Framework for Implementing New Tuberculosis Diagnostics

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Executive Summary

Care of patients with tuberculosis (TB) starts with a quality assured diagnosis. Research on new TB diagnostic tools has been accelerated over the last few years and the diagnostic pipeline has been growing rapidly as a result. At the same time, an unprecedented effort to improve and expand TB laboratory capacity currently being lead by WHO and the Stop TB Partnership Global Laboratory Initiative (GLI) together with the GLI network of international collaborators (http://www.stoptb.org/wg/gli).

Uptake of TB diagnostic technologies requires appropriate laboratory infrastructure and adequate policy reform at country level to enable their effective use in TB screening and diagnostic algorithms. WHO has established a structured, systematic process to rapidly review the evidence base for new TB diagnostics, ensuring that new tools meet the required performance standards. Nevertheless, even the best tools are bound to fail if all core elements of laboratory services are not addressed at the same time. These include:

- Laboratory infrastructure, appropriate biosafety measures and maintenance;
- Equipment validation and maintenance;
- Specimen transport and referral mechanisms;
- Management of laboratory commodities and supplies;
- Laboratory information and data management systems;
- Laboratory quality management systems;
- Appropriate, adequate strategies and funding for laboratory human resource development.

Several GLI resources are available to facilitate this process, including the GLI Roadmap for Strengthening TB Laboratory Services within the context of national strategic plans for laboratory services. The specialised nature of TB technical procedures, the need for adequate biosafety, appropriate laboratory management and administration, and the need to ensure laboratory quality require different levels of laboratory testing, with clear specimen referral mechanisms.

Because of the complexities of TB laboratory strengthening, the involvement of an expert laboratory consultant is recommended to guide the implementation process at country level. This document provides the policy framework for such implementation, outlining current TB technologies endorsed by WHO and their place at different levels of tiered laboratory services. It explains the advantages and disadvantages of available tests and methods, as well as the biosafety and laboratory infrastructure requirements.

Lastly, the document provides WHO-recommended diagnostic algorithms using different new TB technologies and outlines the time to diagnosis of multidrug-resistant and extensively drug resistant TB using different testing modalities.
Framework for Implementing New Tuberculosis Diagnostics

Introduction

Care of patients with tuberculosis (TB) starts with a quality assured diagnosis. Successful DOTS expansion, as well as programmatic management of drug-resistant and HIV-associated TB therefore require - at its core - a robust network of TB laboratories with adequate biosafety, modern methods for diagnosis, standard operating procedures and appropriate quality assurance.

Arguably the weakest component of health systems, laboratory services have historically been grossly neglected, under-staffed and underfunded. Diagnostic capacity is therefore a major bottleneck for scaling up management and control of drug-resistant and HIV-associated TB, largely as a result of:

- Slow policy change and technology transfer, especially in low- and middle-income countries;
- Insufficient and underfunded laboratory strengthening plans;
- Inadequate laboratory infrastructure and biosafety;
- Vastly inadequate numbers of skilled staff;
- Insufficient technical assistance.

Strengthening TB laboratory services offer one of the best avenues for overall laboratory improvement as an essential health systems activity. Fundamental to this activity is collaboration between TB control programmes and public health laboratory services at country level, as adequate laboratory capacity consists of several essential elements which need to be addressed simultaneously, within comprehensive strategies and national laboratory strengthening plans.

An unprecedented effort to improve and expand TB laboratory capacity is currently under-way, spearheaded by the WHO and Stop TB Partnership Global Laboratory Initiative (GLI) and its network of international collaborators (http://www.stoptb.org/wg/gli). At the same time research on new TB diagnostic tools has been accelerated and the diagnostic pipeline is now rapidly growing.¹

Robust, point-of-care diagnostic tests for TB are not expected before 2015; therefore, uptake of existing WHO-recommended technologies must be accelerated, which requires adequate laboratory infrastructure and clear policies at country level on their use in TB screening and diagnostic algorithms. Because of the complexity of laboratory strengthening, the involvement of an expert laboratory consultant is recommended to guide the implementation process at country level.

WHO policy on new technologies is evidence-driven

The landscape of new TB diagnostics is changing rapidly. Policy formulation therefore needs to be a dynamic and ongoing process, both at global and country level. New TB technologies are

regularly assessed by WHO with a view towards rapid policy development, using a systematic, structured process:\(^2\)

### WHO policy formulation process

- **Identifying the need for policy change**
  - The need to formulate new or revised policies may arise from WHO’s ongoing monitoring of technical developments or from interested parties submitting requests with supporting documentation for policy or guideline development. WHO receives information about a new technology or approach via many channels, with the most direct lines coming from national TB programs and researchers themselves. To consider a global policy change, WHO must have solid evidence, including clinical trials or field evaluations in high TB prevalence settings.

