IMPLEMENTING TUBERCULOSIS DIAGNOSTICS

Policy framework

World Health Organization

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Abbreviations

AFB	acid-fast bacilli
CRI	colorimetric redox indicator
DST	drug-susceptibility testing
FIND	Foundation for Innovative New Diagnostics
GLI	Global Laboratory Initiative
GRADE	Grading of Recommendations Assessment, Development and Evaluation
IGRA	interferon-gamma release assay
ISO	International Organization for Standardization
LED	light-emitting diode
LPA	line-probe assay
LTBI	latent TB infection
MDR-TB	multidrug-resistant tuberculosis
MODS	microscopic observation drug-susceptibility assay
MTBC	*Mycobacterium tuberculosis complex*
NAAT	nucleic acid amplification test
NRA	nitrate reductase assay
NTM	non-tuberculosis mycobacteria
PCR	polymerase chain reaction
QMS	quality-management system
*rpo*B	gene encoding for the ß-subunit of the DNA-dependent RNA polymerase of *Mycobacterium tuberculosis*
RR-TB	rifampicin-resistant tuberculosis
SLIPTA	stepwise laboratory quality improvement process towards accreditation
SLMTA	strengthening laboratory management towards accreditation
SOP	standard operating procedure
STAG-TB	Strategic and Technical Advisory Group for Tuberculosis
TB	tuberculosis
TST	tuberculin skin test
WHO	World Health Organization
XDR-TB	extensively drug-resistant tuberculosis

Introduction

Tuberculosis (TB) laboratories play a critical part in national TB programmes, providing clinicians with invaluable information that is used to diagnose and guide the care of patients. Because of the specialized nature of the different technical procedures needed to diagnose TB, and the need for quality assurance and effective laboratory management, TB control programmes require a tiered network of laboratories in which different tiers use complementary diagnostic tools and mechanisms for referring specimens. Establishing, equipping and maintaining a laboratory network to ensure that there are timely and universal access to quality-assured diagnostics is challenging, complex and expensive, and the following core elements must be addressed simultaneously:

- planning for the implementation of diagnostic services;
- developing laboratory infrastructure and plans for maintaining the infrastructure, as well as implementing appropriate biosafety measures;
- developing schedules for equipment validation and maintenance;
- establishing mechanisms for specimen collection, transport and referral;
- establishing systems for managing laboratory commodities and supplies;
- developing systems for managing the laboratory and the data collected;
- establishing systems for assessing the quality of each laboratory's services; and
- ensuring there are appropriate strategies for managing human resources and adequate funding for human resources.

Diagnostic capacity continues to be a major bottleneck in TB control, including scaling up management and control efforts to tackle drug-resistant TB and TB associated with HIV. An unprecedented effort to improve and expand the capacity of TB laboratories is under way, coordinated by the World Health Organization's (WHO's) Global TB Programme and with the active involvement of the Global Laboratory Initiative (GLI), a working group of the Stop TB Partnership (for more information, see http://www.stoptb.org/wg/gli).

The targets for laboratory strengthening in *The global plan to stop TB 2011–2015* include ensuring:[1]

- there is 1 microscopy centre per 100 000 population (for smear examinations for acid-fast bacilli [AFB]);
- there is 1 laboratory per 5 000 000 population to perform culture testing;
- that 50% of tests for drug resistance for new TB patients and more than 90% of tests for previously treated patients are done using rapid TB diagnostic tests.

However, with the roll-out of the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, United States), the number of microscopy centres and facilities offering culture and drug-susceptibility testing (DST) will need to be adjusted depending on the extent of the roll-out in different settings and the epidemiology of TB, multidrug-resistant TB (MDR-TB) and HIV infection. Smear examinations for AFB remain essential for monitoring patients' responses to treatment, as well as for the initial evaluation of patients with suspected pulmonary TB when Xpert MTB/RIF testing is not available. Provided that molecular methods are made available, the priority for using culture and DST would be to monitor response to treatment for MDR-TB, as well as to determine whether there is susceptibility to second-line anti-TB agents.

WHO's global strategy for TB prevention, care and control for 2015–2035 (known as the End

TB Strategy) prioritizes the early diagnosis of TB, which should include the universal availability of DST, and systematic screening of contacts and high-risk groups.[2] Therefore, all national TB control programmes should prioritize the development of a robust network of TB laboratories that have adequate biosafety standards, use modern methods of diagnosis, use standard operating procedures (SOPs) and appropriate quality assurance processes, and that have qualified and sufficient human resources; these priorities should be comprehensively addressed in national strategic plans.

Overall, the development landscape for TB diagnostics is promising: many different organizations are developing products, and there is a robust pipeline of technologies. The range of technologies that may replace sputum-smear microscopy continues to expand, and smaller, simpler and more robust products are expected to become available in the coming years. Several technologies aim to deliver results in less than 1 hour, including DST results; this should improve the time to treatment, enable point-of-care testing programmes and provide greater access to DST.[3]

Until these new technologies become available, the use of existing WHO-recommended diagnostic techniques must be accelerated and strengthened. This will require ensuring that laboratories have adequate infrastructure and human-resources capacity. Additionally, there must be clear country-level policies on using these recommended tests in the most effective screening and diagnostic algorithms, depending on each country's specific epidemiology and resources.

2. Purpose of this document

This document aims to provide a structured framework for introducing WHO's recommended diagnostic techniques for TB. It is expected that countries will adapt this generic policy framework within the contexts of their own epidemiological situation and resources. No single policy framework can address all issues in detail due to the diversity of resources and needs in different countries, and geographical variation in the epidemiology of TB, HIV-associated TB and drug-resistant TB. Therefore, this document aims at providing a generic framework or template for implementing TB diagnostics; it encompasses the managerial, technical and operational processes required for developing and implementing a national strategy for TB laboratories to ensure the early diagnosis of TB and universal access to DST, as well as to ensure there is systematic screening of contacts of people with TB and high-risk groups. These issues should be addressed within national strategic plans for laboratory strengthening.

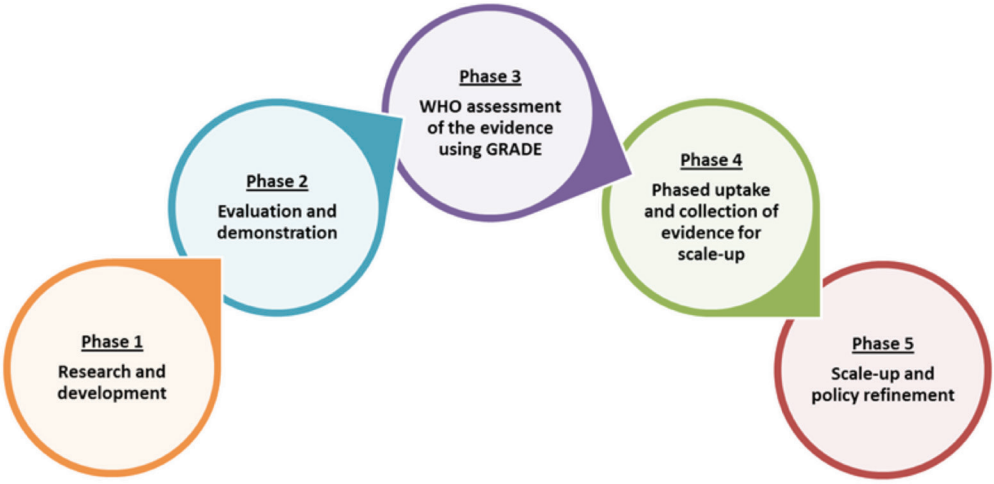
3. Who should use this document?

This document has been developed as a reference for everyone involved in activities aimed at strengthening laboratory systems and the diagnosis of TB, such as laboratory managers and technicians, programme managers, technical advisers, procurement officers, warehouse managers, service providers, government officials, implementing partners and donor agencies. The document will assist managers of national TB control programmes and laboratory managers in coordinating with external laboratory consultants, donors and other decision-makers to help set priorities to address the specific requirements for scaling up the capacities of TB laboratories. The document provides guidance for strategic planning committees about how to address issues associated with TB within a broader national plan for laboratory strengthening. Anyone who may be responsible for programme planning, budgeting and mobilizing resources for diagnostic services will also benefit from this document.

4. WHO's process for developing policies on TB diagnostics

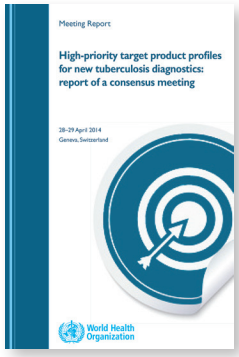
The landscape of TB diagnostics has changed dramatically during the past 10 years, and in response WHO's Global TB Programme has implemented a systematic and dynamic process that is used to evaluate technologies and develop policies (*Figure 1*). A summary of the evidence assessed by WHO's Global TB Programme when evaluating diagnostic technologies is presented in *Figure 1*, and each of the phases shown in the figure is described below. WHO uses the GRADE (Grading of Recommendations Assessment, Development and Evaluation) process to systematically assess the evidence.[4]

Figure 1. The phases of TB diagnostics development and assessment for WHO recommendation using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) process



Phase 1: Research and development

This is the discovery phase for new diagnostic technologies. It includes a feasibility assessment aimed at developing a final version of the technology (the design-locked product) that can be used in subsequent evaluations. The developers of diagnostic tests are encouraged to engage in early discussions with WHO to ensure that the new technology will be appropriate for the end-users. Priority target product profiles (TPP) for new diagnostics, developed following a consensus building process, are described in the TPP meeting report.[5]



http://www.who.int/tb/publications/tpp_report/en/

Phase 2: Evaluation and demonstration

Controlled laboratory trials, or evaluation studies, are often conducted at the level of reference laboratories and should be performed in three to five sites in different countries that have a high burden of TB and varying epidemiology in terms of TB, HIV infection and MDR-TB. Data generated from these initial trials are often used for product registration with global or national regulatory authorities, or both. It should be noted that WHO is not a regulatory authority.

Following the initial validation of a technology in evaluation studies, field demonstration studies are required in 5–10 sites to validate the specifications and performance characteristics in the intended settings of use. These studies also should be undertaken in high-burden countries in which the burden of TB, HIV infection and MDR-TB varies epidemiologically.

Phase 3: Evidence assessment by WHO

During this phase, WHO evaluates a dossier about new technologies or new indications for an existing technology, provided the technology is not intended for use only in a specific country. The dossier contains data from phase 1 and phase 2 (*Figure 1*).

Data on new technologies (or new indications for the use of technologies already recommended by WHO) from controlled evaluations and field-demonstration studies, as well as from operational research studies and cost–effectiveness analyses, are systematically assessed by an independent group of experts convened by WHO.

The expert group uses the GRADE process as a systematic, structured framework to evaluate the diagnostic accuracy of new tools and their effect on patients and public health. The expert group synthesizes the evidence using systematic reviews and meta-analyses (where possible), in accordance with WHO's standards.

For generic versions of technologies already recommended by WHO, comparative data are needed to determine their non-inferiority (that is, their equivalence) in performance. Non-inferiority studies must be multicentre, blinded and conducted independently by at least three members of WHO's TB Supranational Reference Laboratory Network.

The end result of the process is the development of policy guidance based on the outcome of the expert group's consensus meeting together with input from the Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) for dissemination to Member States and other stakeholders.

Phase 4: Phased uptake and evidence for scale-up

The new technology is implemented in routine TB services including in high burden TB and HIV settings. WHO subsequently evaluates operational issues associated with implementation, as well as the cost effectiveness of a new technology, by engaging with early implementers in different countries and settings.

Phase 5: Scale-up and policy refinement

WHO's process for policy development is a dynamic mechanism, and diagnostic policies are regularly reviewed (every 3 to 5 years); during these reviews, additional evidence is evaluated, allowing initial guidance to be updated and refined for further country-level scale-up.

