Implementation of new diagnostic approaches and methods: operational considerations

Servicio Micobacterias
Instituto Nacional de Enfermedades Infecciosas
ANLIS “Dr Carlos G. Malbran”
Argentina

WHO/IUATLD Supranational Reference Laboratory Coordinator of Latin American Supranational Laboratory Network
LED microscopy

MDR TB rapid screening by
- Nitrate reductase assay (NRA)
- Colorimetric redox indicators (CRI)
- Line probe molecular assays (LPA)

Rapid culture and drug susceptibility testing (DST) in (automated) liquid culture
Adoption and selection of methods should be based on the analysis of initial investment but also on:

- existing resources and organization of the laboratory network
- users acceptance and technical know-how
- long-term guarantee of:
  - basic logistics
  - recurrent budgeting
  - maintenance of laboratory infrastructure/biosafety/equipment
  - quality of laboratory results
Long-term interventions and thus long-term sustainable diagnostics are needed to fight tuberculosis.

Long-term sustainability of new diagnostics has yet to be demonstrated in tuberculosis endemic areas.

In the meantime, fast growth of rapid diagnosis demand should be satisfied following a careful risk-benefit analysis of alternative approaches.
LED microscopy

Laboratory infrastructure/Biosafety requirements

The technology does not originate novel risks and can be employed in the biosafety level required for conventional microscopy.
<table>
<thead>
<tr>
<th>Smear microscopy</th>
<th>Open bench, separated from other benches in an laboratory with restricted access and adequate ventilation (open windows and a fan or a mechanical systems that provide inward flow of air without recirculation in the room)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>If an autoclave is not available decontamination may be performed in a pressure cooker or by incineration</td>
</tr>
</tbody>
</table>
LED microscopy

Equipment

LED microscopes /attachments are available from several manufacturers that have already created a commercial space in the developing world.

Gradual incorporation of this shared platform seems to be feasible during the processes of:

- continual renovation of microscopes/accessories
- expansion of microscopy capacity

Performance of LED modules depends on quality of the microscopes to which they are attached.
LED microscopy

Supplies

Reagents are easy to procure.

Quality of auramine is less variable than that of fucshin.

Stability of auramine solutions under field conditions has been questioned and should be further evaluated.
LED microscopy

Intensive training and QA activities are essential requirements


technicians are generally unfamiliar with fluorescence microscopy in developing countries

Global guidance for fluorescence microscopy EQA is still required

Challenges

Instability of stained smears upon prolonged storage
Slides restraining practices
Rapid DST

MDR TB rapid screening by
- Nitrate reductase assay (NRA)
- Colorimetric redox indicators (CRI)
- Line probe molecular assays (LPAs)

Rapid first and second line DST in (automated) liquid culture
Addressing common barriers for rapid DST implementation
Poor basic logistics connecting the DST laboratory to the health system

Availability and use of proper forms to identify patients that will benefit from the new test

Appropriate shippers and labeling

Regularly scheduled transportation of specimens/isolates

Contamination resulting from delays is relatively more critical for methods employing liquid media

Reduced bacilli viability result in invalid or even false results

Telecommunication systems and information networking
Laboratory infrastructure / biosafety

Improvement is needed in most laboratories performing culture and/or DST in developing countries
Guidance on Bio-safety related to TB laboratory diagnostic procedures
Geneva, Switzerland 8-9 April 2009

## Processing

### Specimen manipulation for microscopy and culture and/or LPAs DST
- Administrative & physical separation
- Restricted access
- Impervious surfaces
- Class I or IIA BSC (regularly maintained/re-certified, UPS and backup generator)
- Closed windows
- No air re-recirculation to other areas
- On site autoclave
- Centrifuge with aerosol tight buckets

### Post-culture for ID and DST
- Plus
  - Containment (double door entry)
  - BSCs vented outside (preferred or necessary if there is no other system to create directional airflow)

