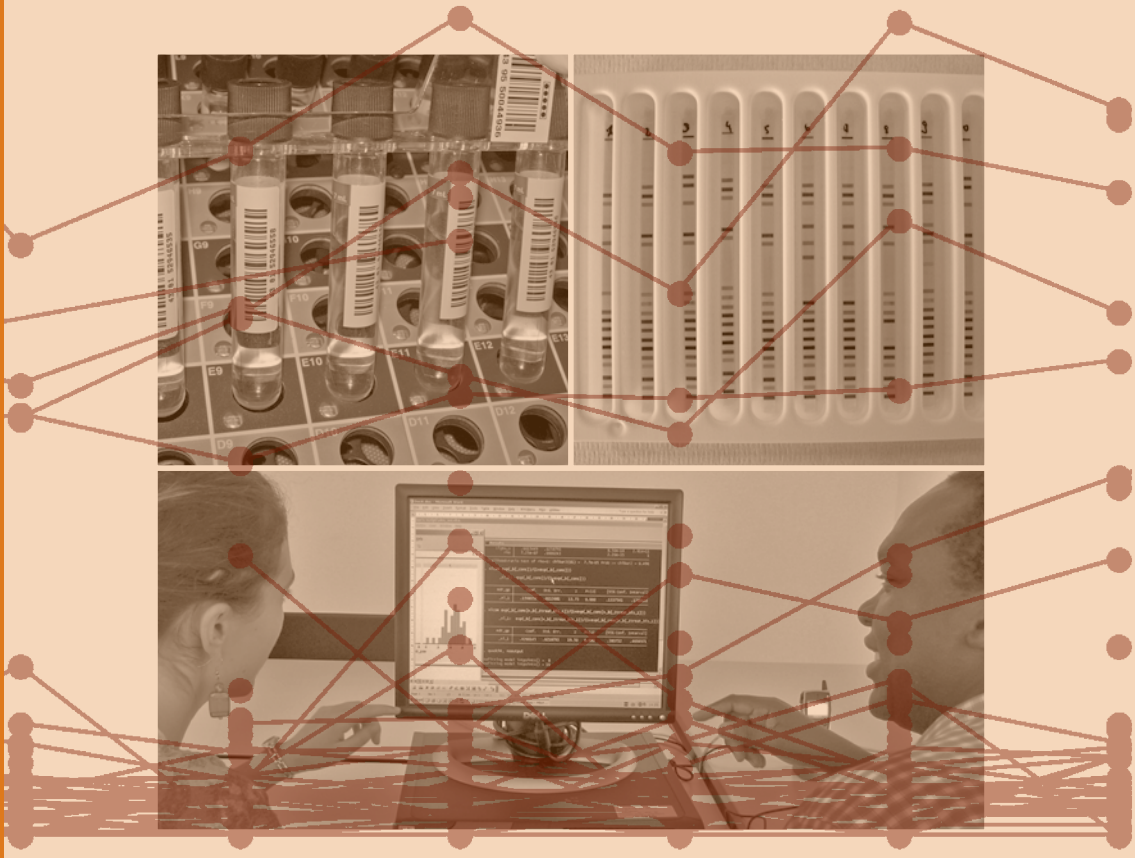
Guidelines for surveillance of drug resistance in tuberculosis

FOURTH EDITION



World Health Organization

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Introduction

This fourth edition of World Health Organization (WHO) *Guidelines for surveillance of drug resistance in tuberculosis* is an updated version of earlier editions published in 1994 (*1*), 1997 (*2*) and 2003 (*3*). These guidelines incorporate the 2007 WHO *Interim recommendations for the surveillance of drug resistance in tuberculosis* (*4*), as well as the conclusions of an Expert Committee Meeting on Anti-Tuberculosis Drug Resistance Surveys held in Geneva in September 2008. In addition, the guidelines take into account recent advancements in laboratory diagnostics and subsequent WHO guidance, including the 2008 WHO *Policy guidance on drug-susceptibility testing (DST) of second-line anti-tuberculosis drugs* (*5*) and the 2008 WHO Policy Statement, *Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis* (*6*).

Furthermore, these updated guidelines incorporate the wealth of experience gained from 15 years of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance (*7–16*) (hereinafter, referred to as the Global Project), a project initiated by WHO and the International Union Against Tuberculosis and Lung Disease (The Union).

Since its launch in 1994, the Global Project has collected and analysed data on drug resistance from surveys of sampled patients and from national surveillance systems from an ever increasing number of settings around the world. The 4th Global Report *Anti-tuberculosis drug resistance in the world* (*15*), published in 2008, included data provided from over 100 geographical settings.

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 also quantified the growing global burden of multidrug-resistant tuberculosis (MDR-TB)[1] and, in recent years has started to document the spread of extensively drug-resistant tuberculosis (XDR-TB).[2] Since the inclusion of MDR-TB management in the new and comprehensive Stop TB Strategy (*17, 18*), a new and fundamental role of the Global Project has been to assist

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

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

countries in planning the scale-up of MDR-TB management with essential data on national burden and prevalence of drug resistance patterns.

The aim of the current guidelines 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, but moving towards surveillance systems based on routine drug susceptibility testing (DST). Mechanisms for carrying out surveillance vary from country to country, but 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 these guidelines is the NTP, and in particular a coordination team for surveillance composed of the NTP manager, laboratory specialist, logistics specialist, epidemiologist, and statistician.

This edition of the guidelines is divided into two parts. Part I describes the principles of the Global Project that should be considered fundamental to both surveillance systems and periodic surveys. Part II describes the steps needed to plan and implement a survey, as well as manage and interpret the collected data.

Drug resistance surveillance in the Global Plan to STOP TB (2006–2015) and in the 2007 and 2009 World Health Assembly resolutions

Worldwide capacity to conduct drug resistance surveillance has increased since the initiation of the Global Project, but large gaps still exist. As part of the Global Plan to STOP TB (2006–2015) (*19*), the Stop TB Partnership's Working Group on MDR-TB has established a set of five specific objectives for MDR-TB control by 2015, two of which provide targets for drug-resistance surveillance. Firstly, by 2015, representative and reliable data should be available on the global magnitude of MDR-TB, trends in high MDR-TB prevalence countries, and the relationship between MDR-TB and HIV/AIDS. Secondly, by 2015, all countries should carry out drug DST for all previously treated TB patients. In the Eastern European Region, where MDR prevalence is highest, DST should also be done for all new TB patients, while in the Latin American, South-East Asian and Western Pacific Regions, DST should be done for a subset of new TB patients, focused on people at increased risk of MDR-TB.

At the 2007 World Health Assembly, resolution WHA60.19 "*Tuberculosis control: progress and long-term planning*" recognized "the importance of the situation and the trends of multidrug-resistant and extensively drug-resistant tuberculosis as barriers to the achievement of the Global Plan's objectives by 2015, and the need for an increased number of Member States participating in the network of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance and for the required additional resources to accomplish its task" (*20*).

The need for strengthening surveillance for drug-resistant TB was reiterated

by the 2009 World Health Assembly resolution WHA62.15 "*Prevention and control of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis*", that 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" (*21*).

Changes from previous editions of the Guidelines

Readers familiar with the 2003 edition of the *Guidelines* will notice the following updates and clarifications in surveillance methodology are incorporated into the current edition:

1. Establishment of surveillance based on routine DST of previously treated TB cases[1] is a priority in all settings, with country-specific prioritization of patient subcategories based on history of previous treatment.
2. In settings that do not yet have sufficient capacity for surveillance based on routine DST of all *new* TB cases, surveys should be conducted periodically among new cases, e.g. every three to five years. In settings currently lacking capacity for surveillance based on routine DST of all *previously treated* TB cases, separate sampling of previously treated cases should be considered in the design of periodic surveys.
3. New rapid phenotypic and genotypic laboratory drug susceptibility testing methods endorsed by WHO may be used for surveillance purposes. Integration of line probe assays into the TB diagnostic algorithm can allow for rapid screening of patients for resistance to rifampicin and, as a proxy, for MDR-TB. Resistance to isoniazid can also be detected using line probe assays, though resistance may be underestimated due to lower sensitivity of this tool.
4. At a minimum, surveillance should evaluate susceptibility to the following drugs:
   a) isoniazid and rifampicin;
   b) If resistance is detected to rifampicin, then susceptibility to the fluoroquinolones and second-line injectable agents most often used in the setting should also be tested. Testing for susceptibility to the first-line drug ethambutol should also be considered.
5. Surveys should be conducted at a minimum on smear positive pulmonary cases; inclusion of smear negative cases requires consideration of the implications for logistics and laboratory capacity, given that smear negative cases are less likely to be culture positive.

[1] For definitions of new and previously treated cases, see section 2.1 *Patient treatment history classifications*.

6. Establishment of sentinel surveillance networks for estimating the burden of resistance requires the establishment of systems free of significant bias. Periodic surveys should be considered the preferred alternative until capacity is reached for establishment of a surveillance system based on routine DST that produces data representative of a geographically-defined population. A sentinel system could be used as an interim approach for countries expanding routine DST to all retreatment cases.
7. Drug resistance surveillance activities, generally conducted only in the public sector under the NTP, can be enhanced via initiatives that gradually involve health care providers and laboratories outside of the NTP.
8. Inclusion of HIV testing in anti-tuberculosis drug resistance surveillance can produce valuable information for both the national TB control programme and individual patients. Therefore, in close collaboration with national AIDS programmes and other HIV stakeholders, all possible efforts must be made in order to make such information available as part of anti-tuberculosis drug resistance surveillance activities. Standard and rapid methods of HIV testing (e.g. oral tests) should be used in line with existing global and national HIV testing guidelines.
9. Statistical and epidemiological methodology is a fundamental aspect of designing surveys that sample patients, and appropriate technical assistance should be received in the early stages of planning. In particular, for surveys that use cluster-based sampling methods, results should be adjusted to correct for biases introduced by these sampling techniques. Missing values should also be accounted for, e.g. using multiple imputation techniques when possible.
10. MDR-TB management is a component of the Stop TB Strategy and WHO Member States have committed themselves to achieve universal access to diagnosis and treatment by 2015. Therefore, all drug resistance surveillance activities should be linked to patient treatment and care. Planning a comprehensive treatment programme for patients identified during a survey as having drug-resistant TB should run in parallel to planning the survey itself.
11. Survey protocols should be reviewed and approved by ethical committees or institutional review boards.

Part I.

Principles of anti-tuberculosis drug resistance surveillance in the Global Project

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

from the International Health Regulations (2005), adopted by the 58th World Health Assembly

The Global Project was initiated in 1994 with the aim of collecting and evaluating data on anti-tuberculosis drug resistance in a systematic and ongoing way around the world following three main principles:

1. Reported data should be representative of the TB patients in the country/geographical setting under study. In surveys using sampling, the sample size should be determined to permit standard epidemiological analysis;
2. The patient's history should be carefully obtained and available medical records reviewed to clearly determine whether the patient has previously received anti-tuberculosis drugs. This is essential to distinguish between drug resistance among new cases and drug resistance among previously treated cases;
3. The laboratory methods for anti-tuberculosis drug susceptibility testing should be selected from among those that are WHO-recommended, and all laboratory processes should be quality-assured in cooperation with a partner Supranational Reference Laboratory (SRL).

Within the standardized methodological framework designed for the Project, two main mechanisms of surveillance are able to collect data on drug resistance representative of a geographically-defined population to allow for comparison across settings and within settings over time: surveillance based on routine DST of all TB patients and periodic surveys of sampled TB patients.

The mechanism of surveillance that is more strongly able to fulfil *systematic* and *ongoing* requisites is a system that continuously collects DST data. As part of the Global Plan to Stop TB 2006–2015, it is an aim that all countries will be carrying out DST for all previously treated TB patients by 2015. In the WHO-

defined European Region the aim is to achieve DST among all new TB patients. In the rest of the world, the aim is to achieve DST for new TB patients who are at higher risk of poor treatment outcomes.

In the meantime, as capacity for surveillance based on routine DST grows, it is clear that alternative measures are needed in many parts of the world in order to evaluate the magnitude of drug resistance in the most systematic and ongoing manner as possible, given region-specific and country-specific circumstances and capacity. Therefore, in many countries, periodic surveys of sampled TB patients currently form the basis of surveillance.

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

It should be noted that the Global Project measures resistance only in newly registered episodes of TB (among new and/or previously treated cases), the results of which can be used in the estimation of incidence of MDR-TB. The Global Project does not measure the proportion of prevalent TB cases with resistance. This means the results cannot be used to directly calculate the number of existing drug-resistant cases, or consequently the need for second-line anti-tuberculosis drugs. Nevertheless, knowing how many new episodes of MDR-TB arise each year can be a valuable tool in planning a response to MDR-TB. Readers interested in a more in-depth discussion of the limitations of the current drug resistance surveillance methodology are referred to other sources (*22*, *23*).

1.1 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, as well as localized outbreaks. Approx-

Expanding coverage of surveillance by including health care providers outside of the NTP

When designing a mechanism of surveillance for a geographically-defined population of TB cases, one should consider the roles of all relevant health care providers not formally linked to the national TB control programme (NTP) (public, voluntary, private and corporate) in the diagnosis and treatment of TB, including drug-resistant TB. Laboratories outside the NTP undertaking mycobacterial culture and DST ideally should be included in surveillance activities. Inclusion of care providers functioning outside of the NTP in surveillance will require particular attention to assuring quality standards in diagnostics, sampling, and data recording and reporting. Surveillance should initially be conducted in the public sector and private-sector laboratories in collaboration with the NTP for quality assurance. Public-private mix initiatives can serve as platforms to gradually involve the private-sector laboratories in drug resistance surveillance activities.

imately half of the countries currently reporting data to the Global Project have surveillance systems with quality-assured laboratories that can provide such routine DST data on all TB cases (or that miss only a negligible number of such cases). Due to the resources required to maintain such a system, these surveillance systems are typically found in higher income countries. In these countries, DST results usually form the basis of the clinical management of drug-resistant TB using tailored or individualized treatment regimens.

Ideally, in the near future every country should have in place universal DST for both clinical care and surveillance purposes. However, in settings where capacity is currently not available for routine DST of all TB patients, a surveillance system should be organized with priority on establishment of routine DST of cases at high risk for drug-resistant TB. **At a minimum, a system of routine DST should be established among all previously treated TB cases, with country-specific prioritization of patient subcategories**. Subcategories include cases after treatment failure, return cases after default, relapses, and other previously treated cases (For more details on subcategories, see section 2.1 *Patient treatment history classifications*).

Significantly, in a number of countries that provide routine DST to patients, 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 significantly less than perfect coverage. Data from these surveillance systems are not included in the Global Project. However, significant efforts are currently being made in many settings to improve quality, which will allow for a growing number of countries to supply continuous surveillance data to the Global Project.

1.2 Periodic surveys

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 sample of patients representative of the geographically-defined population under study. When properly constructed 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 surveillance system, but they are less effective in detecting localized outbreaks, may produce results with margins of error that prevent meaningful analysis or determination of trends, and are suspect 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 correct classification of patients by treatment history. Surveys can also provide a platform for operational and other types of research, including study-

ing 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 smaller administrative levels should also be considered in large countries or when national capacity is insufficient. The size and scope of the survey should be determined by the ability of the NTP to ensure quality, and furthermore depends on the specific objectives of the survey. Starting at smaller administrative levels such as cities or districts, and then expanding to nationwide surveys is one way of developing capacity while ensuring quality. However, results from surveys at the subnational level should not be extrapolated to estimate the burden at the national level.

In settings without capacity for surveillance based on routine DST of new TB cases, surveys of new TB cases should be conducted periodically, e.g. every three to five years. Surveys can be conducted more frequently in settings if there is reason to believe that rates of drug resistance are changing. This could be due to introduction of new treatment regimens, programmatic changes in the NTP, any significant socioeconomic disturbance, or past observed trends in drug resistance. However, in order to detect a significant difference in proportions between two surveys conducted with a short time interval in between, a very large sample size would be required. A single survey can provide critical information to the national TB control programme on the burden of resistance and common patient drug resistance profiles at a certain point in time. However, it should be noted that without plans for repetition at regular intervals, such an activity cannot by definition be considered surveillance.

As described earlier, surveillance based on routine DST of all previously treated cases should be established as a priority in all countries.

1.3 Sentinel surveillance systems

Some countries with well-established laboratory networks have opted for a sentinel system for surveillance. This type of system continuously reports DST results of all TB cases from a selection of laboratory or hospital sites, and therefore can be useful in documenting trends and detecting outbreaks or localized epidemics of drug resistance.

