

CHAPTER 6

Laboratory aspects

6.1 Chapter objectives

This chapter describes laboratory services needed to diagnose and treat all forms of drug-resistant TB. It expands on information published in guidelines by WHO (1–3) and the IUATLD (4) on laboratory services for TB control.

6.2 General considerations

Optimal management of drug-resistant TB requires both mycobacterial and clinical laboratory services. At a minimum, the mycobacteriology reference laboratory should provide: culture; confirmation of the species as *M. tuberculosis*, *M. bovis* or nontuberculous mycobacteria (NTM) and testing for susceptibility to isoniazid and rifampicin. Clinical laboratory services, including basic haematology, biochemistry, serology and urine analysis, are required for the proper evaluation and monitoring of patients (see Chapter 11). A comprehensive, routine system of internal quality control and external quality assurance is mandatory.

In addition to diagnostic services, laboratories have a critical role in surveillance of prevailing drug resistance patterns and trends (1). The network of supranational TB reference laboratories provides quality assurance through validation of drug susceptibility data. Central reference laboratories supporting DR-TB control programmes should establish formal links with a supranational TB reference laboratory to help ensure the quality of laboratory services and validation of DST results. Usually, an external quality assurance programme with a supranational TB reference laboratory consists of an initial assessment visit by the laboratory, proficiency testing with a panel of coded isolates and then periodic rechecking of isolates obtained within the programme. This system should be negotiated with the supranational TB reference laboratory before the start of the DR-TB control programme.

Quality assurance goes beyond the relationship with the supranational laboratory and includes good infection control measures and internal methods to document the validity of results. These aspects are discussed below.

6.3 Organization and development of the laboratory network

The laboratory network has a pyramidal structure based on a large number of Level I laboratories accessible to all TB suspects and patients, a moderate number of Level II laboratories located in mid-sized population centres and health facilities and a few (or even a single) apex Level III laboratories at the provincial, state or national level. Table 6.1 describes the different functions and responsibilities of the three different levels of laboratory services. This chapter concentrates on Level III laboratories; the organization and operation of Level I and II laboratories are well described in other publications (1–4).

Each DR-TB control programme must have a rapid, reliable means of collecting and transferring specimens, cultures and information from the patient and physician to each level of the laboratory service and for returning the results. There should be no financial barrier between the patient and the TB diagnostic services at any of these three levels. A country or region can control and prevent drug-resistant TB only if infectious patients are detected and cured without delay. Ready access to microscopy for acid-fast bacilli (AFB), culture and DST free of charge to the patient are essential elements of political commitment to control drug-resistant TB.

DST of at least isoniazid and rifampicin is needed in any programme for control of drug-resistant TB; DST of streptomycin and ethambutol is also desirable, although less essential. In the initial phase of treatment implementation for drug-resistant TB, DST of second-line drugs is best left to supranational or other TB reference laboratories with documented capacity, expertise and proficiency. Once DST of first-line drugs operates at a consistently high level of proficiency, laboratories serving populations and patients with significant previous exposure to second-line drugs may consider extending their services to DST of second-line drugs (see section 6.5).

6.4 Microscopy, culture and identification of *M. tuberculosis* in DR-TB control programmes

Detailed information on sputum smear examination and culture can be found in the WHO manuals *Laboratory services in tuberculosis control. Parts I, II and III* (2).

6.4.1 Microscopy

Microscopy for AFB cannot distinguish between drug-susceptible and drug-resistant *M. tuberculosis* or between different species of mycobacteria. The main uses of microscopy for drug-resistant TB are therefore limited to assessing the infectiousness of patients, triaging specimens to different methods of culture and DST, and confirming that microbes growing on (or in) artificial media are mycobacteria rather than contaminants.

