



**Policy Framework
for Implementing New Tuberculosis Diagnostics**

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March 2010

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Executive summary

Strengthening TB laboratory services is one of the best means for overall improvement of laboratories as an essential part of health systems. Fundamental to this activity is collaboration between TB control programmes and public health laboratory services at country level. Adequate laboratory capacity consists of several essential elements that must be addressed simultaneously within comprehensive strategies and national plans.

An unprecedented effort to improve and expand TB laboratory capacity is under way, spearheaded by WHO and the Stop TB Partnership Global Laboratory Initiative 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 expanding rapidly.

Establishing, equipping and maintaining laboratory networks is challenging, complex and expensive. The introduction of new techniques is bound to fail if the 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; and appropriate, adequate strategies and funding for human resources.

The specialized nature of technical procedures, of laboratory management and administration and of laboratory quality control require different levels of laboratory testing, with clear specimen referral mechanisms. Laboratory and diagnostic algorithms should start with appropriate screening to identify people suspected of having TB. Current WHO case-finding strategies recommend screening of all persons with a cough that has lasted more than 2 weeks.

Laboratory policies and diagnostic algorithms at country level are largely dictated by the prevalence of HIV- and drug-resistant TB. In settings with a high HIV burden, a substantial investment in culture capacity is required, given the absence of means to diagnose smear-negative TB. In settings with a high burden of MDR-TB, laboratory diagnostic algorithms based on groups at greatest risk of drug-resistant TB (including those with HIV infection) represent 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. As the risk categories of drug-resistant TB vary widely among countries, careful assessment at country level is essential. Algorithms for testing patients suspected of having drug-resistant TB depend on the local epidemiology, local treatment policies, the existing laboratory capacity, specimen referral and transport mechanisms and human and financial resources.

Abbreviations

CRI	colorimetric redox indicator
DOTS	directly observed treatment (short course)
DST	drug-susceptibility testing
GRADE	grades of recommendation assessment, development and evaluation
LED	light-emitting diode
LPA	line-probe assay
MDR	multidrug-resistant(ce)
MODS	microscopic observation of drug susceptibility
NRA	nitrate reductase assay
STAG-TB	Strategic and Technical Advisory Group for Tuberculosis
TB	tuberculosis
WHO	World Health Organization
XDR	extensively drug-resistant

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Introduction

Care of patients with tuberculosis (TB) starts with a quality assured diagnosis. Successful expansion of directly observed treatment (short course) (DOTS) and programmatic management of drug-resistant and HIV-associated TB therefore require a robust network of TB laboratories with adequate biosafety, modern methods of diagnosis, standard operating procedures and appropriate quality assurance.

Arguably, the weakest component of health systems is laboratory services, which have been grossly neglected, understaffed and underfunded over time. 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 plans for strengthening laboratories;
- inadequate laboratory infrastructure and biosafety;
- vastly inadequate numbers of skilled staff; and
- insufficient technical assistance.

Strengthening TB laboratory services is one of the best means for overall improvement of laboratories as an essential part of health systems. Fundamental to this activity is collaboration between TB control programmes and public health laboratory services at country level. Adequate laboratory capacity consists of several essential elements that must be addressed simultaneously within comprehensive strategies and national plans.

An unprecedented effort to improve and expand TB laboratory capacity is under way, spearheaded by WHO and the Stop TB Partnership Global Laboratory Initiative 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 expanding rapidly (1).

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

WHO policy on new technology is evidence-driven.

As TB diagnostics are changing rapidly, policy formulation must be dynamic and continuous, both at global and country level. New TB technology is regularly assessed by WHO in a systematic, structured way for rapid policy development (2; Figure 1).

In accordance with current WHO standards for evidence in policy recommendations, the grades of recommendation assessment, development and evaluation (GRADE) system (3) is used to assess the findings of Expert Groups. This approach provides a systematic, structured framework for evaluating the accuracy of new interventions and their effect on patients and public health.

The findings of expert groups and GRADE evaluations are presented to the WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB), an independent advisory body to WHO. After endorsement by this Group, detailed policy recommendations are drawn up by WHO (3). Techniques and methods currently recommended by WHO are summarized in Annex 1.

Figure 1: WHO TB diagnostic policy formulation process



Techniques must be used in appropriate laboratory services.

Establishing, equipping and maintaining laboratory networks is challenging, complex and expensive. The introduction of new techniques is bound to fail if the 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; and
- appropriate, adequate strategies and funding for human resources.