For countries where resources, the health care system structure, or geographical features preclude routine DST of all patients or surveys of sampled patients, the establishment of a sentinel surveillance system may be an option. A sentinel system could be a useful interim approach for countries intending to expand routine DST to all retreatment cases while moving towards this goal. The implementation of a sentinel network requires good planning in order to produce data that are useful for planning and monitoring, even if not strictly representative. Key issues to consider include whether the characteristics of the TB cases from the reporting units risk being biased (e.g. if there is an excess of urban settings

or places where individuals at risk for drug-resistance congregate) when compared with the population of interest, and whether the units are likely to detect and report a majority of cases occurring in their areas.

1.4 Regimen surveys

'Regimen surveys' measure first-line and/or second-line drug resistance among a group of selected patients that cannot be considered representative of a patient population. These surveys can help determine the predominant patterns of drug resistance, and can be useful in providing guidance on appropriate regimens for MDR-TB treatment for particular patient groups. These include return cases after treatment failure, chronic cases and symptomatic contacts of MDR-TB cases. Regimen surveys should be conducted in the process of developing MDR-TB treatment programmes, or within selected centres or diagnostic units that regularly address high-risk cases.

Regimen surveys do not need to be nationwide in scope. Organization of these surveys can help build capacity for later surveillance based on routine DST of high-risk cases. Due to their design, regimen surveys cannot provide data that is representative of a geographically-defined population, nor can they provide accurate data on trends.

2.

Standardized stratification of results by patient categories

2.1 Patient treatment history classifications

The Global Project measures resistance in newly registered episodes of TB (including among new and previously treated patients) stratified by the patients' TB treatment histories. Forms in Annex 1A and 1B collect aggregated data on resistance to first-line and second-line drugs respectively, by treatment history category. Careful classification of treatment history is critical to allow for proper and accurate interpretation of data. The fourth edition of WHO *Guidelines for treatment of tuberculosis* (*24*) 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 denies having had any prior anti-tuberculosis treatment (for up to one month), and in countries where adequate documentation is available, for whom there is no evidence of such history.

Determining the proportion of drug resistance among new cases is vital in the assessment of recent transmission.

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 admits having been treated for TB for one month or more, or, in countries where adequate documentation is available, there is evidence of such history. Chemoprophylaxis should not be considered treatment for TB.

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

Subcategories of previously treated cases

Definition: "Relapse" – a patient whose most recent treatment outcome was "cured" or "treatment completed", and who is subsequently diagnosed with bacteriologically positive TB by sputum smear or culture.

Primary resistance: Patients with TB resistant to one or more anti-tuberculosis drugs, but who have never been previously treated for TB, are said to have "primary resistance" (or "initial resistance") due to transmission of a drug-resistant strain. Primary drug resistance is a theoretical concept, as history of prior anti-tuberculosis treatment is often difficult to accurately ascertain (*25*). Resistance among new cases (defined as cases with no or < one month history of treatment) has been selected as a proxy to estimate primary resistance.

Acquired resistance: Patients diagnosed with TB who start anti-tuberculosis treatment and subsequently acquire resistance to one or more of the drugs used during the treatment, are said to have developed "acquired resistance". In the past, resistance among previously treated cases (defined as cases with ≥ one month history of treatment) was used as a proxy for acquired resistance; however, this patient category is now known to also be comprised of patients who have been re-infected with a resistant strain, and patients who were primarily infected with a resistant strain and subsequently failed therapy or relapsed.

Therefore, resistance among previously treated cases is not a useful proxy for truly acquired resistance (*26*, *27*). Truly acquired resistance can be ascertained only if the drug susceptibility pattern is determined before the start of treatment for any newly registered episode, as well as at a later point in treatment or at the end of treatment. Furthermore, to avoid misclassifying re-infection with a resistant strain as a case of acquired resistance, molecular fingerprinting of strains would be required (*25*).

Definition: "Treatment after failure" – a patient who is started on a retreatment regimen after having failed previous treatment for TB. Failure is defined as sputum smear positive at five months or later during treatment. The treatment course failed (an initial treatment course with first-line drugs, a retreatment course with first-line drugs, or a treatment course using second-line drugs) should be specified.

Definition: "Treatment after default" – a patient who returns to treatment, bacteriologically positive by sputum smear microscopy or culture, following interruption of treatment for two or more consecutive months.

Definition: "Other retreatment" – all cases that do not fit the above definitions. This includes patients who were previously treated:

- but the outcome of their previous treatment is unknown, and/or;
- who have returned to treatment with smear negative pulmonary TB or bacteriologically negative extrapulmonary disease.

Note: Patients who are bacteriologically-positive at the end of (or returning from) a second or subsequent course of treatment are no longer defined as "chronic". Instead, they are classified by the outcome of their most recent re-treatment course: relapsed, defaulted or failed (according to the fourth edition of WHO *Guidelines for treatment of tuberculosis* (*24*)).

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 resistant to one or more drugs, and are usually the group from which patients are screened for inclusion in drug-resistant TB treatment programmes. The information gained from surveillance can be used for regimen development and evaluation. Therefore, developing 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 achieved 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

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 are more likely to be harbouring older infections.

The form in Annex 1C collects information for the Global Project on MDR-TB by age groups and sex.

2.2.2 HIV status

Incorporation of HIV testing in anti-tuberculosis drug resistance surveillance may yield important information to the national TB control programme on the relationship between HIV and drug resistant TB at the population level. It can also provide critically important individual benefits to HIV-infected patients, including better access to testing, early detection and rapid placement on treatment. Provider-initiated HIV testing is recommended for all TB patients, and patients presenting with signs and systems of TB (*28*). Therefore, whenever possible, regardless of the state of the HIV epidemic in a particular setting, HIV testing should be encouraged and HIV status information should be included for all patients enrolled in anti-tuberculosis drug resistance surveillance. However, HIV testing should be considered an integral part of anti-tuberculosis drug resistance surveys in HIV-prevalent settings, which are defined as settings with an HIV prevalence of ≥1% in pregnant women or ≥5% in TB patients.

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 AIDS programme should be involved in the planning and execution of the surveillance from the beginning. Rapid HIV tests (e.g. oral tests), in accordance with national HIV testing and surveil-

The association between anti-tuberculosis drug resistance and HIV

Outbreaks of drug-resistant TB among HIV-positive patients have been widely documented in nosocomial and other congregate settings, but little information is available about the association of HIV and drug-resistant TB on a population level (*30*, *31*). The 4th Report on the *Global project on anti-tuberculosis drug resistance surveillance* reported a significant association between HIV-positive status and MDR-TB in two settings (*15*).

There are two main reasons why drug-resistant TB may be associated with HIV. The first is the documented acquisition of isolated rifampicin resistance among HIV-positive people under treatment for TB, although this may also be due to disruptions in therapy. Anti-tuberculosis drug malabsorption has also been documented in patient cohorts in settings of high HIV prevalence, which suggests that HIV-positive TB patients may be at greater risk of acquiring drug resistance. The second reason relates to exposure. HIV-positive patients and drug-resistant TB patients may have similar risk factors, such as history of hospitalization, which may mean that HIV-positive TB patients are at a higher risk of exposure to resistant forms of disease.

The epidemiological impact of HIV on the epidemic of drug-resistant TB is not known and may depend on several factors. HIV-positive TB cases are more likely to be smear negative. In addition, delayed diagnosis of drug resistance and unavailability of treatment (particularly in previous years) have led to high death rates in people living with HIV. Both of these factors (smear negativity and short duration of disease due to mortality) may suggest a lower rate of general transmission. However, HIV-positive cases progress more rapidly to disease, and in settings where MDR-TB is prevalent (either in the general population or in the local population such as a hospital or a district), this may lead to rapid development of a pool of drug-resistant TB patients or an outbreak.

lance policies, are preferred methods of HIV testing compared with plasma or serum-based tests (e.g. ELISA) (*29*).

In settings with a low burden of either MDR-TB or HIV, incorporating HIV testing into surveillance may not allow for statistically significant determination of the relative risk of resistance in HIV-positive compared with HIV-negative TB patients. Furthermore, surveillance incorporating HIV testing should take into account the limitations in interpretation of data due to incomplete information as a result of testing coverage, and proportion of patients opting out. Surveillance should be designed to differentiate between negative test results and tests not performed.

The form in Annex 1D collects information for the Global Project on MDR-TB by patient HIV status.

2.2.3 Other patient biographical and clinical factors

Inclusion of other patient biographical and clinical information to collect and measure is optional, and selection should be based on the objectives of surveillance and planned data analysis. Optional variables to measure associations between drug resistance and stratified patient groups include:

- sub-geographic location
- country of origin
- history of incarceration
- drug use
- alcohol abuse
- tobacco use
- other country-specific factors.

Using surveys to study risk factors for drug-resistant tuberculosis

Surveys can serve as a valuable platform for studying the country- or setting-specific causes of drug resistance and for identifying the most important targets for intervention (*32*). Surveys can be designed to include a series of questions investigating potential risk factors, to be asked to patients or reviewed from medical records at the time of enrolment. Furthermore, a study seeking to investigate risk factors for acquisition or amplification of drug resistance can test drug susceptibility, not only before treatment, but also after failure of a treatment course.

Risk factors that may be evaluated include patient HIV status and use of antiretroviral treatment; *M. tuberculosis* genotype; type and quality of previous (and/or current) treatment and treatment supervision; previous (and/or current) infection control practices; composition of previous (and/or current) treatment regimens including use of rifampicin in the continuation phase; previous (and/or current) source of drugs used; usage of fixed-dose combinations. Risk factors for evaluation can also include possible social determinants* including socioeconomic status, education level, employment, etc. and more direct risk factors such as malnutrition, crowding, diabetes, alcohol abuse, drug use, smoking, etc. It should be noted that multiple risk factors for acquisition, amplification and transmission of drug resistance may operate simultaneously in a setting.

Evaluation of these potential risk factors should be a considered a research activity, and should not compromise the quality of the survey and attainment of its primary objectives.

* For examples of how to design questions to measure social determinants, see Lönnroth et al. (*33*), or Annex 13 of *Assessing tuberculosis prevalence through population-based surveys*. WHO Western Pacific Region, 2007 (available online at http://www.wpro.who.int/health_topics/tuberculosis/). Note: the examples provided may require adjustment based on local conditions and the population under study.

3.

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

In settings around the world, laboratory capacity has repeatedly turned out to be the weakest link in developing a reliable system of surveillance of anti-tuberculosis drug resistance. Establishment of quality-assured bacteriology using WHO-recommended methods is necessary for surveillance, and introduction of rapid methods for DST into the diagnostic algorithm 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, and can significantly reduce the delay between detection of TB and diagnosis of first-line and second-line drug resistance. Rapid methods of DST allow for the timely design of appropriate treatment regimens based on patients' drug resistance profiles using diagnostics that can be feasibly implemented in settings worldwide. This increased capacity for DST also translates into increased capacity for surveillance.

After comprehensive review, WHO has endorsed certain new DST methodologies, including molecular line probe assays and liquid culture systems. Due to the dynamic nature of research and development, new technologies other than those described below may have been endorsed by WHO since the time of writing of this publication.

3.1.1 Solid culture methods

Although newer liquid culture and certain genotypic methods for DST have been endorsed by WHO and are being established in national TB control programmes worldwide, conventional phenotypic methods using solid media are still more commonly used.

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 inexpensive and highly standardized for testing susceptibility to many drugs, but they have the serious 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 (*5*).

Methodology of each of the three methods is well-described elsewhere (*3*, *34*–*37*), as are instructions for the preparation of the most commonly used egg-based media, Löwenstein-Jensen and Ogawa (*38*).

3.1.2 Liquid culture methods

Compared with solid culture methods, liquid culture methods significantly reduce the turnaround time for results, while also moderately increasing sensitivity. With liquid culture, confirmation of pulmonary TB can be obtained in less than two weeks, and DST results in an additional one to two weeks. Use of liquid culture methods is possible for susceptibility testing for both first-line and second-line drugs. WHO has endorsed the use of liquid culture and DST in low- and medium-income settings, provided that the required infrastructure and biosafety measures are in place, and that affordability and sustainability are ensured (*39*). 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), and the need for strict quality control measures to prevent contamination. Reading of commercial liquid culture systems is now partially or fully automated, preventing human error and contamination to some degree.

Radiometric liquid culture systems are highly sensitive, fast, and used effectively in many settings, but are being phased out. This is due to their high cost and problems related to the disposal of a large volume of radioactive material.

3.1.3 Molecular line probe assays

Molecular line probe assays are a genotypic method of DST that are used to detect the most common mutations of *M. tuberculosis* DNA that confer resistance to anti-tuberculosis drugs. Validated line probe assays have been endorsed by WHO for use in screening patients for isoniazid and rifampicin resistance, provided that technical expertise on molecular techniques and proper facilities are in place and sustainability is ensured (*6*). Such assays can be used for surveillance purposes.

Line probe assays have the great advantage of being able to produce results within 24 to 48 hours, thus representing a revolutionary step forward in the ability to rapidly confirm or rule out MDR-TB. Furthermore, they can be used directly on smear-positive sputum specimens, therefore providing lower biohazard

risks. Line probe assays are relatively simple to perform and require only basic expertise in polymerase chain reaction (PCR) techniques. Data from systematic reviews and meta-analyses to evaluate assay performance results against conventional DST methods have shown that line probe assays are highly sensitive and specific for the detection of rifampicin resistance, alone or in combination with isoniazid, on isolates of *M. tuberculosis* and on smear positive sputum specimens (*6*, *40*). However, given the lower sensitivity of line probe assays in detection of isoniazid resistance, resistance to isoniazid may be underestimated using this method.

The integration of line probe assays into MDR-TB screening algorithms can significantly reduce the demand on conventional culture and DST laboratory capacity. Use of line probe assays also decreases costs of shipping from diagnostic centres to laboratory facilities, if used only on smear positive specimens.

However, line probe assays are not a complete replacement for liquid or solid culture and DST. Culture is still required for smear negative specimens. In addition, conventional methods of DST are still necessary to detect resistance to second-line drugs, as well as ethambutol and streptomycin, as assays that detect genetic mutations conferring resistance to these drugs are still under development. Another barrier to widespread use of line probe assays is the cost of the equipment and consumables, as well as the potential need for laboratory renovations in order to establish the separate rooms required to prevent contamination. Procedures should be performed strictly according to the manufacturer's instructions.

3.2 Consensus on critical concentrations for first- and second-line drug susceptibility testing

There has long been a consensus on the methodologies, critical drug concentrations, and reliability and reproducibility of testing using conventional methods for DST of first-line anti-tuberculosis drugs. However, consensus on DST of second-line drugs has only recently been tentatively established, with the publication of WHO's *Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs* (*5*). The policy guidance is based on a robust assessment of published studies combined with laboratory experience and expert opinion. It systematically evaluated available DST methods for all second-line drugs and established consensuses on the reliability and reproducibility of DST for first-line and second-line anti-tuberculosis drugs (see Table 1). However, additional research is needed to assess the role of second-line DST results in guiding treatment regimen design.

Drugs are grouped in DST categories based on the following broad criteria that were used to assess the strength of available evidence. This was based on two or more criteria having been met for assigning a drug to a specific DST category between I and V:

Table 1. Current status of DST methodology and critical concentrations for first-line and second-line DST

Drug group[a]	Drug	DST category	DST method available	DST crtitical concentration (µg/ml)				
				Löwenstein-Jensen[b]	Middlebrook 7H10[b]	Middlebrook 7H11[b]	BACTEC460	MGIT960
Group 1 First-line oral anti-TB agents	Isoniazid	I	Solid, liquid	0.2	0.2	0.2	0.1	0.1
	Rifampicin	I	Solid, liquid	40.0	1.0	1.0	2.0	1.0
	Ethambutol	II	Solid, liquid	2.0	5.0	7.5	2.5	5.0
	Pyrazinamide	II	Liquid	–	–	–	100.0	100.0
Group 2 Injectable anti-TB agents	Streptomycin	II	Solid, liquid	4.0	2.0	2.0	2.0	1.0
	Kanamycin	II	Solid, liquid	30.0	5.0	6.0	4.0	–
	Amikacin	II	Liquid	–	–	–	1.0	1.0
	Capreomycin	II	Solid, liquid	40.0	10.0	10.0	1.25	2.5
	Viomycin	V	None	–	–	–	–	–
Group 3 Fluoroquinolones	Ciprofloxacin[d]	III	Solid, liquid	2.0	2.0	2.0	2.0	1.0
	Ofloxacin	III	Solid, liquid	2.0	2.0	2.0	2.0	2.0
	Levofloxacin	IV	Solid, liquid	–	2.0	–	–	2.0
	Moxifloxacin	IV	Liquid	–	–	–	0.5	0.25
	Gatifloxacin[e]	IV	Solid	–	1.0	–	–	–
Group 4[c] Oral bacteriostatic second-line anti-TB agents	Ethionamide	IV	Solid, liquid	40.0	5.0	10.0	2.5	5.0
	Prothionamide	IV	Solid, liquid	40.0	–	–	1.25	2.5
	Cycloserine	IV	Solid	40.0	–	–	–	–
	Terizidone	IV	None	–	–	–	–	–
	P-aminosalicylic acid	IV	Solid, liquid	1.0	2.0	8.0	2.0	–
	Thioacetazone	V	None	–	–	–	–	–
Group 5[c] Antituberculosis agents with unclear efficacy (not recommended by WHO for routine use in MDR-TB patients)	Clofazimine	V	Liquid	–	–	–	4.0	–
	Amoxicillin/clavulanate	V	None	–	–	–	–	–
	Clarithromycin	V	None	–	–	–	–	–
	Linezolid	V	Liquid	–	–	–	1.0	1.0

[a] WHO *Guidelines for the programmatic management of drug-resistant tuberculosis* (*25*).
[b] Indirect proportion method recommended. Other solid media methods (resistance ratio, absolute concentration) have not been adequately validated for second-line drugs.
[c] Routine DST for group 4 and 5 drugs is not recommended.
[d] Ciprofloxacin is no longer recommended to treat drug-susceptible or drug-resistant TB (*25*).
[d] Gatifloxacin only to be used in exceptional circumstances (*25*).