5. Components of a high-quality network of TB laboratories

Establishing, equipping and maintaining a network of laboratories is challenging, complex and resource-intensive. The introduction of new techniques is bound to fail if the core elements of laboratory services are not addressed at the same time.

5.1 Planning for the implementation of diagnostic services

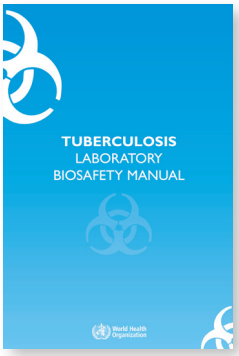
The process of determining which laboratory services are needed to support a comprehensive national TB programme is based on:

- an estimate of the total population to be served;
- an assessment of the epidemiological situation (TB, MDR-TB and HIV);
- the establishment of a national algorithm (or algorithms) for testing (including information such as the groups being targeted for testing, and the other TB tests in use);
- an analysis of existing laboratory services and available resources to identify gaps in funding for the laboratory network;
- a country's diagnostic policy and the arrangement of the network:
  - the level where a specific diagnostic technique will be placed;
  - the structure and functionality of the referral network for samples – that is, the geographical distribution of laboratories and testing sites;
- the technologies being used:
  - the capacity of technologies and their performance characteristics;
  - the maintenance requirements for equipment;
  - the expected lifetime of the equipment;
- the stage of implementation:
  - whether the technology has recently been introduced (early stage) or is routinely being used (later stage);
- the regulations regarding labour:
  - the number and proficiency of the personnel;
  - the number of hours personnel are permitted to work;
  - the number of working days per week, month and year.

5.2 Ensuring appropriate laboratory infrastructure and its maintenance, as well as biosafety measures

Each laboratory needs to have sufficient space to ensure the quality, safety and efficiency of the services provided to the clients whose samples are tested and to ensure the safety of laboratory personnel, patients and visitors.[6]

Handling specimens and cultures containing *Mycobacterium tuberculosis* poses various risks. Therefore, WHO has developed a risk-based approach that should be implemented worldwide to improve TB laboratory biosafety. WHO's *Tuberculosis laboratory biosafety manual* is the result of consensus guidelines developed to specify the minimum standards required for the safe use of TB procedures in a laboratory; the manual has been published in Chinese, English, French, Portuguese, Russian and Spanish.[6]



http://www.who.int/tb/publications/2012/tb_biosafety/en/

This risk-based approach considers the bacillary load of materials (such as specimens and cultures), the viability of the bacilli, whether the material might generate aerosols, the number of manoeuvres in each technique that might generate infectious aerosols, the workload of laboratory staff, the epidemiological characteristics of the population that the laboratory serves, as well as any additional precautions that may be needed to protect laboratory workers who have an increased susceptibility to TB. Three levels of risk have been identified for TB laboratories:

- low-risk TB laboratories;
- moderate-risk TB laboratories;
- high-risk laboratories (that is, TB-containment laboratories).

Explanations of the risk levels, the associated activities, and assessments of the risks are presented in *Table 1*.

Table 1. Description of risk levels for TB laboratories, by activity and associated assessment of risk

Risk level for TB laboratory[a]	Laboratory activities	Assessment of risk
Low risk	Direct sputum-smear microscopy; preparation of specimens for use in an automated nucleic acid amplification test (such as the Xpert MTB/RIF assay)	Low risk of generating infectious aerosols from specimens; low concentration of infectious particles
Moderate risk	Processing and concentration of specimens for inoculation onto primary culture media; direct DST (for example, line-probe assays on processed sputum)	Moderate risk of generating infectious aerosols from specimens; low concentration of infectious particles
High risk (TB-containment laboratory)	Culture manipulation for species identification; DST or line-probe assays on cultured isolates	High risk of generating infectious aerosols from specimens; high concentration of infectious particles

DST, drug-susceptibility testing.

[a] The risk level refers to how likely it is that someone in the laboratory will become infected with TB as a result of procedures performed in the laboratory.

5.3 Validating and maintaining equipment

Validating and maintaining equipment are necessary to ensure effective functioning. These activities should be scheduled routinely and documented using standard operating procedures.[7]

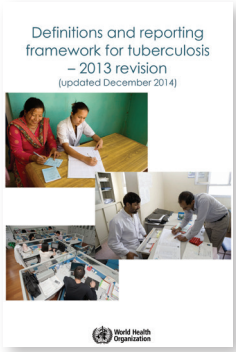
Validation is important to show that each piece of equipment is able to perform the tasks expected and that it delivers the appropriate results.

A schedule of preventive maintenance and calibration should be designed for each piece of equipment. If calibration and maintenance are easy to perform, then a staff member or a designated equipment officer may perform the tasks (with or without additional technical training). If the equipment is sensitive and maintenance or calibration is complex, it is better to hire an external, specialized company to perform these tasks. In some cases, manufacturers offer maintenance and calibration services.

5.4 Collecting, transporting and referring specimens

Good quality specimens are necessary to ensure that laboratories properly diagnose TB. The national TB programme or the national TB reference laboratory, or both, should determine what demographic information must be included on each specimen container and the request for examination form in order for the specimen

to be accepted and processed. The WHO revised definitions and reporting framework for TB suggests what information should be included and provides samples of request forms.[8] The national TB programme and the national TB reference laboratory should monitor and offer advice to provincial health units.



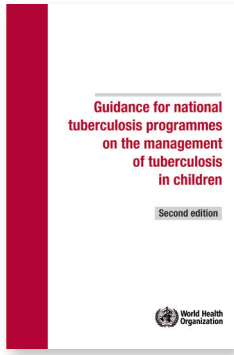
http://www.who.int/tb/publications/definitions/en/

The sputum-collection process represents a significant hazard since coughing produces potentially infectious aerosols. Whenever possible, sputum specimens should be collected outdoors where the infectious droplets will be rapidly diluted and ultraviolet light can inactivate the TB bacilli. Specimens should never be collected in enclosed spaces that have not been specifically designed and equipped for sputum collection, such as laboratories. When ventilated sputum-collection rooms are used and maintained correctly, they are a safe alternative to outdoor collection.

Staff must be trained to provide patients with easy-to-follow instructions about how to collect a good quality specimen. Patients must be provided with appropriate supplies, including wide-mouthed screw-capped sterile containers that are clear and leak-proof. Providing instructions on posters and in leaflets in designated sputum-collection areas can be helpful. Nevertheless, it is recommended that a staff member should supervise a patient's first attempt to collect a specimen to help the patient understand the protocol and ensure that an appropriate specimen is collected.

Staff should ensure that specimen containers are transported to a laboratory within 24 hours of collection or sooner, using packaging that is appropriate to safely transport infectious material. If delays in transport are anticipated to last longer than 24 hours, specimens should be refrigerated at 4 °C and transported to the laboratory in a cool box. This is especially relevant for specimens for TB culture and DST.

Children with TB may swallow mucus that contains *M. tuberculosis*. Gastric aspirates are used for collection of samples for microscopy and mycobacterial cultures in young children when sputa cannot be either spontaneously expectorated or induced using hypertonic saline. Annex 4 in the WHO Guidance for national TB programmes on the management of TB in children describes the procedures for obtaining samples from children.[9]



http://www.who.int/tb/publications/childtb_guidelines/en/

Developing a reliable system for transporting specimens will ensure that the full benefit of testing is gained by reducing diagnostic delay. Some options for delivering specimens to TB testing laboratories include:

- using government-run or clinic-operated courier services (which may include motorized vehicles, motor bikes or bicycles);
- asking medical personnel who regularly travel to remote clinics and subsequently return to health facilities in population centres to transport specimens;
- contracting or otherwise making a formal agreement with drivers of commercial delivery vehicles that travel between cities and remote villages to transport specimens.

5.5 Managing laboratory equipment and supplies

The effective management of laboratory equipment and supplies in any setting (public or private) at any level (local, regional, provincial or national) requires planning, understanding the past use of supplies, and anticipating changes in the workload.

Laboratories cannot operate successfully unless they have uninterrupted access to the relevant supplies. A laboratory may be forced to temporarily discontinue services if access is interrupted to media, reagents or other supplies. Offering intermittent service is worse than not offering service at all since those who rely on the laboratory cannot predict whether testing will be available.

Although it is detrimental not to have sufficient supplies, overstocking can be equally problematic, especially for supplies that have expiry dates. Additionally, supplies must be stored in clean, dry, well-ventilated and well-organized storage areas; and requirements for appropriate temperatures, humidity levels and light must be met. Managing a laboratory's commodities involves careful planning and coordination, and should follow a well- known cycle of selection, procurement, distribution and use.

To ensure ease of procurement, each laboratory should have comprehensive lists of the required equipment, reagents and disposables needed for tests, along with detailed specifications for each item.

5.6 Implementing systems to manage laboratory information and data

Each laboratory needs a system for collecting, processing, recording, reporting, storing and retrieving data from examinations and other information. This system must be validated, protected from unauthorized access, safeguarded against tampering or loss, and maintained in a manner that ensures the integrity of the data and complies with national and international requirements for data protection.

Laboratory-information and data-management systems should be able to monitor:

- the requisition, receipt, scheduling and performance of tests;
- the collection and management of samples, and the chain of custody;
- the distribution of test results to clinicians;
- inventory;
- general laboratory reporting (including billing and contracts);
- workload statistics and laboratory performance;
- human resources development, including training;
- quality control and external quality assessment processes;
- laboratory biosafety measures;
- and additional functions as necessary.

The laboratory-information and data-management systems may be paper-based or electronic. Since the mid-1990s there has been increased use of and interest in the electronic recording and reporting of TB data, including data produced by laboratories.

Compared with paper-based reporting, there are potential benefits to capturing and storing TB data electronically. The immediate benefits include:

- improving data quality by, for example, highlighting values that are outside the normal limits for a particular category;
- decreasing the workload – for example, data may be entered once instead of on multiple forms;
- facilitating access to data;
- ensuring the timeliness of information;

- having flexibility to modify the recording or reporting format; and
- facilitating data analysis and reporting, and making it easier to manage complex data.

Additional information can be found in WHO's *Electronic recording and reporting for tuberculosis care and control*.[10]

http://who.int/tb/publications/electronic_recording_reporting/en/

Software platforms with an open application programming interface can be integrated with any web-based database or monitoring and evaluation system. The objective is to connect TB diagnostic devices to routine monitoring and evaluation systems. These interfaces may be able to work with a number of diagnostic technologies, including automated liquid culture systems, line-probe assays (LPA) and the GeneXpert platform. Assuming that there is Internet connectivity, these interfaces can provide updates to monitoring and evaluation systems within several seconds of the diagnostic result becoming available in order to ensure the exchange of real-time information and updates.

Whether a laboratory's information and data-management systems are electronic or paper-based, they need to be integrated with the data-management systems used by the national TB control programme. When paper-based systems are used, data should be regularly cross-checked to ensure internal consistency.

5.7 Implementing quality-management systems for a laboratory

A quality-management system (QMS) affects each process in a laboratory and consists of several levels, often visualized together as a pyramid (*Figure 2*).

Figure 2. Representation of the quality-management system as a pyramid

The base of the pyramid, or the basis of a quality-management system, consists of inspection. At this level of the pyramid, laboratory technologists routinely inspect their own work, and whether errors are detected depends on their own alertness.

In comparison, at the next level of the pyramid, quality assurance is an active, structured, conscious and rational inspection process. This process is undertaken by the laboratory to monitor whether tasks are performed correctly and to monitor the quality of the laboratory's output; it includes implementing quality-assurance steps at strategic, logical points in each process. This enables the laboratory to be certain that its final output – that is, the test result – is of good quality.

