



WHO/IUATLD GLOBAL WORKING GROUP
ON ANTITUBERCULOSIS
DRUG RESISTANCE SURVEILLANCE



GUIDELINES FOR SURVEILLANCE OF DRUG RESISTANCE IN TUBERCULOSIS

1997

Global Tuberculosis Programme
World Health Organisation
WHO Geneva

and

International Union Against
Tuberculosis and Lung Disease
IUATLD Paris

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INTRODUCTION

There is much anecdotal, but little rigorously documented evidence that drug resistance is becoming an increasing threat to the effectiveness of national tuberculosis programmes. The available information suggests there may be high and increasing levels of drug resistance in many parts of the world^{1 2 3 4 5}. These guidelines have been developed to assist national tuberculosis programmes in adopting standardised methods for drug resistance surveillance. This document provides a standard tool for tuberculosis control programmes to evaluate and improve performance. Standardised data generated according to these guidelines in different countries will be directly comparable. The use of drug resistance tests for monitoring and guiding tuberculosis treatment programmes was recommended many years ago⁶. Owing to the practical difficulties in collecting comparable data, WHO and IUATLD proposed a global surveillance project through various centres worldwide which would function as Supranational Reference Laboratories⁷. Subsequently, several countries established national surveillance projects and some adopted a standardised methodology for susceptibility testing with the assistance of these Supranational Reference Laboratories. In establishing surveillance of drug resistance at country level, three principles should be strictly adhered to:

1. The sample of specimens should be representative of the patients from the area under study and the sample size should be determined to permit standard epidemiological analyses.
2. The patient's history should be carefully obtained and available medical records reviewed to clearly determine whether or not the patient has received prior anti-tuberculosis drugs in order to distinguish between primary and acquired drug resistance.
3. The laboratory methods for susceptibility testing of anti-tuberculosis drugs should be selected from among those that are internationally recommended.

BACKGROUND AND RATIONALE

Knowledge of the prevalence of anti-tuberculosis drug resistance is essential for evaluating and improving national tuberculosis control efforts⁸. The global surveillance network proposed in this document will measure susceptibility to commonly used drugs, and the standardisation of methods will ensure that the results are comparable both within and among participating countries. As the decisions on treatment regimens and programme management can only be made at country level, prevalence of drug resistance should be monitored primarily at that level. Any global or regional surveys should be based on the results of these national surveillance efforts. This requires the adoption of standardised surveillance methods in all countries.

Causes of drug resistance

The main cause of drug resistance is the failure to ensure correct treatment of each patient with tuberculosis. Programmes are often at fault due to: 1) improper prescription of treatment regimens, 2) inadequate drug supply, 3) poor case holding, 4) poor quality of drugs, and 5) failure to ensure that patients follow the prescribed regimens. Once selected, drug resistant strains of *Mycobacterium tuberculosis* may be transmitted in the community. This effect may be enhanced by HIV infection and inadequate infection control.

Choice of drugs

Four anti-tuberculosis drugs, isoniazid, rifampicin, streptomycin, and ethambutol, should be tested by all countries adopting these guidelines. These drugs were chosen because they are and have been widely used throughout the world, their susceptibilities can be reliably measured by standardised techniques, have been studied for many years, and background knowledge already exists to which new information can be added. Given the difficulties in standardising susceptibility testing for pyrazinamide (due to the instability of the drug at low pH), this drug should not be routinely included in the panel of antituberculosis drugs to be tested for surveillance purposes.

DEFINITIONS OF RESISTANCE

Resistance to each of the four anti-tuberculosis drugs is defined according to the results of bacteriologic testing (see Susceptibility Testing, page 17). Multidrug resistance is defined as resistance to both isoniazid and rifampicin, with or without resistance to other agents.

Acquired resistance to antituberculosis drugs

Patients diagnosed with tuberculosis who start antituberculosis treatment and acquire resistance to one or more of the drugs used during the treatment are said to have developed "acquired drug resistance". This can be ascertained only if the drug susceptibility pattern to the drugs used is known before treatment and at a second determination while the patient is still on these drugs. Acquired drug resistance is thus a sensitive indicator of combined physician's and patient's adherence to internationally recommended treatment regimens. Such an approach is only

possible to some extent in countries with resources to perform serial susceptibility testing. In most of the world, a systematic evaluation is not usually feasible, and an alternative approach to estimate acquired drug resistance needs to be pragmatic.

Proxy for the prevalence of acquired drug resistance: patients with history of previous anti-tuberculosis treatment

Because acquired drug resistance depends on the use of anti-tuberculosis drugs, patients with a previous history of anti-tuberculosis treatment are thus grouped as patients with a "history of previous anti-tuberculosis treatment for at least one month". This group includes patients in one of the four following groups which should be reported separately whenever feasible:

- Patients failing anti-tuberculosis treatment, i.e., patients who begin treatment for smear positive pulmonary tuberculosis and who remain or become smear positive again at five months or later during the course of treatment (treatment failure);
- Patients who become smear positive again after having been treated for tuberculosis and declared cured after the completion of their treatment (relapse case);
- Patients who have interrupted their treatment for more than two months after having received a total of at least one month of antituberculosis treatment and who return with bacteriologically confirmed tuberculosis (return after default);
- Patients who continue to be smear positive after the completion of a retreatment regimen (chronic case).

