Guidelines for surveillance of drug resistance in tuberculosis

5th Edition

World Health Organization

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Abbreviations

CPC	cetylpyridinium chloride
DST	drug susceptibility testing
Global Project	Global Project on Anti-Tuberculosis Drug Resistance Surveillance
LPA	line probe assay
MAR	missing at random
MDR-TB	multidrug-resistant tuberculosis
NAAT	new nucleic acid amplification tests
PPS	probability proportional to size
SRL	Supranational Reference Laboratory
SRLN	Supranational Reference Laboratory Network
TB	tuberculosis
USAID	United States Agency for International Development
XDR-TB	extensively drug-resistant tuberculosis

Introduction

This fifth edition of the *Guidelines for surveillance of drug resistance in tuberculosis* is an updated version of earlier editions published in 1994 (1), 1997 (2), 2003 (3) and 2009 (4). The document takes into account recent advancements in laboratory diagnostics and subsequent WHO guidance, including *Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis* (5), *Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children* (6) and *Xpert MTB/RIF implementation manual* (7).

These updated guidelines also incorporate experience gained from 20 years of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance (hereafter referred to as the Global Project), a project initiated by WHO and the International Union Against Tuberculosis and Lung Disease (The Union). This is the oldest and largest project for the surveillance of anti-microbial drug resistance in the world. Since its launch in 1994, the Global Project has collected and analysed data on drug resistance from national surveillance systems and from surveys of sampled patients from 144 countries. Drug resistance surveillance data have been disseminated on regular basis in a number of reports (8–12). Since 2012, data have been published annually within the Global Tuberculosis Report. The Global Tuberculosis Report 2014 (13) encompassed data provided by 144 WHO Member States, accounting for 95% of the world population and estimated TB cases.

The Global Project has served as a common platform for country, regional and global level evaluation of the magnitude and trends in anti-tuberculosis drug resistance. It has quantified the global burden of multidrug-resistant tuberculosis (MDR-TB)[1] and of extensively drug-resistant tuberculosis (XDR-TB)[2]. More importantly, it has assisted countries in planning the scale-up of MDR-TB management by providing essential data on national burden and distribution of drug resistance patterns.

The aim of this document is to assist national tuberculosis control programmes (NTPs) in developing the strongest possible mechanisms of surveillance, starting from periodic country-specific surveys of sampled patients. The ultimate goal will be to establish routine continuous surveillance systems based on systematic drug susceptibility testing (DST). Although mechanisms for carrying out surveillance vary from country to country, these guidelines promote certain standardized criteria for surveillance within the Global Project to ensure that results are comparable between participating countries, as well as within countries over time.

The target audience of this document is NTPs and, in particular, a coordination team for surveillance ideally composed of the NTP manager, laboratory specialist, logistics specialist, epidemiologist, and statistician.

1 MDR-TB: *Mycobacterium tuberculosis* with resistance to rifampicin and isoniazid.

2 XDR-TB: *Mycobacterium tuberculosis* with resistance to rifampicin and isoniazid (MDR-TB), plus additional resistance to a fluoroquinolone and a second-line injectable drug.

This document is divided into three parts. Part I describes the principles of the Global Project that should be considered fundamental to continuous surveillance systems and surveys. Part II describes the steps needed to plan and implement a survey in order to determine the burden of MDR-TB in a given area, as well as to manage and interpret the collected data. Part III describes an approach to monitoring trends in drug resistance over time, which is relevant to countries for which baseline MDR-TB burden data already exist from surveys.

The need for strengthening surveillance for drug-resistant TB has been reiterated by the 2009 World Health Assembly resolution WHA62.15 "*Prevention and control of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis*". This urges all Member States to "achieve universal access to diagnosis and treatment of multidrug-resistant and extensively drug-resistant tuberculosis", including by means of "strengthening health information and surveillance systems to ensure detection and monitoring of the epidemiological profile of multidrug-resistant and extensively drug-resistant tuberculosis and monitor achievement in its prevention and control".

Changes from previous editions

Readers familiar with the 2009 edition of the *Guidelines for surveillance of drug resistance in tuberculosis* will notice the following updates in surveillance methodology are incorporated into the current edition:

- The incorporation of molecular technologies into surveys is recommended, either alone or as a screening tool prior to culture-based methods. Any WHO-endorsed testing method can be incorporated into surveillance systems and surveys. A spectrum of different diagnostic testing algorithms is presented; the choice of algorithm will depend upon the objectives of the survey and the resources available.
- For countries with recent, high quality survey data on the burden of MDR-TB but without the capacity for routine continuous surveillance based on diagnostic DST of all patients, the monitoring of trends in drug resistance over time is recommended using rapid molecular technologies in sentinel sites.

Part 1

Principles of anti-tuberculosis drug resistance surveillance

1 Mechanisms of surveillance that produce data representative of a geographically-defined population

"Surveillance" means the systematic ongoing collection, collation and analysis of data for public health purposes and the timely dissemination of public health information for assessment and public health response as necessary

WHO International Health Regulations (2005)

The Global Project for Anti-TB Drug Resistance Surveillance was initiated in 1994 with the aim of collecting and evaluating data on anti-tuberculosis drug resistance in a systematic and ongoing manner across the world. Within the standardized methodological framework designed for the Project, two main approaches to surveillance are capable of collecting data on drug resistance representative of a geographically-defined population to allow for comparison across settings and within settings over time. These two approaches are continuous surveillance based on routine DST of all TB patients, and periodic surveys of sampled TB patients.

A continuous surveillance system based on routine DST is best able to meet the criteria of *systematic* and *ongoing*. *WHO Standards and Benchmarks for TB Surveillance* (14) sets a benchmark that rifampicin susceptibility testing results should be documented for at least 75% of new pulmonary TB cases. This threshold was established based on surveillance systems in Europe and North America.

However, capacity for continuous surveillance based on routine DST is still insufficient and it is clear that alternative measures are needed in many parts of the world where this benchmark has not yet been reached, in light of region- and country-specific characteristics and capacities. Therefore, in many countries, periodic surveys of randomly selected TB patients remain the basis of drug resistance surveillance.

Each country should take a long-term view of surveillance, and design a system that best fits its needs. This system should be based on capacity that is sustainable, and ideally allow the evaluation of trends over time—an inherent objective of surveillance. Countries may combine components from the two key surveillance mechanisms in order to meet their specific needs and capacities.

The Global Project measures resistance only in newly registered episodes of TB (among new and/or previously treated cases - see section *6.1 Inclusion and exclusion criteria*), which can be used to estimate the number of MDR-TB cases expected to occur among pulmonary TB patients notified in a country. This is

valuable for planning the response to MDR-TB. The Global Project does not measure resistance among prevalent TB cases.

1.1 Continuous surveillance systems based on routine drug susceptibility testing

A surveillance system based on routine DST of all TB cases is able to provide continuous information on drug resistance patterns among patient groups, and is therefore able to accurately detect trends over time, as well as localized outbreaks. Approximately half of the countries currently reporting data to the Global Project have continuous surveillance systems with quality-assured laboratories that can provide routine DST data on most TB cases. Due to the resources required to maintain such a system, these surveillance systems are typically found in high-income countries. In these countries, DST results usually form the basis of the clinical management of TB using tailored or individualized treatment regimens.

In settings where capacity is currently not available for systematic DST of all TB patients, a system should prioritize routine DST of cases at high risk of drug-resistant TB. At a minimum, systematic DST should be established among all previously treated TB cases and a periodic survey should be conducted regularly among new cases every five years (see section *1.2 Periodic surveys for estimating the burden of drug resistance*).

In a number of countries that provide routine DST to patients, continuous surveillance continues to be substandard due to low quality of laboratory processes, weaknesses in data recording and reporting, a lack of standardization in patient classifications, and incomplete patient coverage. Data from these surveillance systems are not included in the Global Project. However, significant efforts are underway in many settings to improve quality, which will allow a growing number of countries to rely on their continuous surveillance data to monitor drug resistance.

1.2 Periodic surveys for estimating the burden of drug resistance

In resource-constrained settings where capacity is currently not available for routine DST of all TB cases, surveys can be conducted to measure drug resistance among a random sample of patients which is representative of the geographically-defined population under study. When properly designed and periodically conducted, such surveys provide a sound estimation of the resistance profile of all TB cases in the population under study and can detect general trends over time. Approximately half of the countries currently reporting data to the Global Project provide data from surveys.

Periodic surveys can provide much of the same critical information provided by a continuous surveillance system, but they are unable to detect localized outbreaks, may produce results with margins of error that prevent meaningful analysis or determination of trends, and are subject to biases inherent in sampling. However, when considering secondary benefits, conducting surveys can

strengthen laboratory capacity, transport and referral systems, as well as evaluate the accuracy of classification of patients by treatment history. Surveys can also provide a platform for studying risk factors for drug resistance (see section *2.2.3 Other patient biographical and clinical factors*).

Nationwide surveys are desirable for programmatic reasons, but surveys at other administrative levels could also be considered in large countries, when national capacity is insufficient, or for specific reasons such as the suspicion of an increasing burden of drug-resistant TB in certain regions. The size and scope of the survey depends on the specific objectives of the survey , and should be determined by the ability of the NTP to ensure quality. Starting at lower administrative levels such as cities or provinces before expanding to nationwide surveys is one way of developing capacity while ensuring quality. However, results from surveys at the subnational level cannot be extrapolated to estimate the burden at the national level.

In settings without capacity for continuous surveillance based on routine DST of new TB cases, surveys of new TB cases should be conducted at least every five years. A survey can provide critical information for the NTP on the burden of resistance and common patient drug resistance profiles at a point in time.

1.3 Sentinel surveillance systems for monitoring trends over time

For countries where limited resources, health care system structure, or geographical features preclude routine DST of all patients in surveillance systems, the establishment of a sentinel surveillance system may be an option for monitoring trends in drug resistance over time.

A sentinel system could be a useful interim approach for countries intending to expand routine DST to all cases. The implementation of a sentinel system requires careful planning in order to produce data that are useful for monitoring trends over time. It also has several important limitations. Unlike national surveys, the data are not nationwide and therefore cannot be used to estimate the proportion of drug resistance in the country. Additionally, the data cannot be used to make inferences on trends in the rest of the country. **A sentinel system is therefore only recommended for countries which have high quality data from a recent survey (within the previous 5 years) and are moving towards establishing a national system for routine surveillance.**

Annex 1 provides a comparison of three possible approaches to the surveillance of drug resistance, which differ in their design (national survey versus subnational sentinel system) and the laboratory methods employed (culture-based, molecular, or a combination of the two). These are given as examples only, and should be adapted to match the surveillance objectives and capacity for laboratory testing in the country.

2 Standardized stratification of results by patient characteristcs

2.1 Patient treatment history classifications

Careful classification of treatment history is critical to proper and accurate interpretation of surveillance data. The 2013 revision of WHO's *Definitions and reporting framework for tuberculosis* (15) defines patient registration groups by history of previous treatment.

Definition: "New case"

For the purpose of surveillance, a "new case" is defined as a newly registered episode of TB in a patient who, in response to direct questioning, reports never having been treated for TB or reports having taken anti-TB drugs for less than one month; or, in countries where adequate documentation is available, for whom there is no evidence of having taken anti-TB drugs for one month or more.

Definition: "Previously treated case"

For the purpose of surveillance, a "previously treated case" is defined as a newly registered episode of TB in a patient who, in response to direct questioning, reports having received one month or more of anti-TB drugs in the past; or, in countries where adequate documentation is available, there is evidence of having received one month or more of anti-TB drugs.

Previously treated cases (also referred to as "retreatment cases") are a heterogeneous group composed of several subcategories:

Subcategories of previously treated cases

Relapse patients have previously been treated for TB, were declared *cured or treatment completed* at the end of their most recent course of treatment, and have now been diagnosed with a recurrent episode of TB (either a true relapse or a new episode of TB caused by reinfection).

Treatment after failure patients are those who have previously been treated for TB and whose *treatment failed* at the end of their most recent course of treatment.

Treatment after loss to follow-up patients have previously been treated for TB and were declared *lost to follow-up* at the end of their most recent course of treatment (previously known as *treatment after default* patients.)

Other previously treated patients are those who have previously been treated for TB but whose outcome after their most recent course of treatment is unknown or undocumented.