The Global Laboratory Initiative has prepared a 'Roadmap for TB laboratory strengthening' with the aim of ensuring high-quality TB diagnostics in appropriate laboratory services in the context of national laboratory strategic plans (4).

Mycobacterium tuberculosis is a 'risk group 3' pathogen, and handling of specimens poses various risks. WHO and the United States Centers for Disease Control and Prevention therefore convened an expert group to achieve consensus on the minimum requirements for safe laboratory use of TB laboratory procedures, using a risk-based approach. Draft policy guidance and a manual for laboratory biosafety are under peer review; the final documents are expected in late 2010.

The risk assessment approach includes the bacillary load of materials (specimens, cultures), the viability of bacilli, whether the material handled might generate aerosols, the number of manoeuvres in each technique that might generate infectious aerosols, 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.

TB risk level 1

Purpose: Preparation of direct smears on the open laboratory bench for acid-fast bacilli microscopy.

Minimum requirements

- adequate ventilation*;
- laboratory separated from other areas;
- access to the laboratory restricted to authorized persons; and
- 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 obviate window opening, consideration should be given to mechanical ventilation systems that provide an inward flow of air without recirculation in the room.

TB risk level 2

Purpose: Processing of sputum specimens for primary culture inoculation, direct nitrate reductase assay (NRA), direct microscopic observation of drug susceptibility (MODS) assay or direct line-probe assay (LPA)

Minimum requirements

- laboratory separated from other areas;
- access to the laboratory restricted to authorized persons;
- floors, walls, ceilings and benches and furniture with 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, class I (EN12469/NSF49), class IIA2 (NSF49) or class II (EN12469), equipped with H14 high-efficiency particulate air filters;
- biological safety cabinets designed by certified manufacturers, properly installed, regularly maintained and re-certified at least annually on site; and
- a controlled ventilation system that maintains a directional airflow into the laboratory from functionally clean to dirty areas, with a minimum of 6 and up to 12 air changes per hour*.

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

TB risk level 3

Purpose: Manipulation of cultures for identification and drug-susceptibility testing (DST) with indirect phenotypic methods and/or LPAs

Minimum requirements

Meet all requirements for a TB risk level 2 laboratory. In addition:

- designed as a containment laboratory, with double-door entry; and
- autoclave available on site and close to the laboratory for safe waste disposal.

Techniques must be suitable for different levels of laboratory services.

The specialized nature of technical procedures, of laboratory management and administration and of laboratory quality control require different levels of laboratory testing, with clear specimen referral mechanisms. Conventional tiered laboratory services for TB diagnosis are described in many documents (5). Three main levels of laboratory services are common in most countries:

- *peripheral (typically district) level:* Perform sputum smear microscopy; refer specimens or patients requiring further tests to higher-level laboratories
- *intermediate (typically regional) level:* Perform smear microscopy and conventional culture, with or without species identification and first-line DST; refer cultures requiring further tests (e.g. second-line DST) to higher-level laboratories.
- *central (typically national or reference) level:* Perform sputum smear microscopy, conventional and rapid culture and DST and molecular tests; refer isolates requiring further tests (e.g. second-line DST or molecular sequencing) to supranational reference laboratories in other countries or regions.

Techniques must be used in appropriate strategies for case-finding.

Laboratory and diagnostic algorithms should start with appropriate screening to identify people suspected of having TB, with microscopy services as the entry point. Current WHO case-finding strategies recommend screening of all persons with a cough that has lasted more than 2 weeks (6).

Good-quality microscopy of two sputum specimens is adequate to identify the vast majority (95–98%) of smear-positive TB patients. The 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 microscopy. A case is defined in such settings as one positive smear, i.e. one or more acid-fast bacillus, in at least 100 microscopic fields (7).

Recent WHO policy guidance confirmed the diagnostic accuracy of examining two consecutive smears in a 'same-day diagnosis' approach, in which treatment is started during the first visit of a patient to health services (7).

Case-finding strategies for drug-resistant TB have been published (8) and the role of DST in identifying multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB outlined (9). Detection of HIV-associated drug-resistant TB requires appropriate laboratory diagnostic algorithms based on patient groups at greatest risk, to ensure cost-effective use of scarce laboratory and diagnostic resources. Such algorithms are highly country-specific and depend on the factors described below.

WHO-recommended techniques

Microscopy

Mycobacteria can be distinguished from other microorganisms by their thick, lipid-containing cell walls, which retain biochemical stains despite decolourization by acid-containing reagents (so-called 'acid-fastness').

