Assessing tuberculosis prevalence through population-based surveys

World Health Organization
Western Pacific Region

Acknowledgements

Contents

Executive Summary

Tuberculosis (TB) prevalence surveys are most valuable in areas where notification data obtained through routine surveillance are of unproven accuracy or incomplete, and in areas with an estimated prevalence of bacteriologically confirmed TB of more than 100 per 100 000. To help in assessing the performance of TB control programmes, to provide information for planning, and to assess trends of the disease burden over time, data on TB can be collected through standard methods in a well-defined study population. This document gives overall guidance in conducting cross-sectional surveys of pulmonary TB disease. It is intended for TB experts and advisers at national and international levels, and investigators involved in prevalence surveys.

In general, the primary objective of TB prevalence surveys is to determine the prevalence of pulmonary TB in the general population aged 15 years and above. Such surveys can also be used to establish the prevalence of different TB risk factors and to study the determinants of TB disease. Objectives involving testing for HIV or TB drug sensitivity are discussed here as well but are not systematically recommended because of ethical, statistical, and logistical limitations. A specific limitation of TB prevalence surveys is their inability to estimate the burden of childhood TB due to the unsuitability of the currently available methods for diagnosing that form of the disease.

Because of the nature of TB and its low rate of occurrence in general populations, survey samples will be large (typically more than 10 000 individuals), and the sampling methodology will most often involve the selection of clusters from a population. Besides being at least 15 years old, the participants in a TB prevalence survey must meet residency criteria. A simple criterion renders eligible any individual of the targeted age group who slept in a selected household the night before the first visit of the survey investigators.

Screening is based on symptom assessment using a standardized questionnaire, chest X-ray examination, and bacteriological examination of sputum samples. Four alternative strategies in this regard can be considered:

Strategy 1. Identifying **all** smear- or culture-positive individuals by collecting two sputum samples for microscopic examination and culture from all eligible individuals. This strategy does not involve screening. It provides high-quality information but has been used in only a few small-scale studies because of the high costs and the significant demand for laboratory capacity. Besides sputum collection, a chest X-ray and the completion of a symptom questionnaire by all eligible individuals are recommended to provide more information and allow a comparison of the results of the survey with those of other surveys.

Strategy 2. Identifying **all** smear-positive individuals by requiring all eligible individuals to submit two sputum samples for microscopy examination and to undergo chest X-ray and symptom assessment. The sputum samples of all individuals who show any abnormality on any of these three tests (one or more smear-positive results, abnormality on chest X-ray, or presence of symptoms) are cultured. No chest X-ray abnormality is sensitive and specific enough for a definite diagnosis of TB. Therefore, individuals with abnormalities on their chest X ray should be considered suspect pulmonary TB cases. A symptom that has been used to identify suspects in TB prevalence surveys is cough that lasts for three weeks or more. A limitation of this screening strategy is that some individuals with culture-positive pulmonary TB may not be identified through chest X-ray examination, symptom assessment, and sputum smear screening. Furthermore, the sputum examination must be rapidly done because of the high number of sputum examinations needed. Fluorescence microscopy of sputum samples is a rapid method that has been shown to be more sensitive than light microscopy and to have equal specificity in diagnosing TB patients in the health system.