I. Extensive published studies, extensive multicentre laboratory review, broad intermethod agreement, high stability of drug powder in vitro, consistent DST reliability and reproducibility, extensive clinical outcome data.

II. Extensive published studies, extensive multicentre laboratory review, limited intermethod agreement, variable DST reproducibility (and therefore reliability), variable stability of drug powder in vitro, less extensive clinical outcome data.

III. Less extensive published studies, limited multicentre laboratory review, limited intermethod agreement, limited data on DST reproducibility and reliability, limited data on drug powder stability in vitro, limited clinical outcome data.

IV. Limited or no published studies, limited multicentre laboratory review, limited data or questionable DST reproducibility (and therefore reliability), instability of drug powder in vitro, no clinical outcome data.

V. No published studies, no multicentre laboratory review, reproducibility and reliability impossible to assess, unknown stability of drug powder in vitro, no clinical outcome data.

3.3 Selection of drugs to be tested for susceptibility

Due to the difficulties in treating patients with MDR-TB, determining the proportion of TB cases with isoniazid and rifampicin resistance is extremely important. Furthermore, resistance to both of these drugs can be reliably measured by standardized techniques, resulting in high sensitivities and specificities.

- Therefore, susceptibility testing for both isoniazid and rifampicin should form the backbone of all drug-resistance surveillance. Among the other first-line anti-tuberculosis drugs, testing for streptomycin and ethambutol may also be included in surveillance, as these drugs are widely used throughout the world and are tested for in rounds of proficiency testing among the Supranational Reference Laboratory Network (SRLN). However, SRLN reliability of susceptibility tests for streptomycin and ethambutol are lower than reliability corresponding to isoniazid and rifampicin (*41*). It is difficult to standardize susceptibility for pyrazinamide on solid media and the cost of liquid DST is high. Therefore, this drug should not be routinely included in the selection of drugs to be tested for surveillance purposes.

Furthermore, due to the extreme obstacles in treatment options for XDR-TB, evaluation of the proportion of MDR-TB cases that have XDR-TB strains should also become a component of drug resistance surveillance.

- Therefore, in TB cases with detected rifampicin resistance (a strong surrogate marker for MDR-TB, especially in areas with high prevalence of MDR-TB[1]), susceptibility to the fluoroquinolones and second-line injectable agents[2] most often used in the setting should also be measured in surveillance. In such cases, resistance to ethambutol could also be measured in surveillance. This is because ethambutol is the only first-line oral agent for which susceptibility can be somewhat reliably tested, and which could be part of a treatment regimen for MDR-TB.

In summary, at a minimum, drug resistance surveillance should measure susceptibility to the following drugs:

a. Isoniazid and rifampicin;
b. If resistance is detected to rifampicin, then susceptibility to the fluoroquinolones and second-line injectable agents most often used in the setting should also be tested. Testing for susceptibility to the first-line drug ethambutol should also be considered.

Testing for susceptibility to other drugs should be considered based on perceived use in a setting, country capacity for testing, and known reliability of susceptibility results.

For further guidance on selecting second-line drugs for DST, including information on cross-resistance and known reliability and reproducibility of second-line DST, see WHO *Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs* (5).

3.4 Quality assurance of drug susceptibility testing

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

3.4.1 Internal quality control

Standardized procedures and registers must be employed; whether the proportion method, resistance ratio method, absolute concentration method, liquid culture methods, or other method is used for susceptibility testing and for formulation of media. As a part of internal quality control, the quality of the medium should be controlled for each batch. Drugs added to the medium must be pure substances

[1] It should be noted that rifampicin resistance unaccompanied by isoniazid resistance is rare; presence of such a phenomenon in more than 3% of TB cases is an indication that errors are likely present in either rifampicin or isoniazid testing.

[2] Second-line injectable agents include kanamycin, amikacin, and capreomycin. Streptomycin is classified as a first-line injectable agent (Fourth edition of the WHO *Guidelines for treatment of tuberculosis* (24)).

obtained from a reputable firm, the percentage of potency must be clearly indicated, and they must be properly stored. Drug dilutions and their addition to the medium should be performed in accordance with accepted standards.

For conventional solid culture methods, susceptibility testing should be performed on the standard $H_{37}Rv$ strain in each new batch of drug-free and drug-containing medium. It is also recommended that this internal quality control include a combination of strains with known resistance to two or three drugs, but avoiding MDR and particularly XDR strains. Since medium batches will be consumed quickly, it may be necessary to include these reference strains with each batch of survey strains processed for DST. Moreover, the usual internal quality assurance 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.

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

External quality assessment is composed of several components: proficiency testing, rechecking of strains, and onsite evaluations of laboratories; all conducted in cooperation with a partner external 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 these guidelines, there were 28 Supranational Reference Laboratories (SRLs) in the network (see Annex 2).

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 (isoniazid, rifampicin, ethambutol, streptomycin) and, as of 2008, to second-line drugs (kanamycin, amikacin, capreomycin, ofloxacin). The panels of strains are subsequently used to assess the proficiency of National Reference Laboratories (NRLs), as well as 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 a NRL's accuracy at 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 coded strains from a partner SRL to be tested for susceptibility to first-line and, if applicable, to second-line drugs. For each strain, the NRL indicates the susceptibility to each of the four first-line drugs (as shown in Annex 3A) and each of the selected second-line drugs (as shown in Annex 3B). The NRL and SRL should agree beforehand on which second-line drugs

to test. The test results of the NRL should be compared with the coded results of the judicial consensus of the SRLN, which can be considered a "gold standard". The procedure should be double-blinded.

The minimum required agreement should be defined for each drug and should be at least 95% for isoniazid and rifampicin. Sensitivity, specificity, and reproducibility of susceptibility testing are calculated for each of the four first-line drugs tested; a sample analysis sheet is shown in Annex 4A. If an NRL also tests for susceptibility to second-line drugs, an additional analysis sheet is used, as shown in Annex 4B. Similar methodology can be applied for external quality assessment from the NRL to regional laboratories in countries where such laboratories are also performing susceptibility testing. Note: when applying to the Green Light Committee Initiative (GLC) for access to high-quality second-line anti-TB drugs at concessional prices for the treatment of MDR-TB, applicants are asked for their latest proficiency testing results.

- *From the NRL to the SRL (quality assessment of results, also known as "rechecking"):* 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. The results should be compared for agreement with respect to each drug. Sample analysis sheets for first-line drugs and second-line drugs are shown in Annexes 5A and 5B, respectively. For information on how rechecking should be included in the design of a survey, see section 6.4.4 *Susceptibility testing, including rechecking*. In countries where exporting strains is not permitted, a rechecking exercise should take place with another laboratory participating in proficiency testing exercises with an SRL. National and international rules and regulations and turnaround times for shipment to the SRL must be considered for planning purposes.

Quality assessment indicators for DST of *Mycobacterium tuberculosis* in the Supranational Reference Laboratory Network	
Sensitivity	Ability to detect true resistance; i.e. the proportion of resistant strains that are detected
Specificity	Ability to detect true susceptibility; i.e. the proportion of susceptible strains that are detected
Efficiency or Accuracy	The proportion of total strains that are correctly detected as being resistant or susceptible
Predictive value for resistance	The proportion of total measured resistance that is true resistance
Predictive value for susceptibility	The proportion of total measured susceptibility that is true susceptibility
Reproducibility or reliability	Intra-laboratory agreement between duplicate cultures expressed as a percentage

At the time of publication, quality assurance systems for molecular and liquid media DST were being developed by WHO.

4.
Ethical considerations

Information obtained from anti-tuberculosis drug resistance surveillance is crucial for planning a robust MDR-TB control programme. The overall 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 (*42*).

Some 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 as just one or the other. Research ethics and public health ethics are grounded in similar principles, but the application of these principles will not always be identical (*43*). 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 (*42*, *44*, *45*):

- **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 as far as 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. Special care should be taken that marginalized groups (e.g. children, women, prisoners, migrants, refugees, etc.) do not feel forced or pressured to participate, and that they are protected against potential stigma resulting from participating. In contrast to the usual practise in medical research, obtainment of individual informed consent is not usually neces-

sary for routine surveillance, especially when obtaining information from an entire population is essential to achieving critical public health objectives. Even when obtaining individual consent is deemed unnecessary, individuals should be given information about the nature and purposes of the surveillance to the extent this is possible.

- **Access to treatment** – In contrast with surveillance of standard tuberculosis indicators, surveillance of drug-resistance in tuberculosis raises a particular ethical dilemma when surveillance activities are conducted in settings where there is no capacity to properly treat patients that may be identified as having drug-resistant strains. The results of the testing should be communicated to participants, and those in need should receive appropriate treatment with second-line anti-tuberculosis drugs. If appropriate treatment options are not available in a setting, the necessary measures to initiate an MDR-TB treatment programme should be planned in parallel with planning for a survey. The national or regional TB control programme should consider preparing an application to the Green Light Committee (GLC) Initiative[1] to gain access to quality-assured second-line drugs for the treatment of MDR-TB.

For assistance in developing an ethical review protocol for reviewing a MDR-TB surveillance activity, contact the Global Project secretariat at TBDRS@who.int

[1] In addition to enabling access to high-quality second-line anti-TB drugs at concessional prices for the treatment of MDR-TB, the GLC ensures effective treatment of MDR-TB patients in accordance with WHO *Guidelines for the programmatic management of drug-resistant tuberculosis* (*25*), and furthermore provides access to technical assistance to facilitate rapid scale-up of MDR-TB management. For more information, see http://www.who.int/tb/challenges/mdr/greenlightcommittee/en/.

Part II.

Conducting a survey

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 sample must be carefully designed, and measures must be in place to ensure that the data collected is properly categorized and the DST is based on quality-assured bacteriology. All of this requires comprehensive and accurate planning of logistics, including pre-survey budgeting of all planned expenses.

5.1 Setting specific objectives

A critical component of the initial planning process is to clearly identify the specific survey objectives in order to guide the development of a survey that will be able to collect meaningful information. Specific objectives may include:

- to determine the proportion of new TB cases in a geographical setting that have resistance to selected first-line anti-tuberculosis drugs;
- to determine the proportion of previously treated cases in a geographical setting that have resistance to selected first-line anti-tuberculosis drugs;
- to determine the proportions and pattern of drug resistance to fluoroquinolones and second-line injectable agents among strains with confirmed resistance to isoniazid and rifampicin;
- to evaluate associations between drug resistance and age groups and sex;
- to evaluate associations between drug resistance and HIV status;
- to evaluate associations between drug resistance and country of origin;
- to speciate mycobacteria isolated from sputum smear positive pulmonary TB cases in the country;
- to establish the foundation for routine surveillance of drug resistance in order to observe trends over time;
- to evaluate associations between drug resistance and risk factors including history of incarceration, smoking, alcohol abuse and/or drug abuse.

At the same time, conducting a survey can contribute to establishing or strengthening a quality-assured laboratory network in a country.

5.2 Development of a protocol and time schedule

A survey protocol should be developed that describes all aspects of the survey: the coordination team and individual members' roles and responsibilities; objectives; sample size and design; logistics; training; ethical considerations; laboratory capacity and quality assessment of drug susceptibility results; data management; and budget. Once the diagnostic centres participating in the survey are identified by the chosen sampling method, a schedule can be established, taking into account logistics, climatic conditions, and the Central Reference Laboratory workload. All laboratory methods and the system of quality assurance should be discussed and agreed 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 relevant ethical review panels.

A checklist for a survey protocol is included in Annex 6. 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 requisites have been considered and described comprehensively, quality control measures are in place, and the data collected would be representative of the geographically-defined population under study. Once finalized, such a protocol should be distributed to all coordination team members and health officers participating in the survey.

5.3 Minimum required facilities for a survey area

The country, state, province, or city selected to be a survey's geographical area should have at least one quality-assured central laboratory for culture and DST (i.e. a Central Reference Laboratory, which is usually the National Reference Laboratory) linked by mail or messenger with 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 centres

Diagnostic centres include all institutions where decisions on diagnosis are made and patients suspected of having TB are registered. Most diagnostic centres in TB control programmes with limited means are non-specialized health centres and clinics or outpatient departments of hospitals operated by the government or by nongovernmental organizations. Private sector institutions and general practitioners are not included in survey activities unless their activities are based on some agreement with the national TB control programme (public-private mix initiatives), and they are following national guidelines for diagnosis and treatment.

A basic set of TB infection control measures should be implemented in all facilities involved in a drug resistance survey. In particular, attention should be paid to control the spread of pathogens and minimize time patients suspected of having TB spend in health-care facilities.[1]

Diagnostic centres may or may not have the ability to conduct smear microscopy and culture. Quality-assured microscopy together with adequate referral systems for culture and DST are prerequisites for the implementation of a drug resistance survey.

Central Reference Laboratory

The Central Reference Laboratory prepares cultures from the sputum samples received by the diagnostic centres, and undertakes the identification of *M. tuberculosis* strains, as well as DST. If there are intermediate culture laboratories in the network, mycobacterial isolates, rather than sputum specimens, can be sent to the Central Reference Laboratory for testing.

One of the main tasks of the Central Reference Laboratory is to ensure the quality of smear microscopy, 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, organized in cooperation with a partner SRL, will validate the results of susceptibility tests done by the Central Reference Laboratory.

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 conducting culture and DST are safe, and appropriately equipped with trained staff working with clear standard operating procedures and producing quality-assured data. Processing of specimens for culture and DST must be performed under appropriate biosafety conditions, the guidelines for which are currently being developed by WHO. It is important to note that drug resistance surveys will heavily increase the workload of the reference laboratory, and should only be undertaken where capacity is sufficient.

5.4 Sampling of cases

Statistical methodology should be considered a fundamental aspect of the design of surveys that sample patients, and an experienced statistician should be involved throughout the survey from the early planning stages.

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[1] For more information see: *WHO policy on TB infection control in health-care facilities, congregate settings and households*. Geneva, World Health Organization, 2009, (document WHO/HTM/TB/2009.419).

Sampling of previously treated cases

Accurate evaluation of resistance in previously treated cases provides crucial information for programme management, including information for regimen development and evaluation. However, the design of a representative survey among previously treated cases is challenging. As part of the Global Plan to STOP TB (2006–2015), establishment of routine DST for all previously treated TB cases is a priority, with the aim of all regions having this capacity by 2015. In the meantime, in settings where routine DST of previously treated cases is not yet feasible, ideally a separate, appropriately-sized sample for previously treated cases should be devised for a survey. However, in most settings, reaching a sample size calculated for previously treated cases would not be feasible due to the small number of previously treated cases notified annually.

On the other hand, simply enrolling previously treated patients during the intake period for new cases may result in a sample size that does not allow for a sufficiently precise estimate of MDR prevalence, due to the likely small number of previously treated cases enrolled. It is therefore advisable to extend the intake period for previously treated cases beyond the intake period for new cases. The duration of such an extension will depend on local resources and capacity, and could serve as a basis to establish an ongoing surveillance system for previously treated cases. However, any extension in the recruitment of previously treated cases should be identical across all diagnostic centres selected as clusters, to ensure that the sample remains geographically representative. If the sample is self-weighted (probability-proportional to size cluster sampling), extensions in the recruitment of previously treated cases should also be identical across clusters.