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

Disadvantages: Direct smear microscopy is relatively insensitive, as at least 5000 bacilli per millilitre of sputum are required for a positive result. The sensitivity is further reduced in patients with extrapulmonary TB, those with HIV coinfection and those with disease due to nontuberculous mycobacteria.

Limitations: Microscopy for acid-fast bacilli cannot distinguish *M. tuberculosis* from nontuberculous mycobacteria, viable from nonviable organisms or drug-susceptible from drug-resistant strains.

Conventional light microscopy

Ziehl-Neelsen light microscopy performed directly on sputum specimens is suitable for all levels of laboratory services, including peripheral laboratories at primary health care centres or district hospitals.

There is insufficient evidence that processed (e.g. concentrated or chemically treated) sputum specimens give better results than direct smear microscopy. Use of such methods in programmatic settings is therefore not recommended.

The number of Ziehl-Neelsen smears examined by one microscopist per day should not exceed 20, as visual fatigue leads to a deterioration of reading quality. Nevertheless, proficiency in reading such smears can be maintained only by examining at least 10–15 smears per week (10).

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

Conventional fluorescence microscopy

In conventional fluorescence microscopy, quartz–halogen or high-pressure mercury vapour lamps are typically used as light sources. As a lower-magnification objective is used to scan smears, a much larger area of the smear can be seen, and therefore less time is needed than with Ziehl-Neelsen microscopy.

Conventional fluorescence microscopy is on average 10% more sensitive than Ziehl-Neelsen microscopy but requires considerable technical expertise; the capital and running costs are also considerably higher. Conventional fluorescence microscopy is therefore recommended by WHO for use at intermediate laboratory level where more than 100 smears are examined per day (10).

Light-emitting diode fluorescence microscopy

Light-emitting diodes (LEDs) provide a much less expensive light source for fluorescence microscopy. LED microscopes or attachments require less power and can run on batteries, and the bulbs have a long half-life and do not release potentially toxic products if broken.

In a recent WHO evaluation, the diagnostic accuracy of LED microscopy was found to be comparable to that of conventional fluorescence microscopy and superior to that of conventional Ziehl-Neelsen 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 Ziehl-Neelsen light microscopy in both high- and low-volume laboratories (11).

Culture and species identification

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

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

Limitations: Specimens must be decontaminated before culture to prevent overgrowth by other microorganisms. All decontamination methods are to some extent also harmful to mycobacteria, and culture is therefore not 100% sensitive. Good laboratory practice maintains a delicate balance between the yield of mycobacteria and contamination by other microorganisms.

Solid and liquid culture methods are suitable for central reference laboratories (or regional laboratories in large countries). Usually, one culture laboratory is adequate to cover a population of 500 000–1 million. Solid culture methods are less expensive than liquid systems, but the results are invariably delayed because of the slow growth of mycobacteria. Several culture methods are recommended (12). Liquid culture increases the case yield by 10% over solid media, and automated systems reduce the diagnostic delay to days rather than weeks (13). Liquid systems are, however, more prone to contamination, and the manipulation of large volumes of infectious material mandates appropriate, adequate biosafety measures.

Positive cultures must be identified to differentiate *M. tuberculosis* from nontuberculous mycobacteria, which are more common in HIV-infected patients, with a prevalence that varies from country to country. Diseases due to nontuberculous mycobacteria are treated entirely differently from drug-resistant TB. As a minimum, laboratories performing DST must differentiate *M. tuberculosis* from nontuberculous mycobacteria; further speciation is not recommended at programme level.

Confirmation is usually done from the biological characteristics of the culture growth and with selected molecular or biochemical tests (which invariably delay the final result) (12). Rapid immunochromatographic assays (so-called strip speciation tests) for species identification on culture isolates provide a definitive identification of *M. tuberculosis* in 15 min and are recommended (13). 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 techniques are available:

- Phenotypic methods involve culturing *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 are 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. The results obtained with the three methods do not differ significantly for first-line anti-TB drugs.

Disadvantages: DST methods are suitable for use at central reference laboratory level only, given the need for appropriate laboratory infrastructure (particularly biosafety) and the technical complexity of the available techniques and 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 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 for treating MDR-TB patients should establish laboratory capacity to detect MDR. Rifampicin resistance is a valid and reliable indicator or proxy of MDR. Rapid DST is essential for identifying patients at risk for MDR-TB, as a first priority. Automated liquid systems and molecular LPAs (see later) for first-line DST are the current gold standard (13). Once MDR-TB has been confirmed, additional first- and second-line drug susceptibility results should be obtained following current WHO recommendations (14).

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 (14).