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┌──────────────────────┐         ┌──────────────────────┐         ┌──────────────┐
│ Symptom screening    │    ┌───→ │ No Symptoms          │ ───→    │ No culture   │
│ Chest X-ray screening│    │     │ Normal chest X-ray   │         │              │
│ Sputum smear         │────┤     │ Negative smear       │         │              │
│ examination          │    │     └──────────────────────┘         └──────────────┘
└──────────────────────┘    │
                            │     ┌──────────────────────┐         ┌──────────────┐
                            │     │ Symptoms             │         │              │
                            └───→ │ or                   │ ───→    │ Culture      │
                                  │ Abnormal chest X-ray │         │              │
                                  │ or                   │         │              │
                                  │ Positive smear       │         └──────────────┘
                                  └──────────────────────┘
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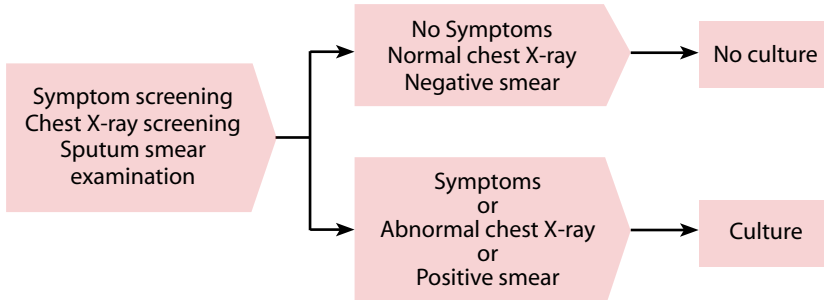
Strategy 3.   Identifying smear- and culture-positive individuals by administering a questionnaire on symptoms and taking chest X-rays of all eligible individuals, and obtaining two sputum samples for microscopic examination and culture from all individuals who show symptoms or abnormalities of any kind on their chest X-rays. Those with no abnormalities during screening are not considered tuberculous suspects and do not have to submit sputum samples. Screening populations for suspects has the advantage of limiting the number of individuals who are asked to provide sputum for microscopic examination (to ± 10% of the total surveyed population), thus allowing the data collectors to pay special attention to these individuals and reducing the workload of the laboratory.

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┌──────────────┐              ┌──────────────────┐         ┌──────────────┐
│ Symptom      │      ┌────→   │ No Symptoms      │ ───→    │ No smear     │
│ screening    │      │        │ Normal chest     │         │ microscopy   │
│ Chest X-ray  │──────┤        │ X-ray            │         │ No culture   │
│ screening    │      │        └──────────────────┘         └──────────────┘
└──────────────┘      │
                      │        ┌──────────────────┐         ┌──────────────┐
                      │        │ Symptoms         │         │ Smear        │
                      └────→   │ or               │ ───→    │ microscopy   │
                               │ Abnormal chest   │         │ Culture      │
                               │ X-ray            │         │              │
                               └──────────────────┘         └──────────────┘
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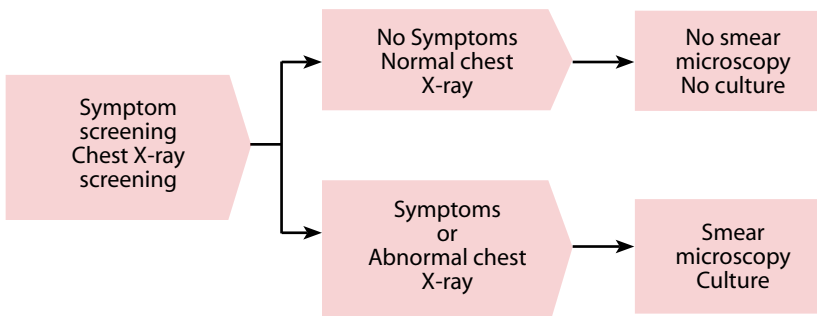
Strategy 4.   (If neither chest X-ray nor culture is available) Leaving out the screening step and collecting sputum samples from all eligible individuals for microscopic examination.

The following table shows the degree of success of the alternative strategies in identifying bacteriologically confirmed pulmonary tuberculosis. The strategies are sorted in descending order of epidemiological information, logistical complications, and cost.

Strategies for identifying bacteriologically confirmed pulmonary tuberculosis			
Strategy	**Identified Cases**	**Missed Cases**	**Comments**
Strategy 1	all S(+); all C(+)	None	Very intensive lab and CXR requirements
Strategy 2	all S(+); most C(+)	S(−) C(+) sym(−) CXR(−)	Very intensive lab requirements
Strategy 3	most S(+); most C(+)	S(+) sym(−) CXR(−); S(−) C(+) sym(-) CXR(-)	Most common screening method
Strategy 4	all S(+)	S(−) C(+)	May be considered where infrastructure is very limited

C(+) = culture-positive, CXR = chest X-ray, CXR(−) = normal chest X-ray, S(+) = smear-positive, S(−) = smear-negative, sym(−) = no symptom

During the fieldwork for a tuberculosis prevalence survey, data collectors should strive to collect all information according to the protocol. Missing information complicates the interpretation of the results. The number of those who refuse to participate can be reduced by clearly informing the community and the individual about the purpose of the survey, the benefits of participating, and the risks of not doing so. Persons who are not present during data collection should be traced. Inconclusive test results can be prevented by giving adequate training to investigators who will perform the diagnosis, using good-quality equipment, and systematically implementing quality assurance procedures. Each measurement tool (questionnaire, chest radiograph, bacteriological examination) has its own characteristics such as sensitivity, specificity, and positive and negative predictive values. These tools may be used for clinical or diagnostic purposes and for epidemiological measurement.