The three important elements of quality assurance are (*i*) internal quality control; (*ii*) external quality assessment and (*iii*) continuous quality improvement.

However, quality-control processes detect errors only after they have happened. A distinctive feature of quality-management systems is that not only is attention paid to detecting and correcting problems, but it is also paid to preventing problems. One way in which this is achieved is through standardization. Standardization requires that all critical processes in a laboratory are validated and that they are documented as standard operating procedures (SOPs). By implementing SOPs and continually following them, the laboratory ensures that processes are always carried out in the same, correct way. Hence, the quality of those processes is ensured.

Many activities are involved in controlling and ensuring quality. An organizational structure known as the quality system is necessary to ensure that:

- these activities are always carried out at the right time and by the right person;
- the right number of appropriately trained staff are available;
- the necessary equipment and consumables are available;
- manuals, guidelines, forms and SOPs are in place so that processes are always carried out correctly.

Quality management encompasses all of the planning, leadership and management activities necessary to ensure that an organization functions correctly – that is, to ensure that the quality system functions properly. These activities are at the top level of the quality-management system pyramid.

There are four main sources of information and guidance that can be used to assist TB laboratories in developing and maintaining a QMS:

- The International Organization for Standardization (ISO) standard 15189:2012;
- *The GLI stepwise process towards TB laboratory accreditation: quality management*;
- The framework known as the *WHO guide for the stepwise laboratory improvement process toward accreditation in the African Region* (SLIPTA);
- The framework known as the *Strengthening laboratory management towards accreditation* (SLMTA).

ISO standard 15189:2012

The most recent edition of this standard (*15189:2012 Medical laboratories – requirements for quality and competence*) contains a description of the components necessary for a QMS to be effective in a medical laboratory.[11] These components have been further organized into 12 groups of quality-system essentials by the Clinical and Laboratory Standards Institute.[12]

GLI *stepwise process towards TB laboratory accreditation: quality management*

The *GLI stepwise process towards TB laboratory accreditation* is an online tool available at http://www.gliquality.org/ [13] This computer-based interactive tool can help national TB reference laboratories implement in a gradual manner all of the requirements of ISO 15189:2012, which will lead to the

development of a properly functioning QMS. The use of this tool may require the involvement of a consultant or group of individuals to act as the driving force to encourage a laboratory to work through the tool systematically, as well as to provide technical support to the implementing laboratory. Embarking on this process will require additional dedicated human resources capacity, which must be considered.

WHO guide for the stepwise laboratory improvement process towards accreditation in the African Region

SLIPTA was originally developed by WHO's Regional Office for Africa. This checklist is based on the ISO 15189:2007 standard and the Clinical and Laboratory Standards Institute's guideline GP26-A4:2011). The checklist was developed to monitor progress in improvement of a laboratory's QMS. It is based on the 12 quality-system essentials, and is applicable to all laboratory settings and disciplines.[14] This tool has been modified by FIND to address specific elements for TB laboratories and is referred to as TB-SLIPTA.[15]

Strengthening laboratory management towards accreditation

This is a task-based mentoring programme developed by the United States Centers for Disease Control and Prevention, to enable laboratories to implement the different components of a QMS, with the ultimate goal of achieving accreditation as a practicing laboratory.[16] The Foundation for Innovative New Diagnostics (FIND) has developed the *TB stepwise laboratory management towards accreditation training programme*. This is a modified version of the SLMTA programme, called TB-SLMTA, that includes TB-specific modules and guidance (in areas such as safety, sputum collection and specimen transport) and TB-specific activities, examples and tools.[17]

Accreditation is a means of recognizing a laboratory's quality and competence. Accreditation is achieved when a laboratory has a QMS in place that complies with the requirements of ISO standard 15189:2012. An independent accreditation body assesses the laboratory to determine whether the QMS functions as it should and whether it complies with the ISO standard. This accreditation body must operate according to ISO standard 17021:2011 (*Conformity assessment – requirements for bodies providing audit and certification of management systems*)[18] and must be an affiliate or member of the International Laboratory Accreditation Cooperation.[19]

National level reference laboratories are encouraged to meet all of the requirements in the ISO standard 15189:2012. At a minimum, lower-level laboratories are required to implement internal quality control and external quality-assessment processes. All diagnostic techniques used in a laboratory should be quality assured. It is important that supervision and technical support are routinely provided from the upper level of the laboratory network to the lower level to ensure that the network operates effectively. The TB microscopy network assessment tool provides a framework for evaluating a laboratory network and uses 11 standards covering all aspects required for optimal functioning of a laboratory network. The tool is primary targeted at microscopy networks, but can be adapted to evaluate the implementation of other diagnostic technologies (e.g. Xpert MTB/RIF) at lower levels of the laboratory networks.[20]

5.8 Ensuring appropriate strategies for managing human resources and adequate funding for human resources

The workforce is a critical and essential part of a health-care system. The development of functional TB laboratory networks are at different stages in different countries, and countries possess variable capacities for improving the skills of and maintaining their workforce; however, there are common challenges, including developing strategies for improving the recruitment, education, training and distribution of personnel; enhancing their productivity and performance; and improving retention of skilled staff.

6. Using diagnostic techniques in a tiered laboratory network

Most countries have three levels of laboratory services within their networks (*Figure 3*), and different tests are performed at different levels of the network. (Details of the tests performed at each level are shown in *Table 2*) Additionally, clear mechanisms are required for referring specimens for additional testing. This stratification is the result of the specialized nature of the technical procedures, the structure of laboratory management and administration, and the need for quality control.

Figure 3. The three tiers of a network of TB laboratories and the responsibilities of each level of the network (details of the tests performed at each of the different levels are shown in *Table 2*)

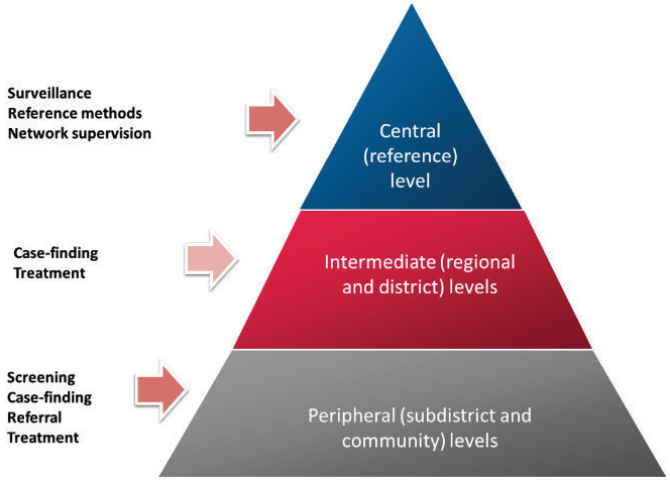


Table 2. WHO's recommendations for appropriate testing at different levels of a TB laboratory network

Laboratory level	Function	Tests
Peripheral (subdistrict[a] and community)	Screening[b], case-finding, referral, treatment	AFB smear exams using either Ziehl–Neelsen stain with light microscopy, or fluorochrome stain with fluorescence microscopy (preferably with LED illumination); Xpert MTB/RIF assay
Intermediate (regional and district[c])	Case-finding, treatment follow up	All tests performed at the peripheral level and possibly culture on solid media and LPA directly from AFB smear-positive sputum
Central (reference)	Case-finding, treatment follow up, surveillance, development and provision of reference methods and standards, supervision of laboratories in the network	All tests performed at the peripheral and intermediate levels plus liquid culture, DST for first-line and second-line anti-TB agents (including fluoroquinolones and injectable agents) on solid or in liquid media; LPA on positive cultures and AFB-positive sputum; and rapid speciation tests

AFB, acid-fast bacilli; LED, light-emitting diode; LPA, line-probe assay; DST, drug-susceptibility testing.
[a] In some settings subdistrict level can be part of the intermediate tier of TB laboratory network.
[b] In some settings at the community level, screening activities may be part of TB case-finding, most frequently assuming use of non-laboratory tools such as patient questionnaires. This may be followed by referral to higher levels of the network where sputum microscopy or Xpert MTB/RIF diagnostic techniques, or both, are available. If TB is diagnosed, treatment should be initiated either at the community or higher levels.
[c] In some settings district level can be part of the peripheral tier of TB laboratory network.

7. WHO's recommended techniques for diagnosing TB

7.1 Microscopy

Mycobacteria can be visually distinguished from other microorganisms by their thick lipid-containing cell walls, which retain biochemical stains despite decolourization by acid-containing reagents (known as 'acid fastness'). Given that the examination of two sputum specimens is adequate to identify the majority (95-98%) of smear-positive TB patients, WHO's current policy on case-finding using microscopy recommends that in settings with appropriate external quality assessment and documented good-quality microscopy two specimens should be examined. In settings with appropriate quality assurance procedures, a case is defined as someone with one positive smear – that is, at least 1 acid-fast bacillus in at least 100 microscopic fields.[21]

In 2010, WHO confirmed the diagnostic accuracy of examining two consecutive smears on the same day to diagnose TB, so that treatment can be started during the patient's first visit to a health-care facility.[22]

Advantages

- Microscopy of sputum smears is simple and inexpensive, and allows rapid detection of the most infectious cases of pulmonary TB. Sputum specimens from patients with pulmonary TB, especially those with cavitary disease, often contain sufficiently large numbers of AFB to be detected by microscopy.
- Microscopy is suitable for peripheral-level and higher-level laboratories.
- Microscopy can be done safely in a laboratory that has implemented only a low level of precautions to mitigate the risk of laboratory acquired TB infection.
- It is a simple, rapid and inexpensive test and is necessary for treatment follow up of patients with susceptible TB.

Disadvantages

- Direct sputum-smear microscopy is relatively insensitive: at least 5 000 bacilli per ml of sputum are required for a positive result. The sensitivity is further reduced in patients with extrapulmonary TB, children and in those who are coinfected with HIV.
- A comprehensive quality assurance programme is necessary; although this may be challenging to implement, it is necessary to ensure high-quality test results.

Limitations

- Microscopy for AFB cannot distinguish Mycobacterium tuberculosis complex from nontuberculous mycobacteria; it cannot distinguish viable from nonviable organisms; and it cannot distinguish drug-susceptible strains from drug-resistant strains.
- Smears that have been stained with auramine will need to be stained again if they are to be rechecked as part of an external quality assessment programme.

7.1.1 Conventional light microscopy

Direct Ziehl–Neelsen staining of sputum specimens and examination using light microscopy is suitable for use at all levels of laboratory, including peripheral laboratories at primary health-care centres or district hospitals. There is insufficient evidence that processed sputum specimens (for example, those that are concentrated or chemically treated) give better results than direct smear microscopy. Therefore, the use of such methods is not recommended.[23]

The number of Ziehl–Neelsen smears examined by 1 microscopist each day should not exceed 20–25 because visual fatigue can occur and lead to a deterioration in quality.

In general, it is sufficient for there to be 1 centre using Ziehl–Neelsen staining and light

microscopy per 100 000 population;[1] however, if services are expanded, then it is important to consider the location of the centre, the workload of the technicians, the accessibility of the centre to the population as well as the effectiveness of specimen transportation.

7.1.2 Light-emitting diode fluorescence microscopy

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

Evidence shows that the diagnostic accuracy of LED microscopy is comparable to that of conventional fluorescence microscopy and it surpasses that of conventional Ziehl–Neelsen microscopy (by an average of 10%). Therefore, WHO recommends replacing conventional fluorescence microscopy with LED microscopy, and that LED microscopy should be phased in as an alternative to conventional Ziehl–Neelsen light microscopy in all settings, prioritizing high-volume laboratories.[24]

7.2 Culture and species identification

Mycobacteria can be cultured in specific solid or liquid media. Bacterial growth can be identified visually (that is, by identifying specific characteristics) or by automated detection of its metabolism. All positive mycobacterial cultures must be tested to confirm the identification of *M. tuberculosis* complex (MTBC).