Routine second-line DST is not recommended unless the required laboratory infrastructure and capacity have been established, rigorous quality assurance is in place and sustainable proficiency has been demonstrated (14). In order to retain proficiency and expertise, it is recommended that second-line DST be performed only 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 the reliability and reproducibility of laboratory testing cannot be guaranteed.

Noncommercial methods

Noncommercial culture and DST methods are less expensive than commercial systems but are prone to errors due to 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 must be implemented and enforced.

The evidence base for selected noncommercial 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 or national laboratories in selected settings* (15). The methods include MODS, colorimetric redox indicator (CRI) methods and the NRA. Recommendations for their respective use are:

- **MODS:** a microcolony method in liquid culture, based on inoculation of specimens into 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 on a microtitre plate after exposure of *M. tuberculosis* strains to anti-TB drugs in vitro
Recommended as indirect tests on *M. tuberculosis* isolates from patients suspected of having MDR-TB, although the time to detection of MDR is not faster (but less expensive) than conventional DST methods with commercial liquid culture or molecular LPAs (see below)
- **NRA:** A direct or indirect method on solid culture based on the ability of *M. tuberculosis* to reduce nitrate, which is detected by a colour reaction
Recommended as direct or indirect tests for screening patients suspected of having MDR-TB, although the time to detection of MDR in indirect application is not faster than conventional DST methods with liquid culture (see below).

Both commercial and noncommercial culture and DST systems and methods are suitable for use at central or 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, standardized testing, potentially high through-put and reduced requirements for biosafety. The ultimate aim should be to use molecular assays (such as the LPA or other molecular platforms that may be endorsed by WHO in the future) for rapid first-step identification of MDR.

Molecular LPAs allow rapid detection of resistance to rifampicin (alone or in combination with isoniazid) and were endorsed by WHO in 2008, with detailed policy guidance on their introduction at country level (16).

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 of *M. tuberculosis* isolates grown from smear-negative specimens by conventional culture methods.

Limitations: LPAs are suitable for use at central or national reference laboratory level, with potential for decentralization to regional level if the appropriate infrastructure can be ensured.

Annex 2 gives a summary of the characteristics and laboratory requirements for currently WHO-recommended techniques and methods.

Selecting appropriate algorithms, techniques and methods

1. The currently available techniques are not mutually exclusive. Molecular LPAs and selected noncommercial 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 LPAs 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. Use of new techniques and methods for detecting TB should be decided by ministries of health in 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.