Definition

For the purpose of surveillance, the following definition is used: **Acquired resistance study group**, i.e. tuberculosis patients who have been treated for one month or more. "**Acquired drug resistance**" is, therefore, defined as the presence of resistant strains of *M. tuberculosis* in a patient who, in response to direct questioning, admits having been treated for tuberculosis for one month or more, or, in countries where adequate documentation exists, documented evidence of such a history exists. These patients include cases classified as relapse, return after default, failure, and failure after retreatment. The definitions of these terms are in agreement with those described in the document Framework for effective Tuberculosis Control, WHO/TB/94.179⁹, and the Tuberculosis Guide, IUATLD¹⁰.

Primary resistance to antituberculosis drugs

Even more so than with acquired drug resistance, the definition of primary drug resistance is a theoretical concept. Primary drug resistance refers to patients who have never been treated for tuberculosis or who have been treated for less than one month and who harbour organisms resistant to one or more anti-tuberculosis

drugs. As history of prior anti-tuberculosis treatment can never be entirely accurate, an alternative approach to estimate primary drug resistance has to be pragmatic in most instances. "Initial drug resistance" has thus been proposed to refer to patients presenting with an organism resistant to any anti-tuberculosis drug prior to commencement of therapy. However, the systematic utilisation of this term may encourage inadequate enquiries into prior anti-tuberculosis treatment history.

Proxy for the prevalence of primary drug resistance: patients with no previous anti-tuberculosis treatment

Patients should be interviewed in a standardised manner to exclude prior history of up to one month of anti-tuberculosis treatment. Patients who claim never to have received such treatment (preferably verified by checking tuberculosis registers) are said not to have been previously treated.

Definition

For the purpose of surveillance, the following definition is used: **Primary resistance study group**, i.e. patients who have never been treated for tuberculosis or have been treated for less than one month. "**Primary drug resistance**" is, therefore, defined as the presence of resistant strains of *M. tuberculosis* in a patient who, in response to direct questioning, denies having had any prior anti-tuberculosis treatment, and, in countries where adequate documentation is available, no documented evidence of such treatment exists. Under certain circumstances, an accurate history or documentation of prior treatment cannot be obtained in spite of direct questioning. The presence of resistant strains in these patients should be classified as resistance of uncertain origin. The combination of this category and primary drug resistance is defined as "**initial drug resistance**". Since prior anti-tuberculosis treatment is defined as the evidence that anti-tuberculosis drugs have been taken for at least one month, when the duration of previous treatment is unknown, cases should be classified as prior anti-tuberculosis treatment.

Significance of distinguishing patients who have been previously treated from those not previously treated

The pragmatic approach of separating patients with and without history of at least one month of anti-tuberculosis treatment has repercussions not only on classification for the purpose of surveillance of drug resistance, but it also has programmatic relevance in deciding what type of treatment a patient is to receive.

Cluster sampling

Cluster sampling methods are best used in situations where there are logistic difficulties involved in covering the entire area of the country and where the number of tuberculosis cases is high. With this design, centres are randomly selected, and all sputum smear positive patients newly registered during a defined period of time at these selected centres are included in the survey. A defined intake period, identical for all centres included, should result in a balanced sample, as centres are represented according to their burden of cases to the control programme. This allows direct estimation of the prevalence of resistance from the proportion calculated in the sample. To obtain an unbiased estimate of the prevalence of drug resistance, it is essential to include a minimum number of centres (i.e. 30), so as to have a good probability of including centres of different types, such as clinics, dispensaries and hospitals of different sizes scattered throughout the country. Indeed, while the main advantage of this technique is its simplicity, the main disadvantage is the risk of missing the largest diagnostic centres, resulting in a non-representative assessment of resistance prevalence despite randomisation.

The desirable number of centres can be selected from the list of all diagnostic centres in the country, either by simple random sampling assigning a sequential number to each from 1 to X, in any order, and selecting at random n numbers between 0 and X, or by systematic sampling. For systematic sampling, a sampling interval should be calculated by dividing the total number of centres X by the number n. The first centre is selected from the list by finding a number between 0 and X/n, the second is found on the list by adding the sampling interval X/n to this number, and so on consecutively. The interest of this selection procedure is that if the list of centres is ordered according to centre size, i.e. the patient load, systematic sampling from the list would result in a sample stratified by centre size. However, it is always possible to miss some important centres, particularly in situations where centre sizes vary widely and where the total number of centres is not large¹³.

Population proportionate cluster sampling

To avoid the risk of missing the largest diagnostic centres when drawing the sample a weighted cluster sampling technique can be used. 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 is picked between one and the sampling interval. This random number 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 twice or three times more patients per year, then the sampling interval may well be more than one cluster per diagnostic centre.

