Primary Barrier

Personal protections equipment (PPE)

- Gloves
- Coat/apron
- Shoe covers
- Respirators
- Face shields
- Safety glasses
- And more
Primary Barrier

- Biological safety cabinets
  - Class I, Class II, Class III
- Safety cups
- Pipettes
- Sharp containers
- Transport containers
- and more
Biological Safety Cabinets

Biological Safety Cabinets

• Class I
  – Open front, inflow air,
  – exhaust air HEPA filtered
  – Personal protection only

• Class II
  – Open front, vertical laminar air flow,
  – Internal and exhaust air HEPA filtered
  – Personal and material protection

• Class III
  – Glove box
Class I safety cabinet  
Class II safety cabinet
Validation of Cabinets

HEPA filter leak test

Flow velocity test

When?
- At initial installation and annually thereafter
- After moving
- After replacing filters
Centrifuges with safety cups
Precondition to perform culture isolation

Well trained technicians
  • theoretically
  • practically

Internal quality control
External quality control
Culture Media

**Solid Culture Media**

- Löwenstein-Jensen
- Ogawa
- Stonebrink
- 7H10 or 7H11 Agar (Middlebrook)
- Coletsos
Liquid Culture Media

- BACTEC 460
- MGIT
- MGIT 960
- MB Redox
- BacT/Alert
Time to growth detection of *M. tuberculosis*

- **Liquid media automated systems**
- **Growth on solid media**

Specimen: 1 2 3 4 5 6 7 8 9 10

Weeks: 1 2 3 4 5 6 7 8 9
Liquid Culture Media

**Advantages** compared to solid media:
- more rapid
- high quality of media
- fully automated system
- safety: plastic tubes

**Disadvantages:**
- only NALC-NaOH decontamination
- higher contamination rate
- expensive
- dependency on a company
Differentiation
<table>
<thead>
<tr>
<th>$M.\ tuberculosi$s complex</th>
<th>atypical Mycobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOTT = Mycobacteria Other Than Tuberculosis</td>
<td></td>
</tr>
<tr>
<td>NTM = Non-Tuberculous Mycobacteria</td>
<td></td>
</tr>
</tbody>
</table>
Identifying *M.tuberculosis* complex

- Niacin, nitrate, 68° C catalase
- Paranitrobenzoic acid inhibition
- NAP in BACTEC (inhibition)
- Genetic probes (DNA, rRNA) with single probe or multiple probes
- HPLC
<table>
<thead>
<tr>
<th>Test</th>
<th>Image 1</th>
<th>Image 2</th>
<th>Image 3</th>
<th>Image 4</th>
<th>Image 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacin test</td>
<td>![Image](Image 1)</td>
<td>![Image](Image 2)</td>
<td>![Image](Image 3)</td>
<td>![Image](Image 4)</td>
<td>![Image](Image 5)</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>![Image](Image 1)</td>
<td>![Image](Image 2)</td>
<td>![Image](Image 3)</td>
<td>![Image](Image 4)</td>
<td>![Image](Image 5)</td>
</tr>
<tr>
<td>Catalase foam test</td>
<td>![Image](Image 1)</td>
<td>![Image](Image 2)</td>
<td>![Image](Image 3)</td>
<td>![Image](Image 4)</td>
<td>![Image](Image 5)</td>
</tr>
<tr>
<td>Bromcresol medium</td>
<td>![Image](Image 1)</td>
<td>![Image](Image 2)</td>
<td>![Image](Image 3)</td>
<td>![Image](Image 4)</td>
<td>![Image](Image 5)</td>
</tr>
<tr>
<td>Lebek medium</td>
<td>![Image](Image 1)</td>
<td>![Image](Image 2)</td>
<td>![Image](Image 3)</td>
<td>![Image](Image 4)</td>
<td>![Image](Image 5)</td>
</tr>
</tbody>
</table>

Left: *M. bovis* / right: *M. tuberculosis*
Molecular Techniques for Differentiation of Mycobacteria

DNA probes (AccuProbe)
- MTBC
- *M. avium* complex
- *M. kansasii*
- *M. gordonae*

Sequencing
- all mycobacteria

INNO-LiPA MYCOBACTERIA
- MTBC and 16 species

GenoType® Mycobacterium
- CM: MTBC + 13 species
- AS: 16 species

HPLC
- all mycobacteria
Resistance
Resistance

Georges Canetti (1965): “Bacterial resistance is as old as anti-tuberculosis chemotherapy, aprox. 20 years”
Drug resistance of *M. tuberculosis*

Resistance is defined as decrease in sensitivity of sufficient degree to be reasonably certain that the strain concerned is different from sample of wild strains of human type that have never come into contact with drug.

*D.A. Mitchinson*
Drug resistance of *M. tuberculosis*  

- is a spontaneous genetic event

and worse

- a man-made amplification of a natural phenomenon
WHO Meetings in 1961 and 1968

• Susceptible strain
  …never been exposed to the main anti-TB drugs (“wild strain”), responds to these drugs, generally in a remarkably uniform manner.