To be able to assign an epidemiological outcome to an individual, the person may have to be traced and follow-up examinations done. In the medical follow-up, every potential patient should be given the necessary care. Scenarios should be prepared to standardize case management and ensure correct medical care. If drug susceptibility is tested in the survey, those individuals identified as having drug resistance should receive the proper treatment free of charge.

Proposed survey protocols must be reviewed by appropriate ethical review committees, which must grant approval before the survey can be conducted. The survey funding agency should obtain from the ethical review committee a statement that the survey is organized and operates according to applicable laws and regulations. Documented approval should also be obtained. Typical ethical requirements are informed consent to participate and confidentiality.

Recommendations on the survey organization, the roles and responsibilities of investigators as well as survey monitoring and advisory bodies, and the conduct of field operations including training, data management, reporting, and data analysis, are provided in detail in this guide with a view to standardizing the surveys, ensuring their safe implementation, and maximizing the quality of the results. Standard laboratory methods are recommended. Quality assurance systems are needed for chest X-ray assessments, laboratory determinations, and other components such as questionnaires. A budget complete with itemized services should be prepared.

1.   Introduction

1.1   Purpose of the document

This publication provides countries with practical guidelines for planning population-based surveys to estimate the prevalence of tuberculosis (TB disease) at a national level. TB prevalence surveys yield useful information in areas where notification data obtained through routine surveillance are incomplete or of unproven accuracy, and in areas with an estimated TB prevalence of more than 100 per 100 000. These surveys are used to evaluate the performance of the TB programme and to assess trends over time. To achieve this objective, data are collected through standard methods in a well-defined study population. This document is meant to provide information on the core survey methods, including diagnostic tests for TB, screening strategies, and case definitions. The target audience includes TB experts and advisers at national and international levels, and investigators involved in prevalence surveys.

TB prevalence surveys have inherent limitations. They have limited capacity to provide prevalence estimates in sub-populations, e.g., specific population groups or geographic areas. Because TB is a relatively rare disease, very large numbers of individuals need to be surveyed to obtain a reasonably precise estimate of prevalence. This document emphasizes standard methods that will allow meaningful comparisons between countries or between different time points within countries. The core methods include standardized questionnaires, chest radiographs, and bacteriological examination of sputum specimens, with the results of bacteriological examination providing the basis for case definition. The methods described here do not include tools for assessing the magnitude of TB in children; rather, the methods focus on measuring TB in those who are at least 15 years old. Most TB prevalence surveys do not include children because bacteriological confirmation of TB in children is difficult especially in very young children (Van der Werf and Borgdorff 2007). Furthermore, published surveys indicate that the prevalence of TB in children, estimated with the standard tools, is not especially high. Previous surveys that included individuals below 15 years of age identified few smear-positive cases in this group. Only 3% of the total prevalent culture-positive TB cases found in a prevalence survey in South India (1984–1986) (Tuberculosis Research Center 2001) were among 10- to 14-year olds. In Bangladesh no cases were identified among 12 - to 14-year-olds (Hamid Salim et al. 2004). The yield of sputum in younger age

groups is very low and is negligible when compared with that for older age groups. (Hong 1998; Suryanasayam 1999) Hence, children aged 0–14 years are usually excluded from TB prevalence surveys.   However, the fact that children are excluded from the survey should be considered a limitation of the survey.

More experience will be gained as more prevalence surveys are implemented, and it is anticipated that this document will need to be revised on the basis of that experience.

1.2   Background

TB is one of the most frequent causes of death among adults despite being nearly 100% curable. The current global strategy of TB control is to prevent infection by efficient case finding and treatment and to stop the infection from progressing to an active disease. Control of TB continues to elude mankind more than a century after the causative organism was identified and more than half a century since effective anti-TB treatment was introduced. The majority of the cases are in the 15–54 age group, representing an economic burden to the largely poor countries where the disease is most frequent. The burden of TB is higher among males than among females. Globally, the burden of TB in 2005 was estimated to be 8.9 million new cases, of which about 3.9 million cases were sputum smear-positive (Dye 2006). The TB epidemic has worsened because of the association of TB with the HIV epidemic and the emergence of multidrug-resistant TB.