To determine the number of patients per cluster the required total sample size is divided by 30. 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 required for one or more clusters is reached.

See Annex 1 for a practical example of how to handle cluster selection and calculation of confidence interval.

Sampling for acquired drug resistance

As the proportion of patients with acquired resistance, i.e., patients with history of prior treatment, is usually only a small fraction of the total, the confidence intervals around their estimated levels of resistance may be so broad as to make the estimate for them virtually useless. This would suggest that data on the retreatment cases have to be collected over a longer period to increase their proportion of the total. Alternatively, representative sampling could be used in new cases, while 100% sampling is used for retreatment cases over a long period of time (one year if feasible). In addition, particular care should be ensured not to enrol twice patients with history of prior treatment (such as recurrent defaulters frequently seeking to recommence treatment).

Trend monitoring

In most national programmes the monitoring of trends in the prevalence of drug resistance, rather than a single survey, should be considered the long term strategy. Repeat surveys on drug resistance will be particularly useful in evaluating the impact of intervening changes in control policies in a given region or country. To observe trends, surveys on the prevalence of drug resistance - as described in these guidelines - should be repeated every 3-5 years, depending on the resources available to the national tuberculosis programmes. The establishment of an ongoing surveillance programme may be a better option in some settings.

ORGANISATION AND SURVEY OUTLINE

Suitable survey areas

The country or state or province considered as a survey area should have at least one functioning central culture laboratory linked by mail or messenger with the majority of tuberculosis diagnostic centres. Within the survey area some centres or remote areas may have to be excluded because of logistic reasons. That must be

decided before the sampling. In order to avoid a serious bias, exclusion should never be based on the quality of programme performance.

National co-ordination team

A survey on the prevalence of anti-tuberculosis drug resistance involves three major operational issues:

1. programme management (logistics, training, collection of clinical information)
2. laboratory techniques
3. epidemiology (sampling, data-entry and data-analysis)

A national co-ordination team, including one person from each of these fields, should be established. In general, the head of the National Tuberculosis Programme and the head of the National Reference Laboratory, or a person designated by them, will be assigned for these tasks, and an epidemiologist should be identified. The co-ordinating team needs strong official backing by the authority in charge of health services. The co-ordination team is responsible for the preparation of the survey, the link with the Supranational Reference Laboratory, the supervision and quality control during the survey and the final collection and reporting of results.

Preparatory phase

Before the actual survey starts, the following issues should be addressed by the co-ordinating team: sampling, training, logistics, and funding. It is recommended that all technical, administrative and logistic procedures involved be described in a simple survey manual or protocol, which is distributed to the health officers participating in the study. It is also of great importance to assure the quality of susceptibility testing done at the National Reference Laboratory. In general, staff from the Supranational Reference Laboratory should visit the National Reference Laboratory prior to the beginning of the survey and international quality control of susceptibility testing should be established (see page 19).

Sampling : once the diagnostic centres participating in the survey are identified by a sampling method described under the previous chapter, a time schedule taking into account the logistics, climatic conditions, and workload at the Laboratory can be established.

Training : training should focus on the three essential parts of the survey: (1) enrolment of patients into the survey; (2) obtaining reliable and comparable data on previous treatment; and (3) the laboratory techniques. The training activities must be planned carefully and include, if possible, each health worker who will be directly involved in the survey. As far as the intake of patients is concerned, it is strongly

advised to collect the same minimal set of clinical information in every country in order to allow international comparison. However, the co-ordinating team may adapt the intake form to the needs of the country by adding some relevant questions. The medical officers in charge of the intake of patients and of the interviews should be identified in each diagnostic centre involved in the survey and be properly instructed. In general, a meeting is an efficient way to inform, train and motivate the officers involved, making them realise the need for reliable and comparable data in a national survey. As far as the peripheral laboratories are concerned, training should focus on preparation and reading of smears, decontamination of sputum samples, storage and transport of samples and proper registration.

Logistics : special attention must be given to the transport of the sputum samples in order to minimise the transport time and prevent breakage and contamination. All the material necessary for the survey such as sputum containers, forms, and laboratory equipment should be available in sufficient quantities in each centre before the start of the survey. National and international guidelines on shipment of infectious material should be followed (see Annex 2).

Funding : budget and funding must be arranged well in advance to ensure completion of the survey.

Depending on the local conditions, it could be useful to organise a pilot trial in a district for a limited time to test logistics and quality of training, and to identify and solve unexpected problems.

Quality control of the survey

Quality control of the survey should be organised to detect operational or systematic errors and to improve compliance with the survey procedures. It should concern each essential part of the survey including: 1) the sampling, i.e. the selection of the patients included in the study); 2) the clinical information, i.e. the distinction between never treated and previously treated patients; and 3) the laboratory techniques involved at the peripheral level and at the National Reference Laboratory.