5.4.1 Defining the sampling frame

The sampling frame for a survey to measure the proportion of new cases (i.e. cases with a history of less than one month of previous TB treatment or no previous TB treatment) having anti-tuberculosis drug resistance should include all new sputum smear positive pulmonary TB patients in the setting.[1]

Surveys are usually based on 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 smear positive or smear negative. However, HIV-infected cases with a higher likelihood of being paucibacillary or smear negative may be exposed to different risk factors.
2. The culture yield from smear negative patients is relatively low compared to smear positive cases (*46*). 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 smear negative cases should strongly consider the implications for logistics and laboratory capacity.

[1] In settings that conduct routine culture on all new cases, the sampling frame should include all new culture-positive pulmonary TB patients in the setting.

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.

The sampling frame should include, as a minimum, all patients registered in the public sector under the NTP. Via established initiatives, patients treated in health care facilities outside of the NTP could be included in the sampling frame.

5.4.2 Sample size

For surveys measuring the proportion of new (not previously treated) cases that have anti-tuberculosis drug resistance,[1] the calculation of an appropriate sample size should be based on the following (*47*):

- the total number of new sputum smear positive cases registered in the previous year in the country or in the geographical setting to be studied;
- expected proportion of resistance to rifampicin[2] from available data (in the absence of available data, the best guess of investigators should be used);
- the desired precision, meaning to what extent a measured proportion may err on either side. As a general rule, precision corresponds to the percentage of uncertainty one is willing to accept. For example, an absolute precision of 0.01 would give a proportion within one percentage point (±1%) of the actual proportion of rifampicin resistance. The precision value should be as low as possible, while ensuring that obtaining the calculated sample is logistically feasible. However, it should never should be more than 20% of the value of the expected proportion of rifampicin resistance. For example, if rifampicin resistance is expected to be 4%, then precision should be no more than 0.8% (an absolute precision of 0.008);
- a confidence interval of 95% should be used for the measured proportion.

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

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

[1] An analogous calculation should be considered for previously treated cases, if routine DST of such cases is not yet established.

[2] Resistance to rifampicin can serve as a proxy for MDR-TB.

where:

N = total number of new smear positive 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. 0.01 or 0.02);

p = expected proportion of rifampicin resistance in the target population (as a decimal, e.g. 0.05 or 0.15).

If the cluster sampling method is adopted, the cluster design effect needs to be taken into account. Unless the cluster design effect can be estimated from previous surveys, an effect of 2 should be assumed (which is realistic, but erring on the side of being conservative), and therefore, the calculated sample size obtained from the equation above needs to be multiplied by 2.

Finally, the calculated sample size needs to be increased by 15–20% to account for expected losses. Losses include patients diagnosed as smear positive who do not return to the diagnostic centres or do not produce an adequate sample for the survey, patients whose culture is contaminated or does not grow, and patients whose susceptibility testing does not give interpretable results (unreadable or too few colonies).

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 in the proportions of rifampicin resistance found in the previous survey, and 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. The assistance of a statistician is needed to determine an appropriate sample size for a subsequent survey.

5.4.3 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, a randomization step is essential (48, 49). Simple random sampling of individual patients is not practical in TB diagnostic centres. This is mainly because routines that are usually identical for most patients would be disrupted, and compliance of staff and patients would consequently be low and the quality of data poor. Involving all diagnostic centres can also give rise to logistic problems and high costs. Randomization can take place at the level either of diagnostic centres or possibly of health districts. In this way, routines would be slightly changed for some diagnostic centres, for a period of time, but would remain identical for all newly registered smear positive patients in a particular centre. If each individual patient in the sampling

frame has equal probability of being included in the sample, the sample will be "self-weighted", i.e. weights will not be needed in the analysis. The most useful sampling strategies are described below.

100% sampling of diagnostic centres

This sampling method is most suitable for small countries with relatively small numbers of TB diagnostic units, good infrastructure, and facilities to transport samples from all diagnostic centres to the Central Reference Laboratory. All eligible patients enrolling at each diagnostic centre within the same limited intake period are included.

The self-weighted character of this design is ensured by the inclusion of all diagnostic centres and by the use of the same enrolment period for each of them. Large and small diagnostic centres are equally represented without the need for a complicated sampling method. Under this sampling method, individuals are considered selected by using simple random sampling. The intake period is calculated by dividing the sample size by the total number of sputum smear positive patients per year in the country. For example, if around 7000 eligible patients are diagnosed per year, and if a sample size of 600 patients is required, 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 smear positive patients.

The enrolment could be done either during the same month or on rotation – for example, centres in area 1 during the first month, centres in area 2 during the next month, and so on. In this way, the number of sputum samples sent to the Central Reference Laboratory for culture and DST 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.

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 TB diagnostic centres is high. With this design, centres are randomly selected. To avoid the risk of drawing a sample that misses the largest diagnostic centres, a weighted (probability-proportional to size) cluster sampling technique should be used.

The optimal number of clusters to select depends on the expected inter-cluster variance of the prevalence of drug resistance, and the ratio of the cost of starting a new cluster compared with the cost of adding one patient to an existing cluster (*50*). A minimum number of 30 clusters is recommended. A recommended cluster size between 10 and 40 patients ensures that clusters are not too small,

resulting in high cost and logistic difficulties, or too large, resulting in sampling inefficiency.

Based on a list of all diagnostic centres with the numbers of newly registered patients per year, a cumulative population list is compiled. Assuming the minimum recommended number of 30 clusters is selected, the total number of patients registered per year in all the centres is divided by 30 to obtain the sampling interval. A random number between 1 and the sampling interval is picked, and this determines the first diagnostic centre on the cumulative list to be selected. The sampling interval is sequentially added to the random number to obtain 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 (see Annex 7).

To determine the number of patients per cluster, the required total sample size is divided by the number of clusters. If there is more than one cluster in a diagnostic centre, the number of clusters needed is multiplied by the size of the cluster to calculate the total number of patients needed from that centre. In all selected diagnostic centres, consecutive patients are included in the survey until the number of new cases required is reached. See Annex 7 for a practical example of how to handle cluster selection.

5.5 Formation of a national coordination team

A survey involves three major operational issues:

- programme management (logistics, training, collection of clinical information, supervision of survey);
- standardized laboratory techniques;
- 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 head of the national TB control programme and the head of the Central Reference Laboratory (or persons designated by them), an epidemiologist and a statistician. This team is responsible for the preparation of the survey, for close coordination with the SRL, for supervision and quality assurance during the survey, and for 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.

5.6 Budgeting

The required budget must be carefully calculated in order to ensure the smooth running of a survey and avoid any interruption during implementation. Funds must be available before a 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 between $US 100 000 and $US 150 000 based on an average sample size of approximately 1 000 patients.

All budgets requiring the services of SRLs should include the costs for technical assistance from them, costs of retesting isolates and all lab work, and costs of shipments to and from SRLs for quality assessment of specimens/isolates. Average costs of such items are updated regularly in the WHO tool *Planning and budgeting for TB control activities* (http://www.who.int/tb/dots/planning_budgeting_tool/en/index.html). There may also be important costs associated with the human resources required to process specimens and/or lab running costs. The SRL should be asked to provide the specific costs for these items.

If a partner agency is partially supporting the cost of the survey, then the budget should indicate the source of the resources. Additional information to help in budgeting surveys for grant applications, in particular for the Global Fund to Fight AIDS, Tuberculosis and Malaria, can be found on the following website: http://www.who.int/tb/dots/planningframeworks/gf_tb_proposals_preparation/en/index.html

See Annex 8 for a template of a survey budget.

5.7 Training

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

- enrolment of 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 to the patient, if relevant);
- data entry 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 the intake of patients and of the interviews should be identified and

properly instructed in each diagnostic centre involved in the survey. In general, a meeting can be an efficient way to inform, train and motivate the officers involved.

Training or refresher courses at the peripheral laboratories should be considered on registration of samples, preparation and reading of smears, decontamination of sputum samples, and storage and transport of samples.

5.8 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), 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.

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 culture and DST. All appropriate biosafety measures must be in place before implementation of a survey.

All SRLs have agreed on the basic procedures of drug resistance surveillance as laid out in the guidelines of the Global Project, and are familiar with all standard methods of culture and DST. The partner SRL can guide and advise the national coordinator during the preparation (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 also train staff if required.

The SRLN has developed judicial results for a panel of strains for testing susceptibility to both first- and second-line drugs; any country planning to conduct DST for second-line drugs may now undergo proficiency testing with a partner SRL. If other DST laboratories in addition to the Central Reference Laboratory are included in the survey, they should participate in an initial round of proficiency testing coordinated by an SRL. In subsequent rounds, this process should be taken over by the Central Reference Laboratory.

Proficiency testing in cooperation with a SRL must be completed with good results (i.e. at least 95% agreement for isoniazid and rifampicin) before a survey is implemented. At a minimum, Central Reference Laboratories should have no more than one error for either isoniazid or rifampicin. Laboratories with substandard performance in proficiency testing should implement quality improve-

ment 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 appear 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.

5.9 Pilot study

Depending on the local conditions, it can be useful to organize a time-limited pilot trial in a subregion or district to test logistics (including patient identification and classification, sputum collection, processing and shipment, recovery rate of primary culture, documentation and coordination) and quality of training. The pilot study can serve to identify and solve unexpected problems.

6.
Survey logistics

The following logistics are described for surveys based on a sampling frame of newly registered smear positive cases (new and/or previously treated). Logistics may vary, depending on the capacity of diagnostic centres for smear and culture, the capacity of the Central Reference Laboratory for DST, and the availability of rapid DST methods for direct testing of clinical specimens.

6.1 Inclusion and exclusion criteria

In general, a patient is eligible to be included in the survey if registered as a new sputum smear positive case (or, if the survey has been designed to also include previously treated cases as a newly registered smear positive case with history of previous treatment), according to WHO/Union definitions of smear positivity during the established intake period.

Children under 15 years old who meet the admission criteria may also be included, in accordance with local laws stipulating parental consent.

Extrapulmonary TB and smear negative cases usually are excluded from surveys. New patients who have already started TB treatment should also be excluded. The reason for this exclusion is that after several days of treatment, a significant proportion of patients' cultures would fail to grow. In addition, patients who submit sputum samples after starting treatment, and in whom a positive smear is observed, would more likely be found to be harbouring initially drug resistant strains, thus introducing bias. Likewise, if a survey includes previously treated patients, they should be excluded if he/she has already started a retreatment regimen after being re-registered.

6.2 Patient intake

Each patient who meets the inclusion criteria should be assigned a serial identification number that will be used on all patient forms, including the clinical information form and all laboratory forms (e.g. sputum shipment and laboratory results forms). The serial number permits identification at the diagnostic centre in case the patient has a resistant strain or when additional information is required. As a measure of quality control, when consecutive patients are to be

included, completeness of enrolment should be checked against the TB District Register and the TB Laboratory Register.

Patients should be enrolled in the survey and submit one sputum sample for use in the survey when they are registered for treatment, before treatment has actually started (or, if the survey includes previously treated patients, when the patients are re-registered for retreatment, before retreatment has actually started).

If a probability-proportional to size cluster sampling design has been chosen, the protocol should be adhered to in order to ensure that the target cluster size is reached in each cluster. This will simplify later data analysis.

6.2.1 Clinical information form

The main objective of the clinical information form is to correctly identify any past treatment of a patient. The clinical information form (see Annex 9) consists of four sets 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;
- final decision on history of previous treatment for TB.

This form collects a minimal set of information necessary for programme monitoring, and for allowing analysis of determinants of drug resistance. Therefore, this information should be collected in every survey.

Countries may decide to collect additional information such as HIV status, country or region of origin, place of previous treatment, etc. In principle, only information that is obtainable, reliable, and useful from a programmatic perspective should be added, in a way that allows analysis. The denominator must be known for each variable collected. For example, if TB patients are to be stratified by country of origin, all must be asked to provide such information. If a decision is made to obtain all patients' HIV statuses, a detailed protocol should be prepared in line with existing national guidelines in order to ensure confidentiality and counselling for all patients (*51*).

The number of eligible patients (computed from TB registries) and the number of patients actually included in each cluster should be compared regularly during the enrolment period to identify reasons why some patients may not have been enrolled in the survey, and to reduce the likelihood that eligible patients could be missed from being included in the survey.

A copy of the completed clinical information form should be sent to the coordination team, while the original should be 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 subsequent 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 intake forms to help elicit an accurate treatment history of patients. The collected interview 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%) can be re-interviewed by someone assigned by the coordination team to evaluate the accuracy of treatment histories recorded. Furthermore, all patients with MDR-TB should be re-interviewed – particularly new patients. Verification of treatment history is particularly essential in places where the practise is to provide incentives only to new patients, 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 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, due to the comprehensive patient treatment history recorded in surveys.

6.3 Sputum collection, processing and transport

The correct collection, processing and timely transportation of samples to the Central Reference Laboratory (or other selected culture laboratories) is essential to ensure that results are accurate and reliable.

Diagnostic centres should send one of the initial sputum samples used for diagnosis to the Central Reference Laboratory; ideally it should be a morning specimen. **Treatment for any period of time will reduce the chance of culture positivity. Therefore, samples must be obtained before treatment is started**.

Collecting a good sputum sample requires that the patient be given clear instructions. 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 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 crushing 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 a refrigerator at +4 °C. For homogenization of the mucus and organic debris

and for decontamination on transit, an amount of 0.6% cetylpyridinium bromide (CPB) or 1% cetylpyridinium chloride (CPC), equal to the volume of the sputum, should be added if it is likely that the samples may be exposed to room temperature for extended periods between collection and processing in the culture laboratory. Note: CPB and CPC is strictly not permitted for liquid media.

The patient's serial number from the centre's register should be written on the container (not on the lid).

The local forms used by laboratories to accompany sputum specimens during shipment and request laboratory analysis should be used in a survey and modified as needed.

6.4 Central reference laboratory processes

6.4.1 Decontamination

Decontamination of sputum specimens has two objectives:

- destruction of bacteria other than mycobacteria;
- homogenization.

The aim of decontamination is to kill as much of the contaminating flora as possible while harming as few mycobacteria as possible. Theoretically, many different techniques are available, but none of them is ideal.

The CPB/CPC and trisodium phosphate methods were proposed as a means of digesting and decontaminating sputum in transit (during transportation from peripheral diagnostic centres to the Central Reference Laboratory), but cannot be used for inoculation in liquid media. 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 (*38*). The use of both cetylpyridinium chloride and the Petroff method on the same sputum sample may be harmful to the mycobacteria. Therefore, the Petroff method in combination with CPB/CPC should not be used.

6.4.2 Cultures

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, when a transport medium containing CPC or CPB is used, specimens should not be stored in a refrigerator because of the likelihood of crystallization at cool ambient temperature. Once crystallized, CPC/CPB cannot protect specimens from contamination and will inhibit the growth of *M. tuberculosis* if transferred onto culture medium. Before inoculation of media, CPC/CPB in the specimens should be discarded by centrifugation, which should be carried out without refrigeration to prevent crystallization.

Biosafety measures

All procedures involving the handling of specimens for culture and DST should be carried out in a certified and well-maintained biosafety cabinet. 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 10.

Specimens other than those placed in transport media are decontaminated and homogenized by the modified Petroff method, with an equal volume of 4% NaOH, mixed in a screw-capped tube, digested for 15 minutes with occasional shaking, and then centrifuged at 2000–3000g for 15 minutes. Sediment is then washed with 15 ml of saline or distilled water, and centrifuged for another 15 minutes. (The total contact time between NaOH and the specimen should not exceed 30 minutes unless the specimen is strongly contaminated, in which case the incubation time may be extended by 15 minutes.)

In a simpler method, NaOH-decontaminated specimens are inoculated directly onto acid-buffered medium (such as acidified Ogawa medium) without centrifugation and neutralization. This method is recommended for laboratories that do not have enough resources and biosafety conditions to implement the conventional Petroff method. Sputum specimens collected for drug resistance surveillance are smear positive, contain numerous bacilli, and may therefore not require centrifugation to concentrate the TB bacilli; however, this method may be more prone to contamination than the Petroff method.

Decontamination with N-acetyl–L-cysteine sodium hydroxide (NALC-NaOH) is recommended for automated detection culture systems. However, fast transport of specimens is a prerequisite to minimize contamination.

All positive cultures should be kept until rechecking at the SRL has been completed or the strain has been excluded from further testing. Ideally, they should be stored in a deep-freezer at –20 °C, but they can also be kept for some time in a refrigerator at +4 °C.

6.4.3 Identification

A tube with 500 µg/mL para-nitro benzoic acid should be inoculated together with the drug-containing tubes of every DST set up. TB complex bacilli will not grow (except with too heavy inoculation, as shown by the controls), while virtually all other mycobacteria will grow.