The *M. tuberculosis* complex comprises eight distinct closely related organisms, the most common and important agent of human disease is *M. tuberculosis*. The complex includes *M. bovis* (the bovine tubercle bacillus—characteristically resistant to pyrazinamide, once an important cause of TB transmitted by unpasteurized milk, and currently the cause of a small percentage of human cases worldwide), *M. caprae* (related to M. bovis), *M. africanum* (isolated from cases in West, Central, and East Africa), *M. microti* (the "vole" bacillus, a less virulent and rarely encountered organism), *M. pinnipedii* (a bacillus infecting seals and sea lions in the Southern Hemisphere and recently isolated from humans), *M. mungi* (isolated from banded mongooses in southern Africa), *M. orygis* (described recently in oryxes and other Bovidae in Africa and Asia and a potential cause of infection in humans), and *M. canetti* (a rare isolate from East African cases that produces unusual smooth colonies on solid media and is considered closely related to a supposed progenitor type).[25]

Differentiation of the members of the MTBC is necessary for the treatment of individual patients and for epidemiological purposes, especially in areas of the world where tuberculosis has reached epidemic proportions or wherever the transmission of *M. bovis* between animals or animal products and humans is a problem. In addition, it can be important to rapidly identify isolates of *M. bovis* bacillus Calmette-Guérin (BCG) recovered from immunocompromised patients. Differentiation of species with the MTBC can be achieved using either phenotypic[26] and/ or genotypic methods.[27]

The use of rapid immunochromatographic assays (or strip tests for speciation) to identify cultured isolates is recommended because they provide definitive identification of all members of the MTBC (including *M. bovis*) in 15 minutes.[28]

Much remains to be understood about the pathogenesis of non-tuberculosis mycobacteria (NTM) infection and disease in humans. There is no evidence to suggest either animal-to-human or human-to-human transmission of NTM and it is assumed that most persons are infected by NTM from the environment. NTM may cause both asymptomatic infection and symptomatic disease in humans. Several factors increase the likelihood of clinical significance of NTM isolates, including the recovery from multiple specimens or sites, recovery of the organism in large quantities (AFB smear–positive specimens), or recovery of

an NTM isolate from a normally sterile site such as blood.

Awareness of the context from which an NTM isolate is obtained can be critically important in determining the need for speciation of that isolate. Hence, communication between the clinician and laboratorian is essential for determining the importance and extent of identification and for drug susceptibility testing of an NTM isolate. Differentiation of NTM species can be achieved using a variety of phenotypic or genotypic methods.[29]

Conventional solid or liquid culture is required to monitor the treatment of patients with MDR-TB.

Advantages

- Culture and identification of *M. tuberculosis* provide a definitive diagnosis of TB as well as significantly increasing in the number of cases identified when compared with microscopy: there is often an increase of 30–50%.
- Culture also provides the necessary isolates for conventional DST.

Disadvantages

- Culture is more complex and expensive than microscopy; it also takes longer, requiring facilities for preparing media, processing specimens and encouraging the growth of organisms.
- Culture also requires specific laboratory equipment, technicians with additional skills, and appropriate biosafety conditions.

Limitations

- Specimens must be decontaminated before culture to prevent overgrowth by other microorganisms. To some extent, all decontamination methods are also harmful to mycobacteria; therefore, culture is not 100% sensitive.
- Good laboratory practices must maintain a delicate balance between the yield of mycobacteria and contamination by other microorganisms.

Solid and liquid culture methods are suitable for central reference laboratories (regional laboratories in large countries) or intermediate-level laboratories. Solid culture methods are less expensive than liquid, but the results are invariably delayed because of the slow growth of mycobacteria. Liquid culture increases the case yield by approximately 10% over solid media, and automated systems reduce the diagnostic delay to days rather than weeks. However, liquid systems are more prone to contamination, and the manipulation of large volumes of infectious material mandates appropriate additional biosafety measures.[30]

7.3 Drug-susceptibility testing

DST determines whether a strain is susceptible to particular anti-TB agents: a result indicating that the strain is sensitive to particular agents means that treatment with those agents will most likely be successful; a result indicating that a strain is resistant means that there is a high possibility that treatment with those agents will fail and, therefore, other agents should be used. Thus, using standardized and reliable DST for *M. tuberculosis* provides guidance on treating a patient.

Different techniques are available.

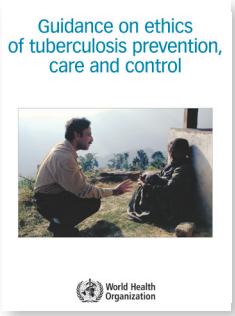
- Phenotypic methods involve culturing *M. tuberculosis* in the presence of anti-TB agents to detect growth (which indicates resistance) or inhibition of growth (which indicates susceptibility).
- Genotypic methods target specific molecular mutations associated with resistance against individual anti-TB agents (for more details, see section 7.4).

Phenotypic DST methods are performed as direct or indirect tests in solid or liquid media. *Direct testing* involves inoculating drug-containing and drug-free media directly with a concentrated specimen. *Indirect testing* involves inoculating drug-containing media with a pure culture grown from the original specimen.

Indirect phenotypic tests have been extensively validated. Three methods are commonly used: the proportion, absolute concentration and resistance ratio methods. For first-line anti-TB agents, the results obtained do not differ significantly among the three methods.

Liquid culture systems for DST reduce the time to result to as little as 10 days, compared with the 28–42 days needed for conventional solid media. Because liquid culture systems have increased sensitivity and reduce delays in diagnosis, they may contribute significantly to improving patient management.[30]

WHO recommends that formal links be established between the TB Supranational Reference Laboratory (SRL) Network and national reference laboratories to ensure that DST is available for both first-line and second-line anti-TB agents. Countries wishing to offer DST should seek advice from the TB Supranational Reference Laboratory Network to ensure they have continual, adequate expert input into the requirements for laboratory design, the transportation of specimens, processes, biosafety standards, SOPs, schedules for maintaining equipment, and processes for external quality assessment. The absence of capacity to treat patients with MDR-TB should not deter countries to build capacity for DST, as it is ethically justified.[31]



http://www.who.int/ethics/publications/9789241500531/en/

Advantages

DST provides a definitive diagnosis of drug-resistant TB.

Disadvantages

- Non-molecular DST methods take longer to provide results.
- These methods are suitable for use only at the central reference laboratory level, given the need for appropriate laboratory infrastructure (particularly biosafety precautions) and the technical complexity of the techniques and methods.
- Liquid DST fails to detect some clinically relevant "borderline rifampicin resistant strains" with *rpo*B mutations.[32]

Limitations

- The accuracy of phenotypic DST varies according to the anti-TB agent being tested.

7.3.1 Drug-susceptibility testing for first-line anti-TB agents

DST is most accurate in detecting susceptibility to rifampicin and isoniazid; results are less reliable and reproducible for streptomycin, ethambutol and pyrazinamide.

At a minimum, national TB-control programmes should establish sufficient laboratory capacity to detect rifampicin-resistant TB (RR-TB) or MDR-TB (MDR-TB is TB that is resistant to at least isoniazid and rifampicin). In many settings and for many groups of patients, rifampicin resistance is a valid indicator of or proxy for MDR-TB. Persons at risk for MDR-TB should be targeted as a priority for rapid DST. Phenotypic culture-based DST methods, using the critical concentrations recommended by WHO in the updated table, are the current reference standards for rifampicin resistance.[33] However, a number of recent studies have raised concerns about using phenotypic DST to detect rifampicin resistance, in particular the automated liquid system.[34] If rifampicin resistance has been detected, DST for resistance to isoniazid and second-line anti-TB agents should be performed, following WHO's recommendations.[35] WHO will be updating policy recommendations on DST in 2016.

7.3.2 Drug-susceptibility testing for second-line anti-TB agents

Commercial liquid methods and the proportion method used on solid media have been studied; methods for the absolute concentration or resistance ratio methods on solid media for second-line anti-TB agents have not been validated. The recommended gold standard for DST for second-line anti-TB agents is the automated liquid system.[36] Routine DST for second-line agents is not recommended unless laboratory infrastructure and capacity have been established, rigorous quality assurance is in place and sustained proficiency has been demonstrated.[37]

Phenotypic DST for second-line injectable agents (kanamycin, amikacin, capreomycin) and fluoroquinolones (ofloxacin, levofloxacin, moxifloxacin, gatifloxacin) is generally reliable and reproducible across various settings.[37] The susceptibility of *M. tuberculosis* to all fluoroquinolones used by a national TB programmes should be tested to guide the choice of the most appropriate agent for treatment.

Current molecular methods cannot replace phenotypic DST for second-line agents because there is incomplete cross-resistance among second-line injectable agents. Current molecular methods cannot identify resistance to specific second-line injectable agents; thus, they cannot be used to guide the choice of second-line agents included in individualized MDR-TB regimens.[38]

Routine DST for other second-line agents (such as ethionamide, prothionamide, cycloserine, terizidone, *p*-aminosalicylic acid, clofazimine, amoxicillin/clavulanic acid, clarithromycin and linezolid) is not recommended because the reliability and reproducibility tests for these anti-TB agents cannot be guaranteed.

The WHO SRL network is currently developing and validating DST methods for the new and re-purposed second-line agents (bedaquiline, delamanid, clofazimine, linezolid).

7.3.3 Non-commercial methods

Non-commercial methods of culture and DST are less expensive than commercial systems but are prone to errors due to a lack of standardization and to local variations in the methods. The performance of these methods is highly operator-dependent; therefore, it is imperative that good laboratory practices are followed, good microbiological techniques are used, and there is adequate quality assurance, supported by adequate training. Similar to the conditions needed with commercial systems, non-commercial systems require the implementation and enforcement of stringent laboratory protocols, SOPs and internal quality controls.

The evidence base for selected non-commercial methods of culture and DST has been reviewed by WHO, and the performance of these methods has been found to be acceptable *in reference or national laboratories in selected settings only when stringent laboratory protocols are followed*.[39] The methods evaluated include the microscopic observation drug-susceptibility (MODS) assay, colorimetric redox indicator (CRI) methods, and the nitrate reductase assay (NRA). The recommendations for their use are listed below.

- **MODS** is a microcolony method that uses liquid culture. Drug-free and drug-containing media are inoculated, and this is followed by microscopic examination of early growth.

  MODS is recommended as a direct or indirect test for rapid screening of patients suspected of having MDR-TB.

- **CRI methods** are indirect methods. A coloured indicator is added to liquid culture medium on a microtitre plate after *M. tuberculosis* strains have been exposed to anti-TB agents in vitro. Resistance is detected by a change in the colour of the indicator, which is proportional to the number of viable mycobacteria in the medium.

CRI methods are recommended for use as indirect tests on *M. tuberculosis* isolates from patients suspected of having MDR-TB; however, the method is slower in detecting MDR-TB than conventional DST methods using commercial liquid culture and molecular LPAs, but it is less expensive (see section 7.4.1).

- **NRAs** can be used as direct or indirect methods on solid culture. NRAs are based on the ability of *M. tuberculosis* to reduce nitrate, which is detected by a colour reaction.

  NRAs are recommended for use as direct or indirect tests to screen patients suspected of having MDR-TB; however, indirect NRA is not faster in detecting MDR-TB than conventional DST using solid culture (see section 7.3).

Both commercial and non-commercial culture and DST systems and methods are suitable for use only by central or regional reference laboratories. Non-commercial methods are recommended for use only as an interim option while capacity is being developed for rapid genotypic DST. Furthermore, non-commercial methods have not been validated for use with second line agents.