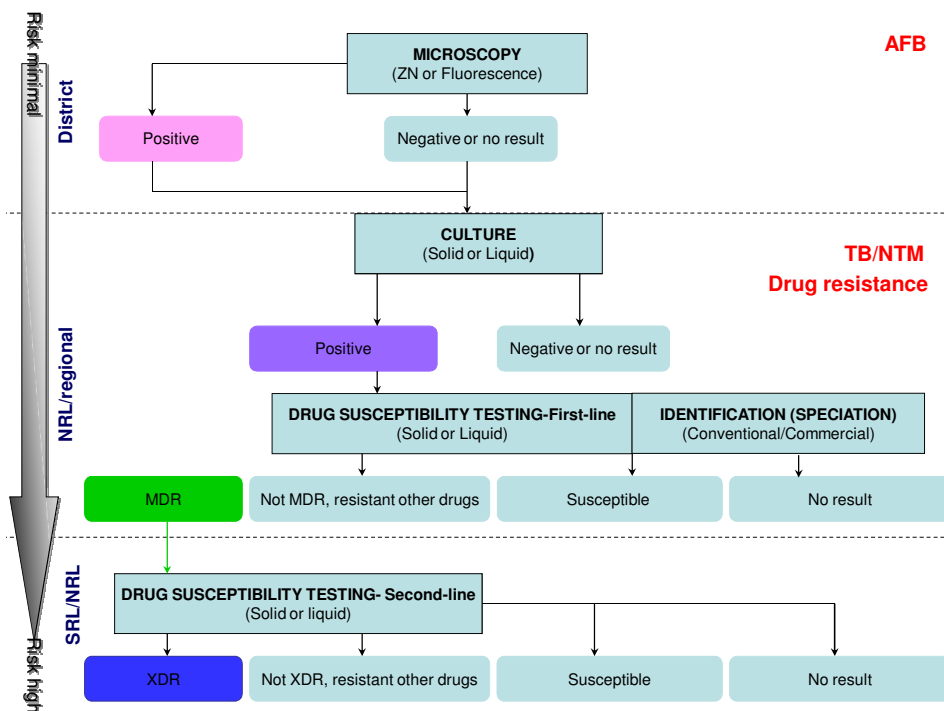
Diagnostic testing algorithms

Laboratory policies and diagnostic algorithms at country level are largely dictated by the prevalence of HIV- and drug-resistant TB. Management of HIV-associated and drug-resistant TB also requires concurrent clinical laboratory capacity (e.g. biochemistry, haematology, general microbiology) to monitor treatment and associated comorbid conditions. In settings with a high HIV burden, a substantial investment in culture capacity is required, given the absence of means to diagnose smear-negative TB. In settings with a high burden of MDR-TB, laboratory diagnostic algorithms based on groups at greatest risk of drug-resistant TB (including those with HIV infection) represent 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. As the risk categories of drug-resistant TB vary widely among countries, careful assessment at country level is essential. Algorithms for testing patients suspected of having drug-resistant TB depend on the local epidemiology, local treatment policies, the existing laboratory capacity, specimen referral and transport mechanisms and human and financial resources.

Figure 2 presents an algorithm-based approach based on WHO-endorsed global standards for microscopy, culture and conventional DST, within appropriate norms for laboratory infrastructure and biosafety.

Figure 2: Algorithm for use of conventional microscopy, culture (solid or liquid) and drug susceptibility testing

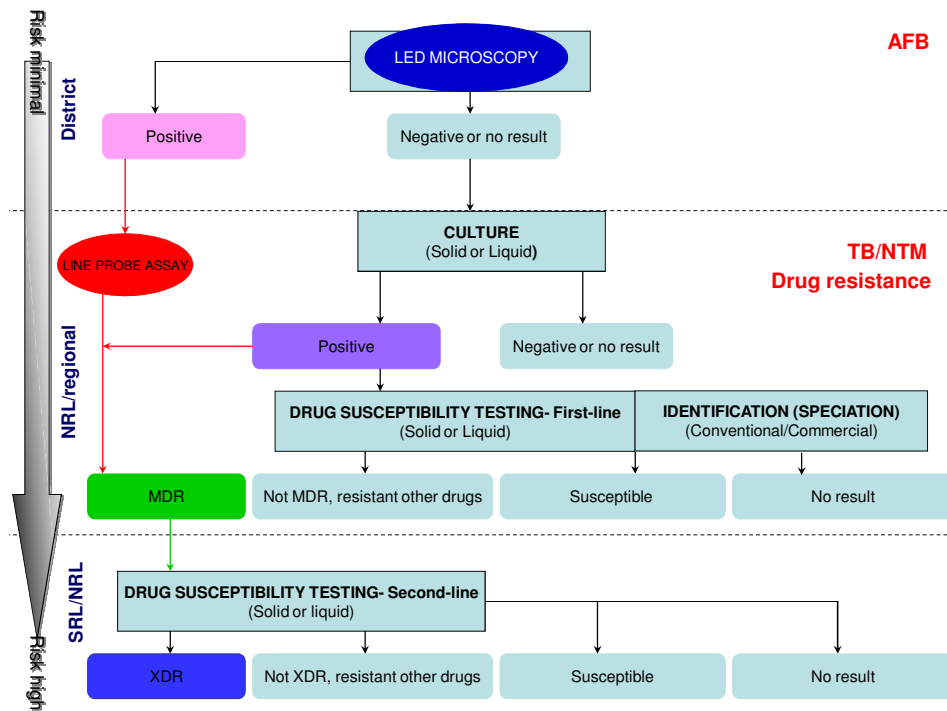


Key considerations

- Microscopy is suitable for peripheral (typically district) and higher-level laboratories.
- Microscopy can be done safely under TB risk level 1 conditions.
- Microscopy has limited sensitivity, which is further reduced in HIV-positive individuals.
- Microscopy can be used to identify acid-fast bacilli but not *M. tuberculosis*.
- Microscopy cannot distinguish between viable and nonviable organisms or between drug-susceptible and drug-resistant organisms.
- Culture is suitable for intermediate- (typically national or regional) level laboratories.
- Solid culture requires biosafety level 2 conditions and is less expensive than liquid culture, but results are delayed because of slow growth of mycobacteria.
- Liquid culture requires biosafety level 3 conditions and is more expensive than solid culture, but the 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 or exclude drug resistance of *M. tuberculosis* isolates.
- DST is suitable for intermediate- (typically national or regional) level laboratories.
- DST requires biosafety level 3 conditions.
- First-line DST should be done to confirm MDR in all *M. tuberculosis* isolates.
- Second-line DST should be done on all MDR *M. tuberculosis* isolates.