• Resistant strain
  …shows a decrease in sensitivity of sufficient degree to be reasonably certain that it is different from wild strain … that has never come into contact with the drug.
Main conclusion

“A diminished clinical response may occur when resistance in the above-mentioned bacteriological sense is demonstrated in the laboratory”
Methods for DST

- Proportion method
- Absolute concentration method
- Resistance ratio method
- DST in liquid media
- Molecular techniques
Proportion method
Methods for Drug Susceptibility Testing

Proportion method with Löwenstein-Jensen medium
H, R, E, S, PTH, CM, OFL, CS, NSA (instead of P)

MGIT 960
For all drugs except cycloserine

BACTEC 460TB
For all drugs except cycloserine
Conclusion: DST on automated systems

**Advantages** compared to solid media:
- more rapid
- high quality of media
- fully automated system
- testing of 1st line, 2nd line and new drugs
- safety: plastic tubes

**Disadvantages:**
- expensive
- higher contamination rate
- dependency on a company
- no DST for Cycloserine
Drug Susceptibility Testing

Highly important in countries with recognized drug resistance problem

- First usable culture from specimens before initiation of treatment
- Retreatment cases – increased probability for resistance
- Treatment failure – resistant
Drug Susceptibility Testing

*In vitro* vs *In vivo*

The laboratory results detailing the activity of an antimicrobial agent against a patient's isolate are just suggestions.
Drug Susceptibility Testing

In vitro vs In vivo

When selecting antimicrobial drug for treatment Clinicians have take into consideration site of infection, pharmacological properties of the drug, the body’s ability to metabolize the drug when metabolic function is normal and when it is impaired.
Drug resistance of *M. tuberculosis*

- Primary drug resistance – drug resistant TB case in which the patient denies any history of previous chemotherapy.
- Infected with drug resistant strain somewhere in community
Drug resistance of *M. tuberculosis*

- Acquired drug resistance in which the emergence of drug resistance is known to be result of chemotherapy
- Result of improper treatment regimen (combination of drugs, duration, absorption problems, compliance)
<table>
<thead>
<tr>
<th>Medication</th>
<th>L-J slant</th>
<th>BACTEC MGIT 960</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>4 μg/ml</td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>0,2 μg/ml, 1 μg/ml</td>
<td>0,1 μg/ml</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>40 μg/ml</td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>2 μg/ml</td>
<td>5 μg/ml</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td></td>
<td>100 μg/ml</td>
</tr>
<tr>
<td>Kanamycin</td>
<td></td>
<td></td>
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<tr>
<td>PAS</td>
<td></td>
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<tr>
<td>Cycloserine</td>
<td></td>
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<tr>
<td>Tioacetzone</td>
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</tr>
<tr>
<td>Ethionamide</td>
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<tr>
<td>Capreomycin</td>
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<tr>
<td>Ofloxacin</td>
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<tr>
<td>Moxifloxacin</td>
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<tr>
<td>Linezolid</td>
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</tbody>
</table>
Cross resistance

- Streptomycin resistant strains usually are not resistant to amikacin, kanamycin, and capreomycin
- Kanamycin resistant strains can exhibit resistance to streptomycin
- Known cross-resistance between kanamycin and amikacin
- Unpredictable cross-resistance between kanamycin, amikacin and capreomycin
Cross resistance

- There was reported PAS cross-resistance with thiacetazon
Rapid detection of MDR-TB

- Things to consider before implementing new techniques:
  - Rapid delivery of specimens to point of testing
  - Rapid reporting system
  - QA and QC
Methods

• Ultra rapid (1-2 days) INNO – LiPA Rif TB, GenoType MTBDR; FastPlaque – Rif resistance detection, should be smear positive specimens

• Rapid (1–2 weeks) adaptations of conventional microbiology methods – direct BACTEC, quad (direct) method on 7H10 agar, MODS, Griess (Nitrat RA) method on modified LJ
Time to MDR detection

Ultra rapid methods

Rapid methods

indirect BACTEC

indirect uz LJ
Molecular DST

- No single mutation in *M. tuberculosis* results in resistance to >1 first line drug
- No single genetic alteration has yet been found that results in the MDR phenotype
- The differing locations for the mutations have made detection of MDR by molecular methods more challenging
- Resistance to certain drugs can involve a mutation in one or several possible genes or gene complex
Mission

• **FIND** is a leading non-profit organization dedicated solely to the development of rapid, accurate and affordable diagnostic tests for poverty-related diseases in the developing world. Its mission is driven by the conviction that good health is central to winning the war against poverty and that correct diagnosis is a crucial first step towards establishing health and thus efficient use of resources.

Question for discussion?

• What laboratory diagnosis issues has been neglected?