7.4 Molecular testing

Genotypic methods have considerable advantages when the programmatic management of drug-resistant TB is being scaled up, in particular with regard to their speed, the standardization of testing, their potentially high throughput and the reduced requirements for biosafety. The ultimate aim should be to use molecular assays – including LPAs, Xpert MTB/RIF, and any other molecular platform that may be recommended by WHO in the future – for rapid first-step identification of RR-TB and MDR-TB.

7.4.1 Line-probe assays

Performing an LPA involves extracting DNA from *M. tuberculosis* isolates or directly from clinical specimens and using polymerase chain reaction (PCR) to amplify the resistance-determining region of the *rpo*B gene using biotinylated primers. Subsequently, labelled PCR products are hybridized with specific oligonucleotide probes immobilized on a strip. Colorimetric development of the captured and labelled hybrids enables the presence of *M. tuberculosis* complex to be detected as well as the presence of wild-type *M. tuberculosis*. It also detects mutations associated with drug resistance. If a mutation is present in one of the target regions, the amplicon will not hybridize with the relevant probe. Therefore, mutations are detected by a lack of binding to wild-type probes as well as by binding to specific probes for the most commonly occurring mutations. The post-hybridization reaction leads to the development of coloured bands on the strip at the site of probe binding, and it can be read by the laboratory technician.[40] In 2015, WHO plans to update the policy recommendations on LPA for the detection of rifampicin resistance conferring mutations as well as utility of LPA in detection resistance to fluoroquinolones (FQ) and second-line injectable anti-TB agents.

Advantages

- Molecular LPAs enable rapid detection (in less than 48 hours) of resistance to rifampicin (alone or in combination with resistance to isoniazid); they were endorsed by WHO in 2008, and WHO has provided detailed policy guidance on introducing them at the country level.[40]

- LPAs are a high throughput technology, allowing up to 48 specimens to be processed simultaneously and enabling several batches of tests to be done each day.

Disadvantages

- LPAs do not eliminate the need for conventional culture and DST.

- Available LPAs are recommended for use only on smear-positive sputum specimens and isolates of *M. tuberculosis*.

- Current LPAs cannot replace phenotypic DST for second-line anti-TB agents. There is

incomplete cross-resistance among second-line injectable agents. LPAs cannot identify resistance to specific second-line injectable agents; thus, they cannot be used to guide the choice of second-line agents included in individualized MDR-TB regimens.[38]

Limitations

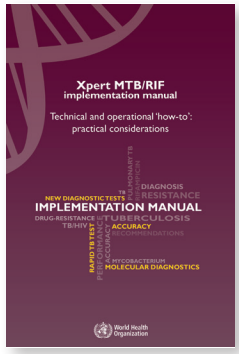
- LPAs are suitable for use at the central or national reference laboratory level; they have the potential to be used at the regional level if the appropriate infrastructure can be ensured (three separate rooms are required).
- The sensitivity of LPAs to detect resistance to isoniazid is lower (approximately 85%) than that of culture methods.

7.4.2 Xpert MTB/RIF assay

The Xpert MTB/RIF assay is an automated, cartridge-based nucleic acid amplification test (NAAT) that uses the multidisease GeneXpert platform. The Xpert MTB/RIF assay is performed directly on sputum, processed sputum sediment and selected extrapulmonary specimens from adults and children. GeneXpert instruments are modular, and options include systems with the capacity to have 1, 2, 4, 16, 48 or 80 independently functioning modules.

http://www.who.int/tb/laboratory/xpert_policyupdate/en/

The technology was first recommended by WHO in 2010, and a policy update was issued in 2013 following the meeting of an expert group to assess its use for detecting pulmonary and extrapulmonary TB and rifampicin resistance in adults and children.[35, 41] The "how to" Xpert MTB/RIF implementation manual was updated in 2014; it describes the operational aspects of and practical considerations associated with introducing and using the system.



http://www.who.int/tb/publications/xpert_implem_manual/en/

Advantages

- The Xpert MTB/RIF assay simultaneously detects *M. tuberculosis* and rifampicin resistance in less than 2 hours.
- The sensitivity of the Xpert MTB/RIF assay for detecting TB is similar to that of to liquid culture (sensitivity, 88% when compared with liquid culture as a reference standard); the specificity is also high (99%).
- For smear-negative culture-positive TB, the pooled sensitivity of Xpert MTB/RIF has been found to be 68%.[41] The superior performance of Xpert MTB/RIF in detecting TB over that of microscopy makes it a particularly useful tool for case-finding among people living with HIV. As a tool for detecting rifampicin resistance, Xpert MTB/RIF has a sensitivity of 95% and specificity of 98% when compared with phenotypic reference standards.
- The biosafety precautions required for Xpert MTB/RIF are similar to those for smear microscopy, and the training is minimal, which allows the technology to be used at relatively low levels in a laboratory network.

Disadvantages

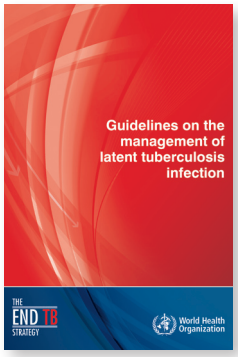
- A stable uninterruptable electrical supply is needed; in settings where extended power outages may occur, uninterrupted power devices (UPS) and/or additional batteries may be needed to provide up to 2 hours of power.
- The ambient operating temperature of the instrument cannot exceed 30 °C, and cartridges must be stored at less than 28 °C.
- The shelf-life of the cartridges must be monitored to prevent them from expiring before they are used; thus, careful planning and management of supplies are essential.
- Security measures must be put in place to prevent the theft of the accompanying laptop or desktop computer.

Limitations

- The modules require annual calibration; if modules fail the calibration test, using a specific calibration cartridge, they must be exchanged, which entails the importation of additional modules and exportation of the faulty modules.
- The use of Xpert MTB/RIF does not eliminate the need for conventional microscopy, culture and DST, which are required to monitor the progress of treatment and to detect resistance to anti-TB agents other than rifampicin.
- In patients who are not at risk for drug resistance but who initially test positive for rifampicin resistance by Xpert MTB/RIF, a second Xpert MTB/RIF test should be performed to control for preanalytical and postanalytical errors, and to improve the clinician's confidence in the diagnosis.[35, 41]
- An increasing amount of evidence has shown that the infrequent occurrence of false-positive results may be linked to the detection by Xpert MTB/RIF of strains that are truly resistant to rifampicin but for which resistance is not detected by phenotypic culture-based DST, which is the present reference standard. Such strains appear to have clinically relevant mutations in the region conferring resistance to rifampicin, causing disease for which first-line treatment is likely to fail. In cases where discordant results are obtained from Xpert MTB/RIF and phenotypic DST or LPA, the culture isolate should be referred to a reference laboratory for DNA sequencing; while awaiting the results, a clinical decision should be made whether to continue the MDR-TB regimen.[41]

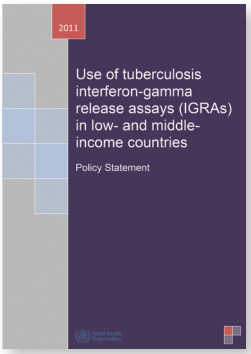
7.5 Testing for latent TB infection

Persons with latent TB infection (LTBI) do not have active TB disease but may develop it in the near or remote future, a process called TB reactivation. The lifetime risk of TB reactivation for a person with documented LTBI is estimated to be 5–10%, with the majority developing TB disease within the first five years after initial infection.[42, 43]



http://www.who.int/tb/publications/ltbi_document_page/en/

A direct measurement tool for *M. tuberculosis* infection in humans is currently unavailable, hence, there is no gold standard for the diagnosis of LTBI. The tuberculin skin test (TST) and Interferon-gamma release assays (IGRAs) indirectly measure TB infection by detecting memory T-cell response signifying the presence of host sensitization to *M. tuberculosis* antigens.



http://www.who.int/tb/features_archive/policy_statement_igra_oct2011.pdf

WHO recommends that either TST or IGRA can be used to test for LTBI in high-income and upper middle-income countries with estimated TB incidence less than 100 per 100 000 population.

IGRA should not replace TST in low-income and other middle-income countries.

Advantages

- IGRAs require a single patient visit, results are available in 24-48 hours, and prior BCG vaccination does not cause false-positive results.
- TST is widely used, not expensive, and does not require any special laboratory infrastructure or supplies.

Disadvantages

- TST requires two patient visits, results are available in 48-72 hours, and requires an injection into the skin, and adequately trained staff. Moreover, it has poor specificity in BCG-vaccinated populations, cross-reactivity with non-tuberculous mycobacteria and poor sensitivity in immunocompromised persons.
- IGRAs are expensive, require blood to be drawn, special laboratory infrastructure and supplies, and adequately trained staff. Given comparable performance but increased cost, replacing TST by IGRAs as a public health intervention in resource-constrained settings is not recommended.

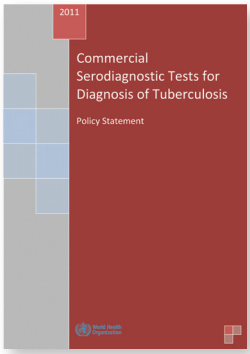
Limitations

- IGRAs and the TST cannot accurately predict the risk of infected individuals developing active TB disease.
- Neither IGRAs nor the TST should be used for the diagnosis of active TB disease.

8. Techniques not recommended by WHO for the diagnosis of active TB

8.1 Commercial serodiagnostic tests for diagnosis of active TB disease

It is strongly recommended that commercial serodiagnostic tests not be used for the diagnosis of pulmonary and extra-pulmonary TB. Currently available commercial serodiagnostic tests (also referred to as serological tests) provide inconsistent and imprecise findings. There is no evidence that existing commercial serological assays improve patient outcomes, and high proportions of false-positive and false-negative results may have an adverse impact on the health of patients.[44]



http://whqlibdoc.who.int/publications/2011/9789241502054_eng.pdf?ua=1

8.2 IGRA for diagnosis of active TB disease

There is no consistent evidence that IGRAs are more sensitive than TST for diagnosis of active TB disease. Studies evaluating the incremental value of IGRAs to conventional microbiological tests show no meaningful contribution of IGRAs to the diagnosis of active TB. IGRAs are considered inadequate as rule-out or rule-in tests for active TB, especially in the context of HIV infection. IGRAs should not be used for the diagnosis of active TB disease.

9. Algorithms for diagnostic testing

According to international standards for TB care, all patients, including children, who have an unexplained cough lasting 2 weeks or longer or who have unexplained findings on chest radiographs that are suggestive of TB should be evaluated for TB.[45] People living with HIV or at risk for HIV who report any one of the symptoms of current cough, fever, weight loss or night sweats should be evaluated for TB and other diseases.[46]

Case-finding strategies for drug-resistant TB have been published, and the role of DST in identifying MDR-TB and extensively drug-resistant TB (XDR-TB) have been outlined.[47] Detecting HIV-associated TB and drug-resistant TB requires the use of appropriate diagnostic algorithms for laboratory testing that take account of the groups of patients who are at greatest risk to ensure the cost-effective use of limited laboratory and diagnostic resources. Such algorithms will be highly specific to each country and depend on the factors described below.

Choosing one diagnostic technique does not rule out using others as well.