Figure 3 presents an algorithm-based approach based on WHO-endorsed global standards, including LED microscopy and LPAs, within appropriate norms for laboratory infrastructure and biosafety.

Figure 3: Algorithm for use of line-probe assays in conjunction with conventional culture (solid or liquid) and drug susceptibility testing

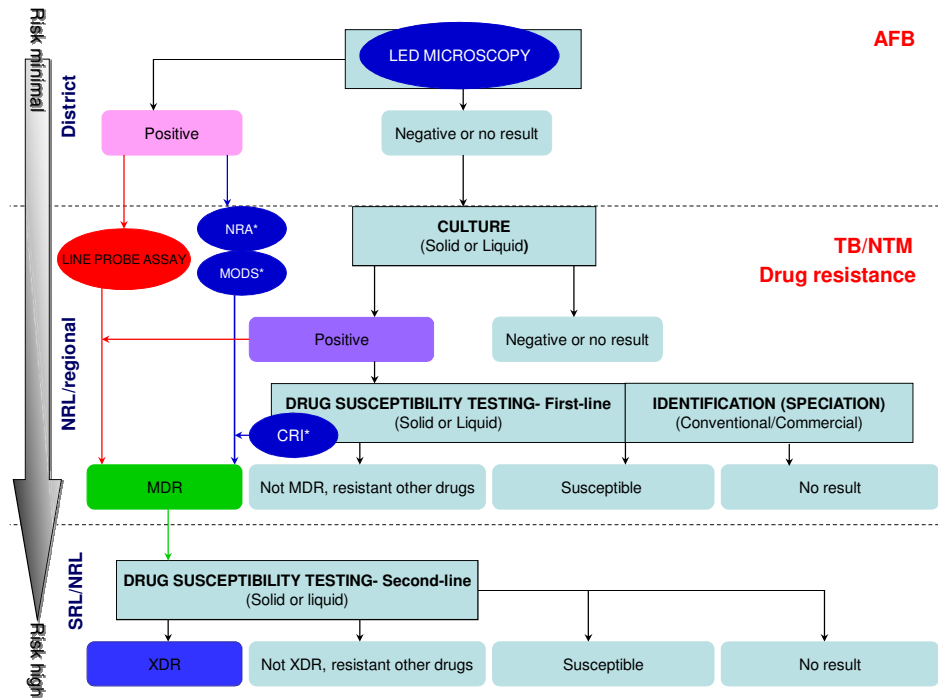


Key considerations

- LPA is suitable for intermediate (typically national or regional) level laboratories.
- LPA is registered for use on smear-positive specimens and *M. tuberculosis* isolates only; therefore, smear-negative specimens require conventional culture (solid or liquid) and speciation before LPA testing, with appropriate TB risk level conditions.
- LPA requires at least three separate rooms to avoid cross-contamination.
- LPA detects only MDR; conventional DST is required to detect XDR-TB.
- Conventional culture (solid or liquid) is required to monitor treatment (culture conversion) of MDR-TB patients.

Figure 4 presents an algorithm-based approach based on WHO-endorsed noncommercial culture and DST methods, within appropriate norms for laboratory infrastructure and biosafety.

Figure 4: Algorithm for use of selected noncommercial culture and drug susceptibility testing methods



* NRA – nitrate reductase assay, MODS – Microscopic observation of drug susceptibility, CRI – Colorimetric redox indicator

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 before testing, with appropriate TB risk level conditions.
- CRI methods are recommended as indirect tests only; therefore, all specimens require conventional culture (solid or liquid) and speciation before testing, with appropriate biosafety conditions.
- NRA, MODS and CRI methods detect 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 to accord with the local situation, e.g. using microscopy and LPAs together in resource-constrained settings with a high MDR-TB burden and a low HIV prevalence.

Decisions about appropriate algorithms are highly country-specific and should be taken by the TB control programmes in consultation with laboratory experts, on the basis of the existing infrastructure and available resources.

Time to detection

Multidrug-resistant tuberculosis

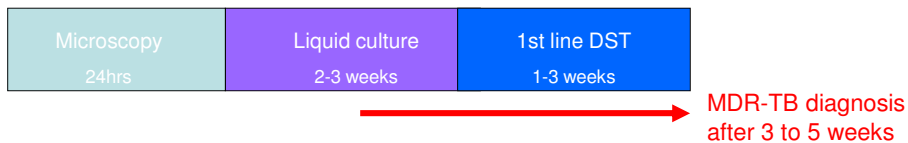
New diagnostics allow a definitive diagnosis of MDR-TB within a few days. Figures 5–7 show the advantages of direct tests for rapidly screening patients suspected of having MDR-TB and the delay in diagnosis with indirect testing.