- **Reviewing the evidence**
  - WHO may carry out or commission a review of the documentation of the technology’s clinical or programmatic performance, including newly published and “grey” research or reviews, “proof of principle” reports, large-scale field trials, and demonstration projects in different resource settings. Standardized evaluation criteria have been and are being developed by the New Diagnostics, New Drugs, and New Vaccines Working Groups of the Stop-TB Partnership.

- **Convening an Expert Panel**
  - If the evidence base is compelling, WHO will convene an external panel of experts, excluding all original principal investigators from the studies. The panel will review the evidence and make a recommendation or propose draft policies or guidelines to WHO’s Strategic and Technical Advisory Group for Tuberculosis (STAG-TB).

- **Assessing draft policy and guidelines**
  - STAG-TB provides objective, ongoing technical and strategic advice to WHO on TB care and control. STAG-TB’s objectives are to provide the Director-General, through the Stop TB Department, with an independent evaluation of the strategic, scientific, and technical aspects of WHO’s TB activities; review progress and challenges in WHO’s TB-related core functions; review and make recommendations on committees and working groups; and make recommendations on WHO’s TB activity priorities. STAG-TBreviews the policy drafts and supporting documentation during its annual meeting. STAG-TB may endorse the policy recommendation with or without revisions, request additional information and re-review the evidence in subsequent years, or reject the recommendation.

- **Formulating and disseminating policy**
  - New WHO policies and guidelines will be disseminated through different channels to Member States, including through the World Health Assembly, WHO Web site, listener, and journal publications. WHO also disseminates its recommendations to other agencies and donors engaged in TB control activities.

In accordance with current WHO standards for evidence in policy recommendations, the GRADE system (http://www.gradeworkinggroup.org), is used to assess the findings of Expert Groups. The GRADE approach provides a systematic, structured framework for evaluating both the accuracy and the patient/public health impact of new interventions.

Expert Group findings and final GRADE evaluations are presented to the WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB), an independent advisory body to WHO (http://www.who.int/tb/advisory_bodies/stag/en/index.html). STAG-TB endorsement is followed by detailed WHO policy recommendations.\(^3\) A summary of currently recommended WHO technologies/methods is provided in Annex 1.

### Technologies must be used in appropriate laboratory services

Establishing, equipping and maintaining laboratory networks are challenging, complex and expensive. Introducing new technologies is bound to fail if all core elements of laboratory services are not addressed at the same time. These include:

- Laboratory infrastructure, appropriate biosafety measures and maintenance;
- Equipment validation and maintenance;

\(^2\) World Health Organization. *Moving research findings into new WHO policies.* Available at: http://www.who.int/tb/dots/laboratory/policy/en
• Specimen transport and referral mechanisms;
• Management of laboratory commodities and supplies;
• Laboratory information and data management systems;
• Laboratory quality management systems;
• Appropriate, adequate strategies and funding for laboratory human resource development.

The GLI has developed a Roadmap for TB laboratory strengthening aimed at ensuring quality TB diagnostics in appropriately laboratory services within the context of national laboratory strategic plans, available at http://www.who.int/tb/dots/laboratory/policy/en.

Laboratory biosafety

*M. tuberculosis* is classified as a Risk Group 3 pathogen but handling of specimens poses different risks based on the methods employed. WHO and the US Centers for Disease Control have therefore convened an Expert Group to define consensus on minimum requirements for laboratory biosafety using a risk-based approach for different TB laboratory procedures. Draft policy guidance and a Manual for TB Laboratory Biosafety are currently under peer review and final documents are expected in late 2010.

Using a risk based assessment of different technical procedures performed in a TB laboratory permitted the development of a set of minimum requirements for laboratory facilities. The risk assessment approach considers the bacillary load of materials (specimens, cultures), the viability of bacilli, whether the material handled is prone to generate aerosols, the number of manoeuvres generating infectious aerosols with each technique, the workload of the laboratory, the epidemiological characteristics of patients, and the medical fitness of the laboratory workers. A summary of relative risks follows below:

Preparing direct smears for AFB microscopy (low TB risk precautions)

Minimum requirements

- Adequate ventilation*;
- Laboratory separated from other areas;
- Access to the laboratory restricted to authorized persons;
- The bench for smear-preparation separated from other work benches in the laboratory.