Preliminary identification of the isolates will be based on acid-fastness and cord formation. If colonial morphology is consistent with *M. tuberculosis* complex, only one culture per patient needs to be identified.

Conventional identification of *M. tuberculosis* will be based on at least one conventional biochemical test (for example, the niacin production test, the nitrate reduction test, and/or the catalase tests).

Alternatively, rapid species identification based on molecular tests, such as nucleic acid probe tests or immunoassay is possible, and is required when liquid culture is used. Within 15 minutes, this simple lateral flow immunechromatographic strip test can confirm *M. tuberculosis* from culture, and is easy to use (dipstick format). Conventional phenotypic and biochemical methods take several weeks to obtain results, and require a high degree of technical proficiency and manipulation of infectious cultures, an added biohazard risk.

Lack of growth on para-nitro benzoic acid, and species confirmation by either biochemical identification or strip test technology confirms *M. tuberculosis*. Mycobacterial strains other than *M. tuberculosis* will not be further considered for the purpose of the survey.

6.4.4 Internal quality assurance at the survey laboratory

The laboratory that processes the survey strains should monitor the rates of contaminated and false negative cultures monthly. Feedback based on this monitoring should be given to the survey coordinator in case high rates are observed for particular sampling units, i.e. in combination with excessively long transit times. More attention may then be needed to assure fast transport, or CPB/CPC may be needed, or in cases of high false negative rates, patients may already have been given TB drugs before the sample was taken.

The contamination rates can be expressed as the percentage of contaminated tubes with all inoculated tubes of the period as the denominator; or, less sensitive but of more practical importance, as the number of samples for which all tubes were contaminated as a percentage of all specimens put on culture.

False negative rates apply only to new smear positive patients and smear positive relapses as the denominator, with those for which all or at least one tube stayed negative and none were positive as the nominator.

The lab should monitor culture-positive results and resistant results by cluster for evidence of possible cross-contamination.

6.4.5 Susceptibility testing, including rechecking

Susceptibility testing should be performed *on only one isolate for each patient*. Participating laboratories should use the DST method with which they are most familiar, provided that it is one of the WHO-recommended methods described in the previous section 3.1 *WHO-recommended methods of drug susceptibility testing*. This is to eliminate variability which would arise from changing to a new testing procedure.

Prior to a survey, the participating laboratories should have proven evidence of proficiency by participating in at least one round of DST proficiency testing

with an SRL. The laboratory should have a well-established system of quality assurance, as described in section 3.4 *Quality assurance of drug susceptibility testing*.

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 coordination team and to the diagnostic centre.

Quality assessment of drug susceptibility results, also known as "rechecking"

In implementing a drug resistance survey, a sample of strains isolated should be sent from the Central Reference Laboratory to the partner SRL to be retested as a measure of quality assurance. The results should be compared for agreement with respect to each drug. Sample analysis sheets for first-line drugs and second-line drugs are shown in Annexes 5A and 5B, respectively.

The following groups should be considered when computing sample size for sample rechecking to assess quality of drug susceptibility test results for isoniazid and rifampicin at the Central Reference Laboratory:

- Group 1: strains diagnosed as MDR by the Central Reference Laboratory;
- Group 2: strains diagnosed with resistance to isoniazid or rifampicin but not MDR by the Central Reference Laboratory;
- Group 3: strains diagnosed fully sensitive to both isoniazid and rifampicin by the Central Reference Laboratory.

For rechecking purposes, a randomly selected sample of strains from each of groups 1–3 should be sent to the SRL. Annex 11 details sample size computations which should be done separately for each of the three groups.

If performance of the Central Reference Laboratory is found to be unsatisfactory for any group, then consider retesting all strains from that group (or a large random sample of strains from that group) at the SRL. Report SRL results instead of those from the Central Reference Laboratory.

In countries where exportation of strains is not permitted, a rechecking exercise should take place with another laboratory participating in proficiency testing exercises with an SRL.

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, manipulating, analysing, and storing/archiving data from the start of the study to its completion. Data management systems should address:

- data acquisition;
- confidentiality of data;
- electronic data capture;
- data management training for investigators and staff;
- completion of questionnaires and other survey-related documents, and procedures for correcting errors in these documents;
- coding/terminology for patient characteristics and medical history (data dictionaries);
- data entry/verification (double entry) and data processing (including laboratory data);
- database closure;
- database validation;
- 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 development of a centrally-managed database.[1] 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

[1] On the WHO drug resistance surveillance website (http://www.who.int/tb/challenges/mdr/surveillance), one can find a specification document describing the necessary features of a database for a drug resistance survey.

procedures and processes for creating accurate, complete, verifiable data with source documents (primary data), and data that follow exactly the data protocols in the survey, as well as for making this data available for analysis. The plan should include the following: monitoring the survey and then transferring, sorting and filing, entering, validating, and cleaning the data, and finally making the data available for data analysis. The use of barcodes is recommended to match data for the same patient from different data sources and even physical locations (TB clinic, laboratory, Supranational Reference Laboratory).

At regular intervals (not exceeding two to three months) during the intake period, the coordination team should tabulate all data produced by the diagnostic centres and the Central Reference Laboratory. The coordination team's epidemiologist should make regular reports based on these tables to the managers of the national TB control programme and the Central Reference Laboratory. These reports should include information on the enrolment of patients, quality of clinical information collected, transport or logistic problems, and contamination of samples. If the data or comments suggest that a significant problem has occurred, the national coordinator and the managers of the national TB control programme and Central Reference Laboratory should analyse the situation and develop a plan of action. Missing information should be requested from the respective centres; the sooner after receipt of a specimen, the better.

About halfway through the survey, the national coordinator and the managers of the national TB contol programme and the Central Reference Laboratory should meet to discuss the quality of data collection, laboratory procedures, quality control results, and preliminary survey results, including interpretation.

7.2 Data analysis

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 diagnostic centre (cluster) with the expected number based on the sampling method (the target sample size is usually based on new cases, not previously treated cases), disaggregated by treatment history. Tabulations of data by cluster allow to explore the extent of missing data for each of the outcome and individual characteristic variables.
- *Analysis of missing value patterns*. Missing values should be described for each drug tested (and other important variables such as treatment history) and tabulated by cluster. Typically, when a drug susceptibility result is missing, then results for all first-line drugs are also missing. This is often because cultures failed to grow. The percentage of individuals for whom data on drug resistance to isoniazid and/or rifampicin is missing should be summarized by age group, sex, treatment history, and cluster.

- *Analysis of drug resistance patterns*. A table describing the proportions of patients with resistance to individual drugs, and to different combinations of drugs (the most important being the combination of resistance to isoniazid and to rifampicin), among patients classified as new and those classified as previously treated patients is essential. Tables of aggregated numbers of cases are shown in Annexes 1A and 1B for susceptibility to first-line and second-line drugs, respectively, among new and previously treated patients. These tables include subcategories of previously treated patients, allowing for evaluation of proportions among this heterogeneous group. These analyses should be done both with, and without, multiple imputation for missing data.

  The tables in Annexes 1A and 1B should only be used to report results when a 100% sampling approach is used and the number of missing data is negligible. In this case, to calculate the proportion of resistance to a drug, the denominator is the number of cases for which drug susceptibility results are available and confidence intervals for calculated proportions can be computed using standard simple methods. Otherwise, when cluster sampling has been used and/or when a significant number of cases have missing data, procedures must be implemented to take into consideration sampling design effects and missing data. These procedures will result only in *proportions* (not absolute numbers) of patients having various drug resistance patterns. In such cases, the adjusted proportions and confidence limits should be reported.

- *Analysis of determinants of resistance*. Depending on the patient biographical and clinical data collected, further comparisons based on sex, age groups, HIV status, country of origin, etc. should be evaluated. The Global Project collects and reports data on MDR-TB stratified by age groups and sex (Annex 1C) and stratified by HIV status (Annex 1D).

Any standard data analysis software can be used for analysis of drug resistance data from routine testing or using simple random sampling of patients, including the 100% sampling of diagnostic centres method. However, specialized statistical software such as Stata (http://www.statacorp.com), R (freely available from http://www.r-project.org) or others, is needed to analyse drug resistance data from national surveys with cluster sampling. The reason for specialized

Cluster sampling design

If a cluster sampling design has been chosen, it is recommended to adopt a probability-proportional to size (PPS) sampling procedure and then to adhere strictly to protocol during the course of the survey, including ensuring that the target cluster size is reached in each cluster. If the sampling was PPS and cluster size is the same or similar among clusters, it is not recommended to use weights, and the analysis will be relatively simple.

software is the need to account for missing data, and sampling design effects on standard errors. **Practical and detailed steps for analyzing an example cluster survey dataset are available for download at: http://www.who.int/tb/publications/mdr_surveillance, using methods for multiple imputation of missing data and logistic regression with robust errors**.

7.2.1 Imputation of missing values

It is important to report numbers of results missing as a result, for example, of contamination, negative cultures, or insufficient growth for susceptibility testing. Patterns of missing data should then be analysed carefully, in particular across diagnostic centres/clusters and in relation to other variables such as age, sex, and history of treatment. It is not unusual to observe higher levels of missing data in remote diagnostic centres/clusters due to a longer time to process sputum samples, resulting in more contaminations or failures of cultures to grow.

A common procedure applied in the past to missing drug resistance data is to perform record-wise deletion; that is, remove records for which drug susceptibility data are not available. The result is a loss of valuable information at best, and severe selection bias at worst (*52*). A procedure based on the concept of "multiple imputation" of missing data, that is not difficult to use although it should be used with care (*53*), is recommended instead.

Missing values in processes that are missing completely at random (MCAR) cannot be predicted any better with observed information. An example of an MCAR process is one in which culture tubes are discarded randomly in the laboratory and therefore, not tested for drug susceptibility. Of course, the MCAR assumption rarely applies: If culture tubes are not tested because cultures failed to grow, then the data are not necessarily MCAR, and failure to grow may be associated with particular cluster or patient characteristics. For missing at random (MAR) processes, the probability that a particular piece of data is missing may depend on observed data, but not on unobserved data. The process would be MAR if missing data patterns can be predicted with other variables in the data set (such as cluster identifiers; distance from cluster to processing laboratory; time to process the sample; and patient characteristics such as age, sex and treatment history). The prediction required is not causal. Finally, if the probability that a particular piece of data is missing depends on the unobserved value of the missing response, the process is nonignorable (NI).

Inferences from analyses using record-wise deletion are relatively inefficient unless MCAR applies. More importantly, they are biased unless MCAR holds. Inferences based on multiple imputation are more efficient than record-wise deletion (since no observed data are discarded), and they are not biased under MCAR or MAR (*54*). Both record-wise deletion and basic multiple imputation approaches can be biased under NI, in which case additional steps must be taken (such as a sensitivity analysis), or different models must be chosen, to ensure

valid inferences. Thus, multiple imputation will normally be better than, and almost always not worse than, record-wise deletion.

Multiple imputation involves assigning *m* values for each missing item and creating *m* completed data sets. Across completed data sets, the observed values are the same, but the missing values are filled in with different imputations to reflect uncertainty levels. That is, for missing cells that the model predicts well, variation across the imputations is small; for other cases the variation may be larger to reflect whatever knowledge and level of certainty is available about the missing information. Analysts can then conveniently apply the statistical method they would have used if there were no missing values to each of the *m* data sets, and use a simple procedure to combine the *m* results (*52*). Usually, the number *m* of multiply-imputed datasets is set at 5 or 10, and 10 is usually sufficient.

7.2.2 Sampling design effects on standard errors

Apart from addressing potential biases created by data that are missing, but not completely at random, the second major feature of a cluster sample survey that must be addressed correctly 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. Similarly, 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, may lead to incorrect interpretations and conclusions.

To account for intra-cluster correlation, robust standard errors should be computed, and it is straightforward to do this using an individual-level analysis of the survey data in a statistical package through logistic regression. If a PPS sampling method was used, and cluster size is constant (or shows little variation), weights should not be applied to individual records.

In an ordinary logistic regression model, the probability model for individual *j* in cluster *i* ignores the clustering:

$$\Pr(y_{ij} = 1) = \text{logit}^{-1}(\alpha + \beta h)$$

where α is the estimated intercept and logit^{-1} is the inverse logit function, defined over the domain of real numbers, such as $\text{logit}^{-1}(x) = \exp(x)/(1+\exp(x))$; h is an indicator variable of treatment history, coded 0 if new and 1 if retreatment; and β is the model coefficient estimate for the treatment history indicator.

The model predicted probability of resistance given absence of past exposure to TB drugs (patient classified as new) is provided by the following equation:

$$\Pr(y = 1 \mid h = 0) = \text{logit}^{-1}(\alpha)$$

The model predicted probability of resistance given past exposure to TB drugs (patient classified as retreatment) is provided by the following equation:

$$\Pr(y = 1 \mid h = 1) = \text{logit}^{-1}(\alpha + \beta)$$

A complication arises from inter-cluster variation, often described by the term over-dispersion. Over-dispersion is measured by comparing the sum of squared standardized residuals, calculated at the level of a cluster, to a $\chi 2$ distribution with $n–k$ degrees of freedom, where n is the number of data points (in this case clusters) and k is the number of estimated model parameters. In quasi-binomial logistic regression models, the standard deviation has the form $\sqrt{\omega np(1-p)}$, where $\omega > 1$ is the over-dispersion parameter, n is cluster size and p is the proportion with the outcome. Without adjustment for over-dispersion, confidence intervals would be too narrow, and the precision of estimates would be overstated. Robust standard errors provide a correction, when $\omega > 1$.

7.2.3 Other considerations for data analysis

For settings in which PPS sampling was selected as the sampling strategy, if some diagnostic units were not able to collect the required amount of cases during the intake period, existing data from these units should be compared with completed units. If actual cluster sizes differ greatly between the units (although this will not happen if study protocol is adhered to), then the actual sampling can no longer be considered to be probability-proportional to size. If there is concern that, when study protocol has not been adhered to, cluster size might be associated with MDR prevalence, then 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 and there is a large variation of actual cluster size due to difficulties in enrolling patients in some clusters, then weights within a given cluster will be equal to 27 divided by the actual number enrolled. Such calculations should be done for all records, regardless of whether drug susceptibility testing was successful and results are available. The analysis should be done with and without weights, and differences in model coefficients should be analysed carefully.

A complication arises when the recruitment period is extended for previously treated patients, particularly if the extension is done differently between clusters. It is important that if the recruitment period is extended for previously treated patients, it needs to be done in exactly the same way across all clusters. This can be achieved by continuing to recruit all previously treated TB cases during the time that a fixed total number of additional TB cases are registered (with this fixed total combining new and previously treated cases). This will ensure that the previously treated patients who are included in the sample survey are representative of the total population of previously treated cases.

It should be noted that the ratio of retreatment to new patients in a survey sample may not reflect the ratio of retreatment patients to new patients notified by the TB programme at national level. Reasons for the difference may include (1) a higher misclassification rate of treatment history under routine practise compared with survey practise; (2) an extended recruitment period for retreatment patients to inflate sample size in that group; (3) random errors due to sampling.

It is not recommended to use weights when assessing potential risk factors for drug resistance such as HIV or age group using multivariable logistic regression models.

7.3 Interpretation of results

The presence of drug resistance among new cases reflects creation and transmission of drug resistant strains over many years, and cannot be readily used to assess the quality and performance of a national TB contol programme. An established national TB control programme that adopts standardized chemotherapy and an effective control programme will see a subsequent reduction in drug resistance among new cases, although this may take a long time to become significant, since patients infected with resistant strains may become ill only after many years. High proportions of resistance among new cases may also indicate that some previously treated patients had been misclassified as new cases. In these instances, important corrections may be possible by re-interviewing all new subjects that show drug resistance. Cross-contamination in laboratory processes may also be the 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.

High proportions of resistance among previously treated cases may indicate a problem with programme performance, particularly when resistance among new cases is also high and subsequent surveys or periodic monitoring do not indicate a declining trend. This would be through acquisition of resistance during treatment. However, acquired resistance can only be shown when both strains before and after treatment can be compared, which is only exceptionally the case for survey strains. Other factors are also in play, and in general not much can be concluded about recent performance without any information on recent trends. High proportions of resistance among previously treated cases may also reflect cases reporting after treatment in the private health sector, particularly where this sector plays an important role in the country and mismanagement of cases is an issue.

Previously treated cases are a heterogeneous group, and differentiation by subcategory can result in stronger analysis and more targeted conclusions and

recommendations. Various factors promote acquired resistance among previously treated cases, including unsupervised treatment; inadequate drug regimens; availability of anti-tuberculosis drugs without physician prescription or oversight; poor quality of the drugs supplied; weaknesses in methods for declaring patients successfully cured; and substandard infection control.