Evaluation of resistance among subcategories of previously treated cases is critical for data interpretation, and provides crucial information for programme management. Previously treated patients are at higher risk of having strains of TB with resistance to one or more drugs, and are usually the group from which patients are screened for inclusion in drug-resistant TB treatment programmes. Information about the size and composition of this population and the patterns of resistance in subcategories of previously treated cases is extremely important for programmatic reasons. This can be collected by establishing a surveillance system based on routine culture and DST of all such cases.

2.2 Age groups, sex, HIV status and other patient biographical and clinical factors

Given the large imbalance in numbers of drug-susceptible and drug-resistant patients in most surveys, it may not be possible to detect significant differences between patient groups. Although a case-control study is a more appropriate design for exploring risk factors, the opportunity provided by drug resistance surveys should not be overlooked. Additionally, basic patient demographic data can be used to inform imputation models which may be used for handling missing data (see section *7.2.1 Imputation of missing values*).

2.2.1 Age groups and sex

Data on drug resistance stratified by age groups and sex can provide insight into risk groups and effectiveness of specific TB control activities. Furthermore, the magnitude of drug resistance among younger age groups is more likely to be indicative of recent transmission than among older age groups, which may be harbouring older infections.

2.2.2 HIV status

Incorporation of HIV testing in anti-tuberculosis drug resistance surveillance may yield important information for the national TB control programme on the relationship between HIV and drug-resistant TB. Provider-initiated HIV testing is recommended for all TB patients and for patients presenting with signs and symptoms of TB.

The specific objectives for including HIV testing should be addressed when developing a surveillance system or should be indicated in a survey protocol. Existing national policies on HIV testing and HIV surveillance should be followed, including the availability of counselling services, ensuring consent and confidentiality procedures. The national HIV/AIDS programme should be involved in the planning and execution of the surveillance from the beginning. Rapid HIV tests in accordance with national HIV testing and surveillance policies are more convenient methods of HIV testing compared with conventional laboratory-based tests (16,17).

2.2.3 Other patient biographical and clinical factors

Inclusion of other patient biographical and clinical information is optional, depending on the objectives of the survey and the availability of resources. Surveys can serve as a valuable platform for studying setting-specific causes of drug resistance and for identifying the most important targets for intervention. They can include a series of questions about potential risk factors, to be answered either by patients or through a review of medical records at the time of enrolment.

Potential risk factors that may be evaluated include: use of antiretroviral treatment in HIV positive patients; *M. tuberculosis* genotype; type of health centre and patient residence (e.g. urban/rural); social determinants including socioeconomic status, education level, or employment; or direct risk factors such as malnutrition, crowding, diabetes, alcohol abuse, injecting drug use, or smoking. For previously treated patients, additional information could include: type and quality of previous treatment and treatment supervision; infection control practices; composition of treatment regimens; or source of drugs used. It should be noted that multiple risk factors for acquisition, amplification and transmission of drug resistance may be present simultaneously in a given setting.

For examples of how to design questions to measure social determinants, see Lönnroth et al (18) or *Annex 5* of the 2011 WHO publication, *Tuberculosis prevalence surveys: a handbook* (19). The examples provided may require modification based on local conditions and the population under study.

3 Quality-assured laboratory methods for determining resistance to first- and second-line drugs

Establishment of quality-assured bacteriology using WHO-recommended methods is essential for reliable surveillance of drug resistance. The introduction of rapid methods into the diagnostic algorithm for identification of *M. tuberculosis* and DST should be considered a priority in all settings.

3.1 WHO-recommended methods of drug susceptibility testing

Recent technological advances in laboratory diagnostics have expanded the list of WHO-recommended methods available for DST, which can significantly reduce the delay between detection of TB and diagnosis of drug resistance. Rapid methods of DST allow for the timely selection of appropriate treatment regimens based on patients' drug resistance profiles using diagnostics that can be feasibly implemented into a variety of settings worldwide. This increased capacity for DST also translates into increased capacity for surveillance.

After comprehensive review, WHO has endorsed certain new nucleic acid amplification tests (NAAT) which will allow greater coverage of drug resistance surveillance, as they can be implemented in countries without the capacity to perform culture-based methods. Due to the dynamic nature of research and development, new technologies other than those described below may have been endorsed by WHO since the publication of this document and could therefore be incorporated into surveillance activities.

More detailed information about the methods described below is given in the *Mycobacteriology Laboratory Manual* (2014) of the Global Laboratory Initiative (20).

3.1.1 Nucleic Acid Amplification Tests (NAAT)

Xpert® MTB/RIF

Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) is a fully-automated assay that integrates sputum processing, DNA extraction and amplification, and detection of *M. tuberculosis* and rifampicin resistance (6). This cartridge-based system detects common mutations in the *rpoB* gene in both smear positive and smear negative sputum specimens. Test results are obtained in approximately 90 minutes. Although Xpert MTB/RIF has dramatically cut the time required in order for patients to receive an accurate diagnosis of tuberculosis and rifampicin resistance, additional DST methods may be needed to investigate resistance to other first-

line and second-line drugs. A training package is available online from the Global Laboratory Initiative (21).

Molecular line probe assays

Molecular line probe assays (LPAs) are a genotypic method of DST used to detect *M. tuberculosis* and the most common mutations that confer resistance to anti-tuberculosis drugs. LPAs have been endorsed by WHO for use in testing smear positive sputum samples or *M. tuberculosis* cultures for rifampicin resistance alone or in combination with isoniazid resistance, provided that technical expertise on molecular techniques and proper facilities are in place (5). Such assays can be used for surveillance purposes when testing sputum smear positive samples. However, LPAs are less sensitive for the detection of isoniazid resistance and therefore may underestimate the level of resistance.

3.1.2 Phenotypic DST

The recommended critical concentrations of drugs to be tested are given in WHO's *Updated interim critical concentrations for first-line and second-line DST* (2012) (22).

Liquid culture

Compared with solid culture methods, liquid culture methods significantly reduce the turnaround time for results and are around 10% more sensitive than culture using solid media. With liquid culture, confirmation of pulmonary TB can usually be obtained within two weeks, and DST results in an additional one to two weeks. Liquid culture methods can be used for susceptibility testing for both first-line and second-line drugs. WHO has endorsed the use of liquid culture and DST, provided that the required infrastructure and biosafety measures are in place. Procedures should be performed strictly according to the manufacturer's instructions. The disadvantages of the liquid culture method include a relatively high cost for equipment and consumables, the need for rapid speciation (since the recovery rate of non-tuberculosis mycobacteria may be high in certain settings), and the requirement for fresh specimens with optimized processing methods to minimize contamination of cultures. Reading of commercial liquid culture systems is now partially or fully automated, reducing human error and contamination to some degree.

Solid culture

Conventional phenotypic methods using solid media remain more common. Three solid culture methods using egg-based or agar-based media continue to be used around the world: the proportion method, the resistance ratio method, and the absolute concentration method. These methods are relatively inexpensive and highly standardized for testing susceptibility to many drugs, but they have the disadvantage of requiring up to eight weeks to produce a definitive confirmation of pulmonary TB, and another six weeks to produce DST results.

Of the three methods, the proportion method is the most commonly used worldwide. DST critical concentrations for second-line drugs have not yet been

adequately validated for the resistance ratio and absolute concentration methods. Methodology is well described elsewhere, as are instructions for the preparation of the most commonly used egg-based media (Löwenstein-Jensen and Ogawa).

3.2 Selection of drugs to be tested for susceptibility

The selection of drugs to be tested will depend upon the survey objectives and logistical considerations.

3.2.1 Determining the MDR-TB burden

If the objective is to determine the burden of MDR-TB, susceptibility testing for both rifampicin and isoniazid is a minimum requirement. However, in countries with limited laboratory capacity, rifampicin susceptibility can be used for initial screening. Strains that are determined to be resistant to rifampicin should then be tested for resistance to isoniazid. Due to the extreme challenges in treatment options for XDR-TB, MDR-TB strains should be tested for susceptibility to fluoroquinolones and the second-line injectable agents available for use in the given setting.

3.2.2 Monitoring trends over time

If the objective of the survey is to monitor drug resistance trends over time and recent, good quality data on the burden of MDR-TB already exist, testing for rifampicin resistance alone is sufficient. This is discussed in more detail in *Part III* and *Annex 1*.

3.2.3 Planning for new treatment regimens

If the country is considering the introduction of new treatment regimens, both new and previously treated patients should be tested for susceptibility to the drugs of interest, e.g. fluoroquinolones or pyrazinamide.

3.3 Quality assurance of drug susceptibility testing

To ensure that the results of DST are reliable, a comprehensive laboratory quality assurance system is fundamental. This system should be designed to continuously monitor internal work practices, technical procedures, equipment and materials (internal quality control), and to systematically assess laboratory capabilities through the support of an external laboratory (external quality assessment).

3.3.1 Internal quality control

Molecular-based DST

- A single verification test must be performed per module upon installation of each GeneXpert instrument and following calibration of instrument modules. Each instrument should be monitored using the following minimum set of indicators to evaluate proper use (7). Number of tests performed per month per module;

- Number and proportion of *M. tuberculosis* positive results;
- Number and proportion of *M. tuberculosis* positive rifampicin resistant results;
- Number and proportion of errors (disaggregated by type of error);
- Number and proportion of indeterminate results; and
- Number and proportion of invalid results.

For LPA, negative and positive controls should be included in each run. The success of the amplification reaction should be verified by the control zones of the testing strips.

Culture-based DST

As a part of internal quality control, the quality of the culture medium should be controlled with each batch of isolates tested. Drugs added to the medium must be pure substances obtained from a reputable firm and properly stored. Drug dilutions and the addition of these to the medium should be performed in accordance with accepted standards.

For conventional solid culture methods, susceptibility testing should be performed on the standard H_{37}Rv *M. tuberculosis* strain and a combination of strains with known resistance to two or three drugs, but avoiding XDR-TB strains. Since batches of media will be consumed quickly, it may be necessary to include these reference strains with each batch of survey strains processed for DST. The usual internal quality control procedures for new batches of drug-free and drug-containing media apply, and results should always be validated by a supervisor who will ascertain that all strains with doubtful results will be re-tested.

3.3.2 External quality assessment and the role of the Supranational Reference Laboratory Network (SRLN)

External quality assessment has several components: proficiency testing, retesting of strains, and onsite evaluations of laboratories, all conducted in cooperation with an external partner laboratory.

The SRLN plays a critical role in capacity strengthening of laboratories worldwide, and is fundamental in the external quality assessment activities that ensure the accuracy of national surveillance of drug resistance. At the time of publication of this document, there were 33 Supranational Reference Laboratories (SRLs) in the network (see full list here: http://www.stoptb.org/wg/gli/srln.asp).

SRLs maintain a high level of quality by participating in annual intra-network proficiency testing of DST. The SRLs judicially determine a consensus on the susceptibilities of selected strains to first-line drugs (rifampicin, isoniazid, ethambutol, pyrazinamide) and to second-line drugs (kanamycin, amikacin, capreomycin, ofloxacin). The panels of strains are subsequently used to assess the proficiency of National Reference Laboratories (NRLs) and any subnational reference laboratories that provide DST results for surveillance systems and drug resistance surveys. SRLs can also provide onsite evaluations of NRLs and training and supervision as necessary.

External quality assessment of an NRL's accuracy at culture-based DST requires an exchange of strains of *M. tuberculosis* in two directions: from the SRL to the NRL, and from the NRL to the SRL.

From the SRL to the NRL (proficiency testing):

An NRL should annually receive a panel of precoded strains from a partner SRL to be tested for susceptibility to first-line and, if applicable, to second-line drugs. The test results of the NRL should be compared with the precoded results of the judicial consensus of the SRL, which can be considered a "gold standard". The procedure should be performed double-blinded. The minimum required level of agreement is 90% for each drug except for rifampicin and isoniazid for which the level is 95%.

From the NRL to the SRL (quality assessment of results, also known as "retesting"):

In order to assure the quality of DST, a sample of strains isolated during surveillance should be sent to a partner SRL to be retested (see section *6.4.1 Culture-based surveys*). The results should be compared for agreement with respect to each drug. National and international rules and regulations and turnaround times for shipment to the SRL must be considered for planning purposes (see *Annex 7*).

4 Ethical considerations

Information obtained from anti-tuberculosis drug resistance surveys or surveillance is crucial in planning a robust MDR-TB control programme. The overarching goal of public health activities is to promote a population's health, but the rights, freedom, privacy and confidentiality of individual patients need to be respected as far as possible in planning and implementing a surveillance system or a survey (23).