*Adequate ventilation can be ensured by opening windows if local climatic conditions allow. An exhaust fan can be used to ensure adequate room air changes. When climatic conditions prevent window opening, consideration should be given to mechanical ventilation systems that provide an inward flow of air without recirculation in the room.*

Processing sputum specimens for primary culture inoculation, direct nitrate reductase assays (NRA), direct MODS or direct line-probe assays (LPA) (moderate TB risk precautions)

Minimum requirements

- Laboratory separated from other areas;
- Access to the laboratory restricted to authorized persons;

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• Floors, walls, ceilings and benches and furniture with have impervious surfaces;
• Windows permanently closed. Air supply either passive or mechanical without recirculation;
• Centrifuge with aerosol tight buckets;
• Handling of specimens in appropriate biological safety cabinets (BSC), class I (EN12469/NSF49) or Class IIA2 (NSF49) or Class II (EN12469) equipped with HEPA filters H14;
• BSCs designed by certified manufacturers, properly installed, regularly maintained and re-certified at least annually on site;
• Controlled ventilation system that maintains a directional airflow into the laboratory from functionally clean to dirty areas, with a minimum of 6 up to 12 air changes per hour*.

*Installation of a controlled ventilation system should be planned with engineering specialists.

Manipulating cultures for identification and drug-susceptibility testing (DST) with indirect phenotypic methods and/or line probe assays (high TB risk precautions)

Minimum requirements:
• Meeting ALL requirements for abovementioned tests, and in addition:
• Containment laboratory with double door entry;
• Autoclave available on site and in close vicinity of the laboratory, for safe waste disposal.

Technologies are suitable for different laboratory service levels

The specialised nature of technical procedures, laboratory management and administration, and ensuring laboratory quality require different levels of laboratory testing, with clear specimen referral mechanisms.

Conventional tiered laboratory services for TB diagnosis are described in many resource documents.4 Three main laboratory service levels are common to the majority of countries:

• Peripheral (typically district) level: Performing sputum smear microscopy; referring specimens or patients in need of further tests to higher level laboratories.
• Intermediate (typically regional) level: Performing smear microscopy and conventional culture, with or without species identification and first-line drug susceptibility testing (DST); referring cultures in need of further tests (eg. second-line DST) to higher level laboratories.
• Central (typically national or reference) level: Performing sputum smear microscopy, conventional and rapid culture and DST, and molecular tests; referring isolates in need of further tests (eg. second-line DST or molecular sequencing) to Supranational Reference Laboratories in other countries or regions.

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Currently recommended TB tests and technologies fit into the following tiered system:

**Technologies must be used in appropriate case finding strategies**

Laboratory/diagnostic algorithms should start with appropriate screening policies to identify persons suspected of having TB, using microscopy services as the entry point. Current WHO case-finding strategies recommend screening of all persons with a cough of longer than two weeks.

Good quality microscopy of two sputum specimens identifies the vast majority (95% - 98%) of smear-positive TB patients. Current WHO policy on case finding by microscopy therefore recommends that two specimens be examined in settings with appropriate external quality assurance and documented good quality of microscopy. The case definition in such settings is defined as one positive smear, ie. one or more acid-fast bacillus in at least 100 microscopic fields [http://www.who.int/tb/dots/laboratory/policy/eng](http://www.who.int/tb/dots/laboratory/policy/eng).
Recent WHO policy guidance confirmed the diagnostic accuracy of examining two consecutive smears in a ‘Same-day-diagnosis’ approach, which allows treatment to be started during the first visit of patients to health services.5

Case finding strategies for drug-resistant TB have been published6 and the role of drug susceptibility testing (DST) in identifying MDR- and XDR-TB patients outlined.7 Detection of HIV-associated and drug-resistant TB requires appropriate laboratory diagnostic algorithms based on patient groups at greatest risk to allow cost-effective use of scarce laboratory and diagnostic resources. Such algorithms are highly country-specific and depend on several factors (see below).

WHO-recommended technologies

MICROSCOPY

Mycobacteria are distinguished from other micro-organisms by thick lipid-containing cell-walls that retain biochemical stains despite decolourisation by acid-containing reagents (so-called 'acid-fastness').

Advantages: Microscopy of sputum smears is simple and inexpensive, quickly detecting infectious cases of pulmonary TB; Sputum specimens from patients with pulmonary TB - especially those with cavitary disease - often contain sufficiently large numbers of acid-fast bacilli to be readily detected by microscopy.

Disadvantages: Direct smear microscopy is relatively insensitive as at least 5,000 bacilli per millilitre of sputum are required for direct microscopy to be positive. Smear sensitivity is further reduced in patients with extra-pulmonary TB, those with HIV-co-infection, and those with disease due to nontuberculous mycobacteria (NTM).

Limitations: Microscopy for acid-fast bacilli (AFB) cannot distinguish Mycobacterium tuberculosis from nontuberculous mycobacteria (NTM)s, nor viable from non-viable organisms, or drug-susceptible from drug-resistant strains.

Conventional light microscopy

Ziehl-Neelsen (ZN) light microscopy performed directly on sputum specimens is suitable for all laboratory service levels, including peripheral laboratories at primary health care centres or districts hospitals.