Periodic survey results and trends should always be interpreted within the context of the overall programme and should consider 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 negative socioeconomic events or drug shortages; and so on. This allows for more robust interpretation of drug resistance surveillance data.

References

1. *Guidelines for surveillance of drug resistance in tuberculosis*. Geneva, World Health Organization, 1994, (document WHO/TB/94.178).
2. *Guidelines for surveillance of drug resistance in tuberculosis*. Geneva, World Health Organization, 1997, (document WHO/TB/96.216).
3. *Guidelines for surveillance of drug resistance in tuberculosis*. Geneva, World Health Organization, 2003, (document WHO/TB/2003.320).
4. *Interim recommendations for the surveillance of drug resistance in tuberculosis*. Geneva, World Health Organization, 2007, (document WHO/HTM/TB/2007.385).
5. *Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs*. Geneva, World Health Organization, 2008, (document WHO/HTM/TB/2008.392).
6. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB): Policy Statement. Geneva, World Health Organization, 27 June 2008; http://www.who.int/tb/dots/laboratory/lpa_policy.pdf, accessed 6 September 2009.
7. *Anti-tuberculosis drug resistance in the world: the WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance*. Geneva, World Health Organization, 1997, (document WHO/TB/97.229).
8. Cohn DL et al. Drug-resistant tuberculosis: review of the worldwide situation and the WHO/IUATLD Global Surveillance Project. International Union Against Tuberculosis and Lung Disease. *Clinical Infectious Diseases*, 1997 Jan, 24(Suppl. 1):S121–30.
9. Tuberculosis. The WHO/IUATLD Global Project on Antituberculosis Drug Resistance Surveillance. *Weekly Epidemiological Records*, 1996, 71:281–286.
10. Pablos-Méndez A et al. Global surveillance for antituberculosis-drug resistance, 1994–1997. World Health Organization-International Union against Tuberculosis and Lung Disease Working Group on Anti-Tuberculosis Drug Resistance Surveillance. *New England Journal of Medicine*, 1998, 338(23):1641–1649.
11. *Anti-tuberculosis drug resistance in the world: the WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Report 2: Prevalence and trends*. Geneva, World Health Organization, 2000, (document WHO/CDS/TB/2000.278).
12. Espinal MA et al. Global trends in resistance to antituberculosis drugs. World Health Organization-International Union against Tuberculosis and Lung Disease Working Group on Anti-Tuberculosis Drug Resistance Surveillance. *New England Journal of Medicine*, 2001, 344(17):1294–1303.
13. *Anti-tuberculosis drug resistance in the world: the WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Third global report*. Geneva, World Health Organization, 2004, (document WHO/HTM/TB/2004.343).

14. Aziz MA et al. WHO/International Union Against Tuberculosis And Lung Disease Global Project on Anti-tuberculosis Drug Resistance Surveillance. Epidemiology of antituberculosis drug resistance (the Global Project on Anti-tuberculosis Drug Resistance Surveillance): an updated analysis. *Lancet*, 2006, 368(9553):2142–2154.
15. *Anti-tuberculosis drug resistance in the world: the WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Fourth global report*. Geneva, World Health Organization, 2008, (document WHO/HTM/TB/2008.394).
16. Wright et al. Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Epidemiology of antituberculosis drug resistance 2002–07: an updated analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance. *Lancet*, 2009, 373(9678):1861–1873.
17. Raviglione MC, Uplekar MW. WHO's new Stop TB Strategy. *Lancet*, 2006, 367(9514): 952–955.
18. Maher D et al. Planning to improve global health: the next decade of tuberculosis control. *Bulletin of the World Health Organization*, 2007, 85(5):341–347.
19. The global plan to stop TB 2006–1015, Stop TB Partnership. World Health Organization, 2006, (document: WHO/HTM/STB/2006.35).
20. Resolution WHA60.19. Tuberculosis control: progress and long-term planning. In: World Health Assembly First Special Session, Geneva, 9 November 2006. Resolutions and decision, annex. Sixtieth World Health Assembly, Geneva, 14–23 May 2007. Resolutions and decisions, annexes. Geneva, World Health Organization, 2007 (document WHASS1/2006-WHA60/2007/REC/1):78–81.
21. Resolution WHA62.15. Prevention and control of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. In: Sixty-Second World Health Assembly, Geneva, 18–22 May 2009. Resolutions and decisions, annexes. Geneva, World Health Organization, 2009; http://apps.who.int/gb/ebwha/pdf_files/A62/A62_R15-en.pdf, accessed 6 September 2009.
22. Cohen T et al. Challenges in estimating the total burden of drug-resistant tuberculosis. *American Journal of Respiratory Critical Care and Medicine*, 2008, 177(12):1302–1306.
23. Cohen T et al. Are survey-based estimates of the burden of drug resistant TB too low? Insight from a simulation study. *PLoS ONE*, 2008, 3e2363.
24. *Guidelines for treatment of tuberculosis. Fourth edition*. Geneva, World Health Organization, 2009, (document WHO/HTM/TB/2009.420).
25. *Guidelines for the programmatic management of drug-resistant tuberculosis*. Geneva, World Health Organization, 2008 (document WHO/HTM/TB/2008.402).
26. Van Deun A et al. Evaluation of tuberculosis control by periodic or routine susceptibility testing in previously treated cases. *International Journal of Tuberculosis and Lung Disease*, 2001, 5(4):329–338.
27. Van Rie A et al. Classification of drug-resistance tuberculosis in an epidemic area. *Lancet*, 2000, 356(9223):22–25.
28. *Guidance on provider-initiated HIV testing and counselling in health facilities*. Geneva, World Health Organization, 2007.
29. *Rapid HIV tests: guidelines for use in HIV testing and counselling services in resource-constrained settings*. Geneva, World Health Organization, 2004.
30. Suchindran S et al. Is HIV infection a risk factor for multi-drug resistant tuberculosis? A systematic review. *PLoS ONE*, 2009, 4(5):e5561.
31. Wells CD et al. HIV infection and multidrug-resistant tuberculosis – the perfect storm. *Journal of Infectious Diseases*, 2007, 196(Supplement):pS86–S107.

32. Espinal MA et al. Determinants of drug-resistant tuberculosis: analysis of 11 countries. *International Journal of Tuberculosis and Lung Disease*, 2001 Oct, 5(10):887–893.
33. Lönnroth K et al. Inclusion of information on risk factors, socio-economic status and health seeking in a tuberculosis prevalence survey. *International Journal of Tuberculosis and Lung Disease*, 2009, 13(2):171–176.
34. Canetti G et al. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. *Bulletin of the World Health Organization*, 1969, 41(1):21–43.
35. *Manual of bacteriology of tuberculosis*. Washington, DC, Pan American Health Organization, 1983.
36. Heifets L. Conventional methods for antimicrobial susceptibility testing of *Mycobacterium tuberculosis*. In: Bastian I, Portaels F, eds. *Resurgent and emerging infectious diseases. Multi-drug resistant tuberculosis*. Dordrecht: Kluwer Academic; 2000:135–141.
37. The public health service national tuberculosis reference laboratory and the national laboratory network: minimum requirements, role and operation in a low-income country. Paris, International Union Against Tuberculosis and Lung Disease, 1998.
38. *Laboratory services in tuberculosis control. Part III: Culture*. Geneva, World Health Organization, 1998, (document WHO/TB/98.258).
39. Use of liquid TB culture and drug susceptibility testing (DST) in low and medium income settings. Summary report of the Expert Group Meeting on the use of liquid culture media. Geneva, World Health Organization, 26 March 2007; http://www.who.int/tb/dots/laboratory/Use of Liquid TB Culture_Summary Report.pdf, accessed 6 September 2009.
40. Ling DI et al. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. *European Respiratory Journal*, 2008, 32(5):1165–1174.
41. Laszlo A et al. Quality assurance programme for drug susceptibility testing of Mycobacterium tuberculosis in the WHO/IUATLD Supranational Reference Laboratory Network: five rounds of proficiency testing, 1994–1998. *International Journal of Tuberculosis and Lung Disease*, 2002, 6(9):748–56.
42. Beyer R, Fairchild A. Ethical issues to be considered in second generation surveillance. 2004 Apr 28; http://www.who.int/hiv/pub/epidemiology/en/sgs_ethical.pdf, accessed 6 September 2009.
43. Beauchamp TL, Childress JF. *New ethics for the public's health*, 1st ed. Oxford: Oxford University Press; 1999.
44. *Ethical considerations in developing a public health response to pandemic influenza*. Geneva, World Health Organization, 2007 (document WHO/CDS/EPR/GIP/2007.2).
45. *International ethical guidelines for epidemiological studies*. Geneva: Council for International Organizations of Medical Sciences, 2009.
46. *Toman's tuberculosis, case detection, treatment, and monitoring – questions and answers. Second Edition*. T. Frieden ed. Geneva: World Health Organization, 2004.
47. Lwanga SK, Lemeshow S. *Sample size determination in health studies: a practical manua*l. Geneva, World Health Organization, 1991.
48. Nunn P, Felten M. Surveillance of resistance to anti-tuberculosis drugs in developing countries. *Tubercle and Lung Disease*, 1994, 75(3):163–167.
49. *Measuring change in nutritional status*. Geneva, World Health Organization, 1983, (Annex 2: Statistical aspects of sampling).
50. Williams B et al. The design effect and cluster samples: optimising tuberculosis prevalence surveys. *International Journal of Tuberculosis and Lung Disease*, 2008, 12(10):1110–1115.

51. *Guidelines for HIV surveillance among tuberculosis patients. Second Edition*. Geneva, World Health Organization, 2004, (document WHO/HTM/TB/2004.339).
52. King G et al. Analyzing incomplete political science data: an alternative algorithm for multiple imputation. *American Political Science Review*, 2001, 95(1):49–69.
53. Sterne JA et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *British Medical Journal*, 2009 Jun 29, 338b2393, doi: 10.1136/bmj.b2393.
54. Little JR, Rubin D. The analysis of social science data with missing values. *Sociological Methods and Research*, 1989, 18(November):292–326.

Annexes

ANNEX 1A

First-line anti-tuberculosis drug resistance results

Note: Resistance to H and R should be recorded, at a minimum. Resistance to E and S may be recorded at the NTP's discretion. Surveillance of resistance to E among MDR-TB cases is recommended.

		Previous anti-TB treatment status							
		New		Previously treated [B]		Unknown		Total	
		N	%	N	%	N	%	N	%
Total patients with DST results (H+R) [A]									
I [C]	Any resistance to isoniazid (H)								
	Any resistance to rifampicin (R)								
	Any resistance to ethambutol (E)								
	Any resistance to streptomycin (S)								
II	Resistance to H only								
	Resistance to R only								
	Resistance to E only								
	Resistance to S only								
	Total mono-resistance								
III	H + R								
	H + R + E								
	H + R + S								
	H + R + E + S								
	Total multidrug resistance (MDR) [D]								
IV	H + E								
	H + S								
	H + E + S								
	R + E								
	R + S								
	R + E + S								
	E + S								
	Total poly-resistance other than MDR [E]								

The numbers in this column should correspond to the numbers entered above for all previously treated cases.

		Previously treated cases					
		Total	Relapse	Failure of an initial treatment course with first-line drugs	Failure of a retreatment course with first-line drugs	Failure of a treatment course using second-line drugs	Return after default, other retreatment, or unknown retreatment
		N	N	N	N	N	N
Total patients with DST results (H+R) [A]							
I	Any resistance to H						
III	H + R						
	H + R + E						
	H + R + S						
	H + R + E + S						
Total MDR [D] among previously treated cases							

Notes to Annex 1A

[A] Total number of cases with DST results for at least isoniazid and rifampicin
[B] Previous anti-TB treatment: ≥ 1 month of treatment with anti-TB drugs excluding preventive chemotherapy
[C] For each drug, total resistant cases (part I) should be equal to the sum of cases resistant to this drug in parts II+III+IV (e.g. "Any resistance to H" = (H)+(H+R)+(H+R+E)+ (H+R+S)+(H+R+E+S)+(H+E)+(H+S)+(H+E+S)
[D] Concomitant resistance to isoniazid and rifampicin, with or without resistance to other anti-TB drugs
[E] Resistance to two or more drugs other than MDR

Note: These tables should not be used to report final data from a survey that used cluster sampling or that had significant numbers of missing data. In such surveys, procedures to adjust for the design effect and missing data are necessary, and the final adjusted data can only be reported as proportions (not absolute numbers) with confidence limits of patients having each drug resistance pattern. See chapter 7.2 *Data analysis for more information*.

ANNEX 1B

Second-line anti-tuberculosis drug resistance results

Most commonly tested fluoroquinolone

Other tested fluoroquinolones (FQ)

Tested 2nd-line injectable agent(s) (2LI)

	Previous anti-TB treatment status									
	New		Previously treated		Unknown		Total			
	N	%	N	%	N	%	N	%		
Total MDR-TB patients with DST results for any fluoroquinolone and any 2nd-line injectable agent										
Susceptible to both FQ and 2LI										
Any resistance to FQ										
Any resistance to 2LI										
Any resistance to both FQ and 2LI (XDR)										

The numbers in this column should correspond to the numbers entered above for all previously treated cases.

		Previously treated cases					
	Total	Relapse	Failure of an initial treatment course with first-line drugs	Failure of a retreatment course with first-line drugs	Failure of a treatment course using second-line drugs	Return after default, other retreatment, or unknown retreatment
	N	N	N	N	N	N
Total MDR-TB patients with DST results for any fluoroquinolone and any 2nd-line injectable agent						
Susceptible to both FQ and 2LI						
Any resistance to FQ						
Any resistance to 2LI						
Any resistance to both FQ and 2LI (XDR)						

ANNEX 1C

Multidrug resistance stratified by age groups and sex

	MDR-TB (Resistant to both H and R)									
	Age group									
	0–4	5–14	15–24	25–34	35–44	45–54	55–64	65+	Unknown	**Total**
Male										
Female										
Sex unknown										
Total										

	Not MDR-TB (Not resistant to both H and R)									
	Age group									
	0–4	5–14	15–24	25–34	35–44	45–54	55–64	65+	Unknown	**Total**
Male										
Female										
Sex unknown										
Total										

ANNEX 1D

Multidrug resistance stratified by patient HIV status

	HIV status			
	+	–	Unknown	**Total**
MDR-TB (Resistant to both H and R)				
Not MDR-TB (Not resistant to both H and R)				
Total				

ANNEX 2

Supranational Reference Laboratory List

COUNTRY	SUPRANATIONAL REFERENCE LABORATORY	CONTACT PERSON
Algeria	Laboratoire de la Tuberculose Institut Pasteur d'Algérie 2, rue Laveran 16015 LE HAMMA	Professor Fadila Boulabahl
Argentina	Servicio Micobacterias Instituto Nacional de Enfermedades Infecciosas ANLIS Dr. Carlos Malbran Avda Velez Sarsfield 563 1281 BUENOS AIRES	Dr Lucia Barrera
Australia	Queensland Mycobacterium Reference Laboratory Pathology Queensland Central Laboratory Floor 5, Block 7 Royal Brisbane and Women's Hospital Herston Road HERSTON QLD 4029	Dr Chris Coulter
Australia	Institute of Medical and Veterinary Science Mycobacterium Reference Laboratory Infectious Diseases Laboratories PO Box 14 Rundle Mall ADELAIDE, South Australia 5000	Dr Ivan Bastian
Belgium	Department of Microbiology Mycobacteriology Unit Prince Leopold Institute of Tropical Medicine Nationalestraat 155 2000- ANTWERPEN	Professor Françoise Portaels
Chile	Instituto de Salud Pública de Chile Avenida Marathon Nº 1000 Ñuñoa Almirante Pastene 150 SANTIAGO	Dr Maritza Velazco
Croatia	Croatian National Institute of Public Health Mycobacteriology Department Rockefellerova 7 10000 ZAGREB	Dr Vera Katalinic-Jankovic
Czech Republic	National Institute of Public Health Center of Epidemiology and Microbiology (CEM), Mycobacteriology Unit and NRL for Mycobacteria Srobárova 48 100 42 PRAHA 10	Dr Marta Havelková