Sampling

Patients included in the survey must be selected according to the sampling method chosen by the co-ordinating team in such a way as to maximise representativeness. If consecutive patients are to be included, this must be checked during supervision comparing the TB District Register, the TB Laboratory Register, and the patients included in the survey. The replacement rate, i.e. the proportion of patients who are replaced in the sample, can be indicative of process errors.

Clinical information

Classification of patients as never treated and previously treated cases is a crucial issue for distinction between primary and acquired drug resistance. Therefore, special efforts are needed to ensure reliable clinical data. First of all,

during the survey, the collected interview forms should be checked carefully for deficiencies. Secondly, the reliability of the information recorded should be assessed regularly during the survey. Several validation methods can be used depending on the characteristics of the country. A representative sample of patients should be reinterviewed separately by somebody assigned by the co-ordinating team; this is particularly important in patients denying prior treatment. Depending on the results of the comparison, additional training may be necessary. A less efficient alternative is that every patient is interviewed by two different medical officers.

Laboratory techniques

At peripheral level the process of collecting sputum samples (including sputum quantity and quality), smear examination, and transport of sputum and forms needs careful supervision.

At the National Reference Laboratory, in co-operation with the Supranational Reference Laboratory, a system of internal and external quality control of culturing and drug-susceptibility testing procedures should be established before starting the survey. In particular, the rate of negative culture results in smear positive specimens and the rate of culture contamination must be evaluate before beginning patient intake. In addition, the quality control system must involve blind drug-susceptibility testing of strains exchanged between national and supranational laboratories initially, periodically, or throughout the survey (see Quality Assurance, page 18).

INTAKE OF PATIENTS

Inclusion criteria

Patients are eligible for inclusion in the survey if they have been newly registered as a sputum smear positive case according to the WHO/IUATLD definitions^{9 10}. Children under the age of 15 years who meet the admission criteria are also routinely included.

Sputum collection

In addition to the initial sputum sample used for smear microscopy, the diagnostic centres selected will send to the central laboratory two other sputum samples, e.g. two spot samples or a spot and an overnight sample, from each smear-positive patients found to be eligible for inclusion. As treatment for any period of time will reduce the chance of a positive culture, the samples must be obtained before starting treatment.

Registration

Each patient meeting the inclusion criteria should be assigned a serial number, which will be recorded on the intake forms. The serial number permits identification at the diagnostic centre in case of a resistant strain or when additional information is required. Three forms must be utilised: 1) Sputum Shipment Form; 2) Clinical Form; and 3) Laboratory Results Form.

Sputum Shipment Form (Annex 3)

This form includes 3 sets of data: identification of the patient, date of collection of the sputum, and the result of the smear examination at the National Reference Laboratory. This form will accompany the sputum sample to the National Reference Laboratory. A copy will be kept at the diagnostic centre.

Clinical Form (Annex 4)

The main objective of this form is to identify correctly the patient as never treated or previously treated for tuberculosis. The form consists of 4 sets of data: patient identification, patient history, documented data on previous treatment episodes, final decision. The form in Annex 4 contains a minimal set of information necessary for programme monitoring and for allowing international comparison on essential data. This minimal set of data should be collected in every survey.

However, countries may decide to collect additional specific information such as country of origin, HIV status, place of previous treatment, etc. In principle, only information which is obtainable, reliable and useful from a programme perspective is to be added in a way that allows analysis, and the denominator must be known for each variable collected. For example, if it is decided to stratify tuberculosis patients for country of origin, all patients must be asked to provide that information. If a decision is made to test all patients for HIV antibody, it is recommended that a detailed protocol is prepared in order to ensure confidentiality and counselling for all patients¹⁴.

To help the patient remember any previous TB treatment and to standardise the questionnaire at the national and international level, a minimal set of questions should be asked of all patients denying previous treatment (Annex 4). However, these data do not need to be analysed at the central level. Their only purpose is to standardise and optimise the interview.

A copy of this form should be sent to the co-ordinating team while the original should be kept at the diagnostic centre. It should not be sent to the Reference Laboratory performing susceptibility testing, as knowledge of previous treatment could bias the technician in the interpretation of the test.

Laboratory Results Form (Annex 5)

This form should include data on: identification of the patient, results of identification of *M. tuberculosis* in the two sputum samples sent to the National Reference Laboratory, and results of susceptibility testing done on only one sample. A copy should be sent to the co-ordinating team and the original should be kept at the National Reference Laboratory.

Transport of sputum samples

Sputum should always be treated with care and should be collected in containers that can be sealed hermetically. This is particularly important if the postal service has to be used. The containers must be rigid to avoid crushing in transit. They should be packed in material that will absorb any leakage caused by accidents. Furthermore, all procedures involving sputum should be carried out in a safety cabinet designed for that purpose. Particular care needs to be taken when bottles are being opened, closed or shaken and when materials are being centrifuged, all of which may all lead to the production of infectious aerosols. The transportation of tuberculosis cultures presents special risks in the event of accidents or breakage of the container (see Annex 2).