As AFB sputum smear microscopy cannot distinguish between viable and non-viable bacilli, its utility for monitoring patient infectiousness and

TABLE 6.1 **Functions and responsibilities of the different levels of laboratory (2)**

LEVEL I
<p>The peripheral (often district) laboratory</p> <ul style="list-style-type: none"> ■ Receipt of specimens ■ Preparation and staining of smears ■ Ziehl-Neelsen microscopy and recording of results ■ Dispatch of results ■ Maintenance of laboratory register ■ Cleaning and maintenance of equipment ■ Management of reagents and laboratory supplies ■ Internal quality control
LEVEL II
<p>The intermediate (often regional) laboratory</p> <ul style="list-style-type: none"> ■ All the functions of Level I laboratory ■ Fluorescence microscopy (optional) ■ Digestion and decontamination of specimens ■ Culture and identification of <i>M. tuberculosis</i> ■ Training of microscopists ■ Support to and supervision of peripheral-level staff with respect to microscopy ■ Preparation and distribution of reagents for microscopy in peripheral laboratories ■ Quality improvement and proficiency testing of microscopy at peripheral laboratories
LEVEL III
<p>The central (often national) laboratory</p> <ul style="list-style-type: none"> ■ All the functions of Level I and II laboratories ■ DST of <i>M. tuberculosis</i> isolates ■ Identification of mycobacteria other than <i>M. tuberculosis</i> ■ Technical control of and repair services for laboratory equipment ■ Updating and dissemination of laboratory manuals, including guidelines on diagnostic methods, on care and maintenance of equipment and on quality assurance ■ Close collaboration with the central level of the national TB control programme ■ Supervision of intermediate laboratories regarding bacteriological methods and their support (particularly training and supervision) to the peripheral laboratories ■ Quality assurance of microscopy and culture performed at intermediate laboratories ■ Training of intermediate-level laboratory staff ■ Organization of antituberculosis drug resistance surveillance ■ Operational and applied research relating to the laboratory network, coordinated with the requirements and needs of national TB control programmes

response to treatment is also limited. For example, even with adequate treatment, specimens from MDR-TB patients may remain sputum smear-positive after they become culture-negative, suggesting that the bacilli are non-viable. Caution is nonetheless recommended for patients who are sputum smear-positive and culture-negative; they should be considered as possibly infectious and evaluated for progression of active disease.

6.4.2 Culture

Quality of laboratory processing is of crucial importance. Delays in specimen transport, excessively harsh or insufficient decontamination, poor-quality

ity culture media or incorrect incubation temperature can adversely affect the culture yield. Laboratory errors, such as mislabelling or cross-contamination between specimens during aerosol-producing procedures, may lead to false-negative or false-positive results. In this context, laboratory findings should always be correlated with the patient's clinical condition and any diagnostic test should be repeated if necessary. Low positive culture results (<10 colonies) are not well correlated with clinical prognosis (5) and should be interpreted with caution, especially if a single culture with low colony counts is reported. However, persistent positive cultures or any positive culture in the setting of clinical deterioration should be regarded as significant.

The pros and cons of different culture media and techniques are discussed in other published references (1–4).

6.4.3 Identification of *M. tuberculosis*

In countries with a high burden of TB, most mycobacterial isolates will be *M. tuberculosis*. The prevalence of NTM varies from country to country and can be more common in patients infected with the human immunodeficiency virus (HIV). Unless the species is confirmed as *M. tuberculosis*, mycobacterial isolates appearing phenotypically resistant to first-line drugs may represent not drug-resistant TB but infection with NTM. Treatment of NTM is entirely different from treatment of drug-resistant TB or MDR-TB. As a minimum, laboratories supporting DR-TB control programmes should be able to conduct niacin and nitrate tests (both are positive in most *M. tuberculosis* strains) or at least two other methods that follow international guidelines.

6.5 Drug susceptibility testing

Identification and treatment of patients with, or at high risk of, drug-resistant TB can be based on a range of strategies (see Chapters 5 and 7). In vitro DST plays a key role in all of these strategies. A widely accepted strategy is to decide treatment based on DST results for the individual patient's bacterial isolate. Programmes that do not test each patient's isolate may choose to treat patients based on the prevailing levels of drug resistance in the population or on the previous exposure to antituberculosis drugs, as indicated by case-based clinical data. However, in this latter strategy, the confirmation of MDR-TB with DST of at least isoniazid and rifampicin is recommended.

The drugs used for susceptibility testing should never be taken from those used for treatment. They must come from pure compounds that are available only from the manufacturer.