There is not sufficient evidence that processed (eg. concentrated or chemically treated) sputum specimens provide superior results to direct smear microscopy. Implementation of such methods in programmatic settings is therefore not recommended.


The number of ZN smears examined per microscopist per day should not exceed 20 as visual fatigue leads to a deterioration of reading quality; on the other hand, proficiency in reading ZN smears can only be maintained by examining at least 10-15 ZN smears per week.\(^8\)

In general, one ZN microscopy centre per 100,000 population is sufficient; however, expansion of ZN microscopy services should also take into account the location and utilisation of existing services, urban/rural population distribution, and specimen transport mechanisms.

**Conventional fluorescent microscopy**

Conventional fluorescence microscopy typically uses quartz-halogen or high-pressure mercury vapour lamps as light sources. A lower magnification objective is used to scan smears, allowing a much larger area of the smear to be seen and therefore taking less time than ZN microscopy.

Conventional fluorescence microscopy is on average 10% more sensitive than ZN microscopy, but requires considerable technical expertise. Capital and running costs are also considerably higher. Conventional fluorescent microscopy has therefore been recommended by WHO at intermediate laboratory level where more than 100 smears are examined per day.\(^9\)

**Light-emitting diode (LED) fluorescent microscopy**

LED technology allows the use of fluorescent microscopy with a much less expensive light source. LED microscopes or -attachments require less power, are able to run on batteries, the bulbs have a very long half-life and do not release potentially toxic products if broken.

Recent WHO evaluation confirmed the diagnostic accuracy of LED microscopy compared to conventional fluorescent microscopy, and superior efficiency of LED over conventional ZN microscopy. It is therefore recommended that conventional fluorescence microscopy be replaced by LED microscopy and that LED microscopy be phased in as an alternative for conventional ZN light microscopy in both high- and low-volume laboratories.\(^10\)

**CULTURE AND SPECIES IDENTIFICATION**

**Advantages:** Mycobacterial culture and identification of *M. tuberculosis* provide a definitive diagnosis of TB, significantly increases the number of cases found (often by 30-50%), and can detect cases earlier (often before they become infectious). Culture also provides the necessary isolates for conventional DST.

**Disadvantages:** Culture is much more complex and expensive than microscopy to perform, requiring facilities for media preparation, specimen processing, growth of organisms, specific laboratory equipment, skilled laboratory technicians, and appropriate biosafety conditions.

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Limitations: Specimens have to be decontaminated prior to being cultured to prevent overgrowth by other micro-organisms. All decontamination methods are to some extent also harmful to mycobacteria, and culture is therefore not 100% sensitive. Good laboratory practices maintain a delicate balance between yield of mycobacteria and contamination by other micro-organisms.

Solid and liquid culture methods are suitable for central/national reference laboratories (or regional laboratories in large countries). Usually, one culture laboratory is adequate to cover 500,000 - 1 million population. Solid culture methods are less expensive than liquid culture systems, but results are invariably delayed due to the slow growth of mycobacteria. Several culture methods are recommended. Liquid culture increases the case yield by 10% over solid media, and automated systems reduce the diagnostic delay to days rather than weeks. Liquid systems are, however, more prone to contamination and the manipulation of large volumes of infectious material mandates appropriate and adequate biosafety measures.

Positive cultures have to be identified to differentiate \textit{M. tuberculosis} from NTM. NTM are more common in HIV-infected patients and the prevalence varies from country to country. Treatment of NTM is entirely different from treatment of drug-resistant TB. As a minimum, laboratories performing DST must differentiate \textit{M. tuberculosis} from other NTM (further speciation is not recommended at programmatic level).

Confirmation is usually done by a combination of biological characteristics of the culture growth and selected molecular or biochemical tests (which invariably delay the final result). Rapid immunochromatographic assays (so-called strip speciation tests) for species identification on culture isolates provide a definitive identification of \textit{M. tuberculosis} in 15 minutes and are recommended. Molecular tests, biochemical methods and strip speciation assays are suitable for laboratories where culture and DST are performed.

**DRUG SUSCEPTIBILITY TESTING**

Advantages: DST provides a definitive diagnosis of drug-resistant TB. A number of different DST techniques are available:

- Phenotypic methods involve culturing of \textit{M. tuberculosis} in the presence of anti-TB drugs to detect growth (indicating drug resistance) or inhibition of growth (indicating drug susceptibility).
- Genotypic methods target specific molecular mutations associated with resistance against individual drugs.

Phenotypic DST methods are performed as direct or indirect tests on solid or liquid media. 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.

Indirect phenotypic tests have been extensively validated and are currently regarded as the gold standard. Three methods are commonly used: proportion, absolute concentration, and resistance ratio. DST results do not differ significantly between the three methods for first-line anti-TB drugs.