Some public health activities can unambiguously be identified as research, and others as routine surveillance, but there is a grey zone of activities that cannot easily be classified. Research ethics and public health ethics are grounded in similar principles, but the application of these principles will not always be identical (24). In order to ensure adherence to ethical standards, survey protocols and new surveillance systems in the planning stage should be reviewed by ethics committees or institutional review boards. Such reviews should include due consideration of the following key concepts for the ethical conduct of surveillance (23,25,26):

- **Confidentiality** - Sensitive patient information should be kept confidential unless its disclosure has been authorized by the person concerned. However, it may be permissible to disclose some medical information without patient consent for legitimate public health purposes (for example, mandatory reporting of certain infectious diseases). In practise, personal data should be shared and revealed to others only where strictly necessary for the functioning of the surveillance system and/or for the promotion of crucial public health goals. Unjustified disclosure of personal information would not only violate the patient's privacy, but could also foster stigma and discrimination.
- **Informed consent** - In the course of a survey, informed consent should be obtained from individuals who have the capacity to make their own decisions, and consent should be obtained from a surrogate decision-maker for incapacitated persons. In contrast to the usual practice in medical research, individual informed consent is not always feasible or appropriate for routine surveillance, especially when obtaining information from an entire population is essential to achieving critical public health objectives. Nonetheless, whenever feasible, public health practitioners should strive to obtain consent from the subjects of surveillance. Even when obtaining individual consent is deemed unfeasible or inappropriate, individuals and/or communities should be informed about the nature and purposes of the surveillance to the extent

this is possible. In the particular situation where DST is offered to patients but treatment for drug-resistant TB is not available, individuals should be informed of the risks and benefits of testing and specifically should be asked if they are willing to consent even though treatment is not available to them (27,28).

- **Access to treatment** - Surveillance of drug resistance in tuberculosis raises a particular ethical dilemma when surveillance activities are conducted in settings where there is limited capacity to properly treat patients identified with drug-resistant strains. The results of the testing should be communicated to subjects, and those in need should, if feasible, be provided with appropriate treatment with second-line anti-tuberculosis drugs. Because MDR-TB treatment has been shown to be cost-effective, WHO recommends treatment with second-line drugs as the standard of care for MDR-TB patients. All countries should therefore already have existing MDR-TB treatment programs, or at least plans to put them in place. In a country with no MDR-TB treatment program (or a plan to put one in place), a programme should be established in light of surveillance results. It is essential that surveillance is used to inform the development of the health system, including the setting of priorities and the establishment of diagnostic and treatment services.

Part 2

Conducting surveys for assessing the proportion of anti-TB drug resistance

5 Survey planning

Conducting a drug resistance survey that will provide accurate, precise, and reliable results requires significant planning. In order to obtain data that are representative of the geographically-defined population under study, the process for selecting patients must be carefully designed. Measures must be in place to ensure that the data collected are properly categorized, checked and validated and that the DST is quality-assured. This requires comprehensive and accurate planning of logistics, including pre-survey budgeting of all planned expenses.

5.1 Forming a national coordination team

A survey involves three major operational areas:

- programme management (logistics, training, collection of clinical information, supervision of survey);
- standardized laboratory techniques; and
- epidemiology/statistics (sampling, data management and analysis).

A national coordination team, including experts from each of the above fields, should be established. In general, the coordination team is composed of the manager of the national TB control programme (or designated persons), the head of the Central Reference Laboratory (or designated persons), an epidemiologist and a statistician. This team is responsible for the preparation of the survey, close coordination with the SRL, supervision and quality assurance during the survey, and the final collection, analysis, and reporting of results. The coordination team will require strong official backing from the authority responsible for health services. A clear outline of team members and specific roles and responsibilities should be developed. The person supervising and coordinating the day-to-day activities of the survey should be hired specifically for that role, without concurrent responsibilities for other activities outside of the survey.

5.2 Setting objectives

Identification of specific survey objectives is a critical component of the initial planning process, because this will guide the development of a survey capable of collecting meaningful information. The objectives must be developed in the context of the available resources, funding and laboratory capacity in the area under study. It is important to carefully define the population of interest, as special approaches may be needed to capture meaningful data relating to certain

subgroups, such as children, prison inmates, or patients seeking care in the private health sector.

A secondary aim of a drug resistance survey should be development or strengthening of a quality-assured laboratory network. A survey should enhance the existing diagnostic capacity in the country and lay the foundation for the establishment of continuous surveillance systems in the future.

Specific objectives of the survey may include:

- to determine the proportion of new sputum smear positive pulmonary TB cases that have resistance to rifampicin and isoniazid (MDR-TB) and/or to other anti-tuberculosis drugs;
- to determine the proportions and patterns of drug resistance to fluoroquinolones and second-line injectable agents among patients with strains with confirmed resistance to rifampicin and isoniazid;
- to evaluate associations between drug resistance and characteristics such as age, sex and HIV status;
- to evaluate associations between drug resistance and possible risk factors such as history of incarceration, smoking, alcohol abuse, injectable drug use and diabetes (see section *2.2 Age groups, sex, HIV status and other patient biographical and clinical factors* for limitations relating to survey design); and/or
- to monitor trends in drug resistance over time (see *Part III*).

5.3 Defining the diagnostic testing algorithm

With the development of new molecular technologies, a variety of options are available for laboratory testing. In light of available resources, funding, and laboratory capacity, a testing algorithm should be defined in consultation with the partner SRL which allows the survey objectives to be achieved. The algorithm can include sputum smear microscopy, culture-based methods and molecular methods, either alone or in combination. A flowchart is often helpful for defining the order in which the different tests should be performed, on which samples, and at which level (diagnostic centre, Central Reference Laboratory or SRL).

Molecular methods are being increasingly used in surveys. They can be used as initial screening tools, or alone without additional conventional methods. For example, Xpert MTB/RIF may be used to screen samples for rifampicin resistance. Culture could then be performed on resistant samples in order to determine the full drug susceptibility pattern. Although a survey with this design will not be able to provide information about resistance not associated with rifampicin, it will significantly reduce the workload for the laboratory (see *Annex 1* for examples of diagnostic algorithms).

5.4 Development of a protocol and time schedule

A survey protocol that describes all aspects of the survey should be developed, to include the following:

- roles and responsibilities of the coordination team and individual members;
- objectives;
- sample size and design;
- patient inclusion criteria;
- logistics;
- training;
- ethical considerations;
- laboratory testing algorithm;
- laboratory capacity;
- quality assessment of drug susceptibility results;
- data management; and
- budget.

Once participating health centres have been identified by the chosen sampling method (see section *5.6 Sampling of cases*), a schedule can be established, taking into account logistics, climatic conditions and laboratory workload. All laboratory methods and the system of quality assurance should be discussed and agreed upon with the partner SRL. Furthermore, the protocol should describe ethical issues, and the established timeline should take into consideration the time required for the protocol to receive necessary approval from ethical review panels. Both an experienced epidemiologist and statistician must contribute to the development of the protocol.

A checklist for a survey protocol is included in *Annex 2*. WHO and other technical partners can assist in the development of a survey protocol, and should be asked to review a survey protocol prior to initiation of a survey. This will ensure that all requirements have been considered and described comprehensively; that quality control measures are in place; and that the data collected will be representative of the geographically-defined population under study. Once finalized, such a protocol should be distributed to all coordination team members and health staff participating in the survey.

5.5 Minimum required facilities for a survey area

The country, state, province or city selected to be a survey's geographical area of study should have at least one quality-assured central laboratory for the selected testing methods (i.e. a Central Reference Laboratory, which is usually the National Reference Laboratory) linked to all intermediate TB laboratories and the majority of TB diagnostic centres. If such a quality-assured central laboratory does not yet exist, the shipping of sputum specimens to an external laboratory may be considered.

Diagnostic and/or treatment centres

Patients should be sampled from institutions where patients suspected of having TB are registered. Most of these will be non-specialized health centres or outpatient departments of hospitals operated by the government or by nongovernmental organizations.

The roles of all relevant health care providers (public, voluntary, private and corporate) not formally linked to the national TB control programme in the diagnosis and treatment of TB should be carefully considered. Inclusion of care providers functioning outside the national control programme will require particular attention to assuring quality standards in diagnostics, sampling, and data recording and reporting. Countries with a sizable private sector should seek to include private health centres in the survey, in order to obtain results representative of the entire population of TB patients. Public-private mix initiatives can serve as platforms to gradually involve the private-sector laboratories in drug resistance surveillance activities. Quality-assured microscopy together with adequate referral systems for molecular testing and/or culture and DST are prerequisites for the implementation of a drug resistance survey.

Central Reference Laboratory

The Central Reference Laboratory undertakes the identification of *M. tuberculosis* and also performs DST, by either molecular or culture-based methods. There may also be other intermediate laboratories in the network capable of performing DST by molecular or culture-based methods. One of the main tasks of the Central Reference Laboratory is to ensure the quality of smear microscopy, molecular testing, and culture and DST performed by regional or peripheral units by establishing a regular "onsite" supervision programme for those units, and by providing training in, and quality assurance systems for, the laboratory procedures. An external quality assessment programme with a partner SRL will validate the results of susceptibility tests done by the Central Reference Laboratory and any other relevant laboratories.

Basic laboratory equipment and materials must be available and functional in the Central Reference Laboratory before the implementation of a survey. Drug resistance surveys should only be undertaken when the laboratories are deemed to have an appropriate level of biosafety (29) and are equipped with trained staff working with clear standard operating procedures and producing quality-assured data. It is important to note that drug resistance surveys will heavily increase the workload of the reference laboratory, and therefore should only be undertaken where capacity is sufficient.

5.6 Sampling of cases

Statistical methodology is a fundamental aspect of the design of surveys. Accordingly, an experienced epidemiologist or statistician should be involved from the early planning stages.

5.6.1 Defining the sampling frame

The sampling frame for a survey depends on the objectives of the survey and the testing methods to be used. For example, to measure the proportion of new cases with anti-tuberculosis drug resistance using culture-based methods, the sampling frame should include all new sputum smear positive pulmonary TB patients in the study area.

Sampling of previously treated cases

Continuous surveillance of drug resistance among previously treated patients should be established as a priority in all countries. Accurate evaluation of resistance in previously treated patients provides crucial information for programme management. In settings where routine DST of previously treated cases is not yet in place, ideally a separate sample size calculation should be devised for a survey of previously treated patients. However, in most settings, achieving this sample size would not be feasible due to the small number of previously treated patients notified annually. Instead, during the survey enrolment period for new patients, it is recommended that all previously treated patients who present to study sites be enrolled. Due to the small number of cases, estimates in previously treated cases are likely to be less precise than estimates in new cases and the analysis of subcategories may not be possible.

Culture-based surveys are usually based on sputum smear positive TB cases for two reasons:

1. There is no strong evidence to indicate that the proportion of cases that have drug resistance varies substantially according to whether the TB case is sputum smear positive or negative.
2. The culture yield from sputum smear negative patients is relatively low compared to sputum smear positive cases (30) and is also more negatively affected by delays in sputum transport. Inclusion of cases with a low culture yield requires a significantly larger sample size, and may increase laboratory workload up to 10 times. Therefore, countries interested in including sputum smear negative cases should strongly consider the implications for logistics and laboratory capacity.

If molecular technologies such as Xpert MTB/RIF are being used, the sampling frame could be expanded to include patients with presumptive TB, regardless of smear microscopy result. In this case, all patients with presumptive TB must be tested instead of only those prioritized by national testing guidelines. The calculation of an appropriate sample size can be difficult as information about the numbers of patients with presumptive TB is often not available centrally. As less TB will be found among sputum smear negative patients with presumptive TB than among smear positive patients, a greater number of patients will need to be tested, resulting in a much higher demand in resources. If a survey aims to determine the relative risk of drug-resistant TB among HIV-positive TB patients

compared with HIV-negative TB patients, a more complex study design would be required, often involving a much larger sample size. Few countries have conducted such studies; therefore, it is important to seek appropriate technical support for advice on survey design and laboratory needs when designing such a protocol.