For diagnosis of suspected MDR-TB, at least two sputum specimens should be submitted to the laboratory for AFB microscopy (smear) and culture. One of the two cultures can then be used for DST. This **indirect method** does DST on a culture grown from the processed sputum specimen. The **direct**

method, which does DST directly from the sputum, requires more sophisticated laboratory expertise, and the sensitivity and specificity are not always as good as for DST done from a culture.

A number of different techniques are available for DST that essentially compare growth of the mycobacterium with a control. Several variations exist for each method. The following is a list of the most common DST techniques:

- proportion method,
- absolute concentration,
- resistance ratio,
- broth (or liquid) methods,
- detection of metabolic changes,
- mycobacteriophage-based,
- molecular.

Several rounds of proficiency testing in the network of supranational TB reference laboratories have shown that the three most commonly used techniques (proportion, absolute concentration and resistance ratio) are highly reliable and reproducible, and that the results do not differ according to the method used.

6.5.1 Limitations of DST

The accuracy of DST (performed under optimal circumstances) varies with the drug tested: it is most accurate for rifampicin and isoniazid and less accurate for streptomycin and ethambutol.

DST of second-line drugs is not as simple as DST of some of the first-line drugs. This is partly because critical drug concentrations defining drug resistance are very close to the minimal inhibitory concentrations (6). The clinical relevance of DST of second-line drugs is less well studied. The calibration of DST methods with representative clinical isolates is in progress. (Better calibration will improve the determination of in vitro test criteria and may improve the ability of second-line DST to predict the clinical effectiveness.)

The clinician needs to understand the limitations of DST and interpret the results accordingly. DST provides an indication of the likelihood of a drug's being effective. Drugs for which the DST results show susceptibility are more likely to be effective than drugs for which the DST shows resistance. When discrepant results are obtained, they must be interpreted with care by a clinician experienced in drug-resistant TB. Chapter 7, section 7.7.3, describes the clinical interpretation of DST.

6.5.2 Choice of drugs used for DST

Each Level III laboratory must decide which drugs to test and how to test them, according to the strategy for designing treatment regimens. Reliable

DST for at least isoniazid and rifampicin is a prerequisite for DR-TB control programmes. Some programmes may choose to have these tests done at a distant laboratory until a local laboratory is able to do them. DST for second-line drugs is not mandatory for such control programmes and is not recommended unless rigorous quality controls are in place, including external proficiency testing by one of the supranational TB reference laboratories. One possible hierarchy of DST capacity is suggested in the following priority-ranked list:

- rifampicin and isoniazid,
- ethambutol, streptomycin,
- pyrazinamide (if broth-based DST systems are available),
- kanamycin (or amikacin) and capreomycin,
- fluoroquinolone (generally, the same fluoroquinolone that is used in treatment),
- ethionamide/protonamide, PAS, cycloserine.

6.5.3 Time for testing and reporting: turnaround time

Growth detection and identification of *M. tuberculosis* may take 3–8 weeks on solid media and 1–2 weeks in broth media. DST of an *M. tuberculosis* isolate takes an additional 2–4 weeks in solid media and 1 week in broth media. To ensure rapid diagnosis of *M. tuberculosis* and drug-resistant TB, laboratories should define standard turnaround times, which should be strictly followed.

6.6 Rapid tests

The several advantages of rapid testing include screening of patients at risk for MDR-TB, earlier identification of patients on inadequate regimens and prompt ability to isolate MDR-TB patients. The use of rapid DST is encouraged when possible. Rapid tests employ a variety of techniques (some rapid tests are being field-tested by the GLC).

6.7 Infection control and biosafety in the laboratory

Transmission of TB – including drug-resistant forms such as MDR-TB – is a recognized risk for laboratory workers. A well-maintained, properly functioning Class I or Class II biological safety cabinet is an indispensable piece of laboratory equipment for the performance of culture and DST of specimens from MDR-TB patients. Masks designed to protect the wearer from tiny (1–5 µm) airborne infectious droplets should always be used. Instructions on safe handling of specimens (*I*) should be scrupulously followed: the most expensive and sophisticated biological safety cabinet will not provide protection against MDR-TB infection that results from poor laboratory technique. Proper maintenance of such cabinets is an essential component of infection control and biosafety.