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Disadvantages: DST methods are suitable for use at central/national reference laboratory level only, given the need for appropriate laboratory infrastructure (particularly biosafety) and the technical complexity of available technologies/methods.

Limitations: The accuracy of DST varies with the drug tested (see below).

For both first- and second-line DST, formal links with one of the laboratories in the Supranational Reference Laboratory (SRL) network is recommended to ensure adequate expert input on laboratory design, specimen and process flow, biosafety, standard operating procedures, maintenance of equipment and external quality assurance.

First-line DST

DST is most accurate for rifampicin and isoniazid and less reliable and reproducible for streptomycin, ethambutol and pyrazinamide.

As a minimum, national TB control programmes treating MDR-TB patients should establish laboratory capacity to detect MDR-TB. Rifampicin resistance is a valid and reliable indicator/proxy of MDR-TB.

Rapid DST is essential for identifying patients at risk of MDR-TB, as the first priority. Automated liquid systems and molecular line probe assays (see later) for first-line DST are recommended as the current gold standard.12

Once MDR-TB has been confirmed, additional first- and second-line drug susceptibility results should be obtained following current WHO recommendations.13

Second-line DST

Second-line DST is complex and expensive. Commercial liquid methods and the proportion method on solid medium have been studied; methods for the absolute concentration or resistance ratio on solid medium have not been validated. Automated liquid systems for second-line DST are recommended as the current gold standard.13

Routine second-line DST is not recommended unless the required laboratory infrastructure and capacity has been established, rigorous quality assurance is in place, and sustainable proficiency has been demonstrated.13 In order to retain proficiency and expertise, it is recommended that second-line DST only be performed if at least 200 specimens from high-risk patients are expected per year.

Aminoglycosides, polypeptides, and fluoroquinolones have been shown to have relatively good reliability and reproducibility, allowing a quality-assured diagnosis of XDR-TB.

Routine DST for other second-line drugs (ethionamide, prothionamide, cycloserine, terizidone, _P_-aminosalicylic acid, clofazimine, amoxicillin-clavulanate, clarithromycin, linezolid) is not recommended as reliability and reproducibility of laboratory testing cannot be guaranteed.

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**DST using non-commercial methods**

Non-commercial culture and DST methods are less expensive than commercial systems; however, non-commercial methods are prone to errors due to a lack of standardization and local variations in methodology. Performance of these methods is highly operator-dependent and good laboratory practice, good microbiological technique, and adequate quality assurance, supported by adequate training, are therefore imperative. As for commercial systems, stringent laboratory protocols, standard operating procedures, and internal quality control mechanisms must be implemented and enforced.

The evidence base for selected non-commercial culture and DST methods has been reviewed by WHO and the performance of these methods found to be acceptable under stringent laboratory protocols in reference/national laboratories in selected settings. These methods include microscopic observation of drug susceptibility (MODS), colorimetric redox indicator (CRI) methods, and the nitrate reductase assay (NRA). Recommendations for their respective use are:

- **MODS**: A microcolony method in liquid culture, based on inoculation of specimens to drug-free and drug-containing media followed by microscopic examination of early growth;
  - Recommended as direct or indirect tests, for rapid screening of patients suspected of having MDR-TB;

- **CRI methods**: Indirect testing methods based on the reduction of a coloured indicator added to liquid culture medium in a microtitre plate after *in vitro* exposure of *M. tuberculosis* strains to anti-TB drugs;
  - Recommended as indirect tests on *M. tuberculosis* isolates from patients suspected of having MDR-TB, and acknowledging that time to detection of MDR-TB is not faster (but less expensive) than conventional DST methods using commercial liquid culture or molecular line probe assays (see below);

- **NRA**: A direct and/or indirect method on solid culture based on the ability of *M. tuberculosis* to reduce nitrate, which is detected by a coloured reaction;
  - Recommended as direct or indirect tests, for screening of patients suspected of having MDR-TB, and acknowledging that time to detection of MDR-TB in indirect application is not faster than conventional DST methods using commercial liquid culture or molecular line probe assays (see below).

Both commercial and non-commercial culture and DST systems/methods are suitable for implementation at central/national reference laboratory level only.

**MOLECULAR TESTING**

*Advantages*: Genotypic methods have considerable advantages for scaling-up programmatic management of drug-resistant TB, in particular with regard to speed, standardised testing, potential for high throughput, and reduced biosafety needs.

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The ultimate aim should be to implement molecular assays (such as the line-probe assay or other WHO-endorsed molecular platforms in the future) for rapid first-step identification of MDR-TB.