- Microscopy is suitable for peripheral and higher-level laboratories. It can be done safely in a laboratory that has implemented only a low level of precautions to mitigate risks of laboratory acquired infections. Microscopy enables rapid detection of the most infectious cases of TB.
- Culture is suitable for intermediate and higher-level laboratories that have implemented a moderate level of precautions to mitigate risks of laboratory acquired infections.
- Selected non-commercial culture and DST methods are suitable for direct use only on smear-positive specimens.
- LPAs are currently limited to detecting resistance to rifampicin and isoniazid, and are suitable only on smear positive specimens.
- The Xpert MTB/RIF assay is suitable for diagnosing TB and detecting RR-TB. It requires laboratory infrastructure and biosafety precautions that are similar to that required for microscopy.
- Capacity is needed for conventional microscopy, culture and DST to monitor the progress of treatment.
- Conventional DST capacity is needed to detect resistance to anti-TB agents other than rifampicin and isoniazid; it is needed to detect XDR-TB.
- Liquid culture, molecular LPAs and the Xpert MTB/RIF assay should be phased in to programmes in a way that ensures that the existing capacity for solid culture and DST is maintained.
- Rapid non-commercial methods of phenotypic DST present an interim option for use in resource-constrained settings while the capacity for genotypic testing is being developed.
- The choice of new techniques and methods to be used for detecting TB should be made by ministries of health in the context of their national strategic plans for laboratory strengthening and with input from laboratory experts.
- The diagnostic capacity for TB should be linked to efforts to ensure access to anti-TB agents and adequate programmatic capacity for treating patients according to appropriate standards of care.

Laboratory policies and diagnostic algorithms devised at the country level are largely dictated by the epidemiology of TB, the prevalence of HIV-associated TB and drug-resistant TB, and the

resources available. The clinical management of HIV-associated TB and drug-resistant TB also require concurrent clinical laboratory capacity (for example, for biochemistry, haematology and general microbiology) to monitor treatment and comorbid conditions.

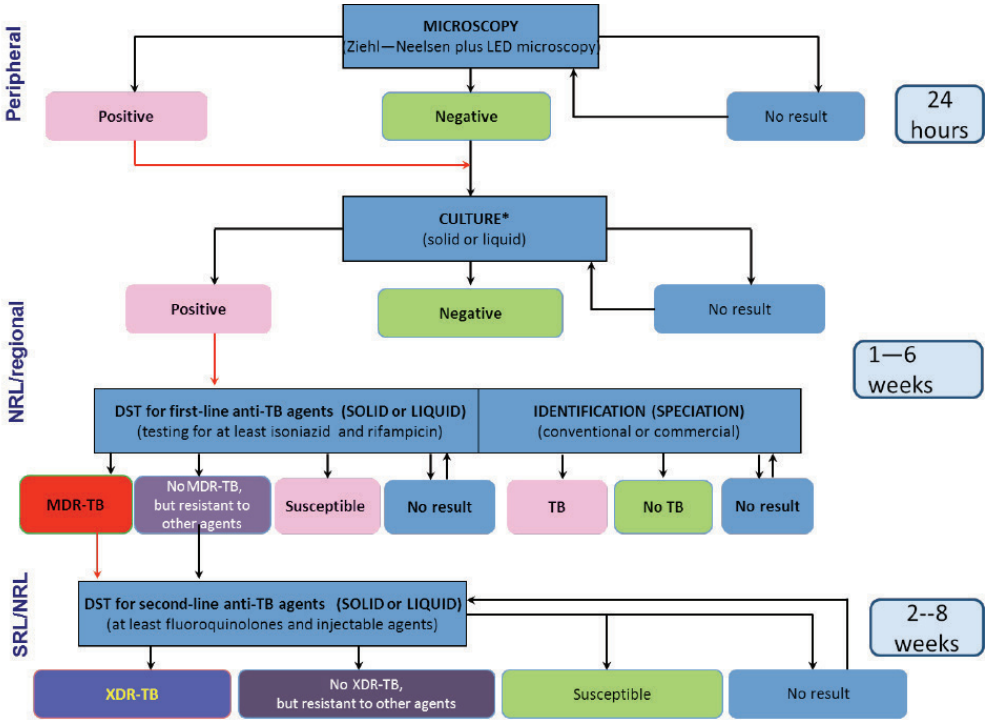
In settings with a high burden of HIV, a substantial investment in Xpert MTB/RIF technology or culture capacity is required in order to effectively diagnose smear-negative pulmonary TB. In settings with a high burden of MDR-TB, diagnostic algorithms for laboratory testing should include as a minimum the groups who are at greatest risk of drug-resistant TB (including previously treated patients, non-converters and contacts of patients with MDR-TB) because this represents the most cost-effective use of limited laboratory and diagnostic resources. In such settings, rapid techniques for detecting drug resistance, including the Xpert MTB/RIF assay and LPAs, should be integrated into diagnostic algorithms in order to minimize delays in starting appropriate treatment. In all settings, countries are encouraged to phase in DST for all new TB cases.

At a minimum, countries embarking on interventions to detect and treat drug-resistant TB should establish laboratory capacity to diagnose MDR-TB (RR-TB) and monitor the culture conversion of patients being treated for MDR-TB. Because the risk categories for drug-resistant TB vary widely among countries, careful assessment of risks at the country level is essential. Algorithms for testing patients suspected of having drug-resistant TB depend on the local epidemiology of TB, local treatment policies, existing laboratory capacity, mechanisms for specimen referral and transport, and human and financial resources.

The algorithms presented here can be adapted to each local situation – for example, diagnostic decisions could be based only on the results of the Xpert MTB/RIF assay if there is no other means of diagnosing TB. The choice of an algorithm depends on the situation in the country. The decision about which algorithm (or algorithms) to use should be made by national TB control programmes in consultation with laboratory experts; the decision should be based on the existing infrastructure and available resources. Whichever diagnostic algorithm is adopted, it will have an impact on the volume of diagnostic tests and, hence, the cost of providing diagnostic services.

The algorithms presented in this section are sample algorithms that show how WHO-recommended testing could be implemented. These algorithms should be adapted by countries to address their specific situations.

Algorithm 1. Using microscopy, solid or liquid culture, species identification and drug-susceptibility testing to diagnose TB



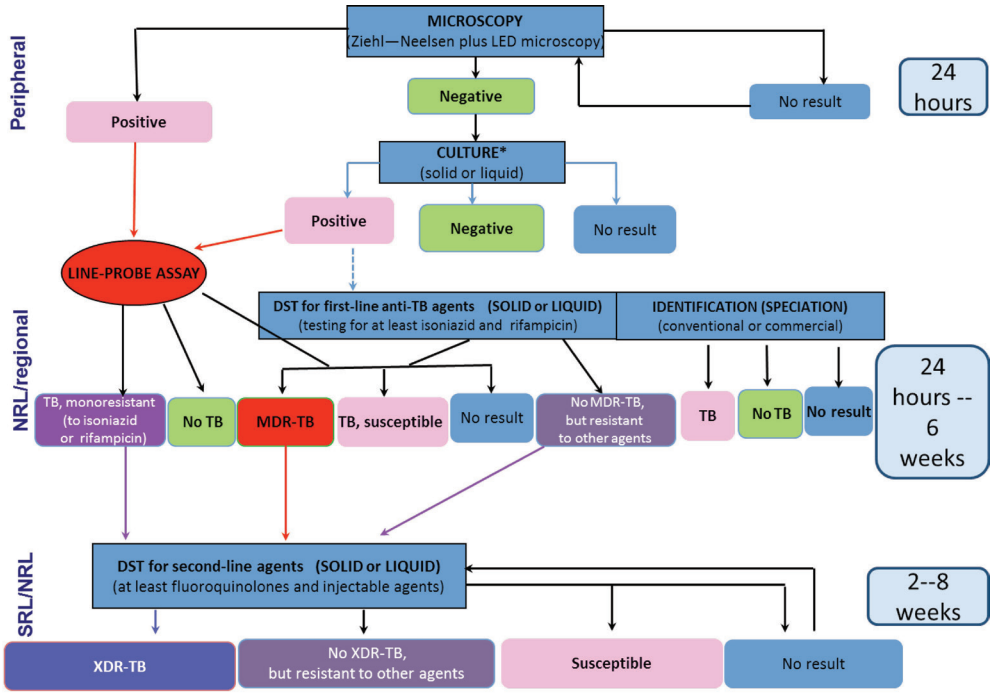
* Implementing this algorithm (culture for diagnosis of all persons suspected to have TB) is highly resource dependant. Efficiency can be improved if culture is performed only for selected smear negative persons with strong clinical suspicion of TB.

Algorithm 1 shows how microscopy, culture and conventional DST could be implemented.

Key considerations

- DST is required to confirm or exclude drug resistance in *M. tuberculosis* isolates.
- DST is suitable for intermediate and higher-level laboratories (typically national or regional level) that have implemented a high level of precautions against risk.
- DST for first-line anti-TB agents (rifampicin and isoniazid) should be done to confirm or exclude MDR-TB.
- DST for second-line anti-TB agents should be done on all *M. tuberculosis* isolates from patients with MDR-TB.

Algorithm 2. Using microscopy and line-probe assays in conjunction with drug-susceptibility testing (with solid or liquid media) to diagnose TB



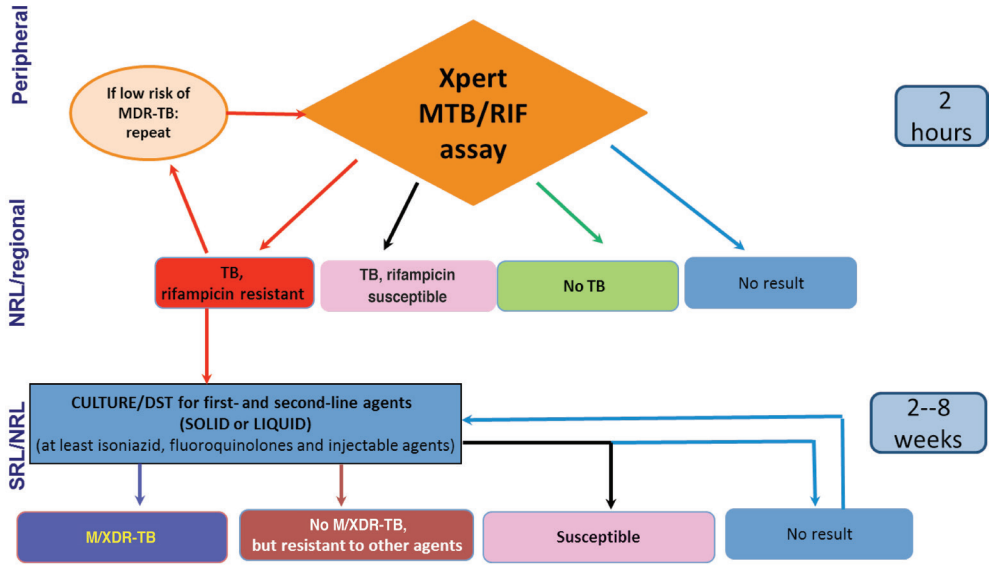
* Implementing this algorithm (culture for diagnosis of all persons suspected to have TB) is highly resource dependant. Efficiency can be improved if culture is performed only for selected smear negative persons with strong clinical suspicion of TB.

Algorithm 2 shows the implementation of WHO-recommended diagnostic techniques, including microscopy (conventional or LED), LPA and DST for second-line anti-TB agents, using the appropriate laboratory infrastructure and biosafety precautions.

Key considerations

- When culture results are positive, either LPA or phenotypic DST can be used based on availability. Where the capacity for molecular tests has been established, LPA should be used in preference to phenotypic DST.
- MDR-TB can be detected in less than 48 hours when LPA is used on smear-positive specimens.
- LPA is suitable for reference-level laboratories (typically national or regional level).
- LPA is recommended for use only on smear-positive specimens and *M. tuberculosis* isolates; therefore, smear-negative specimens require conventional culture (with either solid or liquid media) and speciation before LPA testing. LPAs should be used only in laboratories that have implemented appropriate precautions against risks.
- LPA detects only MDR-TB; conventional DST is required to detect XDR-TB.
- Conventional culture (with either solid or liquid media) is required to monitor treatment success or failure (that is, culture conversion) for MDR-TB patients.