Before transport the sputum samples are kept in a cool place, preferably a refrigerator at +4°C. For homogenisation of the mucus and organic debris and for decontamination on transit, an amount of cetylpyridinium bromide 0.6% or cetylpyridinium chloride 1%, equal to the volume of the sputum, is added, if it is anticipated that between collection and processing in the culture laboratory the samples may be exposed to room temperature for more than 48 hours. The patient's serial number in the centre's register and a simple identification for the two successive specimens from the same patient, such as A and B, are written on the container, not on the lid. The two specimens, together with the sputum shipment form, are sent to the central laboratory. A copy of the form is kept in the patient's file at the diagnostic centre.

Replacement

Patients meeting the inclusion criteria but who cannot be included in the survey should be replaced. For instance, patients may fail to return to the diagnostic centre to give sputum for culture, or samples received in the central laboratory may be spoiled on transport or contaminated, and cultures cannot be obtained. All these events must be recorded in order to facilitate supervision and quality control. If replacement is required, the samples are replaced by those of other patients diagnosed in the centre concerned, according to the sampling method.

NATIONAL REFERENCE LABORATORY

Cultures

Before processing at the central tuberculosis laboratory, the sputum samples should be kept in a refrigerator at +4°C and bacteriological examination should be carried out as soon as possible. The samples are decontaminated and further homogenised, according to Petroff's method, with sodium hydroxide 4% at 37°C, centrifuged at 2000-3000 g for 20 minutes, and the sediment is then neutralised and washed. Total contact time with sodium hydroxide should not exceed 30 minutes. Other standardised methods are acceptable. Acid fast microscopy should be performed on these concentrated samples¹⁵.

The sediment is inoculated on two tubes of Loewenstein-Jensen medium and one tube of egg medium enriched with sodium pyruvate. This last medium is used to optimise growth of *M. bovis*. The cultures are incubated at 37°C until growth of colonies is observed or otherwise for nine weeks. They are first inspected after 48 hours and then weekly, or at least after 21, 28, 42 and 63 days. Each isolate strain will be examined for morphology and pigmentation and the date of appearance of the colonies will be noted. If there is no growth by day 63, or in case of contamination, the cultures are discarded and the laboratory forms completed accordingly. All positive cultures are kept until retesting at the Reference Laboratory has been completed or the strain has been excluded from further testing. The cultures should ideally be stored in a deep freezer at -20°C, but they can also be kept for some time in the refrigerator at +4°C, or even at room temperature.

Identification

The identification of cultures belonging to the *M.tuberculosis* complex will be carried out by determining the susceptibility of the isolates to NAP and or PNB or by standard DNA probe tests. The presence of *M.tuberculosis* will be confirmed by colonial morphology and by positive niacin production and nitrate reduction tests. If the colonial morphology and the other tests confirm the isolate as belonging to the *M.tuberculosis* species, only one culture per patient needs to be identified. Niacin production and nitrate reduction negative isolates of the *M.tuberculosis* complex will be further tested to confirm the presence of *M.bovis*. Mycobacterial strains other than *M. tuberculosis* complex will not be further considered for the purpose of the survey.

Susceptibility testing

Indirect susceptibility testing will be performed on only one isolate for each patient. Drug resistance tests will be performed preferably using the economic variant of the proportion method using Loewenstein-Jensen medium,¹⁶ although the absolute concentration, resistance ratio, and other standardised methods may also be used. The strains' resistance against isoniazid, rifampicin, streptomycin, and ethambutol is routinely tested if these drugs are used in the tuberculosis programme, prescribed by private practitioners, or freely available. Resistance is expressed as the percentage of colonies that grow on critical concentrations of the substances, i.e. 0.2 mg/l for isoniazid, 2 mg/l for ethambutol, 4 mg/l for dihydrostreptomycin sulfate and 40 mg/l for rifampicin if the Loewenstein-Jensen medium has been used. The interpretation will be according to the usual criteria for resistance, i.e. 1% for all drugs. The results of the tests are recorded on the laboratory forms, copies of which are collected by the national co-ordinator for analysis (see Annex 5).

Quality Assurance

To ensure that results of susceptibility testing are reliable and comparable between different countries, a system of quality assurance is recommended. The main components of a quality assurance programme are internal quality control of susceptibility testing, and international quality control of susceptibility testing.

Internal quality control of susceptibility testing

Susceptibility testing should be performed on the standard strain H37RV in each new batch of Loewenstein-Jensen medium and for each drug. It is recommended that internal quality control be performed including a drug resistant strain as well. Standardised procedures should be followed whether proportion method, BACTEC, resistance ratio or other methods are used for susceptibility testing and for formulation of media. As a part of internal quality control the quality of the medium should be controlled batch by batch. Drugs added to the medium must be pure drugs obtained from a reputable firm with the percent of potency clearly indicated. Dilution of drugs and the addition to the medium should be performed following accepted standards.

International quality control of susceptibility testing

International quality control of susceptibility testing should be done by exchanging strains of *M. tuberculosis* strains in two directions: from the Supranational Reference Laboratory to the National Reference Laboratory, and from the National Reference Laboratory to the Supranational Reference Laboratory.