5.6.2 Sample size

For surveys measuring the proportion of new cases with MDR-TB, the calculation of an appropriate sample size should be based on the following (31):

- the total number of new sputum smear positive pulmonary cases registered in the previous year in the country or in the geographical setting to be studied;
- the expected proportion of new sputum smear positive pulmonary cases with MDR-TB, based on available data (in the absence of available data, an informed estimation must be made);
- the desired precision of the estimate, to be expressed as a confidence interval of at least 95%. The sampling uncertainty should be as low as possible, while ensuring that the corresponding calculated sample size is logistically feasible. For example, if the proportion of new cases with MDR-TB is expected to be 4%, an absolute precision of 0.5% (i.e. 0.005) means that the estimate may err within 0.5% of the true proportion, corresponding to a 95% confidence interval from 3.5-4.5%.

The following formula can be used to calculate the sample size under simple random sampling (SRS), with a finite population correction:

$$n = \frac{N * z^2 * p * (1-p)}{d^2 * (N-1) + z^2 * p * (1-p)}$$

where:

N = total number of new sputum smear positive pulmonary cases registered during one year in the country;

z = z-value (from the standard normal distribution) that corresponds to the desired confidence level (if confidence interval =95%, z= 1.96);

d = absolute precision (as a decimal, e.g. 0.5% should be expressed as 0.005);

p = expected proportion of MDR-TB in the target population (as a decimal, e.g. 4% should be expressed as 0.04).

The relative precision can be calculated by $\frac{d}{p} * 100$, and should ideally not be greater than 25% of p. For example, if the absolute precision is 0.005 and the expected proportion of new cases with MDR-TB is 0.04, the relative precision is 0.005 / 0.04 *100 = 12.5%.

If the cluster sampling method is adopted, the correlation between individuals within a cluster needs to be taken into account. In general, the design effect due to clustering in drug resistance surveys ranges from 1.5 to 3. Unless the design effect can be estimated from previous surveys, a design effect of 2 should be assumed.

Therefore, the calculated sample size obtained from the equation above must be multiplied by 2.

Multiple imputation is recommended to reduce bias due to missing data (see section *7.2.1 Imputation of missing values*), but this will reduce the precision of the estimate of the proportion of patients with MDR-TB. It is therefore recommended to increase the calculated sample size to account for potential losses. Losses include patients diagnosed as sputum smear positive who do not provide consent to be enrolled in the survey or do not produce an adequate sample for the survey, patients whose sample is contaminated or negative by molecular testing or culture, and patients whose DST does not give interpretable results. For culture-based surveys, a loss of 10-15% patients should be incorporated into the sample size calculation; for molecular-based surveys, 5-10% is appropriate.

Countries that repeat a survey should aim to document differences in the proportion of patients with drug resistance in comparison with previous surveys. Therefore, the sample size should be calculated so it can detect a significant difference between the proportion of MDR-TB found in the previous survey and the proportion anticipated in the current survey. The sample size then depends on the expected difference and the power of the comparative test. The smaller the difference to detect between the proportions, the larger the sample size required. The assistance of an epidemiologist or statistician is needed to determine an appropriate sample size for a subsequent survey.

As mentioned in section *5.6.1 Designing the sampling frame*, the sample size calculation for previously treated cases is unlikely to be achievable due to the low numbers of notified previously treated cases. Instead, previously treated cases should be consecutively enrolled until the target sample size for new patients is reached.

An Excel spreadsheet is available for download from the WHO Global TB Programme website at http://www.who.int/tb/publications/2015/drs_guidelines/en/ to assist with sample size calculation. This provides a practical tool for exploring the impact of different parameters on the required sample size.

5.6.3 Sampling strategies

Different sampling strategies can be adopted to select a sample of TB patients representative of all TB patients in a geographic setting. In order to select a representative group of newly registered patients, randomisation is essential. However, simple random sampling of individual patients within health centres is rarely feasible. This is mainly because routine diagnostic and treatment activities would be disrupted, resulting in low compliance of staff and patients and poor quality data.

Randomisation can take place at the health centre or, possibly, the health district level. In this way, routine activities would be slightly changed for only selected health centres for a period of time, but would remain identical for each

newly registered sputum smear positive patients in each of these centres. If each individual patient within the sampling frame has an equal probability of being included in the sample, the proportion of drug resistance found in the survey sample provides an unbiased estimation of the true proportion. In this case, the sample will be "self-weighted", i.e. sample weights should not be needed in the statistical analysis. The most useful sampling strategies are described below.

100% sampling of health centres

This sampling method is most suitable for small countries with relatively small numbers of health centres registering TB patients, good infrastructure, and facilities to transport samples to the Central Reference Laboratory. All eligible patients presenting to each health centre in the study area within a defined time period are enrolled.

The self-weighted character of this design is ensured by including all centres and using the same enrolment period for each of them. Large and small centres are equally represented without the need for a complicated sampling method. The intake period is calculated by dividing the sample size by the total number of sputum smear positive patients per year in the study area. For example, if around 7000 eligible patients are diagnosed per year, and if a required sample size of 600 patients has been calculated, the enrolment period will be 600/7000 = 1/11.6 year, i.e. approximately one month. In this case, all consecutive eligible patients enrolled during one month in all centres should be included, which provides approximately a 10% sample of newly registered sputum smear positive patients.

The enrolment could be done either during the same month or on rotation – for example, centres in zone 1 during the first month, centres in zone 2 during the next month, and so on. In this way, the number of sputum samples sent to the Central Reference Laboratory for testing would be approximately the same each month throughout the year. The rotation technique can prevent overload at the Central Reference Laboratory and affords the opportunity to instruct health centre staff in shifts and, where necessary, to correct procedures. The total time to complete the enrolment should not exceed one year. However, the impact of any seasonal differences must be carefully considered before adopting this approach.

Cluster sampling

Cluster sampling methods are best used in situations in which it is logistically difficult to cover the entire area of the country and where the number of health centres where TB patients are registered is high. With this design, health centres are randomly selected. To avoid the risk of drawing a sample that misses the largest centres, a weighted probability-proportional to size (PPS) cluster sampling technique should be used.

The optimal number of clusters depends on the variability of the prevalence of drug resistance between and within clusters, and the cost of including a new cluster compared with the cost of increasing the size of an existing cluster. A minimum of 30 clusters is recommended. Based on previous surveys, a cluster size between 10 and 40 patients ensures that clusters are neither too small nor too large.

Based on a list of the numbers of newly registered patients in all health centres over the course of the previous year, a cumulative case count is compiled. If the inclusion criteria for enrolment in the survey is based on smear positivity, this list should include only sputum smear positive new patients, excluding those that are smear negative. Very small centres with only a low number of notified cases will not come close to reaching the target sample size, and a different approach may be needed (see box on page 29).

Assuming that the minimum recommended number of 30 clusters is selected, the total number of patients registered per year in all of the centres is divided by 30 to obtain the sampling interval. A random number between 1 and the sampling interval is picked, *x*. Using the cumulative case count, the diagnostic centre corresponding to the *xth* patient is selected as the first cluster. The sampling interval is sequentially added to the random number to determine the remaining clusters from the list. If centres are large, with two to three times more patients per year than the average, the sampling interval may well be smaller than the size of the total patient intake for these centres; when this happens, more than one cluster will be selected from such centres. For example, if one centre is chosen twice, thereby contributing two clusters, the sample size will be double that of a centre which serves as only one cluster.

To determine the number of patients per cluster, the required total sample size is divided by the number of clusters. In all selected health centres, consecutive patients are enrolled in the survey until the number of new cases required is reached.

For each new survey, clusters must be reselected using the most recent TB notification data available. Clusters from a previous survey cannot be assumed to be representative of the current situation.

Example. A sample size of 360 new TB patients has been calculated after taking into account the effect of cluster sampling: 30 clusters of 360/30 = 12 new patients to be selected. The following steps must be taken:

a. Establish a list of the health centres with their annual number of patients (see table below).

b. Calculate the cumulative number of new patients and record them in an additional column. Cumulative number for second centre will be (number in first centre) + (number in second centre). Cumulative number for third centre will be (cumulative number for second centre) + (number in third centre), and so on. The total number of patients diagnosed in the country is 6322.

c. Determine the sampling interval: 6322/30 = **211**.

d. Select a number between 0 and 211 at random (using a table of random numbers or the last digits of a currency note, for example). In this case, the number selected is **120**.

e. The first cluster is selected using **120**: it will be in the first centre because 120 falls between 0 and 246 (number of patients in the first centre).

f. Selection of the subsequent clusters is done by adding the sampling interval of 211 to this first number of 120. The next number (120 + 211) = **331** falls between 246 and 1823 (cumulative number of patients for second centre); the second cluster is therefore selected in the second centre. The third number (331 + 211) = **542** also falls between 246 and 1823; the third cluster is therefore also selected in the second centre.

Name of diagnostic centre	No. of new patients diagnosed per year	Cumulative No. of new patients	Cluster No.
A	246	246	1
B	1577	1823	2, 3, 4, 5, 6, 7, 8, 9
C	468	2291	10, 11
D	340	2631	12
E	220	2851	13
F	246	3097	14, 15
G	190	3287	16
H	1124	4411	17, 18, 19, 20, 21
I	61	4472	
J	154	4626	22
K	139	4765	23
K	60	4825	
M	14	4839	
N	38	4877	
O	19	4896	
P	41	4937	
Q	120	5057	24
R	455	5512	25, 26
S	51	5563	
T	26	5589	
U	199	5788	27
V	21	5809	
W	32	5841	28
X	69	5910	
Y	6	5916	
Z	145	6061	29
AA	129	6190	
BB	87	6277	30
CC	10	6287	
DD	35	6322	

Note: Reproduced from: ten Dam HG. Surveillance of tuberculosis by means of tuberculin surveys. Geneva, World Health Organization, 1985, (document WHO/TB/85.145).

Cluster sampling: small health centres

If small centres with only a low number of notified new cases (for example, less than ten notified new patients per year) are selected as clusters, the required sample size will not be reached. If small centres are very rare, these centres can be excluded from the sampling frame prior to cluster selection. However, if small centres are common, their removal can introduce selection bias. In this case, neighbouring small centres should be grouped together and considered as one unit in the sampling frame, prior to cluster selection. If a group is selected as a cluster, all health centres in the group must contribute to the target cluster sample size by enrolling consecutive patients. This will require good communication and coordination between the centres belonging to the same cluster.

5.7 Budgeting

The required budget must be carefully calculated in order to avoid any interruption during implementation of the survey. All necessary funds for the entire survey period (planning, implementation, analysis and dissemination) must be available before the survey is started.

National TB control programmes should consider surveys not only as a means of estimating the magnitude of the drug resistance problem, but also as an important tool for monitoring programme efficiency, and as a means of strengthening the capacity of the Central Reference Laboratory to perform DST. Therefore, allocation of funds for surveys should be an integral part of a programme's budget.

The current average cost of nationwide surveys is around $US 250 000 - 300 000, based on an average sample size of approximately 1000 - 1500 patients. However, this will vary according to the laboratory methods used and country-specific logistics, such as transportation distances.

All budgets requiring the services of SRLs should include the costs for SRL technical assistance, costs of retesting isolates and all other laboratory work, and costs of shipments to and from SRLs for quality assessment of specimens/isolates. There may also be important costs associated with the staffing required to process specimens and/or laboratory running costs. The SRL should be asked to provide the specific costs for these items. Average costs are updated regularly in the WHO *Planning and Budgeting tool* (available for download from the WHO Global TB Programme website at http://www.who.int/topics/tuberculosis/en/).

Costs for the overall coordination of the survey should also be included. This may include salaries of staff, monitoring meetings, supervisory visits to health centres, and communication between the peripheral centres and the central laboratory and data management unit. Costs of external technical assistance should also be included, as appropriate.

See *Annex 5* for a template of a survey budget.

5.8 Training

Training should focus on the following essential parts of the survey:

- enrolment of eligible patients in the survey, and obtaining reliable and comparable data on patient history of previous treatment;
- specimen collection and transportation;
- use of data collection forms;
- laboratory techniques;
- communicating results back to the diagnostic centre (and, in turn, to the patient);
- data entry, validation and analysis.

Training activities must be planned carefully and if possible include each health worker who will be directly involved in the survey. The medical officers/nurses in charge of patient intake and interviews should be identified and properly instructed in each diagnostic centre involved in the survey. A meeting can be an efficient way to inform, train and motivate the officers involved.