Molecular line probe assays (LPAs) focused on rapid detection of rifampicin resistance (alone or in combination with isoniazid) have been endorsed by WHO in 2008, with detailed policy guidance on its introduction at country level.¹⁵

**Disadvantages:** LPAs do not eliminate the need for conventional culture and DST capability. Currently available LPAs are registered for use only on smear-positive sputum specimens *M. tuberculosis* isolates grown from smear-negative specimens by conventional culture methods.

**Limitations:** LPAs are suitable for implementation at central/national reference laboratory level, with potential for decentralisation to regional level if appropriate infrastructure can be ensured.

A summary of characteristics and laboratory requirements for currently recommended WHO technologies/methods is provided in Annex 2.

### Selecting appropriate algorithms and technologies/methods

1. Currently available technologies are not mutually exclusive. Molecular line probe assays and selected non-commercial culture and DST methods are suitable for direct application on smear-positive specimens only. Conventional culture capacity is still required for smear-negative specimens while conventional DST capacity is needed to detect XDR-TB;

2. Liquid culture and molecular line probe assays are regarded as international gold standards, to be phased in without loss of existing solid culture and DST capacity;

3. Rapid phenotypic DST methods present an interim solution, especially in resource-constrained settings, while capacity for genotypic testing is being developed;

4. Implementation of new technologies/methods for TB should be decided by Ministries of Health within the context of national strategic plans for laboratory strengthening and with input from laboratory experts;

5. TB diagnostic capacity should be linked to drug access and programmatic capacity to ensure treatment of patients under appropriate standards of care.

### Diagnostic testing algorithms

The prevalence of HIV and drug-resistant TB to a large extent dictates the use of laboratory policies and diagnostic algorithms at country level. Management of HIV-associated and drug-resistant TB also requires concurrent clinical laboratory capacity (eg. biochemistry, haematology, general microbiology) to monitor treatment and associated co-morbid conditions.

In high HIV-burden settings a substantial investment in culture capacity is required, given the absence of current tools to diagnose smear-negative TB. In high MDR-TB burden settings,

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laboratory diagnostic algorithms based on groups at greatest risk of drug-resistant TB (including those with HIV infection) is the most cost-effective use of scarce laboratory and diagnostic resources.

As a minimum, countries embarking on drug-resistant TB programmes should establish laboratory capacity to diagnose MDR-TB and monitor culture conversion of patients on MDR-TB treatment. Risk categories for drug-resistant TB vary greatly among countries and careful assessment at country level is therefore essential. Algorithms for testing of patients suspected of having drug-resistant tuberculosis are dependent on several factors:

- Local epidemiology
- Local treatment policies
- Existing country laboratory capacity
- Specimen referral and transport mechanisms
- Availability of human and financial resources

Figures 1 presents an algorithm-based approach using WHO-endorsed current global standards for microscopy, culture and conventional DST, within appropriate norms for laboratory infrastructure and biosafety.
**Key considerations**

- Microscopy is suitable for peripheral (typically district) and higher level laboratories
- Microscopy can be done safely in facilities meeting the minimum requirements for low TB risk precautions
- Microscopy has limited sensitivity, which is further reduced in HIV-positive individuals
- Microscopy identifies AFB and not *M. tuberculosis*
- Microscopy cannot distinguish between viable and non-viable organisms, or between drug-susceptible and drug-resistant organisms

- Culture is suitable for intermediate (typically national or regional) level laboratories
- The processing of specimens for the inoculation of solid or liquid media requires facilities which meet the minimum requirements for moderate TB risk precautions.
- Solid culture are less expensive than liquid culture, but results are delayed due to slow growth of mycobacteria
- The manipulation of positive cultures (solid and liquid) for identification or DST requires facilities meeting the minimum requirements for high TB risk precautions.
- Liquid culture are more expensive than solid culture, but results are available more rapidly
- All positive cultures must be speciated to confirm *M. tuberculosis*
Conventional culture (solid or liquid) is required to monitor treatment of MDR-TB patients

- DST is required to confirm/exclude drug resistance of *M. tuberculosis* isolates
- DST is suitable for intermediate (typically national or regional level laboratories)
- DST requires facilities meeting the minimum requirements for high TB risk precautions.
- First-line DST should be done to confirm MDR on all *M. tuberculosis* isolates
- Second-line DST should be done on all MDR *M. tuberculosis* isolates

Figure 2 presents an algorithm-based approach using WHO-endorsed current global standards including LED microscopy and line probe assays, within appropriate norms for laboratory infrastructure and biosafety.

**Figure 2:** Algorithm for use of line probe assays in conjunction with conventional culture (solid or liquid) and drug susceptibility testing

<table>
<thead>
<tr>
<th>Key considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPA is suitable for intermediate (typically national or regional) level laboratories</td>
</tr>
<tr>
<td>LPA is registered for use on smear-positive specimens and <em>M. tuberculosis</em> isolates only;</td>
</tr>
</tbody>
</table>
therefore, smear-negative specimens require conventional culture (solid or liquid) and speciation prior to LPA testing, in facilities meeting the minimum appropriate TB risk precautions.

