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 aboratory 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

Guidance on Bio-safety related to TB laboratory diagnostic procedures
Geneva, Switzerland 8-9 April 2009

Smear microscopy	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) If an autoclave is not available decontamination may be performed in a pressure cooker or by incineration

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

Van Deum A et al. Int J Tuberc Lung Dis 12:1009-14. 2008

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 form 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

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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	LPAs ID+DST
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)	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)

In-house methods		Commercial methods	
NRA	CRI	Liquid culture systems	LPAs
Incubator Inspissator or 80 ℃ incubator (for media preparation)	Incubator Multi & uni channel micropipettes	Automated system for incubation and growth detection	Ultrapure nuclease-free water systems Thermal cycler Sonicator Water bath Heating block Microcentrifuge Micropipettes DNA free hood

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)

	Advantages	Disadvantages	Optional methods
One central laboratory	Training and QA are simpler	NRL may be overburdened Results may be delayed An efficient transportation system is required	(Automated) liquid culture-based methods (High cost) CRI (intermediate cost)
Several selected intermediate laboratories	NRL may be focused on more demanding activities Wider and faster MDR TB detection	Training and QA are complex	LPAs Faster option (High cost) NRA (Low cost)

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

Support	NRA	Liquid culture systems CRI	LPAs
Chemicals procurement	once a year	recurrent	recurrent
Media & reagent preparation	yes, permanent	no	no
Training and experience	Culture & DST in solid media	Culture & DST in liquid media	Specimen processing Molecular testing

Relative running costs (labour and capital costs not included)

Higher LPAs MGIT> CRI> NRA Lower

faster option similar time to produce results

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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

QC/QA

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)