From the Supranational Reference Laboratory to the National Reference Laboratory

The Supranational Reference Laboratory should send a panel of coded strains for blind retesting in the National Reference Laboratory. The results should

be compared with those obtained at the Supranational Reference Laboratory. The minimum required agreement should be defined for each drug and should be higher than 90 % for isoniazid and rifampicin. This component of the international quality control should start before the survey is implemented. A similar methodology can be applied for external quality control from the National Reference Laboratory to Regional Laboratories in those countries where Regional Laboratories are also performing susceptibility testing.

From the National Reference Laboratory to the Supranational Reference Laboratory

A sample of the strains isolated during the survey should be sent to the Supranational Reference Laboratory to be retested without knowledge of the local results. The results should be compared for agreement with respect to each drug. It is recommended that not less than 10 % of the total sample strains be included, without any selection criteria based on results, i.e. resistant or susceptible. The strains could be selected as a percentage from each region of the country or included by their consecutive order of entry. The method must be previously agreed between the National Reference Laboratory and the Supranational Reference Laboratory, and a schedule for the strain exchange should be prepared. This part of the international quality control should preferably be performed during the survey or, if this is not possible, when the survey is completed.

DATA MANAGEMENT AND ANALYSIS

Data Collection

At regular intervals of no longer than two to three months, during the intake period, the co-ordinating team should tabulate all data produced by the diagnostic centres and the central laboratory. Based on these tables, the national co-ordinator will make regular reports to the chiefs of the National Tuberculosis Programme and the National Reference Laboratory. This report should include information on field work, such as enrolment of patients, quality of clinical information collected, transport or logistical problems, and contamination of specimens. If the data or comments suggest that a significant problem has occurred, the national co-ordinator and the chiefs of the National Tuberculosis Programme and National Reference Laboratory should analyse the situation and develop a plan of action.

About half way through the survey, the national co-ordinator and the chiefs of the National Tuberculosis Programme and the National Reference Laboratory should meet to discuss the adequacy of data collection and laboratory procedures, quality control results, and preliminary survey results.

Data Management

Data from the survey is entered and analysed by computer. WHO has produced a simple software based on Epi-Info for entering and analysing data from drug resistance surveys. The software is called Surveillance of Drug Resistance in Tuberculosis (SDRTB), and is available free of charge from the WHO Global Tuberculosis Programme. SDRTB is simple and flexible. A programmed analysis can be run easily and summary tables with the prevalence of drug resistance for each drug and cumulative drugs can be produced.

To ensure accuracy in data entry, the data should be entered twice, preferably by different people, and the two databases compared. This can be done easily using the "Validate" option in SDRTB.

Data Analysis

To calculate the prevalence of drug resistance, the denominator is the number of cases with drug susceptibility results available. However, it is also important to report the number of missing results, e.g. due to contamination, negative cultures, or insufficient growth for susceptibility testing. The following parameters should be included:

Analysis of patient intake: it is useful to make a table comparing the number of patients included from each diagnostic centre with the expected number based on the sampling method.

Analysis of drug resistance patterns: a table should be drawn up describing the proportion of patients with monoresistance to each drug, and to different combinations of drugs, among patients with primary resistance and those with acquired resistance. The presentation of data is based on mutually exclusive categories of resistance (monoresistance and combined resistance). The recommended tabulation is shown in Annex 6. If appropriate, further comparisons based on age, gender, HIV status, country of origin, type of retreatment case, etc., can also be made.

From a public health point of view, the extent of current transmission of drug resistant strains is important. Young people are more likely to have been recently infected than older people. The prevalence of drug resistance in young age groups, therefore, provides more reliable information on recent patterns of transmission of drug resistant TB. For the same reason, assessment of trends in drug resistance is more informative than data collected in a single survey. For countries with a high prevalence of drug resistance, surveys at regular intervals, e.g. 3-5 years, using similar survey methodologies, should be done systematically to monitor the trend in drug resistance.

Interpretation of results

Interpretation of results of a survey on the prevalence of antituberculosis drug resistance depends on local programmatic and epidemiological circumstances. The key indicators of programme performance are the proportions of patients with primary or acquired drug resistance. High levels of resistance may be a serious threat for the National Tuberculosis Programme.

High levels of primary resistance suggest that transmission of *M. tuberculosis* resistant to drugs is occurring in the community. It is an indicator of a National Tuberculosis Programme's performance over many years. In established National Tuberculosis Programmes adopting standardised chemotherapy primary drug resistance is gradually reduced¹⁷. High levels of primary resistance may also indicate that some previously treated patients have been misclassified as new cases.

High levels of acquired resistance to a single drug included in the intensive phase of the retreatment regimen will not significantly increase the failure rate of retreatment. However, high levels of acquired resistance indicate poor programme performance. Even in the absence of results of cohort analysis, corrective action to improve cure rates may be required. Various factors promote acquired resistance. These include unsupervised treatment, use of inadequate drug regimens, free availability of anti-tuberculosis drugs in the market, and poor quality of the drugs supplied.