### LPAs ID+DST
- at least other 3 separate rooms with restricted access
Lack of policy and programme for health surveillance of laboratory personnel

Baseline assessment (tuberculin skin test)
Medical history
Monitoring of tuberculin skin reaction, respiratory signs & symptoms

Incident and accident response

chemoprophylaxis
appropriate medical investigations

Response after accidents with MDR or XDR strains?
Established beliefs

there are no urgencies associated to tuberculosis
Inexorably DST results require at least 4 months since specimen is collected

capacitation programmes should address not only technical issues but also basic topics such as

- distinction of urgent cases/specimens/isolates
- translation of delays introduced by batching, infrequent inspection of cultures and late communication of results to patient outcome
Choosing methods and the appropriate response in a particular scenario
If sustainability of high standards of biosafety is not ensured, the use of methods using liquid culture and, eventually, microplates, should not be encouraged.

High risk of aerosol creation

Eventual spillage
while handling repeatedly microtiter plates that are not tightly sealed
### Equipment

Basic equipment for reagent preparation and specimen processing (water distiller, refrigerators, freezer, electronic balance, vortex mixer, centrifuge, BSCs, UPS, autoclave)

<table>
<thead>
<tr>
<th>In-house methods</th>
<th>Commercial methods</th>
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<tbody>
<tr>
<td>NRA</td>
<td>Liquid culture systems</td>
</tr>
<tr>
<td>Incubator</td>
<td>Automated system for incubation and growth detection</td>
</tr>
<tr>
<td>Inspissator or 80 °C incubator (for media preparation)</td>
<td>Thuopure nuclease-free water systems</td>
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<tr>
<td></td>
<td>Thermal cycler</td>
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<tr>
<td></td>
<td>Sonicator</td>
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<tr>
<td></td>
<td>Water bath</td>
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<tr>
<td></td>
<td>Heating block</td>
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<tr>
<td></td>
<td>Microcentrifuge</td>
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<td></td>
<td>Micropipettes</td>
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<td></td>
<td>DNA free hood</td>
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<td>CRI</td>
<td>LPAs</td>
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<td>Incubator</td>
<td>Ultrapure nuclease-free water systems</td>
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<td>Multi &amp; uni channel micropipettes</td>
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Equipment available in labs performing conventional culture on solid media
No dependency on proprietary equipment/software
Approaches to increase DST capacity
(according to population, MDR-TB burden, geographical extension, accessibility)

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Optional methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>One central laboratory</td>
<td>Training and QA are simpler</td>
<td>NRL may be overburdened</td>
<td>(Automated) liquid culture-based methods (High cost)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Results may be delayed</td>
<td>CRI (intermediate cost)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>An efficient transportation system is required</td>
<td></td>
</tr>
<tr>
<td>Several selected intermediate laboratories</td>
<td>NRL may be focused on more demanding activities</td>
<td>Training and QA are complex</td>
<td>LPAs Faster option (High cost)</td>
</tr>
<tr>
<td></td>
<td>Wider and faster MDR TB detection</td>
<td></td>
<td>NRA (Low cost)</td>
</tr>
</tbody>
</table>
Screening of MDR-TB by rapid methods does not eliminate the need of standard DST.

Screening of MDR-TB should diminish hands-on time in laboratory networks and running costs (if performed by an inexpensive method) compared to standard DST offered to all patients at risk of drug resistance.
Supply logistics

NRA

Reagents and consumables are nonproprietary products relatively cheap locally available from several manufacturers/suppliers.

All chemicals have long shelf-life, even in solution and are stable to temperature fluctuations during transportation and storage (except for antibiotics).

Centralization of procurement and preparation of media/reagents is possible and may be convenient when NRA is performed at the intermediate level of the network. Refrigeration is required for delivery.