COUNTRY	SUPRANATIONAL REFERENCE LABORATORY	CONTACT PERSON
Egypt	National TB Reference Laboratory Central Health Laboratories Ministry of Health and Population 19 El Sheikh Rihan Street, El Tahrir Sq. CAIRO	Dr Mushira Ismail
Germany	Kuratorium Tuberkulose in der Welt e.V. Institut für Mikrobiologie und Laboratoriumsdiagnostik Robert Koch Allee 23 82131- GAUTING	Dr Harald Hoffmann
Germany	National Reference Center for Mycobacteria Forschungszentrum Borstel Parkallee 18 23845 – BORSTEL	Dr Sabine Rüsch-Gerdes
Guadeloupe	TB & Mycobacteria Unit Institut Pasteur de Guadeloupe, Morne Joliviere, BP 484, 97183 Abymes Cedex, GUADELOUPE	Dr Nalin Rastogi
Hong Kong (SAR) China	TB Reference Laboratory Department of Health 7/F, Public Health Laboratory Centre 382 Nam Cheong Street, Shek Kip Mei Kowloon, HONG KONG	Dr Kai Man Kam
India	TB Research Centre Indian Council of Medical Research Mayor VR Ramanathan Road Chetput 6003- CHENNAI 1	Dr Ranjani Ramachandran
Italy	Istituto Superiore di Sanità Dipartimento di Malattie Infettive, Parassitarie e Immunomediate, Viale Regina Elena 299 00161- ROME and San Raffaele del Monte Tabor Foundation (hSR), Emerging bacterial pathogens Via Olgettina 60 20132- MILAN	Dr Lanfranco Fattorini Dr Daniela Cirillo
Japan	Research Institute of Tuberculosis Japan Anti-Tuberculosis Association 3-1-24 Matsuyama Kiyose-Shi 204-8533 TOKYO	Dr Satoshi Mitarai
Latvia	State Centre for TB and Lung Diseases Microbiology Laboratory Miera Iels 80-2 RIGA LV1013	Dr Girts Skenders
Mexico	Departamento de Micobacterias Instituto de Diagnostico y Referencia Epidemiologicos (INDRE) Carpio 470 Colonia Santo Tomas Delegacion Miguel Hidalgo CP 11340 MEXICO DF	Dr Claudia Backer

COUNTRY	SUPRANATIONAL REFERENCE LABORATORY	CONTACT PERSON
Netherlands	National Institute of Public Health and the Environment (RIVM), PO BOX 1 3720 BA BILTHOVEN	Dr Dick van Soolingen
Portugal	Centro de Tuberculose e Micobacterias (CTM) Instituto Nacional de Saude – Delegação do Porto INSA/IBMC Rua do Campo Alegre 823 4150-180 PORTO	Dr Maria Filomena Rodrigues
Republic of Korea	Korean Institute of Tuberculosis 14 Woomyundong, Sochogu SEOUL 137-140	Dr Woojin Lew
South Africa	TB Research Lead Programme Operational and Policy Research The Medical Research Council Cnr Theodore Ave & Soutpansberg Road Private Bag X385 0001 PRETORIA Arcadia	Dr Matsie Mphahlele
Spain	Servicio de Microbiologia Hospital Universitaris, Vall d'Hebron 08035 BARCELONA	Dr Nuria Martin-Casabona
Sweden	Swedish Institute for Infectious Disease Control Nobels väg 18 SE-171 82 SOLNA	Dr Sven Hoffner
Thailand	National TB Reference Laboratory Center Tuberculosis Cluster 3331/116 Sudprasert Road Bangkhlo BANGKOK 10120	Dr Somsak Rienthong
United Kingdom	HPA Mycobacterium Reference Unit, Clinical TB and HIV Group, Centre for Infectious Disease, Barts and The London School of Medicine and Dentistry, 2 Newark Street, LONDON E1 2AT	Dr Francis Drobniewski
United States of America	Department of Public Health Massachusetts State Laboratory Institute 305 South Street JAMAICA PLAIN, MA 02130	Dr Alexander Sloutsky
United States of America	Centers for Disease Control and Prevention Mycobacteriology/ Tuberculosis Laboratory Mail Stop F-08 1600 Clifton Road, N.E. ATLANTA, GA 3033	Dr Beverly Metchock

For the most up-to-date contact information, see the link to the Supranational Reference Laboratory Network on the Global Laboratory Initiative page at http://www.who.int/tb

ANNEX 3A

Example of a proficiency testing results form (first-line drugs)

Sample information written in *italics*

LAB NAME	*NRL of (Country)*			
DATE STRAINS ARRIVED	*28.05.2009*		DATE RESULTS REPORTED	*01.09.2009*
Culture code no.	**SM base** ***4*** **µg/ml**	**H** ***0.2*** **µg/ml**	**R** ***40*** **µg/ml**	**EMB base** ***2*** **µg/ml**
1226	*R*	*R*	*S*	*R*
2644	*S*	*S*	*S*	*S*
2840	*R*	*S*	*S*	*S*
2971	*R*	*S*	*R*	*S*
3019	*R*	*R*	*R*	*S*
3684	*R*	*R*	*S*	*S*
3979	*R*	*R*	*R*	*S*
4011	*R*	*R*	*R*	*S*
4085	*R*	*R*	*R*	*S*
4156	*S*	*R*	*S*	*S*
4452	*S*	*R*	*S*	*R*
4775	*S*	*S*	*S*	*S*
4813	*R*	*S*	*S*	*S*
4933	*R*	*S*	*R*	*S*
4984	*R*	*R*	*R*	*S*
5525	*R*	*R*	*S*	*S*
6058	*R*	*R*	*R*	*S*
6392	*R*	*R*	*R*	*R*
6587	*R*	*R*	*R*	*S*
7110	*S*	*R*	*S*	*S*
7564	*S*	*S*	*R*	*S*
7742	*S*	*S*	*S*	*S*
7823	*S*	*S*	*S*	*S*
8240	*R*	*R*	*S*	*S*
8785	*R*	*S*	*S*	*S*
9219	*S*	*S*	*S*	*S*
9590	*S*	*S*	*S*	*S*
9601	*S*	*S*	*S*	*S*
9606	*R*	*R*	*R*	*S*
9870	*S*	*R*	*R*	*S*

Resistant = R
Susceptible = S
Contamination = C
No growth = NG

ID method used *Niacin test*

DST Method used *Proportion method*
Medium *LJ Medium*

ANNEX 3B

Example of a proficiency testing results form (second-line drugs)

Sample information written in *italics*

LAB NAME	*NRL of (Country)*			
DATE STRAINS ARRIVED	*28.05.2009*		DATE RESULTS REPORTED	*01.09.2009*
Culture code no.	**Kanamycin base** ***30*** **µg/ml**	**Amikacin base** ***Not tested*** **µg/ml**	**Capreomycin base** ***40*** **µg/ml**	**Ofloxacin** ***2*** **µg/ml**
1226	*S*		*R*	*R*
2644	*S*		*R*	*R*
2840	*R*		*R*	*R*
2971	*S*		*R*	*R*
3019	*S*		*S*	*S*
3684	*S*		*S*	*R*
3979	*S*		*R*	*S*
4011	*S*		*S*	*S*
4085	*S*		*R*	*S*
4156	*S*		*R*	*S*
4452	*S*		*R*	*R*
4775	*S*		*R*	*R*
4813	*S*		*R*	*R*
4933	*S*		*R*	*R*
4984	*S*		*S*	*S*
5525	*S*		*S*	*R*
6058	*S*		*R*	*S*
6392	*S*		*S*	*S*
6587	*S*		*R*	*S*
7110	*S*		*R*	*S*
7564	*S*		*S*	*R*
7742	*S*		*S*	*S*
7823	*S*		*S*	*S*
8240	*S*		*R*	*R*
8785	*S*		*S*	*S*
9219	*R*		*R*	*S*
9590	*S*		*S*	*S*
9601	*S*		*R*	*S*
9606	*R*		*R*	*R*
9870	*S*		*S*	*S*

Resistant = R
Susceptible = S
Contamination = C
No growth = NG

ID method used *Niacin test*

DST Method used *Proportion method*
Medium *LJ Medium*

ANNEX 4A

Example of a proficiency testing analysis sheet (first-line drugs)

LAB NAME: *NRL of (Country)*

	Strain code	**Reported test results** R = resistant S = susceptible NG = no growth, or contaminated				**Score** (compared with judicial SRLN results) 1 = correct R = false resistant S = false susceptible			
		DRUGS TESTED				DRUGS TESTED			
		S	**H**	**R**	**E**	**S**	**H**	**R**	**E**
1A	1226	R	R	S	R	R	1	1	1
2A	2644	S	S	S	S	1	1	1	1
3A	2840	R	S	S	S	1	1	1	1
4A	2971	R	S	R	S	1	1	1	1
5A	3019	R	R	R	S	1	1	1	S
6A	3684	R	R	S	S	1	1	1	S
7A	3979	R	R	R	S	1	1	R	1
8A	4011	R	R	R	S	1	1	1	S
9A	4085	R	R	R	S	1	1	1	S
10A	4156	S	R	S	S	1	1	1	1
1B	4452	S	R	S	R	1	1	1	1
2B	4775	S	S	S	S	1	1	1	1
3B	4813	R	S	S	S	1	1	1	1
4B	4933	R	S	R	S	1	1	1	1
5B	4984	R	R	R	S	1	1	1	S
6B	5525	R	R	S	S	1	1	1	S
7B	6058	R	R	R	S	1	1	R	1
8B	6392	R	R	R	R	1	1	1	1
9B	6587	R	R	R	S	1	1	1	S
10B	7110	S	R	S	S	1	1	1	1
11	7564	S	S	R	S	1	1	1	1
12	7742	S	S	S	S	1	1	1	1
13	7823	S	S	S	S	S	1	1	1
14	8240	R	R	S	S	1	1	1	1
15	8785	R	S	S	S	1	1	1	1
16	9219	S	S	S	S	1	1	1	1
17	9590	S	S	S	S	1	1	1	1
18	9601	S	S	S	S	1	1	1	1
19	9606	R	R	R	S	1	1	1	S
20	9870	S	R	R	S	1	1	1	1

Method used: 1*
1* Proportion method LJ
2* Proportion method agar
3* Bactec 460
4* Resistance ratio
5* Absolute conc.
6* MGIT

Arrival:
Strains: *28 May 2009*
Results: *01 Sep 2009*
Turn-over time: *95 days*

Total correct results	**28**	**30**	**28**	**22**
True resistant	**17**	**17**	**11**	**3**
False resistant	**1**	**0**	**2**	**0**
True susceptible	**11**	**13**	**17**	**19**
False susceptible	**1**	**0**	**0**	**8**
Sensitivity	**94%**	**100%**	**100%**	**27%**
Specificity	**92%**	**100%**	**89%**	**100%**
Predictive value res.	**94%**	**100%**	**85%**	**100%**
Predictive value susc.	**92%**	**100%**	**100%**	**70%**
Efficiency	**93%**	**100%**	**93%**	**73%**
Reproducibility	**90%**	**100%**	**100%**	**90%**

ANNEX 4B

Example of a proficiency testing analysis sheet (second-line drugs)

LAB NAME: *NRL of (Country)*

	Strain code	**Reported test results** R = resistant S = susceptible NG = no growth, or contaminated				**Score** (compared with judicial SRLN results) 1 = correct R = false resistant S = false susceptible			
		DRUGS TESTED				DRUGS TESTED			
		Km	**Amk**	**Cm**	**Ofx**	**Km**	**Amk**	**Cm**	**Ofx**
1A	1226	S		R	R	S		1	1
2A	2644	S		R	R	1		1	R
3A	2840	R		R	R	1		1	1
4A	2971	S		R	R	S		1	1
5A	3019	S		S	S	1		1	1
6A	3684	S		S	R	1		1	1
7A	3979	S		R	S	1		1	1
8A	4011	S		S	S	1		S	1
9A	4085	S		R	S	1		1	1
10A	4156	S		R	S	S		1	1
1B	4452	S		R	R	S		1	1
2B	4775	S		R	R	1		1	R
3B	4813	S		R	R	S		1	1
4B	4933	S		R	R	S		1	1
5B	4984	S		S	S	1		1	1
6B	5525	S		S	R	1		1	1
7B	6058	S		R	S	1		R	1
8B	6392	S		S	S	1		1	1
9B	6587	S		R	S	1		1	1
10B	7110	S		R	S	S		1	1
11	7564	S		S	R	1		1	1
12	7742	S		S	S	1		1	1
13	7823	S		S	S	1		1	1
14	8240	S		R	R	1		1	1
15	8785	S		S	S	1		1	1
16	9219	R		R	S	1		1	1
17	9590	S		S	S	1		1	1
18	9601	S		R	S	1		1	1
19	9606	R		R	R	1		1	1
20	9870	S		S	S	S		1	1

Method used: 1*
1* Proportion method LJ
2* Proportion method agar
3* Bactec 460
4* Resistance ratio
5* Absolute conc.
6* MGIT

Arrival:
Strains: *28 May 2009*
Results: *01 Sep 2009*
Turn-over time: *95 days*

Total correct results	**22**	**N/A**	**28**	**28**
True resistant	**3**	**N/A**	**17**	**11**
False resistant	**0**	**N/A**	**1**	**2**
True susceptible	**19**	**N/A**	**11**	**17**
False susceptible	**8**	**N/A**	**1**	**0**
Sensitivity	**27%**	**N/A**	**94%**	**100%**
Specificity	**100%**	**N/A**	**92%**	**89%**
Predictive value res.	**100%**	**N/A**	**94%**	**85%**
Predictive value susc.	**70%**	**N/A**	**92%**	**100%**
Efficiency	**73%**	**N/A**	**93%**	**93%**
Reproducibility	**90%**	**N/A**	**100%**	**100%**

ANNEX 5A

Example of a rechecking analysis sheet (first-line drugs)

LAB NAME: *NRL of (Country)*

Strain code	**Reported test results** R = resistant S = susceptible NG = no growth, or contaminated				**Score** (compared with partner SRL results) 1 = correct R = false resistant S = false susceptible 0 = no valid SRL result NG = no evaluation possible			
	DRUGS TESTED				DRUGS TESTED			
	S	**H**	**R**	**E**	**S**	**H**	**R**	**E**
14	R	S	S	S	1	1	1	S
18	R	R	R	S	1	1	R	1
19	S	R	R	R	0	0	0	0
22	S	R	S	S	1	1	1	S
29	NG	NG	NG	NG	NG	NG	NG	NG
34	S	S	R	S	1	1	1	1
312	S	S	R	S	1	1	1	S
328	S	R	R	S	1	1	1	S
329	NG	NG	NG	NG	0	0	0	0
334	S	R	S	R	1	1	1	1
350	S	S	S	S	1	1	1	1
354	S	S	S	S	0	0	0	0
356	R	R	R	S	1	1	1	1
358	S	S	S	S	1	1	1	S

Method used: 1*
1* Proportion method LJ
2* Proportion method agar
3* Bactec 460
4* Resistance ratio
5* Absolute conc.
6* MGIT

Arrival of strains at SRL:
28 May 2009

Total correct results	**231**	**254**	**254**	**251**
True resistant	**25**	**49**	**5**	**5**
False resistant	**21**	**4**	**1**	**4**
True susceptible	**206**	**205**	**249**	**246**
False susceptible	**7**	**2**	**5**	**5**
Sensitivity	**78%**	**96%**	**50%**	**50%**
Specificity	**91%**	**98%**	**100%**	**98%**
Predictive value res.	**54%**	**92%**	**83%**	**56%**
Predictive value susc.	**97%**	**99%**	**98%**	**98%**
Efficiency	**89%**	**98%**	**98%**	**97%**

ANNEX 5B

Example of a rechecking analysis sheet (second-line drugs)

Sample sheet for a laboratory that has tested the MDR-TB strains included in annex 5A for susceptibility to kanamycin, capreomycin, and ofloxacin

LAB NAME: *NRL of (Country)*

Strain code	Reported test results R = resistant S = susceptible NG = no growth, or contaminated N/A = not tested			Score (compared to partner SRL results) 1 = correct R = false resistant S = false susceptible 0 = no valid SRL result NG = no evaluation possible N/A = not tested		
	DRUGS TESTED			DRUGS TESTED		
	Km	**Cm**	**Ofx**	**Km**	**Cm**	**Ofx**
14	N/A	N/A	N/A	N/A	N/A	N/A
18	R	R	S	1	R	1
19	S	R	R	1	1	1
22	N/A	N/A	N/A	N/A	N/A	N/A
29	N/A	N/A	N/A	N/A	N/A	N/A
34	N/A	N/A	N/A	N/A	N/A	N/A
312	N/A	N/A	N/A	N/A	N/A	N/A
328	S	R	S	1	1	S
329	N/A	N/A	N/A	N/A	N/A	N/A
334	N/A	N/A	N/A	N/A	N/A	N/A
350	N/A	N/A	N/A	N/A	N/A	N/A
354	N/A	N/A	N/A	N/A	N/A	N/A
356	S	S	S	1	1	1
358	N/A	N/A	N/A	N/A	N/A	N/A

Method used: 1*
1* Proportion method LJ
2* Proportion method agar
3* Bactec 460
4* Resistance ratio
5* Absolute conc.
6* MGIT

Arrival of strains at SRL:
28 May 2009

Total correct results	**39**	**48**	**40**
True resistant	**4**	**5**	**18**
False resistant	**1**	**0**	**5**
True susceptible	**35**	**43**	**22**
False susceptible	**2**	**0**	**4**
Sensitivity	**67%**	**100%**	**82%**
Specificity	**97%**	**100%**	**81%**
Predictive value res.	**80%**	**100%**	**78%**
Predictive value susc.	**95%**	**100%**	**55%**
Efficiency	**93%**	**100%**	**82%**

ANNEX 6

Drug resistance survey protocol checklist

In developing a survey protocol (and, if applicable, a proposal for a grant), the following points should be included. The national TB control programme may add any other information deemed necessary.