Figure 5: Expected time to MDR-TB diagnosis with current gold standards

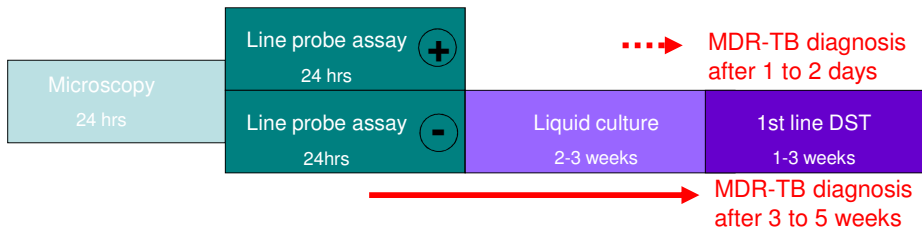
MDR-TB diagnosis with solid culture and DST



MDR-TB diagnosis with liquid culture and DST



MDR-TB diagnosis with line probe assay, liquid culture and DST



Key considerations

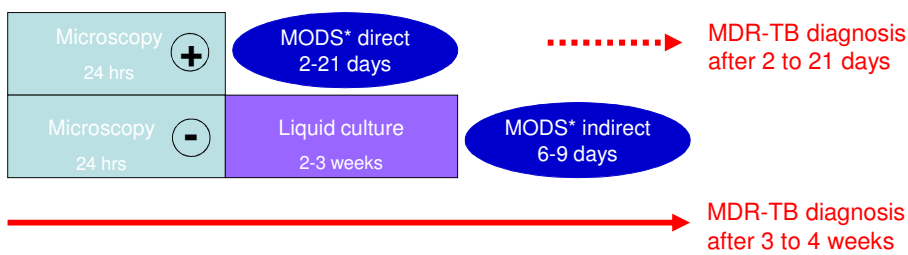
- LPA on smear-positive specimens allows detection of MDR within less than 48 h.
- Smear-negative specimens require conventional culture (solid or liquid) and speciation before LPA testing, with appropriate biosafety conditions.

Figure 6: Expected time to MDR-TB diagnosis with MODS

MDR-TB diagnosis with liquid culture and DST



MDR-TB diagnosis with MODS*



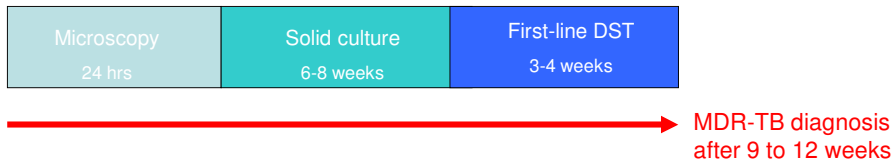
*MODS – microscopic observation of drug susceptibility

Key considerations

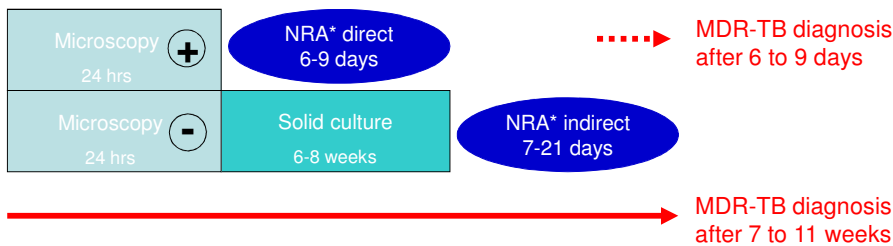
- MODS on smear-positive specimens allows detection of MDR within 2 days to 3 weeks.
- Smear-negative specimens require conventional liquid before MODS testing, with appropriate TB risk level conditions. The time to detection of MDR is not necessarily faster than with liquid culture.

Figure 7: Expected to MDR-TB diagnosis with NRA*

MDR-TB diagnosis with solid culture and DST



MDR-TB diagnosis with NRA*



*NRA – nitrate reductase assay

Key considerations

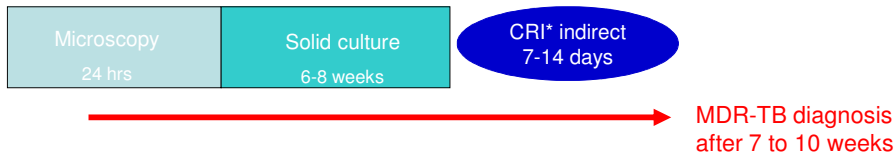
- NRA done on smear-positive specimens allows detection of MDR within 6–9 days
- Smear-negative specimens require conventional solid culture before NRA testing, with appropriate TB risk level conditions. The time to detection of MDR is not necessarily faster than with liquid culture.