Algorithm 3. Using the Xpert MTB/RIF assay as an initial diagnostic test for TB followed by drug-susceptibility testing for second-line anti-TB agents when necessary

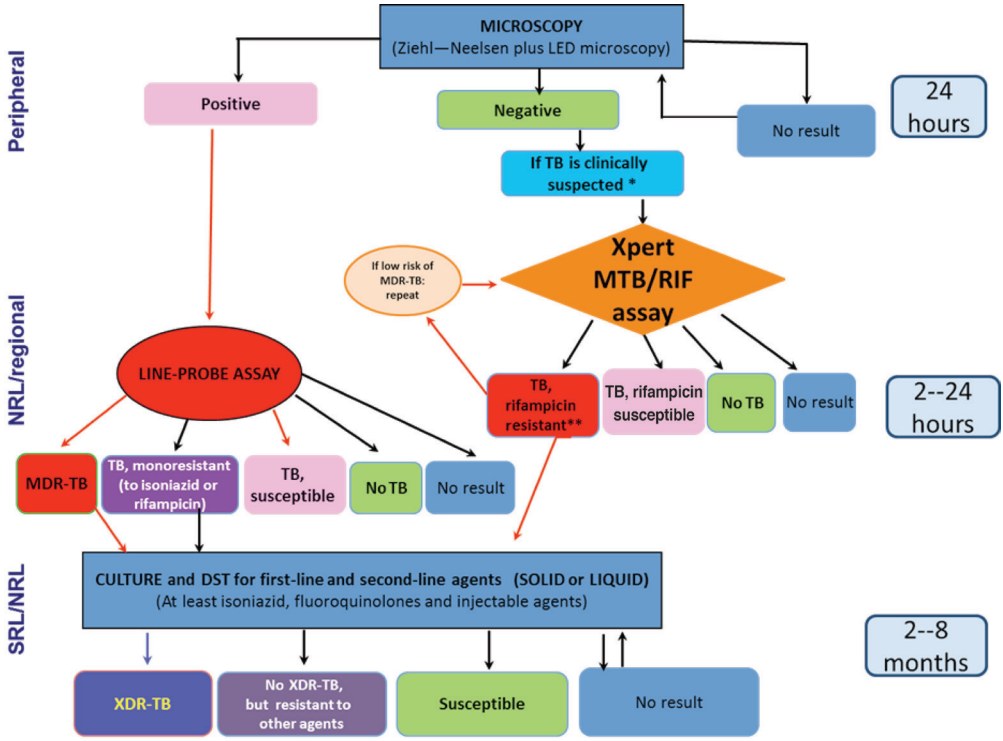


Algorithm 3 uses the WHO-endorsed Xpert MTB/RIF assay as an initial diagnostic test along with DST for second-line anti-TB agents in laboratories that have the appropriate infrastructure and have implemented the appropriate biosafety precautions.

Key considerations

- This algorithm allows for a decentralized approach for rapid detection of DR-TB and for the rapid detection of the majority of culture-positive TB cases.
- The Xpert MTB/RIF assay simultaneously detects *M. tuberculosis* and resistance to rifampicin in less than 2 hours.
- Xpert MTB/RIF should be used as the initial diagnostic test in adults and children who are at risk of MDR-TB or HIV-associated TB.
- Xpert MTB/RIF may be used as the initial diagnostic test in all adults and children who are at risk of TB.
- Xpert MTB/RIF may be used as a follow-on test to microscopy in adults who are suspected of having TB but who are not at risk of MDR-TB or HIV-associated TB.
- Xpert MTB/RIF should be used as the initial diagnostic test for cerebrospinal fluid (CSF) specimens from patients suspected of having TB meningitis.
- Xpert MTB/RIF may be used as a replacement test for usual practice (including microscopy, culture and histopathology) for testing specific nonrespiratory specimens (lymph nodes and other tissues) from patients suspected of having extrapulmonary TB. Xpert MTB/RIF unit can be positioned at the central, intermediate and peripheral levels.
- Specimens from patients in whom rifampicin resistance has been detected should be referred to higher-level laboratories for a complete determination of drug-resistance to second-line anti-TB agents.

Algorithm 4. Using LPA and the Xpert MTB/RIF assay as follow-up diagnostic tests to microscopy for TB with drug-susceptibility testing for second-line anti-TB agents when necessary



* In this scenario the use of Chest X-ray (CXR) in smear negative persons in whom TB is clinically suspected may significantly reduce Xpert MTB/RIF tests needed.

** In patients who are not at risk for drug resistance but who initially test positive for rifampicin resistance by Xpert MTB/RIF, a second Xpert MTB/RIF test should be performed to control for preanalytical and postanalytical errors, and to improve the clinician's confidence in the diagnosis.

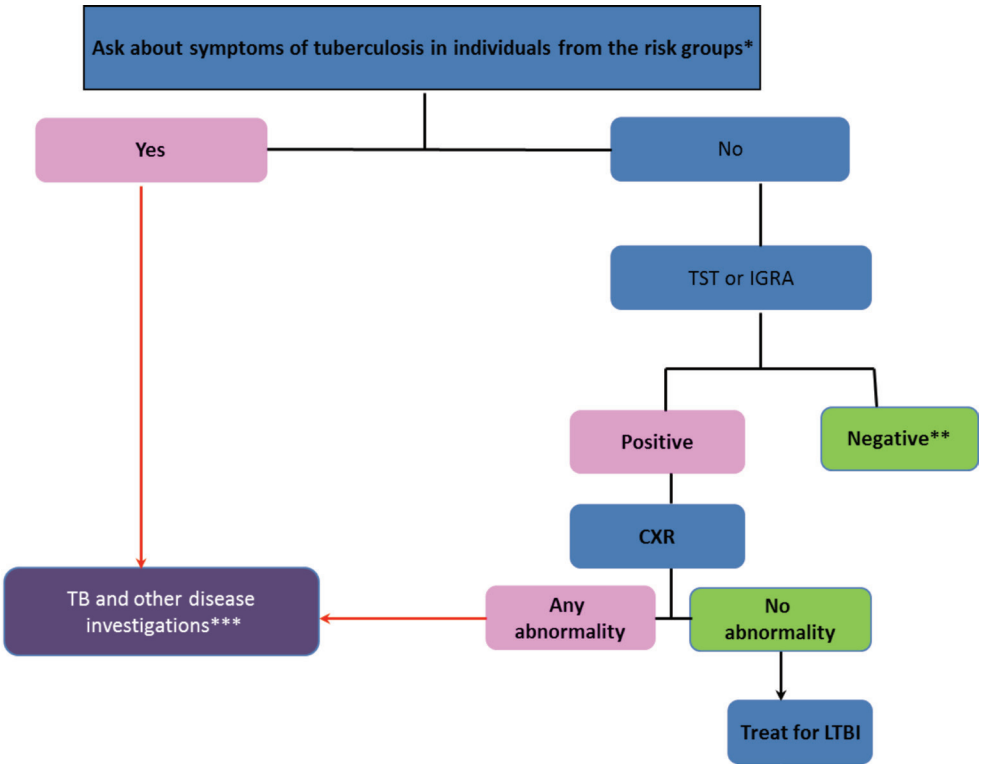
Algorithm 4 uses WHO-endorsed LPA with the Xpert MTB/RIF assay as follow-up diagnostic tests based on the results of sputum-smear microscopy.

Key considerations

- LPA is used on smear-positive specimens whereas the Xpert MTB/RIF assay is used also on smear-negative specimens.
- LPA has a higher throughput (up to 48 tests in 2 days) than a 4-module Gene Xpert unit (which averages 12 tests per day).
- LPA can be positioned at the central and intermediate levels of the laboratory network; the Gene Xpert unit can be positioned at the central, intermediate and peripheral levels.

Algorithm 5. Diagnosis and treatment of LTBI among high risk individuals

Individuals, or their guardians (in the case of small children) should be asked about symptoms of TB before being tested for LTBI. Chest radiography should be done in all individuals with a positive LTBI test, and LTBI treatment should be limited to those who do not have radiological abnormalities. Individuals with TB symptoms or any radiological abnormality should be investigated further for active TB and other conditions.



Key considerations

- (*) Any symptoms of TB include any one of: cough, haemoptysis, fever, night sweats, weight loss, chest pain, shortness of breath, fatigue. HIV test could be offered based on national or local guidelines or clinical judgment. Similarly chest radiographs can be done if efforts are intended also for active TB case finding.
- (**) Patients for whom LTBI treatment is not indicated should be provided information about TB including on the importance of seeking care if symptoms of TB developed.
- (***) National TB guidelines should be followed while investigating for TB. In addition, those individuals in whom TB is excluded after investigations (including individuals with fibrotic radiologic lesions) can be considered for LTBI treatment.

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11. Annex

Annex 1. Summary of characteristics of and laboratory requirements for WHO's recommended techniques for diagnosing TB

Diagnostic tool or method	Laboratory level	Time to detection of MDR-TB		Need for equipment and infrastructure	Need for consumables	Need for training	Infrastructure (risk level of TB laboratory)
		Direct method	Indirect method				
Microscopy	Peripheral, intermediate or central	NA	NA	Minimal	Moderate	Minimal	Low
Solid culture and DST	Central or intermediate	NA	9–12 weeks	Moderate	Moderate	Moderate	Moderate
Commercial liquid culture and DST	Central or intermediate	NA	3–5 weeks	Extensive	Extensive	Extensive	High
Non-commercial culture and DST	Central or intermediate						
MODS		2–21 days	3–4 weeks	Moderate	Moderate	Extensive	Moderate
NRA		6–9 days	7–11 weeks	Minimal	Moderate	Moderate	Moderate
CRI		NA	3–5 weeks (liquid culture) 7–10 weeks (solid culture)	Minimal	Moderate	Extensive	High
LPA for first-line anti-TB agents	Central or intermediate		3–5 weeks (liquid culture) 9–12 weeks (solid culture)	Extensive	Moderate	Moderate	Moderate High
Sputum smear-positive		24–48 hours					
Sputum smear-negative		NA					
Xpert MTB/RIF assay	District, subdistrict, intermediate or central	< 2 hours[a]	NA	Moderate	Extensive	Minimal	Low

MDR-TB, multidrug-resistant TB; NA, not applicable; DST, drug-susceptibility testing; MODS, microscopic observation drug-susceptibility assay; NRA, nitrate reductase assay; CRI, colorimetric redox indicator; LPA, line-probe assay.

[a] The Xpert MTB/RIF assay detects only rifampicin resistance, but this can be used as a proxy for MDR-TB in many settings and for many groups of patients.

Annex 2. WHO Global Tuberculosis Programme Guidance on temporary TB control measures in Ebola-affected countries

1. Background

The Ebola epidemic in Guinea, Liberia and Sierra Leone[1] has seriously impacted already weak health systems and depleted scarce health care personnel. In addition, the widespread fear of Ebola is keeping many people away from health facilities, complicating the management and control of other infectious diseases, including tuberculosis (TB).

The laboratory diagnosis of TB involves the handling of sputum specimens. Health care workers in many countries, including those affected by Ebola, traditionally rely heavily on sputum smear microscopy (SSM) to establish a diagnosis of TB. The fear among health care and laboratory workers of Ebola transmission when collecting and handling sputum specimens is severely affecting TB case detection efforts in Ebola-affected countries.

Together, the above-mentioned factors are expected to result in increased TB transmission during and after the Ebola epidemic, with a likelihood of the magnitude of the TB problem growing in the short and medium-term.

To prevent and mitigate these negative effects on TB control activities in the Ebola-affected countries, this document outlines temporary and pragmatic steps to: i) sustain TB case-finding efforts; ii) ensure prompt treatment of detected TB cases; and iii) manage the ongoing TB epidemic.