Introduction and background

This section should include information on:

- country profile, i.e. geography, population, etc.;
- TB epidemiological situation in the country;
- HIV epidemiological situation in the country;
- information about the national TB control programme, including strategy, operational design, drug regimens used;
- information about the Central Reference Laboratory and the laboratory network in the country, detailing systems for internal and external quality assurance and indicating the relationship with an SRL;
- information about 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;
- a summary of data from the previous cohort analysis (including case-finding and treatment outcome data);
- data from previous DRS, if available;
- management of patients diagnosed with MDR-TB, or plans for development of a treatment programme;
- use of second-line drugs in the country.

Objectives

The objectives should be clearly specified in paragraph or list format.

Materials and methods

- The sampling frame and strategy (e.g. 100% sampling of diagnostic centres, cluster sampling) should be clearly described.
- The statistical basis for the calculation of the sample size must be detailed, and the sample size and expected duration of the survey should be stated.

Intake of patients and logistics

This section should detail:

- intake period (especially if rotating);
- inclusion and exclusion criteria;
- sputum collection process and how specimens will be handled;
- patient interview process;
- recording forms:
  - clinical information form, including measures to assure correct classification of patients by treatment history, i.e. review of records, samples for patient re-interviews;
  - sputum shipment form;
  - laboratory results form;
- transportation of sputum specimens and isolates to the Central Reference Laboratory and other logistics, i.e. frequency of sample pick-ups or shipments.

Laboratory methods

This section should detail:

- the chosen DST method and diagnosis algorithm;
- use of microscopy, media preparation, culture and identification;
- the established system of quality assurance, including internal quality control of DST and other laboratory processes, and external quality assessment of susceptibility testing, including proficiency testing, rechecking of samples, and pre-survey onsite assessments (this section should be developed in cooperation with the partner SRL);
- appropriate biosafety, with particular details on biosafety cabinets and their maintenance, and management of infectious waste.

Training

The training plan for all participating staff should be detailed with regard to responsibilities, timing, location, topics, forms, etc.

Survey monitoring

- supervision
- data monitoring
- re-interviewing of patients.

Data management and analysis

- data collection
- data entry (including double-entry)
- data analysis.

Resources needed (human and financial)

The coordination team and the principal investigator should be identified. Responsibilities should be indicated, including routine supervision during the course of the survey. The budget needed to implement all activities must be detailed.

Ethical considerations

Steps should be described that ensure patients diagnosed with drug-resistant strains during the course of a survey receive the highest possible level of care. Appropriate review by ethical committees should be planned.

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

ANNEX 7

Weighted cluster sampling

Cluster selection

Example. A sample size of 360 TB patients has been calculated after taking into account the effect of cluster sampling: 30 clusters of 360/30 = 12 patients will need to be selected.

The following steps must be taken:

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

b. Calculate the cumulative number of 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 next clusters is done by adding the sampling interval 211 each time to this first number 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 PATIENTS DIAGNOSED PER YEAR	CUMULATIVE NO. OF 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).

ANNEX 8

Survey budget template

ITEM	TYPE OF UNIT	COST/UNIT	# UNITS	TOTAL
Human resources				
Principal investigator				
Laboratory principal investigator				
Information technology specialist				
Laboratory technician(s)				
Logistics staff (e.g. drivers, data entry, secretarial)				
			Subtotal	
Consumables				
General (e.g. stationary, printing, etc.)				
Sputum containers				
Reagents				
Pure substances				
Other ( )				
			Subtotal	
Equipment				
Safety cabinet				
Centrifuge				
Computer				
Other, including inspissator, refrigerators ( )				
			Subtotal	
Meetings				
Initial meeting, follow-up meeting				
Per diem				
Transportation of attendees				
Meeting room costs				
			Subtotal	
Training				
Training of personnel for intake				
Per diem				
Transportation of attendees				
Meeting room costs				
			Subtotal	
Collection and domestic transport of specimens				
Transport containers, packaging				
Transport costs (fuel, air/bus, postage)				
			Subtotal	
Collection and international transport of specimens to SRL*				
Transport containers, packaging				
Transport costs (fuel, air/bus, postage)				
			Subtotal	
SRL costs				
Visit				
Proficiency testing costs				
ID and DST costs, including human resources				
			Subtotal	
Supervision				
Per diem				
Transportation of supervisor				
			Subtotal	
			TOTAL	

* International regulations regarding shipment of biological materials should be taken into consideration.

ANNEX 9

Example of a clinical information form

Diagnostic Centre: .....

Diagnostic Centre Code: .....

A. IDENTIFICATION OF THE PATIENT

Name: .....

Patient identification number: .....

Date registered: ...../...../..... (Day/Mo/Yr)

Sex: ☐ Male ☐ Female

Age: ..... years

Date of sputum collection: A ..... B .....

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

HIV-status .....

Country of origin .....

History of drug-abuse .....

Other risk factors (alcohol abuse, diabetes, smoking, malnutrition, etc.)

.....

B. HISTORY GIVEN BY THE PATIENT

B1. Previously treated for TB? ☐ No ☐ Yes

If the answer is no, go to B2, if yes, go to C.

B2. Standardized history[1]

- For how long have you been sick? .....
- Did you have the same symptoms prior to this episode? .....

[1] Some patients may not immediately recall past treatment for TB or may not be aware that previous treatment was for TB. These questions can be used by the investigator to help 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*.

- Did you have other symptoms of lung disease prior to this episode (haemoptysis, chest pain, cough)? .....
- Did you have sputum examinations prior to this episode? .....
- Did you ever take tuberculosis drugs for more than one month? .....

  If yes, what was the name? .....
- Did you ever have injections for more than one month? .....

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

☐ No ☐ Yes

C. MEDICAL RECORDS

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 Previous TB registration number .....

D. FINAL DECISION

D1. Patient has been previously treated for TB for more than a month:

Yes ☐ (answer to question B1 or B2 and/or C was 'yes')

No ☐ (answer to question B1 and B2 and/or C was 'no')

Doubtful ☐

D2. If yes, what was the outcome of previous treatment?

Cured/treatment completed ☐

Failed new patient regimen using first-line drugs only ☐

Failed retreatment regimen using first-line drugs only ☐

Failed regimen including second-line drugs ☐

Defaulted ☐

Other ☐

Unknown ☐

Responsible Officer: .....

ANNEX 10

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. Most practical for shipping are small 2 ml cryovials, containing a butt or slope of Löwenstein-Jensen medium. If the anticipated transport time is short (i.e. less than one week), no medium or liquid is required for transport of the loopful of bacteria being transported.

Guidance on applicable regulations for the transport of infectious substances, including cultures of *M. tuberculosis*, is available electronically.[1] WHO also offers 1.5 day training courses for shippers of infectious substances through the International Health Regulations Coordination Programme. In addition, an electronic shipper's guide for shipping infectious substances is available to assist shippers with classifying, documenting, marking, labelling, and packaging infectious substances.[2]

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.

[1] http://www.who.int/csr/resources/publications/biosafety/WHO_HSE_EPR_2008_10.pdf.
[2] http://www.who.int/ihr/infectious_substances/en/index.html.

ANNEX 11

Sample size for rechecking TB strains

Motivation

Quality assurance of drug sensitivity testing during national drug resistant surveys typically involves shipping a sample of *Mycobacterium tuberculosis* strains from surveyed TB cases to an SRL for assessment of drug susceptibility testing performance performed at the national laboratory. In the past, all strains with rifampicin resistance and 5–10% of the other strains were often sent for rechecking. However, such practise results in many strains being sent and rechecked at a very high cost and with no formal statistical justification. A statistical framework is hereby proposed for rechecking sample size estimation to assess quality of testing for susceptibility to the two most important first line drugs, isoniazid and rifampicin.

Methods

Strains are first tested by the survey laboratory for sensitivity to first-line anti-TB drugs. A sample of strains is rechecked for external quality assessment by the SRL. Strains are grouped into the following categories according to drug susceptibility test results from the survey laboratory:

1. MDR strains;
2. strains resistant to isoniazid or to rifampicin, but not to both drugs, that is, not MDR;
3. strains sensitive to both isoniazid and rifampicin.

For rechecking purposes, a randomly selected sample of strains from each of groups 1–3 should be sent to the SRL. If more than a specified number (typically zero or one) of drug susceptibility test results are found discordant in a particular group, then the null hypothesis that the true rate of discordance is higher than 5% cannot be rejected, and it is concluded that performance was not satisfactory in that group.

The proposed approach tests whether the data are compatible with a 5% or more level of error in diagnosing true resistance to one drug, and also whether the data are compatible with a 5% or more level of false resistance (strain found resistant to one drug by the Central Reference Laboratory, but found sensitive to that drug by the SRL). If, for each of groups 1-3, all tests are concordant, then all 3 of the null hypotheses (one for each of groups 1, 2, and 3) can be rejected. In

this case, overall performance of the Central Reference Laboratory is interpreted as satisfactory.

The null hypothesis to be tested is:

H_0: $P \geq P_0$ (i.e. the proportion of discordant results ≥ 0.05)

The alternative hypothesis is:

H_a: $P < P_0$ (i.e. the proportion of discordant results < 0.05)

Groups 1–3 are tested separately. In groups 1 and 2, type I error α is the conditional probability of wrongly accepting one batch (group) of strains given an initial resistant result. In group 3, the type I error α is the conditional probability of wrongly accepting the batch of strains given an initial overall susceptible result. Conventionally, α is chosen to be 5%.

If the null hypothesis is rejected in one group, then it is concluded that quality of testing for that group was satisfactory. If the null hypothesis is rejected in all groups, then it is concluded that overall quality of testing at the survey laboratory was satisfactory.

The hypergeometric distribution is important for representing the probability of observing *d* discordant results in a sample of strains of size *n* from a batch of strains of size *N* in which NP_0 strains are hypothesized to show discordant results between the national testing laboratory and the SRL. The hypergeometric distribution accounts for the fact that the probability of selecting a strain with discordant result changes as strains are sampled without replacement.

$$P(d \leq d^*) = \sum_{d=0}^{d^*} \frac{\binom{NP_0}{d}\binom{N(1-P_0)}{n-d}}{\binom{N}{n}} \qquad (1)$$

d^* is a threshold number: if more than d* tests are discordant, then the batch is rejected and quality for the tested group is interpreted as not satisfactory. If the probability of observing d* or fewer discordant samples is small relative to α, where α is the type I error of wrongly accepting the batch, then we can conclude that it is unlikely the proportion of discordant results in the targeted batch is as high as P_0. As a result, the batch would be accepted as of satisfactory testing quality. Conventionally, α is chosen to be 0.05.

The hypergeometric distribution may be used for sample size determination in the sense that we will choose the value of *n* that will yield a hypergeometric probability less than or equal to α given the values of P_0, *d and *N*. N is fixed, and we choose** α, P_0, *and d*. Normally we choose* α=*0.05 and P0=0.05, and d* to be 0 or 1.*

The following R[1] code can be used to compute sample size under a variety of assumptions:

```
nsize <- function (p0 = 0.05, N = 1000, d = 1, alpha = 0.05){
        s <– N
        for (n in N:1){
                m <– N – n
                k <– trunc (p0 * N)
                if (dhyper (d, n, m, k) > alpha) break
                s <– n
                }
        return (s)
        }
```

The above code can be directly pasted or copied into an R console. It generates a function named **nsize**. The function code is minimalist; it does not include checks for improper parameter values.

In the first example below, **nsize** returns a sample size n=71 under the default assumptions P_0=0.05, d=1 and N=140.

In the second example, the returned sample size n=55 corresponds to the assumptions P_0=0.05, d=0 and N=400.

```
> nsize(p0=0.05, N=140, d=1)
[1] 71
> nsize(p0=0.05, N=400, d=0)
[1] 55
```

The following table shows sample sizes under various assumptions.

	P_0=0.05	
N	d^* = 0	d^* = 1
25	24	
50	39	49
100	45	65
200	51	76
300	54	80
500	56	83
800	57	85
1000	57	86

[1] Freely available for download at: http://www.r-project.org.

The table reads as follows: if 800 strains belonged to Group 3 (all strains found fully sensitive to drugs by the survey laboratory), Group 3 sample size for rechecking is then 57 assuming $d^*=0$, that is, quality would be interpreted as not satisfactory if one or more test is found discordant at the SRL, and 85 assuming $d^*=1$, that is, quality would be interpreted as not satisfactory if two or more tests are found discordant at the SRL.

If the size of Group 1 (MDR strains) is smaller than 25, it would be advisable to retest all strains from that group, in which case quality will be interpreted as not satisfactory whenever one or more discordant test results are observed.

It is essential that the strains are randomly selected within groups. A very simple way to draw a random sample in R is to issue the command **sample (x, size)**, where **x** is the size of one group, **size** is the sample size determined from the table above (or the function **nsize**). By default, the command **sample** draws a sample without replacement, that is, the same number cannot be selected twice, and each number has the same probability of being selected. The command will return a random list of numbers from 1 to **x**, of size **size**.

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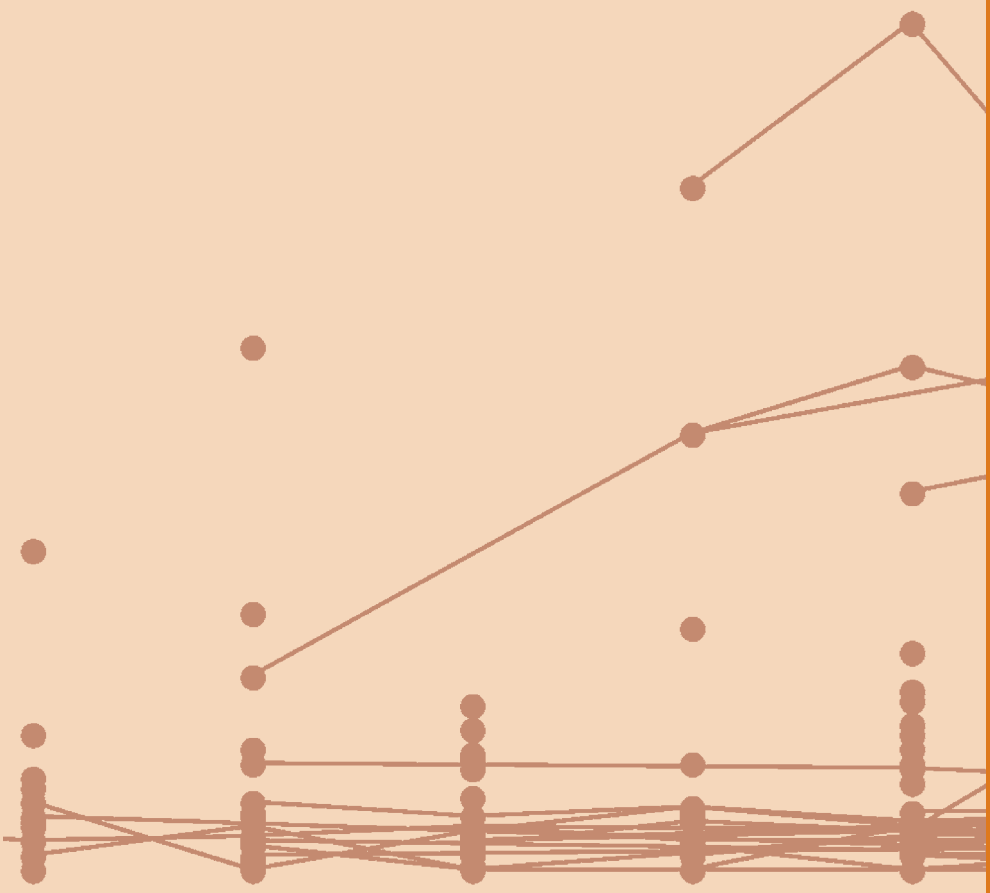

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