Training or refresher courses at peripheral laboratories should be considered for registration of samples, preparation and reading of smears, molecular testing methods, decontamination of sputum samples for culture, storage and transport of samples, and recording of results.

5.9 Laboratory preparedness

Staff from the Central Reference Laboratory should make a supervisory visit to peripheral laboratories before the start of the survey to ensure internal quality control procedures are in place. The collection of sputum samples (including sputum quantity and quality), sputum smear examination, and transport of sputum and forms must be carefully supervised.

Undertaking a survey may place considerable pressures upon the peripheral laboratories and the Central Reference Laboratory. Laboratory logistics, facilities, and resources necessary for a survey must be considered in advance, so that the laboratory network is not overwhelmed by the extra workload and routine activities remain unaffected.

At the Central Reference Laboratory, in cooperation with a partner SRL, a quality assurance system of internal quality control and external quality assessment should be established before the survey is started to ensure quality of testing. All appropriate biosafety measures must be in place before implementation of a survey.

The partner SRL can guide and advise the national coordinator during the planning as well as implementation and evaluation of a survey. Before the start of a survey, experienced staff from the SRL should make an initial assessment of the Central Reference Laboratory regarding standard operating procedures, performance and functioning, quality assurance and biosafety. SRLs can also train staff if required.

Proficiency testing in cooperation with an SRL must be completed with good results (i.e. at least 95% agreement for rifampicin and isoniazid) before a survey based on phenotypic DST is implemented. Laboratories with substandard performance in proficiency testing should implement quality improvement measures, and have all DST results rechecked by the partner SRL during the course of a survey. The relationship between the Central Reference Laboratory and the partner SRL should be continuous and responsive to any substandard performance that may be detected during the course of a survey. An SRL may be required to recheck more or fewer samples, depending on the Central Reference Laboratory's ongoing performance.

5.10 Pilot study

Depending on local conditions, it can be helpful to organize a time-limited (e.g. one month) pilot study in several of the chosen sites in order to test the entire process of patient identification and classification, sputum collection, processing and shipment, laboratory testing, documentation and coordination, and the quality of training. The pilot study can serve to identify and solve unexpected problems before the survey is launched in all sites. If no significant problems are encountered during the pilot period, the data collected can be included as part of the survey and contribute to the necessary sample size.

6 Survey logistics

The logistic aspects of the survey depend on the patient inclusion and exclusion criteria and the diagnostic testing algorithm being used.

6.1 Inclusion and exclusion criteria

The inclusion and exclusion criteria are defined according to the population of interest described in the survey objectives. Most commonly, a patient is eligible to be included in the survey if diagnosed and registered as a new sputum smear positive case (see section *2.1 Patient treatment history classifications*) at a health centre selected to participate in the survey, regardless of whether they will receive treatment at that facility. With the incorporation of Xpert MTB/RIF into diagnostic algorithms, the inclusion of smear negative cases may be considered, although this will greatly increase the required sample size (see section *5.1 Setting objectives*). New patients who have started TB treatment more than one week earlier should be excluded from the survey. This is because patients who submit sputum samples after starting treatment, and in whom a positive sputum smear is observed, may be more likely to be harbouring drug resistant strains, thus introducing bias. Additionally, a significant proportion of cultures would fail to grow in patients on treatment.

Often, previously treated sputum smear positive cases which have been newly registered are also included. Such patients are only eligible if they are beginning a new treatment course. This includes the patient categories of relapse and treatment after loss to follow-up. However, treatment after failure patients should be included only if their first treatment course has failed. Patients whose subsequent treatment courses have failed are excluded.

MDR-TB in children is an indicator of recent transmission of drug-resistant strains from contacts present in their environment (32). Children under 15 years old who meet the admission criteria should therefore be included in surveys, in accordance with local laws stipulating parental consent. The use of Xpert MTB/RIF may reduce the challenges of diagnosis in children (6).

Extrapulmonary TB and sputum smear negative cases are usually excluded from surveys due to difficulties in diagnosis and resource limitations. However, the inclusion of these patients may be more feasible when using Xpert MTB/RIF.

6.2 Patient intake

Each patient who meets the inclusion criteria should be assigned a unique survey identification number that will be used on all patient forms, including the clinical information form, laboratory forms (e.g. sputum shipment and results forms) and the sample container. For example, the survey identification number could be based on a code representing the diagnostic centre where the patient was enrolled, followed by consecutive numbering of each patient enrolled at that centre. Each survey identification number is linked to only one person, and each participant is identified by only one number. Ensuring that basic patient information such as age and sex are recorded on each form can be important for identification, particularly in the event of that the same survey identification number is erroneously attributed to two different patients.

The survey identification number allows data collected on different forms to be linked by the data manager. It also enables the identification of the patient at the diagnostic centre in the event of a drug-resistant strain or when additional information is required. If specific codes are already in use for the identification of administrative areas or health centres, these codes can be included as a component of the unique survey identification number.

All patients meeting the inclusion criteria should be enrolled in the survey and submit sputum samples for use in the survey prior to the commencement of treatment. The number of sputum samples required may vary according to the diagnostic methods being used. As a measure of quality control, the number of eligible patients (computed from TB Registers) and the number of patients actually enrolled in each survey site should be compared regularly during the enrolment period. This can help to identify reasons why some patients may not have been enrolled in the survey, and reduce the likelihood that eligible patients are missed.

6.2.1 Clinical information form

The main objective of the clinical information form is to correctly identify any past TB treatment of the patient.

The clinical information form (see *Annex 6*) consists of four categories of information:

- identification of the patient;
- patient history, including age, sex, and possibly HIV status or other information;
- documented data on history of previous treatment for TB;
- a final decision on history of previous treatment for TB (see box on page 34).

This form collects a minimal set of information necessary for programme monitoring, and for the possible analysis of risk factors drug resistance. This information must be collected in every survey.

Countries may decide to collect additional information such as country or region of origin, or socioeconomic status (see section *2.2 Age groups, sex, HIV status and other patient biographical and clinical factors*). In principle, only information that can assist analysis and is obtainable, reliable, and useful from a programmatic perspective should be added. The denominator must be known for each variable collected. For example, if TB patients are to be stratified by country of origin, all patients must be asked to provide such information. If a decision is made to obtain the HIV status of all patients, a detailed protocol should be prepared in line with existing national guidelines in order to ensure confidentiality and counselling for all patients.

A copy of the completed clinical information form should be sent to the coordination team, with the original kept at the diagnostic centre.

Quality control of classification of history of previous treatment for TB

Classification of patients as being either new or previously treated is critical and has important implications for data analysis and interpretation. Special efforts are therefore needed during the survey to ensure the reliability of clinical data.

Several questions should be included on the clinical information forms to help elicit an accurate treatment history of patients. The collected forms should be checked carefully for deficiencies, and the reliability of the information recorded should be assessed regularly. Re-interviewing patients is one important method to verify treatment history. For example, a representative sample of patients (as a general rule, 10% should suffice) can be re-interviewed by someone assigned by the coordination team to evaluate the accuracy of treatment histories recorded. Furthermore, all patients with rifampicin resistance should be re-interviewed, particularly new patients. Verification of treatment history is particularly essential in places where the common practice is to provide incentives to staff for the detection of new patients only, or where there is any underlying circumstance that would encourage patients to falsify a treatment history. Measures should be taken to provide a comfortable environment for the interview and to eliminate any barriers that may prevent a patient from disclosing a truthful treatment history. It is possible that when patients begin feeling better after starting treatment, they may be more willing to provide details of their treatment history.

It is important to note that the proportion of cases classified as being previously treated is often found to be higher in surveys than in routine programmatic recording. This is because the comprehensive patient treatment history recorded in surveys may reduce patient misclassification.

6.3 Sputum collection, processing and transport

The correct collection, processing and rapid transportation of samples to participating laboratories is essential to ensure that results are accurate and reliable. The number of samples to be sent depends on the testing methods being used.

Health care workers must be well trained in providing patients with clear instructions in order to collect a good sputum sample. Aerosols containing *M. tuberculosis* may be formed when the patient coughs to produce a sputum specimen. Patients should therefore produce sputum (not saliva) either outside in the open air or in special sputum collection rooms with appropriate ventilation and/or other methods to kill bacilli such as ultraviolet irradiation, always away from other people. Sputum collection should not be performed in confined spaces such as a room in the laboratory, or in the toilets.

Sputum should always be treated with care. Suitable containers must be rigid to avoid being crushed in transit, and must have a watertight, wide-mouthed, screw top to prevent leakage and contamination. Containers should be packed in material that will absorb any leakage caused by accidents.

Before transport, sputum samples should be kept in a cool place, preferably refrigerated at +4°C. Cool boxes should be used to transport samples from the health centre to the laboratory. Should an unreliable cold chain and significant delays in transportation (greater than 3-4 days) be anticipated, an amount of 1% cetylpyridinium chloride (CPC) roughly equal to the volume of the sputum should be added. Samples treated with CPC solution should never be refrigerated because of the likelihood of crystallization and inactivation at low temperature. CPC-mixed sputum can only be cultured on egg-based media, not liquid or agar media. Xpert MTB/RIF and line probe assay testing on CPC-mixed sputum produces reliable results and, if transported and stored at ambient temperature, these samples can be tested for at least one month after the sputum was produced.

The patient's unique survey identification number should be written on each container (not on the lid) and the sputum collection and laboratory request forms. The standard laboratory forms which accompany sputum specimens during shipment and to request laboratory analysis should be modified as necessary and used during the survey. To ensure traceability, the health centre should keep a register of the following: survey identification number, date(s) of sample collection, date of sample shipment, date of receipt of laboratory results, date of transmitting results to patient.

6.4 Laboratory methods

The diagnostic testing algorithm defined during the survey planning phase should be followed throughout the survey.

6.4.1 Culture-based surveys

Decontamination

Decontamination of sputum specimens has two objectives:

- destruction of bacteria other than mycobacteria;
- homogenization.

Decontamination aims to kill as much of the contaminating flora as possible while harming as few mycobacteria as possible. Different techniques are available and may impact on the results.

Worldwide, the preferred technique for achieving decontamination with a final maximum sodium hydroxide concentration of up to 2% (using an equal amount of 4% NaOH stock solution and sample) is that of Petroff. However, if CPC was mixed with the sputum for transport, NaOH decontamination should only be used if the delay from sample collection to processing exceeds one week, and the contact time should be reduced to 5-10 minutes. If liquid culture is to be performed, the decontamination method of choice is NaLC-NaOH.

Culture and identification

Before being processed at the reference laboratory, sputum specimens should be kept in a refrigerator at +4 °C, and bacteriological examination should be carried out as soon as possible. However, CPC-mixed specimens should not be stored in a refrigerator. Before inoculation of media, CPC-mixed specimens should be centrifuged without cooling and the liquid decanted as much as possible. Solid and liquid culture and identification should be performed according to WHO recommendations (20,29).

All positive cultures should be kept until retesting at the SRL has been completed or the strain has been excluded from further testing. These should be stored in a deep-freezer at a temperature of at least -20°C. Additionally, all sputum sediments and microscopy slides should be stored until final culture and DST results are available.

Biosafety measures

All procedures involving the handling of specimens for culture and DST should be carried out in a high-risk TB laboratory, as defined in the WHO's *Tuberculosis Laboratory Biosafety Manual* (29). Particular care needs to be taken when bottles are being opened, closed or shaken and when materials are being centrifuged, all of which may lead to the production of infectious aerosols. The transportation of TB cultures presents special risks in the event of accidents or container breakage. It is therefore extremely important that the exchange of strains between the Central Reference Laboratory and the SRL is carried out according to the regulations outlined in *Annex 7.*

Susceptibility testing, including retesting

Susceptibility testing should be performed on only one isolate for each patient. Participating laboratories should use the WHO-recommended method with which they are most familiar. This eliminates variability which may arise from changing testing procedures.

Prior to a survey, laboratories should have demonstrated proficiency by participating in at least one round of DST proficiency testing with an SRL.

In general, if the proportion of MDR-TB is low, all MDR-TB strains and 1-2 susceptible strains enrolled immediately after each MDR-TB patient could be re-tested. If there are many MDR-TB cases, it may be more feasible to re-test 10-30% of MDR-TB strains and 1-2 consecutive susceptible strains.

If DST for second-line drugs is not available in the country or if standards of laboratory performance are unknown, DST for second-line drugs can be conducted outside of the country at an SRL. However, sufficient resources must be obtained to cover the SRL's costs, and the budget for all such work should be agreed before the start of the survey.