2. TB diagnosis and treatment in Ebola-affected countries

The approach to TB testing (adults and children) in Ebola-affected countries should be based on risk assessment and established screening procedures for Ebola virus disease (EBVD) and/or infection, followed by systematic screening for TB as outlined below.

If EBVD is suspected simultaneously with TB, priority should be given to ruling out EBVD. Exclusion of Ebola requires two negative Ebola virus RNA RT PCR test results, and/or by negative Ebola antigen detection test, and/or by negative Ebola Immunoglobulin M (IgM) antibodies test at least 48 hours apart in clinically asymptomatic persons[2].

Essentially, TB diagnostic approaches in Ebola-affected countries apply to two distinct groups of individuals:

Group 1: Persons not considered to be at risk of Ebola infection or where Ebola infection has been ruled out (including contacts of Ebola cases) and individuals being cared for outside ETUs (ie. in general health services);

Group 2: Patients already confirmed to have EBVD and admitted to Ebola Treatment Units (ETUs), or individuals at risk of Ebola infection and being referred to ETUs for follow-up (eg. contacts of confirmed Ebola cases);

Collection and manipulation of specimens (including sputum, tissue samples, secretions and other bodily fluids) for the laboratory diagnosis of pulmonary and extrapulmonary TB involve enhanced risk of Ebola transmission to health care workers. **Therefore it is not recommended to undertake these procedures in any health-care setting which lacks stringent Ebola containment measures.**

3. Outside of Ebola Treatment Units (Algorithm A)

Screening for TB should systematically target all persons (adults and children) accessing health services or encountered during contact tracing for Ebola.

1. Ebola virus disease. Geneva, World Health Organization, September 2014. http://www.who.int/mediacentre/factsheets/fs103/en/
2. WHO Interim Infection Prevention and Control Guidance for Care of Patients with Suspected or Confirmed Filovirus Haemorrhagic Fever in HealthCare Settings, with Focus on Ebola (December 2014). http://apps.who.int/iris/bitstream/10665/130596/1/WHO_HIS_SDS_2014.4_eng.pdf

Initial screening for TB should be based on the identification of any symptom compatible with TB, including cough for 2 weeks or more, haemoptysis, weight loss, fever or night sweats. The recommended algorithm for the diagnosis of TB outside of ETUs in Ebola-affected countries is as follows:

- If clinical symptoms are suggestive of TB, broad-spectrum antimicrobial therapy is recommended for 7-10 days;
- If a positive response is observed after 1 week, no further evaluation for TB is needed;
- If no/partial response is observed after 1 week a chest X-ray (CXR) is recommended if available;

  – If CXR shows TB suggestive abnormalities – initiate first-line TB treatment as per WHO and NTP policies;

  – If CXR does not show TB suggestive abnormalities, consider diagnosis of conditions other than TB;

  – If CXR is not available – initiate first-line TB treatment as per WHO and NTP policies.

Once settings have been declared to be Ebola-free, routine TB control practices should be resumed as soon as possible. These should include the use of appropriate diagnostic tests, including scale-up of the Xpert MTB/RIF assay as per WHO recommendations.

4. In Ebola Treatment Units (Algorithm B)

Initial screening for TB should be based on the identification of any symptom compatible with TB, including cough for 2 weeks or more, haemoptysis, weight loss, fever or night sweats.

If available, the Xpert MTB/RIF assay[3] using the GeneXpert platform **should be prioritized as the initial diagnostic test for active TB in ETUs**. All precautions related to safe handling of infectious biological materials should be followed[4]. Manual manipulation of sputum is minimised by the GeneXpert system, and it is therefore recommended over other TB laboratory tests (sputum microscopy, conventional culture, phenotypic drug-susceptibility testing) to limit the risk of Ebola transmission to laboratory workers.

If Xpert MTB/RIF testing is available, one (preferably early morning) sputum specimen should be collected and treatment initiated based on the test results below. TB treatment should not be started during treatment for EBVD.

Xpert MTB/RIF positive for TB, rifampicin susceptible: Patient is eligible for first-line treatment as per WHO and NTP policies, as soon as s/he is clinically stable and expected to be able to tolerate TB treatment;

Xpert MTB/RIF positive for TB, rifampicin resistant: Patient is eligible for second-line treatment for multidrug-resistant TB (MDR-TB) as per WHO and NTP policies, as soon as s/he is clinically stable and expected to be able to tolerate MDR-TB treatment;

Xpert MTB/RIF negative for TB or result not available: Consider further diagnostic work-up (including repeat Xpert MTB/RIF test) and treatment for diseases other than TB. Should TB still be suspected, a CXR is recommended if available, and broad spectrum antimicrobial therapy for 7-10 days should be considered.

A clinical diagnosis of TB can be made on the basis of the symptoms, CXR and the response to antimicrobial therapy.

Health care workers must wear appropriate personal protective equipment including respiratory protection when collecting sputum specimens. Screw-cap sputum containers

3. For technical and operational considerations on use of Xpert MTB/RIF, see the WHO Implementation Manual on Xpert MTB/RIF (April 2014). http://www.who.int/tb/publications/xpert_implem_manual
4. WHO Interim Guideline: Laboratory Diagnosis of Ebola Virus Disease (September 2014). https://extranet.who.int/iris/restricted/bitstream/10665/134009/1/WHO_EVD_GUIDANCE_LAB_14.1_eng.pdf

are required and sputum collection should be performed in ventilated areas to limit TB transmission. Specimen containers should be decontaminated against Ebola virus (EV) using appropriate routine procedures (e.g. a 0.5% chlorine solution or a solution containing 5000 ppm available free chlorine) before transporting them to the laboratory (1).

All EBVD biosafety as well as respiratory protection requirements should be fulfilled when processing sputum and performing TB laboratory tests. All liquid and solid waste (including used Xpert MTB/RIF cartridges and specimen containers) should be disposed of using established, routine procedures for EBVD waste disposal.

Upon final closure of the ETU, surfaces of the Gene-Xpert machine and accessories (including the computer) should be cleaned and disinfected using standard hospital detergents/disinfectants (e.g. a 0.5% chlorine solution or a solution containing 5 000 ppm available free chlorine (1). The instrument and computer may then be placed in a non-Ebola health care facility for use in TB control activities.

5. Treatment

Necessary measures should be undertaken to assure sufficient drug stocks of both the first and second-line anti-TB drugs.

TB treatment should include co-trimoxazole if the patient is known or suspected to be HIV-positive.

If rifampicin resistant TB (RR-TB) is diagnosed by an Xpert MTB/RIF assay, the treatment of multidrug-resistant TB should be undertaken in accordance with WHO and NTP policies.

Direct observation of treatment (DOT) for both drug-susceptible and drug-resistant TB should be provided as far as possible according to national guidelines. DOT should be planned and combined with the other health related activities, such as health education campaigns, anti-malaria mass drug administration (MDA), and long-lasting insecticide nets distribution (LLINs). A high completion of TB treatment rate is essential to reduce disease transmission and prevent the development of drug resistance.

Disposable gloves, chlorine and disposable masks should be provided for care givers.

6. Monitoring of TB treatment

Microbiological monitoring of TB cases on treatment (using sputum smear microscopy and/or culture) is not recommended due to the risk of Ebola transmission to health care workers while collecting samples and performing laboratory based tests at facilities lacking stringent Ebola containment measures.

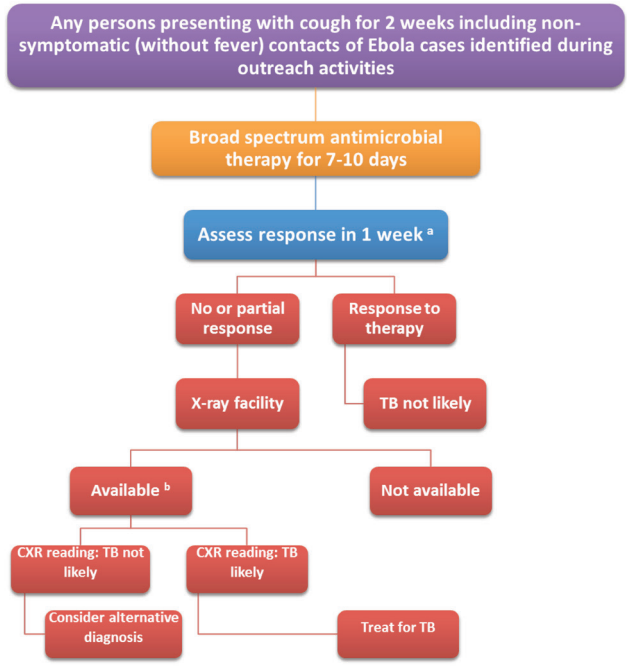
Treatment monitoring based on clinical symptoms only is recommended. If radiology is available, CXR may be taken at regular intervals (eg. every two months) and the dynamics of abnormalities assessed.

7. Occupational health

All health workers, especially those directly involved in household visits, should be trained on the need to follow at all times the following precautions to avoid Ebola transmission:

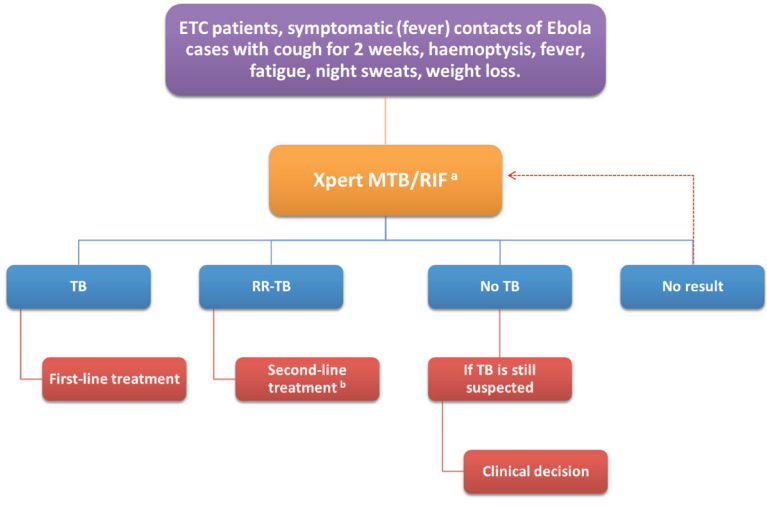
- avoid hand-shaking;
- no touching with bare hands but must use gloves and wash the gloved hands in chlorinated water before removal and disposal;
- use gloves to touch personal items (i.e. plates, cup, utensils) and surfaces in the household, in particular if anyone is sick in the house;
- performing hand hygiene frequently during the drug distribution with either an alcohol based hand-rub solution or, if this is not available, with water and soap;
- maintaining a 1 metre distance and wearing a face mask when providing care to sick people.

Algorithm A. Diagnostic algorithm for tuberculosis outside of ETUs



[a] Antibiotics (except fluoroquinolones) to cover both typical and atypical bacteria should be considered. Assess the response in 3-5 days in patients with danger signs and suspected HIV clinical disease (stage III-IV)
[b] Chest X-ray (CXR) should be done and evaluated at the same time wherever possible in order to decrease the number of visits and speed up the diagnosis

Algorithm B. Diagnostic algorithm for tuberculosis in ETUs



[a] If Xpert MTB/RIF is not available, sputum smear microscopy (if equipment and technician are available) may be performed under stringent EV containing conditions
[b] If rifampicin resistance is detected, see diagnostic algorithm on interpreting Xpert MTB/RIF results in WHO Implementation Manual on Xpert MTB/RIF (April 2014). http://apps.who.int/iris/bitstream/10665/112469/1/9789241506700_eng.pdf#page=23

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