- LPA requires at least three separate rooms to avoid cross-contamination
- LPA detects only MDR only; conventional DST is required to detect XDR-TB
- Conventional culture (solid or liquid) is required to monitor treatment (culture conversion) of MDR-TB patients

Figures 3 presents an algorithm-based approach using WHO-endorsed non-commercial culture and DST methods, within appropriate norms for laboratory infrastructure and biosafety.

**Figure 3: Algorithm for use of selected non-commercial culture and DST methods**

**Key considerations**

- NRA and MODS are recommended as direct tests on smear-positive specimens only; therefore, smear-negative specimens require conventional culture (solid or liquid) and speciation prior to testing, in facilities meeting the minimum appropriate TB risk precautions
- CRI methods are recommended as indirect tests only; therefore all specimens require conventional culture (solid or liquid) and speciation prior to testing, in facilities meeting the minimum appropriate TB risk precautions

- NRA, MODS and CRI methods detects MDR only; conventional DST is required to detect XDR-TB

- Conventional culture (solid or liquid) is required to monitor treatment (culture conversion) of MDR-TB patients

Various permutations of the above algorithms are possible depending on the local situation, eg. using microscopy and line probe assays together in high MDR-TB burden, low HIV-prevalence, resource-constrained settings.

Decisions regarding appropriate algorithms are highly country-specific and need to be taken by the TB control programmes in close consultation with laboratory experts, taking existing infrastructure and available resources into account.

**Time to detection of MDR-TB**

New diagnostics enable a definitive diagnosis of MDR-TB in a few days. Figures 4 to 6 show the advantage of direct tests to rapidly screen patients suspected of MDR-TB, and the delay in diagnosis using new diagnostics in indirect testing:

**Figure 4. Expected time to MDR-TB diagnosis using current gold standards**
Key considerations

- LPA done on smear-positive specimens detects MDR in less than 48 hours
- Smear-negative specimens require conventional culture (solid or liquid) prior to LPA testing, in facilities meeting the minimum appropriate TB risk precautions

Figure 5. Expected time to MDR-TB diagnosis using MODS

MDR-TB diagnosis using liquid culture and DST

<table>
<thead>
<tr>
<th>Microscopy 24h</th>
<th>Liquid culture 2-3w</th>
<th>1st line DST 3-4w</th>
</tr>
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</table>

MDR-TB diagnosis after 3 to 6 weeks

MDR-TB diagnosis using MODS

<table>
<thead>
<tr>
<th>Microscopy 24h</th>
<th>MODS direct 2-21d</th>
<th>MODS indirect 6-9d</th>
</tr>
</thead>
</table>

MDR-TB diagnosis after 2 to 21 days

Key considerations

- MODS done on smear-positive specimens detects MDR between 2 days and three weeks
- Smear-negative specimens require conventional liquid prior to MODS testing, within facilities meeting the minimum appropriate TB risk precautions. The time to detection of MDR is therefore not necessarily faster than liquid culture
Figure 6. Expected time to MDR-TB diagnosis using NRA

MDR-TB diagnosis using solid culture and DST

<table>
<thead>
<tr>
<th>Microscopy</th>
<th>Solid culture</th>
<th>1st line DST</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h</td>
<td>6-8w</td>
<td>3-4w</td>
</tr>
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</table>

MDR-TB diagnosis after 9 to 12 weeks

MDR-TB diagnosis using NRA

<table>
<thead>
<tr>
<th>Microscopy</th>
<th>NRA direct</th>
<th>NRA indirect</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h</td>
<td>14-20d</td>
<td>10-14d</td>
</tr>
</tbody>
</table>

MDR-TB diagnosis after 7 to 10 weeks

MDR-TB diagnosis after 14 to 20 days

Key considerations

- NRA done on smear-positive specimens detects MDR between 14 and 20 days
- Smear-negative specimens require conventional solid culture prior to NRA testing, within facilities meeting the minimum appropriate TB risk precautions, and time to detection of MDR is not necessarily faster than liquid culture
Figure 7. Expected time to MDR-TB diagnosis using CRI methods

<table>
<thead>
<tr>
<th>MDR-TB diagnosis using solid culture and DST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy 24h</td>
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MDR-TB diagnosis after 9 to 12 weeks

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MDR-TB diagnosis after 7 to 10 weeks

<table>
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<tr>
<th>MDR-TB diagnosis using liquid culture and CRI</th>
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<tbody>
<tr>
<td>Microscopy 24h</td>
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</tbody>
</table>

MDR-TB diagnosis after 3 to 5 weeks

Key considerations

- CRI methods done on isolates grown on solid culture detects MDR two weeks earlier
- Time to detection of MDR using CRI methods on isolates grown in liquid culture is not necessarily faster
Time to detection of XDR-TB

The diagnosis of XDR-TB is currently dependent on conventional DST. Figure 8 shows the expected time to diagnosis of XDR-TB using conventional liquid culture and DST, combined with LPA.