Multidrug resistance, i.e. resistance to isoniazid and rifampicin, is a cause of great concern. Such patients require at least individualised treatment with second line anti-tuberculosis drugs in specialised units¹⁸. As the development of combined resistance is usually a stepwise process, serious shortcomings in programme management at several levels have probably occurred¹⁹.

In all situations where high levels of drug resistance occur, reorganisation of the programme, with an emphasis on strict adherence to recommended regimens and supervision of treatment, is urgently needed.

Use of the data for routine management of cases: data derived from the survey are meant primarily for surveillance activities and are not designed for use in individual case management. Use for such case management may be difficult for a number of reasons: results may be delayed due to the necessary procedures for avoiding bias; communication procedures are designed for a survey rather than for routine purposes; and only a sample of patients is tested. Therefore, individual medical officers should follow the policy established by the National Tuberculosis Programme for treatment of tuberculosis patients and for the use of susceptibility testing in routine case management.

ACKNOWLEDGEMENTS

The WHO's Global Tuberculosis Programme and the IUATLD wish to thank all the colleagues participating in the WHO/IUATLD global project on anti-tuberculosis drug resistance surveillance for their contributions to the development of these guidelines. Valuable contributions at the joint WHO/IUATLD Workshop of the Global Working Group on Anti-tuberculosis Drug Resistance Surveillance, which was held on 5-6 October 1996 in Paris, are also gratefully acknowledged. More than 80 participants from 40 different countries participated in the Workshop and contributed with their experience and knowledge.

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ANNEX 1 - CLUSTER SAMPLING

Cluster selection

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

- a) establish the list of the diagnostic centres with their annual number of patients (see table below).
- b) calculate the cumulative numbers of patients and record them in an additional column. Cumulative number for the second centre will be (number in the first centre) + (number in the second centre). Cumulative number for the third centre will be (cumulative number for the second centre) + (number in the third centre) and so on. The total number of patients diagnosed in the country is 6,322.
- c) determine the sampling interval: $6,322 / 30 = 211$
- d) select a number between 0 and 211 at random (with a table of random numbers or by using the last digits of a currency note for example). In this case the number selected is 120.
- e) the first cluster is selected using this number 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 next clusters is performed by adding the sampling interval 211 each time to this first number 120. The next number $(120 + 211) = 331$ falls between 246 and 1,823 (cumulative number of patients for the second centre), therefore the 2nd cluster is selected in the 2nd centre. The 3rd number $(331 + 211) = 542$ falls also between 246 and 1,823, the 3rd cluster is therefore selected in the 3rd centre as well.

Name of diagnostic centre	Number of patients diagnosed per year	Cumulative number of patients	Cluster number
A	246	246	1
B	1,577	1,823	2,3,4,5,6,7,8,9
C	468	2,291	10,11
D	340	2,631	12
E	220	2,851	13
F	246	3,097	14,15
G	190	3,287	16
H	1,124	4,411	17,18,19,20,21
I	61	4,472	
J	154	4,626	22
K	139	4,765	23
K	60	4,825	
M	14	4,839	
N	38	4,877	
O	19	4,896	
P	41	4,937	
Q	120	5,057	24
R	455	5,512	25,26
S	51	5,563	
T	26	5,589	
U	199	5,788	27
V	21	5,809	
W	32	5,841	28
X	69	5,910	
Y	6	5,916	
Z	145	6,061	29
AA	129	6,190	
BB	87	6,277	30
CC	10	6,287	
DD	35	6,322	

Confidence interval calculation

If cluster selection is performed with probability proportional to size (method described above) and if clusters are of the same size, a simplified formula for the confidence interval (CI) around the drug resistance prevalence is :

$$CI = \pm 1.96 \sqrt{\frac{\sum_i (P_i - P)^2}{n(n-1)}}$$

where

P is the prevalence calculated for the total sample,
 P_i is the prevalence calculated in each cluster i
 n is the number of clusters (30)

To calculate the sum of the $(P_i - P)^2$ over all 30 clusters the following table can be used:

Cluster number	P_i	$P_i - P$	$(P_i - P)^2$
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			

The total of the last column can then be used in the formula.

Source: ten Dam H.G. Surveillance of tuberculosis by means of tuberculin surveys. WHO/TB/85.145

ANNEX 2: SAFE SHIPMENT OF INFECTIOUS MATERIAL

For international quality control of susceptibility testing, cultures should be exchanged between the National Reference laboratories and the Supranational Reference Laboratories. Cultures of *M. tuberculosis* are enriched infectious material containing large numbers of viable organisms that can cause disease in humans. The hazard is compounded when cultures of resistant strains are transported.

Some international organisations, such as the Universal Postal Union, the International Civil Aviation Organisation and the International Air Transport Organisation, have developed guidelines and procedures designed to facilitate the safe and expeditious shipment of infectious substances while at the same time ensuring the safety of transport personnel and the general public²⁰. These organisations have also developed agreed common definitions, and packaging and labelling requirements^{21 22}. Information on the documentation requirements should be obtained from the appropriate national authorities of the country where the cultures are sent.