Figure 8: Expected time to MDR-TB diagnosis with CRI* methods

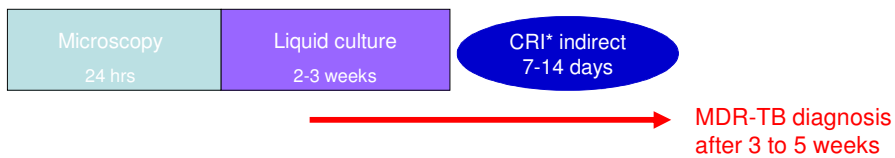
MDR-TB diagnosis using solid culture and DST



MDR-TB diagnosis using solid culture and CRI*



MDR-TB diagnosis using liquid culture and CRI*



*CRI – colorimetric redox indicator

Key considerations

- CRI methods on isolates grown on solid culture allow detection of MDR 2 weeks earlier.
- The time to detection of MDR with CRI methods on isolates grown in liquid culture is not necessarily faster.

Extensively drug-resistant tuberculosis

The diagnosis of XDR-TB is currently done with conventional DST. Figure 9 shows the expected time to diagnosis of XDR-TB with conventional liquid culture and DST, combined with LPA.

Figure 9: Expected time to XDR-TB diagnosis with current gold standards

XDR-TB diagnosis with conventional solid culture and DST



* Methods not validated or standardised

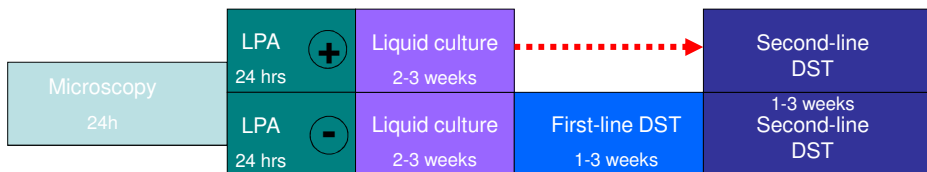
XDR-TB diagnosis
after 12 to 16 weeks

XDR-TB diagnosis with liquid culture and DST



XDR-TB diagnosis
after 4 to 9 weeks

XDR-TB diagnosis with line probe assay, liquid culture and DST



XDR-TB diagnosis
after 4 to 9 weeks

Key considerations

- Conventional solid culture methods for detecting XDR-TB are not recommended.
- Irrespective of the method used to detect MDR, conventional liquid culture and DST capacity are still required to detect XDR-TB.

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Annex 1. Summary of WHO laboratory policies

- 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, thioacetazone, clofazimine, amoxicillin/clavulanat, clarithromycin, linezolid
- 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 solid 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

Available at http://www.who.int/tb/laboratory/policy_statements/en/index.html

Annex 2. Summary of characteristics and laboratory requirements for WHO-recommended techniques

Summary: Characteristics and laboratory requirements of WHO-approved technologies							
Diagnostic tool or method	Laboratory service level	Time to detection of MDR		Equipment	Consumables	Training needs	Infrastructure (Risk category)
		Direct	Indirect				
Microscopy	Peripheral Intermediate Central	n/a	n/a	+	+	Minimal	+
Solid culture & DST	Central Intermediate	n/a	9 - 12 weeks	+	++	Moderate	++
Commercial liquid culture & DST	Central Intermediate	n/a	3 - 5 weeks	+++	+++	Extensive	+++
Non-commercial culture & DST	Central Intermediate						
MODS		2 – 21 days	3 – 4 weeks	++	++	Extensive	++
NRA		6 – 9 days	7 – 11 weeks	+	++	Moderate	++
CLI		n/a	3 – 5 weeks (liquid culture) 7 – 10 weeks (solid culture)	+	++	Extensive	+++
Line probe assay	Central Intermediate			+++	++	Moderate	
SM pos		24-48hrs					++
SM neg		n/a	3 – 5 weeks				+++

Adapted from: World Health Organization, Stop TB Partnership Retooling Task Force, Stop TB Partnership New Diagnostics Working Group. *New Laboratory Diagnostic Tools for Tuberculosis Control*. 2009. Available at: <http://www.stoptb.org/retooling>.

Pre-plate