The Central Reference Laboratory should use its standard laboratory results forms to record the results of culture and susceptibility testing, with any modifications needed for the survey. Results should be sent to the survey coordination team and to the diagnostic centre.

6.4.2 Molecular-based surveys

Any WHO-endorsed laboratory test can be incorporated into a survey. Unlike culture-based methods, some molecular tests can be easily performed at the district or sub-district level, such as Xpert MTB/RIF. The positioning of Xpert machines at the peripheral level significantly reduces logistic challenges for sample transport and processing. Molecular tests can be used alone or in combination with culture-based methods as initial screening tools, as described in section *5.2 Defining the diagnostic testing algorithm*.

6.5 Monitoring and evaluation

A schedule for conducting monitoring visits to all participating health facilities should be developed. A checklist can be helpful for assessing the adherence of the staff to the survey protocol, and could include the following questions:

- Are the patient inclusion and exclusion criteria being followed?
- In comparing the health centre's TB register with the list of patients enrolled in the survey, have all eligible patients been enrolled?
- Is the process for sputum collection, packaging and transport carried out safely and according to the survey protocol?
- How long is the time delay between sample collection and arrival at the laboratory for further testing (average and range in days)?
- Is there a register for ensuring traceability of the samples sent and the laboratory results received, including feedback to the patient?
- Have clinical information forms been completed for all enrolled patients?

At regular intervals (e.g. monthly) during the intake period, all data produced by the health centres and laboratories should be tabulated and reviewed. The coordination team's epidemiologist should make regular reports based on these tables to the survey coordination team. These reports should include the following information:

- Enrolment of patients as a proportion of the target total sample size (and, for cluster-based surveys, enrolment for each cluster in relation to the target cluster sample size);
- Enrolment of patients as a proportion of the total eligible patient population (requires a comparison of the patients recorded in each cluster's routine TB register with a list of patients enrolled in the survey);
- Quality of patient biographical and clinical information collected, including missing data, particularly in relation to treatment history;
- Proportion of samples tested which are negative for *M. tuberculosis* or contaminated;
- Proportion of samples collected for which results are not available; and
- Transport or logistic problems reported by health centres or laboratories.

If significant problems are identified, the national coordinator and the managers of the national TB control programme and Central Reference Laboratory should develop a detailed plan for addressing these. Missing information should be requested from the respective centres as soon as possible after specimen receipt. A supervision team must visit health centres with low patient enrolment, incomplete data collection forms, or delays in sample shipment.

Halfway through the survey, the national survey coordinator and the managers of the national TB control programme and the Central Reference Laboratory should hold a midterm review meeting to discuss the quality of data collection, laboratory procedures, quality control results, and preliminary survey results, including interpretation. Additionally, an external monitoring review should be conducted by experts who are not members of the survey coordination team.

7 Survey data management and analysis

7.1 Data management

Data management is aimed at producing high-quality data on individual characteristics and aggregated indicators, such as the proportion of cases that have MDR-TB. Managing survey data appropriately ensures that the data are complete, reliable, and processed correctly, and that data integrity is preserved. Data management includes all processes and procedures for collecting, handling, cleaning, validating, analyzing, and storing/archiving data throughout the study.

The survey data management systems should address:

- data acquisition;
- confidentiality of data;
- data management training for investigators and staff;
- completion of clinical information forms and other survey-related documents, and procedures for correcting errors in these documents;
- coding/terminology for patient characteristics and medical history (data dictionaries);
- database design and testing;
- data entry and verification (e.g. random checks for errors);
- database validation;
- database closure;
- secure, efficient and accessible data storage; and
- data quality assessment (i.e. reliability of data) and quality assurance.

A database manager should be appointed to take charge of the process, including the development of a centrally-managed database. If the necessary expertise for database design is not available in the national TB control programme, advice should be sought from external partners, such as universities, research institutes, non-governmental organizations or WHO. A plan documenting appropriate data management systems should be developed. The survey coordination team must take responsibility for implementing such systems to ensure that the integrity of survey data is preserved. The data management plan describes the procedures and processes for creating accurate, complete, verifiable data with source documents (primary data) which match the data protocols in the survey, as well as for making these data available for analysis. The plan should include the following: 1)

monitoring the survey; 2) transferring, sorting, entering, validating, and cleaning the data; and finally, 3) making the data available for analysis.

All patients enrolled in the survey should be entered into the database, regardless of whether their laboratory results are available. This includes patients whose samples were lost or contaminated. The data held in the database must be sufficiently comprehensive to allow the analyses specified in the survey protocol to be performed, such as reporting the proportion of patients without DST results and performing multiple imputation of missing data (see section *7.2.1 Imputation of missing values*). All data recorded on forms and in the database should use the same survey identification number to uniquely identify each patient and allow the linkage of different forms. Barcode labels and handheld scanners are recommended, as they reduce transcription errors. Labels should be prepared in advance and attached to the necessary forms and tubes for each patient. Where systems are already in place for the automatic electronic capture of testing results, e.g. from GeneXpert instruments, results can be directly taken from these databases using the unique survey identification number.

A relational database will ensure referential integrity. In such a database, data from the different data collection forms (e.g. patient clinical information form, laboratory results form) can be stored in separate tables, while ensuring that the data are consistently linked between tables using the unique survey identification number of the patient. Automatic validation checks should be built into the database to immediately identify errors during data entry, e.g. placing a restriction on the values which are allowed to be entered into a given field. Additional routine checks that can be regularly run should also be included, such as identifying outlier values requiring further investigation and verification. Microsoft Excel is not a suitable software for entering, storing or managing survey data. For more information about data management, see the 2011 WHO publication, *Tuberculosis prevalence surveys: a handbook* (19).

7.2 Data analysis

The first step in data analysis is the development of a flowchart showing the outcomes of all of the eligible patients enrolled in the study (see example in *Annex 3*). This allows the identification of the steps where eligible patients were lost from the survey, which risks introducing bias. The flowchart should be disaggregated by patient treatment history and should contain boxes for the following: number of patients enrolled; number of patients for whom samples were not available for further testing (e.g. lost samples); number of patients for whom further testing was performed but results were not available (e.g. contaminated samples); and the numbers of patients with final DST results.

The following analyses of drug resistance data should be conducted:

- *Analysis of patient intake.* It is important to make a table comparing the number of patients included from each site with the expected number based on the sampling method, disaggregated by treatment history. Tabulations of data by site allow an assessment of the extent of missing data.

- *Analysis of missing value patterns*. DST results may be missing for a variety of reasons, including lost samples, contaminated samples, negative results for *M. tuberculosis* by molecular methods or culture, or insufficient culture growth for susceptibility testing. The percentage of eligible smear positive patients for whom data on drug resistance to rifampicin and/or isoniazid is missing should be summarized by age group, sex, treatment history, and site. Typically, when a drug susceptibility testing result for one first-line drug is missing in a culture-based survey, results for all other first-line drugs are also missing because cultures failed to grow.
- *Analysis of drug resistance patterns*. It is essential to have a table describing the proportions of new or previously treated patients with resistance to individual drugs and to different combinations of drugs (the most important being the combination of resistance to rifampicin and isoniazid). Tables of aggregated numbers of cases are shown in *Annex 4* among new and previously treated patients. However, an estimate of the proportion of cases with drug resistance that is based only on those patients with a test result (e.g. rifampicin susceptible or rifampicin resistant; non-MDR-TB or MDR-TB) may be biased, as it assumes that patients with results are a random subset of all patients enrolled in the survey, which may not be the case. Therefore, statistical methods such as multiple imputation may be needed to reduce the risk of bias (see section *7.1 Imputation of missing values*).
- *Analysis of determinants of resistance*. Depending on biographical and clinical data collected, further comparisons based on sex, age groups, HIV status, country of origin, etc. should be evaluated (see tables in *Annex 4*).

Specialized statistical software is needed to analyse drug resistance data from national surveys with cluster sampling. The reason for this requirement is to account for missing data and sampling design effects on the estimates and their standard errors.

Practical steps for analyzing a sample cluster survey dataset are available for download from the WHO Global TB Programme website at http://www.who.int/tb/publications/2015/drs_guidelines/en/

7.2.1 Imputation of missing values

As mentioned in section *7.2 Data analysis*, multiple imputation of missing data may reduce bias compared to an analysis based on only those patients for whom a DST result is available. However, multiple imputation should never be considered a substitute for initial collection of high quality data.

In surveys, data are likely to be "missing at random" (MAR). This means that the probability that an individual has missing data for the outcome variable is related to individual characteristics such as age or sex; however, within groups of individuals who have the same age, sex, or other characteristics, the probability of data being missing for the outcome variable is not associated with its value (i.e. MDR-TB or not MDR-TB). For MAR data, multiple imputation of missing values should be performed and the results compared with a non-imputed analysis.

If data are "missing not at random", the probability of an individual having missing outcome data is different for individuals who have drug-susceptible TB compared to those that have drug-resistant TB. In this case, multiple imputation should not be performed and a sensitivity analysis is required, as the survey results may be biased.

Multiple imputation involves using the patterns within the available data to assign outcomes for patients missing data. Analysts can then apply the statistical method they would have used if there were no missing values (e.g. logistic regression). In general, we can be confident of obtaining an unbiased estimate of the proportion of TB cases with drug resistance if (i) the percentage of patients with missing data is low, (ii) the data are MAR, (iii) appropriate imputation models are used, and (iv) the data from imputed datasets are combined in an appropriate way. 95% confidence intervals should be calculated to account for the uncertainty introduced by the imputation.

7.2.2 Sampling design effects on standard errors

Apart from addressing potential biases created by missing data, the second major feature of a cluster sample survey to be addressed in the analysis is the lack of statistical independence of observations from the same cluster. This arises because individuals within clusters are likely to be more similar to each other than to individuals in other clusters. This intra-cluster correlation (equivalent to inter-cluster variation) must be accounted for when computing standard errors (and confidence intervals) for the estimated proportion of MDR-TB. Incorrect interpretations and conclusions could result from subgroup comparisons (e.g. between HIV-infected and HIV non-infected) that do not allow for intra-cluster correlation when conducting statistical tests or calculating confidence intervals.

To account for intra-cluster correlation, robust standard errors should be computed. This can be done through an individual-level analysis of the survey data using logistic regression. With multiple imputation, the confidence intervals can account for both the uncertainty introduced by the imputation as well as the lack of statistical independence of individuals within clusters.

7.2.3 Sampling weights

For settings in which PPS sampling was selected as the sampling strategy but some diagnostic units were not able to collect the required number of cases during the intake period, existing data from these units should be compared with completed units. If the number of patients enrolled in each cluster varies widely (which should not happen if the study protocol is adhered to), then the actual sampling method can no longer be considered to be PPS. If there is concern that number of patients enrolled in a cluster might be associated with the proportion with rifampicin resistance or MDR-TB, it will be important to make a correction in the analysis for this potential bias.

This can be addressed by assigning a weight to each cluster that is proportional to the number of enrolled individuals in each cluster. For instance, if the planned cluster size was 27 new patients and there is a large variation in the actual cluster size due to difficulties in enrolling patients in some clusters, the weight assigned to each new patient within a given cluster will be equal to 27 divided by the actual number of new patients enrolled. Such calculations should be done for all new cases, regardless of whether drug susceptibility testing was successful and results are available. The analysis should be carried out with and without weights, and differences in model coefficients should be examined carefully. Unless a separate sample size was calculated and achieved for previously treated patients, sample weights should not be applied to previously treated cases. Weights should not be used when assessing potential risk factors for drug resistance such as HIV or age group using multivariate logistic regression models.

7.2.4 Other considerations for data analysis

The ratio of previously treated patients to new patients in a survey sample may not reflect the ratio of previously treated patients to new patients notified to the TB programme at the national level. Reasons for this difference may include: (1) misclassification of treatment history under routine practice compared with survey practice; (2) different enrolment periods for new and previously treated cases; or (3) random error due to sampling.

7.3 Interpretation of results

An established national TB control programme that adopts standardized chemotherapy will see a subsequent reduction in drug-resistant TB among new cases. However, this may take a long time, as patients infected with resistant strains may become ill only after many years. High proportions of resistance among new cases may indicate that some previously treated patients were misclassified as new cases. Identification and correction of this error may be possible by re-interviewing all new subjects that are determined to have drug resistance. Cross-contamination in laboratory processes should also be investigated as a possible cause.