Infectious substances and diagnostic specimens likely to contain infectious substances require triple packaging in accordance with the recommendations of the United Nations²². Cultures of mycobacteria should be shipped on solid medium in screwcap tubes or freeze dried in vials as primary watertight containers. Petri dish cultures and cultures in liquid medium must not be shipped. The primary container should be entirely surrounded by at least two cm of absorbant material and enclosed in a second, durable watertight container. The tissue paper or cellulose wadding in the secondary container must be sufficient to absorb all of the fluid in the specimen in case of leakage of the primary container. Several primary containers may be enclosed in a single secondary container, if the total volume of all the primary containers does not exceed 50 ml and there is no contact between them²³. Each set of primary and secondary containers should be enclosed in an outer shipping container made of corrugated fibre board, cardboard, wood or other material of equivalent strength.

One copy of the request forms, letters and other information that identifies or describes the specimen should be taped to the outside of the secondary container. Another copy should be sent by air mail to the receiving laboratory and a third retained by the sender. The outer container must bear the infectious substance (biohazard) label. The label should be about 10 cm wide and printed in red on a white background. In addition to the sender's and recipient's addresses, the telephone numbers and fax numbers if available should also be put on the outside of the package.

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.

ANNEX 3 - FORM 1: SPUTUM SHIPMENT FORM

Country: Diagnostic Centre:

Code:

Code:

IDENTIFICATION OF THE PATIENT

Name:

TB district number: Date registered: | ___ | ___ | ___ |
Day Mo Yr

Sex: | ___ | Male | ___ | Female

Age: Years

Date of sputum collection : A B

Result of smear:

ANNEX 4 - FORM 2: CLINICAL INFORMATION

Country:
Code:

Diagnostic Centre:
Code:

A. IDENTIFICATION OF THE PATIENT

Name:

TB district 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 co-ordinating team):

for example, country of origin |_____|

HIV status |__|

history of drug abuse |__|

B. HISTORY GIVEN BY THE PATIENT

B1 Previously treated for TB? Yes |__| No |__|

If the answer is no, go to B2, if yes, go to B3.

B2 Standardised history

- how long have you been sick ?
- have you had the same symptoms prior to this episode?.....
- have you had other symptoms of lung disease prior to this episode (hemoptysis, chest pain, cough)?.....
- have you had X-ray examinations prior to this episode ?.....
- have you had sputum examinations prior to this episode ?
- have you had drug treatment for more than one month ?
- if yes, what were the name of the drugs ?
- have you ever received injections for more than one month ?

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

Yes |__| No |__|

If yes continue with B3

B3 Information about previous treatment

- where was the patient treated?.....
- when was the patient treated?.....
- how long was the patient treated?.....
- which drugs were used for treatment?.....
- by whom was the patient treated?.....
- how many courses of treatment were given?

- Outcome of the last treatment according to the patient.

cured not cured unknown

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

If "Yes", what was the outcome of the last course of chemotherapy:

cured treatment completed
defaulted failed
transferred-out

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 B1 and B2 and/or C was 'no')
Doubtful

D2 If yes, what was the outcome of previous treatment ?
cured/treatment completed
failed
defaulted
chronic
relapse/defaulter not distinguishable
unknown

Responsible Officer:

ANNEX 5 - FORM 3: RESULTS OF BACTERIOLOGICAL EXAMINATION

Country:

Diagnostic Centre:

Code:

Code:

A. PATIENT

Number:

Date of receipt:

|__| |__| |__|
Day Mo Yr

B. IDENTIFICATION

Sample A:

Sample B:

|__| *M. tuberculosis*

|__| *M. tuberculosis*

|__| *M. bovis*

|__| *M. bovis*

|__| *M. africanum*

|__| *M. africanum*

|__| Negative

|__| Negative

|__| Contaminated

|__| Contaminated

|__| Other

|__| Other

C. SUSCEPTIBILITY OF M. TUBERCULOSIS

Susceptible to:

Resistant to:

|__| Isoniazid

|__| Isoniazid

|__| Rifampicin

|__| Rifampicin

|__| Ethambutol

|__| Ethambutol

|__| Streptomycin

|__| Streptomycin

Date of recording: |__| |__| |__|
Day Mo Yr

Responsible Officer:

This form is to be made out in two copies. The original is to be sent to the diagnostic centre, the copy is filed at the central laboratory.

ANNEX 6: ANTITUBERCULOSIS DRUG RESISTANCE RESULTS

	Primary			Acquired		
	n°	%	95% CI	n°	%	95% CI
Total enrolled						
Total tested						
Fully sensitive						
Any resistance						
Mono-resistance						
H						
R						
E						
S						
H+R resistance						
HR						
HRE						
HRS						
HRSE						
H+ other resistance						
HE						
HS						
HES						
R+ other resistance						
RS						
RE						
RES						
Other multi-resistance						
ES						
Any H resistance						
Any R resistance						

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