Laboratory workers who choose to disclose their HIV-positive status should

be offered safer work responsibilities and should be discouraged from working with MDR-TB specimens. Pregnant women should be reassigned until after childbirth and lactation. Training in laboratory procedures and strict adherence to safety measures should be accompanied by a simple surveillance programme whereby the health status of laboratory staff is monitored regularly.

Routine BCG vaccination is not a substitute for good infection control practices as a means of preventing MDR-TB in laboratory workers. The use of infection control measures is discussed in more detail in Chapter 15.

6.8 Surveillance and surveys using DST

Surveillance of TB antimicrobial resistance is essential for providing information on the magnitude and trends in resistance, for developing treatment guidelines and for monitoring the effect of interventions. WHO and its partners have supported the surveillance of drug-resistant TB in many countries and have provided three global reports (7–9) (see Chapter 1, section 1.7). Surveillance DST of second-line drugs in MDR-TB patients is encouraged provided it is carried out in a quality-assured laboratory. Surveillance systems should be designed according to the needs and capacity of the country. Guidelines for DRS are available from WHO (1).

6.9 Quality control and quality assurance

A comprehensive quality control/quality assurance programme should be developed in each TB laboratory to ensure the accuracy, reliability and reproducibility of the results obtained. Quality control/quality assurance procedures should be performed regularly as an integral part of laboratory operations. Procedures for AFB sputum smear microscopy are described in detail in the WHO manuals *Laboratory services in tuberculosis control. Parts I, II and III* (2). This section will address quality control/quality assurance procedures pertaining to culture and DST.

The procedures for **internal quality control** must be performed during each test round to verify that the test is working correctly. The **external quality control** comprises procedures that are carried out by an external organization to test that the results are correct. **Quality assurance** is control for the entire process of testing, covering all stages from collection of sputum until the result is reported back to the treatment facility.

A manual of standard operating procedures should be available for each of the laboratory operations. Standard operating procedures must be based on precisely how the procedure is carried out in the particular laboratory and incorporate any minor modifications that may have been made when compared with a standard protocol. The manual should also describe a protocol for regular maintenance checks and repairs of equipment.

References

1. *Guidelines for surveillance of drug resistance in tuberculosis*. Geneva, World Health Organization, 2003 (WHO/CDS/TB/2003/320; WHO/CDS/CSR/RMD/2003.3).
2. *Laboratory services in tuberculosis control. Parts I, II and III*. Geneva, World Health Organization, 1998 (WHO/TB/98.258).
3. Laszlo A et al. Quality assurance programme for drug susceptibility testing of *Mycobacterium tuberculosis* in the WHO/IUATLD Supranational Laboratory Network: first round of proficiency testing. *International Journal of Tuberculosis and Lung Disease*, 1997, 1(3):231–238.
4. *The public health service national tuberculosis reference laboratory and the national laboratory network: minimum requirements, roles, and operation in low-income countries*. Paris, International Union Against Tuberculosis and Lung Disease, 1998.
5. Gascoyne-Binzi DM et al. Rapid identification of laboratory contamination with *Mycobacterium tuberculosis* using variable number tandem repeat analysis. *Journal of Clinical Microbiology*, 2001, 39(1):69–74.
6. Kim SJ. Drug-susceptibility testing in tuberculosis: methods and reliability of results. *European Respiratory Journal*, 2005, 25:564–569.
7. *Anti-tuberculosis drug resistance in the world. Third global report. The WHO/IUATLD global project on anti-tuberculosis drug resistance surveillance, 1999–2002*. Geneva, World Health Organization, 2004 (WHO/HTM/TB/2004.343).
8. *Anti-tuberculosis drug resistance in the world. First global report. The WHO/IUATLD global project on anti-tuberculosis drug resistance surveillance, 1994–1997*. Geneva, World Health Organization, 1997 (WHO/TB/97.229).
9. *Anti-tuberculosis drug resistance in the world. Second global report. The WHO/IUATLD global project on anti-tuberculosis drug resistance surveillance, 1997–2000*. Geneva, World Health Organization, 2000 (WHO/CDS/TB/2000.278).