Drug Resistance Surveys
Laboratory requirements and the role of the
TB SRL Network

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26th May 2011

MDR-TB Consultant Course
Lima Peru, 22-26 March 2010
Requirements for survey/surveillance:

- Focus on sputum smear positive cases
- Periodic surveys among new cases
- Continuous surveillance for retreatment cases
- Either phenotypic or genotypic tests could be used
- Drugs to be reported on: Rif, INH → if Rif resistant: FQL, 2nd-line injectables and ethambutol
Functions at the different levels of laboratory Services

- **Supranational Reference Labs**
  - Quality assurance DST
  - Reference methods for Culture and DST

- **Central (often National) Reference Laboratory**
  - Reference methods
  - First line DST
  - Network supervision

- **Regional Level**
  - Microscopy
  - Specimen processing
  - Training

- **District Level**
  - AFB microscopy

- **Peripheral Level**
<table>
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<tr>
<th>RISK GROUP CLASSIFICATION</th>
<th>WORLD HEALTH ORGANIZATION LABORATORY BIOSAFETY MANUAL 3RD EDITION 2004*</th>
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</table>
| Risk Group 1              | (No or low individual and community risk)  
A microorganism that is unlikely to cause human or animal disease. | BSL 1 |
| Risk Group 2              | (Moderate individual risk; low community risk)  
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              | (High individual risk; low community risk)  
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              | (High individual and community risk)  
A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. | |

Where does TB fit?  
? BSL 2  
? BSL 3  
? BSL 4
New approaches are based on Bio-Risk Assessment

- Pathogenicity of the infectious agent
- Route of transmission
- Agent stability and infectious dose
- Concentration of agent
- Type of laboratory procedures to be done
- Availability of effective prophylaxis or therapy
- Skill level and vulnerability of at-risk personnel
Biosafety Guidance:
Manipulating cultures for smear preparation, identification tests, DST, or molecular tests

High risk of generating infectious aerosols during manipulation of liquid suspensions

– Work done in a containment lab which has restricted access and a double door entry

• Impermeable surfaces for easy cleaning
  – Sealing room for fumigation is not required

• Air flows into lab without re-circulation to non-lab areas (directional airflow)
  – 6-12 ACH, mechanical ventilation, sealed windows

• Autoclave available on site
Biosafety Guidance: Essential requirements

- Double door airlock
- Separate air inlet
- Venting of BSC via thimble
- Aerosol containment
- Negative pressure monitoring
- Uni-directional airflow
- PPE
- Autoclave for waste disposal
Mycobacterial Culture (1)

- Specimen processing is critical
  - Transport delay, harsh or inadequate decontamination, poor quality culture media, incorrect incubation temperatures
  - Less important in a DRS
- Laboratory errors
  - Mislabelling, cross contamination
- Liquid culture vs solid media
  - Contamination
  - Time to detection
- Culture essential for performing second-line DST to confirm or exclude XDR-TB
Liquid Culture
• Bacterial contamination rates and recovery rates of NTM need to be analysed separately.

• Contamination rates should be recorded for both liquid and solid media.
  – Bacterial contamination rates between 8-10 per cent are acceptable for liquid culture.
  – Contamination rates between 2-5% are acceptable for solid media.

• Suspicions of laboratory cross-contamination events should be investigated.

• Isolated positive cultures need to be correlated with clinical findings.
Identification of *M. tuberculosis*

- In settings with high burden of TB, most mycobacterial isolates will be MTB.

- Prevalence of NTM varies between countries and use of liquid culture increases NTM isolation.

- Confirmatory ID is important as mycobacteria showing resistance to first line drugs may be NTM and not MTB.

- Minimum requirements:
  - Must have capacity to confirm ID by biochemical ID, or recognised method e.g. capillia /molecular
Drug susceptibility testing

• Phenotypic methods
  – In **direct testing**, a set of drug-containing and drug-free media is inoculated directly with a concentrated specimen.
  – **Indirect testing** involves inoculation of drug-containing media with a pure culture grown from the original specimen.

• Genotypic methods
  – Detect mutations in **rpoB** gene for resistance to rifampicin and a proxy for MDR-TB.

• Gold standard
  – Proportion (solid or liquid), absolute concentration, and resistance ratio.
  – **DST results do not differ between the three methods for first-line anti-TB drugs**.
Line probe assays

Detects resistance to both rifampicin and isoniazid
Labelled for use on AFB smear positive processed sputum specimens and positive cultures
First-line DST

- The minimum pre-requisite for a DR-TB control programme is for quality assured DST of isoniazid and rifampicin.

- The accuracy of DST (performed under optimal circumstances) varies with the drug tested.

- For first-line anti-TB drugs, DST is most accurate for rifampicin and isoniazid and less reliable and reproducible for streptomycin, ethambutol and pyrazinamide

- Rapid rifampicin resistance testing (as a proxy for MDR-TB) is recommended in high-risk MDR-TB settings
Second-Line DST

• Needed to confirm or exclude XDR-TB among cases of MDR.

• MUST have a cultured isolated to perform SL-DST

• Recommend testing of aminoglycosides, polypeptides and fluoroquinolones only.

• Consensus for testing SLD relies on broth or liquid methods and the proportion method on solid media.
Limitations of DST

- Accuracy varies with drug tested
  - Aminoglycosides, polypeptides, fluoroquinolones are reliable and reproducible testing.
- Other drugs are less reliable
  - In vitro drug stability,
  - Critical concentrations close to MIC
- Not all mutations conferring resistance to SLD have been elucidated.
- Cross resistance considerations
  - Limited data on aminoglycosides and polypeptides
  - High cross resistance between AK and K
  - Cross resistance between later generation of fluoroquinolones
    ? vice versa
- QA from an SRL is essential
Role of the SRLN?

- The SRL network should provide technical assistance to countries in establishing national policy on culture and DST
- Build a core group of skilled laboratory personnel
- Implement quality assurance mechanisms for smear microscopy, culture and Drug Susceptibility Testing (DST)
- Assist with implementation of new tools
- Ensure regular Drug Resistance Surveys (DRS)
- Provide laboratory support for MDR-TB diagnosis and treatment monitoring.
- Assist countries with operational research
• The Lot Quality Assurance Scheme method is the recommended method for performing quality assurance of AFB microscopy.

• The widely used system of sampling 100% of positive smears and 10% of negative smears is no longer recommended for the following reasons:

• All stained smears need to be stored in slide boxes in the order they were recorded in the laboratory register or database.

• Each month determine the total number of negative smears examined and the calculated sample size is adjusted, or increased proportional to the positivity rate to yield a sample size of positive and negative smears.

• Slides are collected from the entire lot of slides irrespective or whether the result was positive or negative

• Smears are re-examined in a blinded fashion. (re-stained for FM)
• If any discordant results are found it represents a problem which needs to be investigated.
• 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%
Performance Indicators

- 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
Key messages

- Laboratories must develop DST proficiency for INH and Rif as a minimum.
- Laboratories should develop DST capacity for fluoroquinolones and second-line injectables.
- Appropriate biosafety measures needed for the different levels of testing.
- Quality assurance is critical.