Re-usable glass-tubes may be employed.
Supply logistics
(Automated) liquid culture-based tests and LPAs

Reagents and consumables may be not available in scenarios performing conventional methods on egg-based media, but they should be easy to obtain from local suppliers (some difficulties with NALC and alamar blue).

Media and reagents procurement create dependence on one manufacturer and probably on one local supplier.

Some antibiotics and media enrichment requires refrigeration during transportation.

All chemicals have long shelf-life.
Authorization

Importation and implementation of commercial tests require approval/ registration & licensing processes.

In many developing countries, these processes are not required for the introduction of in-house assays if they are endorsed by the NTP and the NRL.
## Human Resources

<table>
<thead>
<tr>
<th>Support</th>
<th>NRA</th>
<th>Liquid culture systems</th>
<th>LPAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemicals procurement</td>
<td>once a year</td>
<td>recurrent</td>
<td>recurrent</td>
</tr>
<tr>
<td>Media &amp; reagent preparation</td>
<td>yes, permanent</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Training and experience</td>
<td>Culture &amp; DST in solid media</td>
<td>Culture &amp; DST in liquid media</td>
<td>Specimen processing Molecular testing</td>
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</tbody>
</table>
Relative running costs  (labour and capital costs not included)

Higher               LPAs   MGIT> CRI> NRA   Lower
faster option         similar time to produce results

These assay characteristics are critical in health institutions where:

- Budgets are extremely low
- Cost of reagents may be 2-5 higher than catalog price
- Procurement is unreliable and unstructured leading to stock-outs of key supplies
- Support for importation does not exist or is inefficient
- Local distributors may be reluctant to license/import new items especially in markets that are not attractive
- Shipping and customs logistics are complex and time-consuming
- Special storage conditions are not available
Technical considerations

Commercial tests employ simpler procedures

- Reagents/media are almost ready-to-use

- LPAs sample preparation is simple
  
  culture-based methods require suspension dilutions

- Reading of MGIT tubes is simple
  
  in house culture-based methods require repeated addition of reagents

  LPAs require meticulous interpretation
Technical considerations

Introduction of very sensitive methods
(culture in liquid media/molecular tests)

may result in high frequency of (cross) contamination
and even misdiagnosis in some scenarios
(poor experience/implementation/
manipulation of high proportion of positive specimens)

NRA is very easy to implement in scenarios
using the proportion method on LJ and the nitratase test
Technical considerations

Which is the best low-cost and rapid ID method to complement rapid culture-based tests in low-resourced laboratories?

Are commercial lateral flow tests for speciation of *M tuberculosis* applicable to complement in-house assays?

Are they affordable in low-resource scenarios?

Does an additional tube containing PNB work?
QC/QA

Internal quality control of drug-containing media and reagents

(Automated ) liquid media systems and CRI

Rigorous IQC is costly and impractical
(media is prepared for each test
plate-to-plate/ tube-to-tube variation may occur )

NRA LPAs

Daily IQC tests may not be necessary
Testing standard drug-sensitive/resistant strains
with each new batch of media/reagents may be sufficient
External quality control

No particular procedures need to be introduced at country or supranational level to evaluate regularly the competence of laboratories performing new culture-based methods.

Special panels of strains carrying different mutations are to be used for LPAs proficiency testing.
Global guidance and support for the introduction of rapid DST would be helpful

Guidance for matching the choice of an appropriate method with strategies of NTP resources that are available and/or sustainable (even after withdrawal of donors support)
laboratory level
personnel skills

SOPs development
Inclusion of the newly endorsed assays in the WHO training package for culture and DST of tubercle bacilli

Development of standardized protocols for validation at country level

EQA through on-going SRLs PT exercises
Additional operational research would be helpful

Comparative evaluation of alternative tests for rapid screening of MDR-TB

- Implementation and labour costs
- Feasibility of implementation in settings without DST and culture facilities
- Cost-effectiveness, impact on patient management
- Long-term sustainability

(under/ out of the umbrella of demonstration projects)