Younger people are more likely than older people to have been recently infected. The proportions of drug resistance in new cases among younger age groups therefore provide more reliable information on recent patterns of transmission of drug-resistant TB and the quality of a national TB control programme. However, the numbers of patients with drug-resistant-TB in the different age categories may be too small to allow the detection of significant differences.

Previously treated cases are a heterogeneous group. Various factors promote acquired resistance among previously treated cases. These include unsupervised treatment; inadequate drug regimens; availability of anti-tuberculosis drugs without physician prescription or oversight; poor quality of the drugs supplied; and weaknesses in methods for declaring patients successfully cured. A subgroup analysis can result in more targeted conclusions and recommendations, although this may not be possible in surveys due to only small numbers of patients.

Periodic survey results and trends over time should always be interpreted within the context of the overall programme. This includes consideration of other indicators such as treatment outcomes, changes in overall incidence of TB disease, prevalence of HIV, changes in standardized or empirical drug regimens, size of the private sector, major socioeconomic events, drug shortages, and so on. This allows for more robust interpretation of drug resistance surveillance data in a given setting. Surveillance data on resistance to fluoroquinolones and pyrazinamide among new and previously treated TB patients can be used to guide the design of new treatment regimens.

Part 3

Sentinel surveillance for monitoring trends over time

8 Designing a sentinel system

8.1 Setting objectives

A sentinel system can provide valuable information about drug resistance trends over time without the much greater time and resource requirements of a national drug resistance survey. **Such approaches are recommended for countries which already have high quality data available from a recent nationally representative survey (data less than five years old) but do not yet have a continuous surveillance system capable of detecting all drug-resistant cases**. However, the data generated by a sentinel surveillance system do have an important limitation - the results cannot be generalized to the rest of the country, unlike national drug resistance surveys.

A suitable objective for a sentinel surveillance system could be: To monitor the proportion of new and previously treated sputum smear positive pulmonary patients with rifampicin resistance in selected sites over time.

8.2 Defining the diagnostic testing algorithm

Rapid molecular tests, such as Xpert MTB/RIF, are well-suited to a sentinel surveillance system. Depending on the laboratory capacity available in the country, subsequent testing methods could be used to achieve additional objectives. For example, culture and DST for isoniazid could be performed for samples displaying rifampicin resistance by Xpert MTB/RIF, in order to determine the proportion of cases with MDR-TB.

8.3 Sampling of cases

8.3.1 Defining the sampling frame

The selection of sentinel sites depends on the objectives of the system. Some countries may initially only have capacity to monitor trends in areas known to have a high burden of drug-resistant TB. However, in order to be able to monitor trends in both low and high burden settings, sentinel sites should be drawn from a range of geographical and socioeconomic areas. As sentinel sites are selected purposely rather than randomly, the results cannot be extrapolated to the rest of the country. The centres selected as sentinel sites should have the capacity for testing new patients by rapid molecular methods. These should be centres with a moderate to high TB caseload.

Ideally, the sentinel system should function continuously with no limited period of enrolment. However, if countries do not have the resources to establish

a continuous sentinel system, all eligible TB patients in the sentinel sites could be tested according to the predefined testing algorithm over a short time period (for example, three to four months) until the necessary sample size is reached.

8.3.2 Sample size

If the country does not have the resources to establish a continuous sentinel surveillance system, a sample size for sputum smear positive pulmonary patients much be calculated. This depends on the number of sentinel sites, the expected proportion of patients with rifampicin resistance at the time of the previous survey, the anticipated percentage change in this proportion, and the acceptable level of precision. The formula below should be used, which includes a finite population correction:

$$n = \frac{N * z^2 * (1 - g)}{(N - 1) * d^2 g + z^2 * (1 - g)}$$

where:

N = total number of new sputum smear positive pulmonary patients registered in the selected sentinel sites during one year;

z = z-value (from the standard normal distribution) that corresponds to the desired confidence level (narrowing the confidence interval from 95% to 90% will result in some reductions in sample size; if confidence interval =90%, z= 1.65);

d = absolute precision (as a decimal, e.g. 0.01 or 0.02 meaning to err within 1 or 2% of the true proportion);

g = previous estimate of proportion of new cases with rifampicin resistance * (1 + anticipated change of previous estimate). The anticipated change can be considered as the change that the sentinel system should be able to detect. This change is expressed as a decimal, with a negative sign if a decrease is anticipated or a positive sign if an increase is anticipated. For example, a 40% decrease from the previous estimate would be expressed as an anticipated change of -0.4; thus *g* = earlier estimate * (1 - 0.4) = previous estimate * 0.6

Some countries may have capacity for culture and DST at a peripheral level. This testing approach could be used in a sentinel system, provided that all new TB patients are tested rather than only a prioritized group. However, in most countries, testing will be performed onsite with Xpert MTB/RIF. Contaminated or misplaced samples will be less likely than in a culture-based drug resistance survey. There may still be losses due to sputum smear positive patients that fail testing by Xpert MTB/RIF. For this reason, the calculated sample size above should be increased by 5-10%.

An Excel spreadsheet is available for download from the WHO Global TB Programme website at http://www.who.int/tb/publications/2015/drs_guidelines/en/ to assist with sample size calculation. This provides a practical tool for exploring the impact of different parameters on the required sample size. It can also be used throughout the enrolment period to assess whether the number of patients recruited is sufficient.

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Annex 1 -
Approaches for countries without capacity for continuous surveillance

DST – drug susceptibility testing
NTM – Nontuberculous mycobacteria
LPA – line probe assay
Rif - rifampicin
MTB – *Mycobacterium tuberculosis*

	Survey: conventional approach	Survey: molecular screening	Sentinel system
Level	Nationally representative	Nationally representative	Sub-national; not nationally representative
Design	Cluster-based, using probability-proportional-to-size-approach to select health centres	Cluster-based, using probability-proportional-to-size-approach to select health centres	Selection of health centres according to convenience
Laboratory methods	Culture only	Molecular combined with culture	Molecular only
Logistic demands	High	Medium	Low
Frequency	Every 5 years	Every 5 years	Continuous or every year
Requirements	Laboratory capacity to process a large number of samples for culture and first-line phenotypic drug susceptibility testing (DST) and, where indicated, second-line DST	Laboratory capacity to perform culture and first-line phenotypic or molecular DST and, where indicated, second-line DST; GeneXpert instruments	GeneXpert instruments
Resistance patterns	All first-line resistance patterns including MDR-TB, as well as pre-extremely drug-resistant TB (pre-XDR-TB) and XDR-TB	Rifampicin resistance, multidrug-resistant TB (MDR-TB), pre-XDR-TB, XDR-TB	Rifampicin resistance only

Annex 2 -
Drug resistance survey protocol checklist

Introduction and background

- country profile, including geography, population, TB and HIV epidemiological situation in the country
- information about the national TB control programme, including strategy, operational design, drug regimens used
- Central Reference Laboratory and the laboratory network, detailing systems for internal quality control and external quality assessment and indicating the relationship with a Supranational Reference Laboratory (SRL)
- all relevant health care providers not formally linked to the national TB control programme (public, voluntary, private and corporate) and quality-assured non-programme laboratories willing to participate in surveillance activities
- data from previous drug resistance surveys
- data from the previous cohort analysis (including case-finding and treatment outcome data)
- management of patients diagnosed with multidrug-resistant TB (MDR-TB), or plans for development of a treatment programme
- use of second-line drugs

Objectives

- realistic and relevant objectives, in light of the chosen survey design and the country setting

Study design

- sampling frame and strategy (e.g. 100% sampling of health centres, cluster sampling)
- calculation of the sample size
- expected duration of the survey

Patient enrolment and logistics

- survey pilot period
- patient intake period
- inclusion and exclusion criteria
- patient interview process
- sputum collection process and how specimens will be handled, transported and stored

- recording forms:
  - clinical information form, including measures to ensure correct classification of patients by treatment history, e.g. review of records, re-interviewing patients
  - sputum shipment form
  - laboratory results form
  - Health facility register for traceability of survey samples sent and laboratory results received
  - HIV testing and counselling

Laboratory methods

- chosen diagnostic algorithm (a flow-chart is recommended)
- use of microscopy, media preparation, culture and identification, and molecular tests, as appropriate
- established system of quality assurance
- biosafety precautions
- preservation and storage of samples
- feedback of results to health centres

Training

- timing and location of training
- topics to be covered
- participants and their roles and responsibilities

Survey monitoring

- members of the coordination team, and their roles and responsibilities
- frequency of supervisory visits to study sites
- monitoring of data quality
- re-interviewing of a subset of patients to confirm treatment history

Data management and analysis

- data collection forms to be used
- flow of completed forms from health centres and laboratories to data management team
- database design and data entry
- data validation and quality assurance
- statistical analyses to be performed
- dissemination of results to key stakeholders and the scientific community

Human resources

- principal investigator, members of the coordination team, data manager, epidemiologist/statistician
- staff in health centres
- laboratory technicians

Budget (see *Annex 5*)

- consumables and laboratory supplies
- human resources for the entire period of survey planning, implementation, analysis and dissemination of results
- sample transport
- retesting at SRL
- technical assistance

Timeline

- month by month schedule (a Gantt chart is recommended – see page 55) for the planning and implementation of the survey, and analysis and dissemination of results

Ethical considerations

- informed consent
- measures to ensure that patients diagnosed with drug-resistant strains during the course of a survey receive the highest possible level of care
- review of protocol by relevant ethical committees

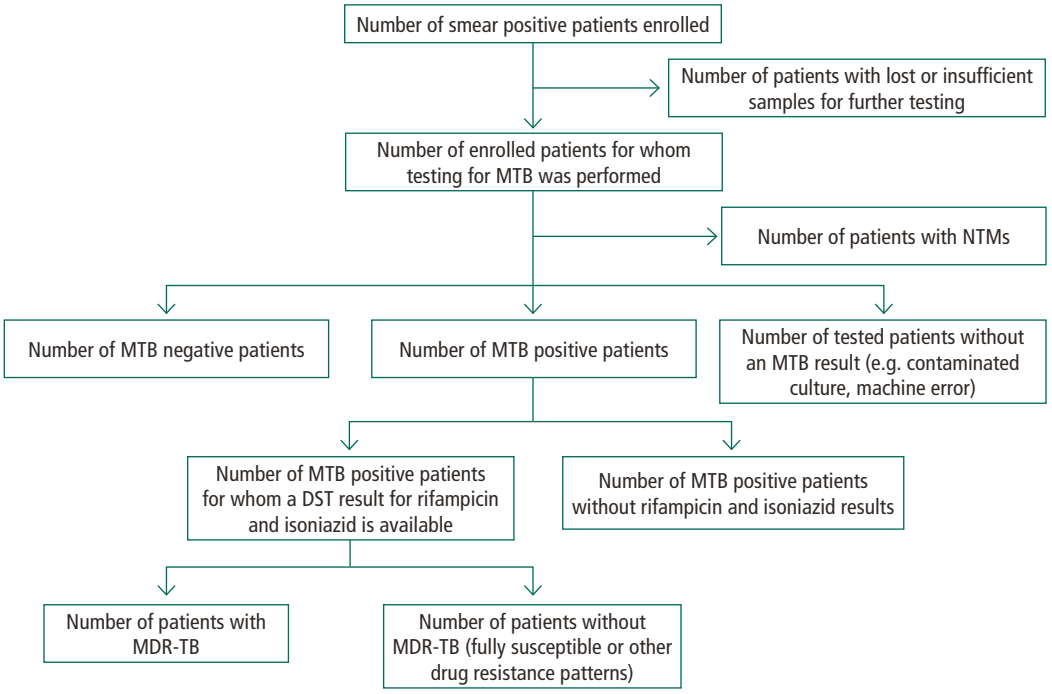
For technical assistance in developing a survey protocol, contact the Global Project secretariat at TB_DRS@who.int

An example of a timeline:

	MONTH*																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Formation of national coordination team																								
Protocol development																								
Ethics approval																								
Procurement and distribution of supplies																								
Database development and testing																								
Training of field staff																								
Pilot in several sites																								
Launch of full survey in all sites																								
Ongoing patient recruitment in all sites																								
Laboratory activities																								
Data management																								
Mid-term external monitoring mission																								
Re-testing of isolates by SRL for external quality assurance																								
Analysis and dissemination of results																								

*The numbers should be replaced with the name of the month and the year, e.g. April 2015.