Figure 8. Expected time to XDR-TB diagnosis using current gold standards

<table>
<thead>
<tr>
<th>XDR-TB diagnosis using conventional solid culture and DST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy 24h</td>
</tr>
<tr>
<td>Solid culture 6-8w</td>
</tr>
<tr>
<td>1st line DST 3-4w</td>
</tr>
<tr>
<td>2nd line DST* 3-4w</td>
</tr>
<tr>
<td>XDR-TB diagnosis after 12 to 16 weeks</td>
</tr>
</tbody>
</table>

* Methods not validated or standardised

<table>
<thead>
<tr>
<th>XDR-TB diagnosis using liquid culture and DST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy 24h</td>
</tr>
<tr>
<td>Liquid culture 2-3w</td>
</tr>
<tr>
<td>1st line DST 1-3w</td>
</tr>
<tr>
<td>2nd line DST 1-3w</td>
</tr>
<tr>
<td>XDR-TB diagnosis after 4 to 9 weeks</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>XDR-TB diagnosis using line probe assay, liquid culture and DST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy 24h</td>
</tr>
<tr>
<td>LPA 24h</td>
</tr>
<tr>
<td>Liquid culture 2-3w</td>
</tr>
<tr>
<td>1st line DST 1-3w</td>
</tr>
<tr>
<td>2nd line DST 1-3w</td>
</tr>
<tr>
<td>XDR-TB diagnosis after 4 to 9 weeks</td>
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</table>

Key considerations

- Conventional solid culture methods for detecting XDR are not recommended
- Irrespective of the method used to detect MDR, conventional liquid culture and DST capacity is still required to detect XDR
### Annex 1. Summary of WHO laboratory policies

#### WHO laboratory policies (1)

- **Automated liquid culture and DST (2007):** Use of liquid culture systems in the context of a comprehensive country plan for strengthening TB laboratory capacity; in a phased manner starting at national/central reference laboratory.

- **Rapid speciation (2007):** Strip speciation for rapid *Mycobacterium tuberculosis* from non-tuberculous mycobacteria; established at regional or central level in combination with liquid culture.

- **Line probe assays (2008):** Use of line probe assays for rapid detection of R resistance within the context of country plans for MDR-TB management, including development of country-specific screening algorithms and timely access to quality-assured second-line anti-tuberculosis drugs; do not eliminate the need for conventional culture and DST capability; should be phased in, starting at national/central reference laboratory or those with proven molecular capability.

- **Second-line drug susceptibility testing (2008):** Reliable and reproducible for injectables and fluoroquinolones; to be conducted in national/central reference laboratories using standardised methodology and drug concentrations; routine DST not recommended for ethionamide, prothionamide, cycloserine, terizidone, PAS, thiacetazone, clofazimine, amoxicillin/clavulanat, clarithromycin, linezolid


#### WHO laboratory policies (2)

- **LED microscopy (2010):** LED microscopy to replace conventional fluorescent microscopy and be phased in as replacement for ZN microscopy

- **MODS (2010):** Recommended for rapid detection of R resistance within the context of country plans for MDR-TB management, including development of country-specific screening algorithms and timely access to quality-assured second-line anti-tuberculosis drugs; do not eliminate the need for conventional culture and DST capability; should be phased in, starting at national/central reference laboratory, under strict laboratory protocols and quality assurance

- **NRA (2010):** Recommended as direct or indirect tests, for screening of patients suspected of having MDR-TB, and acknowledging that time to detection of MDR-TB in indirect application would not be faster than conventional DST methods using liquid culture; to be used within the context of country plans for MDR-TB management, including development of country-specific screening algorithms and timely access to quality-assured second-line anti-tuberculosis drugs; do not eliminate the need for conventional culture and DST capability; should be phased in, starting at national/central reference laboratory, under strict laboratory protocols and quality assurance

- **CRI methods (2010):** Recommended as indirect tests, for screening of patients suspected of having MDR-TB, and acknowledging that time to detection of MDR-TB would not be faster than conventional DST methods using liquid culture; to be used within the context of country plans for MDR-TB management, including development of country-specific screening algorithms and timely access to quality-assured second-line anti-tuberculosis drugs; do not eliminate the need for conventional culture and DST capability; should be phased in, starting at national/central reference laboratory, under strict laboratory protocols and quality assurance