Annex 3 -
Flowchart of enrolled patients



The flowchart should be modified to match the inclusion criteria and laboratory testing methods used. The numbers of patients in each box should be disaggregated by treatment history (new, previously treated, unknown treatment history).

DST – drug susceptibility testing

MDR-TB – multidrug-resistant tuberculosis

MTB – *Mycobacterium tuberculosis*

NTM – Nontuberculous mycobacteria

Annex 4 -
Results summary tables

PROPORTIONS OF CASES WITH DRUG RESISTANCE

	New patients		Previously treated patients	
	proportion (%)	95% confidence interval*	proportion (%)	95% confidence interval*
rifampicin resistance				
isoniazid resistance				
Multidrug-resistant TB (MDR-TB)+				

* As described in section *7.2 Data analysis*, the calculated 95% confidence intervals should account for a clustered design, if relevant.

+ The estimate of the proportion of TB cases with MDR-TB should be calculated based on all enrolled patients, not restricted to only those patients with drug susceptibility testing results. Statistical methods such as multiple imputation of missing values should be performed as appropriate.

NUMBERS OF PATIENTS WITH DRUG RESISTANCE BY KEY PATIENT CHARACTERISTICS

Treatment history

	New	Previously treated	Unknown treatment history	Total
MDR-TB				
Not MDR-TB				
Total				

HIV status

	HIV positive	HIV negative	Unknown HIV status	Total
MDR-TB				
Not MDR-TB				
Total				

Sex

	Male	Female	Unknown	Total
MDR-TB				
Not MDR-TB				
Total				

Age (years)

	0-15	15-24	25-44	45-54	55-64	≥65	Total
MDR-TB							
Not MDR-TB							
Total							

Secondline drug susceptibility testing among MDR-TB patients, by treatment history

	New	Previously treated	Unknown treatment history	Total
(i) Total number of MDR-TB patients with DST results for any fluoroquinolone (FQ) and any second-line injectable agent (2LI)				
(ii) Among MDR-TB patients reported in (i), number of patients susceptible to both FQ and 2LI				
(iii) Among MDR-TB patients reported in (i), number of patients with any resistance to FQ				
(iv) Among MDR-TB patients reported in (i), number of patients with any resistance to 2LI				
(v) Among MDR-TB patients reported in (i), number of patients with any resistance to both FQ and 2LI (XDR-TB)				

Annex 5 - Survey budget template

Item	Type of unit	Cost per unit	Number of units	Total
Human resources				
Principal investigator(s)				
Supervisor of laboratory activities				
Operations coordinator				
Database designer				
Data manager(s)				
Laboratory technician(s)				
Logistics staff (e.g. drivers, secretary)				
Interviewers				
Subtotal				
Coordination meetings (central and peripheral levels)				
Per diem				
Transportation of participants				
Meeting room hire and catering				
Subtotal				
Training courses				
Per diem				
Transportation of participants				
Meeting room hire and catering				
Subtotal				
Monitoring and supervision				
Per diem				
Transportation of supervisors to survey sites				
Subtotal				
Communication				
General (e.g. stationery, printing)				
Computer(s)				
Mobile phone credit				
Subtotal				
Laboratory				
Sputum containers				
Safety cabinet				
Centrifuge				
Reagents, Xpert cartridges, etc.				
Other (e.g. refrigerators)				
Subtotal				

Item	Type of unit	Cost/unit	# units	Total
Collection and domestic transport of specimens				
Transport containers, packaging				
Transport costs				
Subtotal				
Collection and international transport of specimens to SRL				
Transport containers, packaging				
Transport costs				
Subtotal				
SRL technical assistance				
Visits for survey planning and monitoring				
Laboratory proficiency testing costs				
Retesting for external quality assurance of results				
Subtotal				
Epidemiological technical assistance				
Visits for survey planning and monitoring				
Subtotal				
Finalization and dissemination of results				
Data cleaning and analysis				
Report writing and publication				
Dissemination meeting				
Subtotal				
			TOTAL	

Annex 6 -
Example of a clinical information form

Patient's survey identification number: .....

Heath Centre Name: ..... Health Centre Code: .....

Name of interviewer: .....

If already registered, patient's TB register number: .....

A. IDENTIFICATION OF THE PATIENT

1. Name: .....

2. Date of interview: ..... /..... /..... (Day/ Month/ Year)

3. Sex: Male ☐ Female ☐

4. Date of birth: ..... /..... /..... (Day/ Month/ Year) **5.** Age: ..... years

6. Date of sputum collection: sample 1 ..... /..... /..... (Day/ Month/ Year)

sample 2 ..... /..... /..... (Day/ Month/ Year)

Country-specific data (to be decided by the coordinating team), for example:

7. HIV-status: Negative ☐ Positive ☐ Unknown ☐

8. Country of origin: .....

[Additional questions relating to other possible risk factors, e.g. injectable drug use, alcohol abuse, diabetes, smoking, malnutrition, urban/rural]

B. HISTORY GIVEN BY THE PATIENT

9. Previously treated for TB? No ☐ Yes ☐ Unknown ☐

If 'No' to Question 9, go to Question 10[a]. If 'Yes' to Question 9, go to Question 18.

10. For how long have you been sick? .....

11. Did you have the same symptoms prior to this episode?

No ☐ Yes ☐ Unknown ☐

12. Did you have other symptoms of lung disease prior to this episode

(haemoptysis, chest pain, cough)? No ☐ Yes ☐ Unknown ☐

[a] Some patients may not immediately recall past treatment for TB or may not be aware that previous treatment was for TB. Questions 10-16 can be used by the investigator to assist the patient in recalling past treatment. Positive responses should prompt the investigator to follow up on questions to determine whether past treatment could have been for TB. For more information, see section *6.2.1 Clinical information form*. Only the final decision of treatment history (Questions 20-21) needs to be entered into the electronic survey database.

13. Did you have sputum examinations prior to this episode?

No ☐ Yes ☐ Unknown ☐

14. Did you ever take tuberculosis drugs for more than one month?

No ☐ Yes ☐ Unknown ☐

15. If yes, what was the name? .....

16. Did you ever have injections for more than one month?

No ☐ Yes ☐ Unknown ☐

17. Did the patient remember previous treatment for TB after these questions?

No ☐ Yes ☐ Unknown ☐

C. MEDICAL RECORDS

18. After extensive checking through the medical files and other documents available in the health centre, have you discovered that the patient has been registered for tuberculosis treatment before?

No ☐ Yes ☐ Unknown ☐

19. Previous TB registration number .....

D. FINAL DECISION

20. Patient has been previously treated for TB for more than a month:

No ☐

Yes ☐ (answer to Question 9, 17 and/or 18. was 'Yes')

Unknown ☐

21. If 'Yes' to Question 20, what was the outcome of previous treatment ?

Cured/treatment completed ☐

Failed new patient regimen of first-line drugs only ☐

Failed retreatment regimen of first-line drugs only ☐

Failed regimen which included second-line drugs ☐

Lost to follow-up ☐

Other ☐

Unknown ☐

Annex 7 -
Safe shipment of infectious material

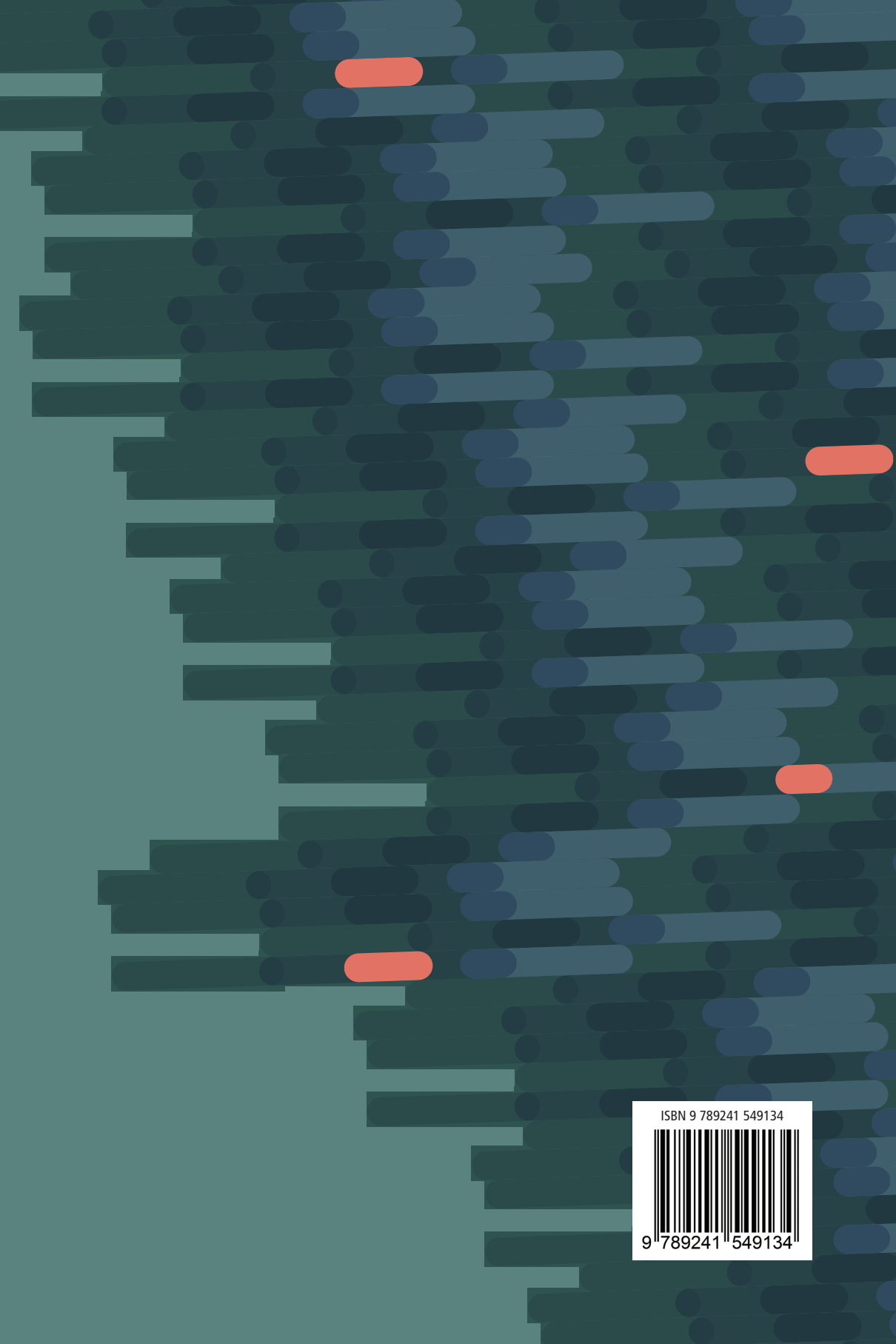
For external quality assessment of susceptibility testing, cultures have to be exchanged between a Central Reference Laboratory and a Supranational Reference Laboratory. Cultures of *M. tuberculosis* are enriched infectious material containing great numbers of viable organisms that can cause disease in humans. The hazard is compounded when cultures of resistant strains are transported.

International regulations on the transport of infectious substances must be followed for their safe and expeditious shipment. The shipment of cultures of *M. tuberculosis* requires shippers to have undergone mandatory, appropriate training (Infectious substance affecting humans, UN2814, Category A).

Cultures of mycobacteria should be shipped on solid medium in screw-cap tubes as primary watertight containers. Petri-dish cultures and cultures in liquid medium must not be shipped. Liquid media often amplify unseen low-grade contamination en route, causing great difficulty at the reference laboratory. If the anticipated transport time is short (i.e. less than one week), no medium or liquid is required for the loopful of bacteria being transported. Shipping in a few drops of sterile water and/or 0.5% cetylpyridinium chloride (CPC) can suffice.

Compliance with the shipment requirements is the responsibility of the shipper, who must be familiar with the regulations. Failure to comply may result in fines and other penalties. Hand carriage of infectious substances is strictly prohibited by international air carriers, as is the use of diplomatic pouches.

For more information, see The Global Laboratory Initiative's *Mycobacteriology Laboratory Manual* (2014) (20) and WHO's *Tuberculosis Laboratory Biosafety Manual* (2012) (29).



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