More and more cases are successfully treated under the WHO-recommended Stop TB strategy, preventing new infections and diminishing prevalence in several settings (WHO 2007). The Tuberculosis Research Centre (TRC) in Chennai, India, has demonstrated in community-based prevalence surveys in south India, where directly observed treatment, short-course (DOTS) was implemented in 1999, that the burden of TB has fallen considerably —a decline attributed to DOTS implementation (Tuberculosis Research Centre 2006a). The results of three tuberculin surveys conducted after DOTS implementation also demonstrated that a DOTS-based programme for TB control was associated with a reduction in prevalence of TB infection among children, compared with the pre-DOTS era (Tuberculosis Research Center 2006b).

The establishment of targets within the framework of the Millennium Development Goals (MDGs) and subsequent targets developed by the Stop TB partnership (see box on page 3) has given greater impetus to the evaluation of TB programmes. National TB control programmes

Goals, Targets, and Indicators for TB Control

Millennium Development Goal 6: Combat HIV/AIDS, malaria, and other diseases

Target 8:   Have halted by 2015 and begun to reverse the incidence of malaria and other major diseases

Indicator 23:   Prevalence and death rates associated with tuberculosis

Indicator 24:   Proportion of tuberculosis cases detected and cured under DOTS (internationally recommended TB control strategy)

Stop TB Partnership Targets

By 2005:   At least 70% of people with infectious TB will be diagnosed (under the DOTS strategy), and at least 85% cured.

By 2015:   The global burden of TB (prevalence and death rates) will be reduced by 50% relative to 1990 levels. This means reducing prevalence to around 150 per 100 000 or lower and deaths to around 15 per 100 000 per year or lower by 2015 (including TB cases co-infected with HIV). The number of people dying from TB in 2015, including those co-infected with HIV, should be less than 1 million.

By 2050:   The global incidence of TB disease will be less than 1 case per million per year (the criterion for TB "elimination" adopted within the USA).

DOTS = directly observed treatment, short-course

Note: The targets in this box were derived from, and are intended to be as consistent as possible with, a series of resolutions and directives issued by various bodies since 1991 (Group of Eight [G8], UN, WHO, Stop TB Partnership, International Union against Tuberculosis and Lung Disease [The Union], US Centers for Disease Control and Prevention [CDC]). However, they are not fully internally consistent from an epidemiological standpoint.

For example, halving the TB death rate by 2015 is much more demanding than the target of ensuring falling incidence by that year. There is an informal agreement within WHO that TB prevalence and deaths should exclude patients co-infected with HIV, mainly to avoid double-counting deaths from AIDS and TB. However, the aim of TB control is to eliminate the disease from whole populations. WHO therefore reports TB statistics and estimates for both the HIV-positive and HIV-negative sub populations. The criterion for elimination in this text box is that defined by the US CDC, and differs from a European recommendation that elimination be defined as less than one smear-positive case per million by 2050.

need to measure more actively the epidemiological impact of control, besides monitoring DOTS implementation. The measurement of DOTS implementation has focused on assessing progress in case detection and treatment success. The evaluation of the impact of TB control requires the measurement of TB prevalence, incidence, and deaths. The success, or failure, of TB control needs to be evaluated for the priority countries and regions with the highest burden of disease, and for the world as a whole.

Prevalence is a relatively sensitive indicator of the impact of chemotherapy that typically changes faster than incidence. The ratio of prevalent (at one point in time) to incident (over a year) cases changes from being often twice as high when no chemotherapy is given to much less than one when case detection and treatment are highly efficient. Alternatively, when services are of poor quality, the ratio may rise even higher than when no chemotherapy is given because of the relatively greater efficiency of saving lives as compared with curing patients.

The prevalence of smear-positive and bacteriologically positive TB can be measured accurately in population-based surveys, though precision is influenced by the large sample size needed for a rare disease like TB. An additional virtue of prevalence surveys is that they can be used to evaluate the quality of surveillance by identifying which patients with active disease have already been captured by the routine reporting system. Questionnaires can be used to explore the reasons why some patients are diagnosed and treated for TB while others are not. In general, prevalence surveys provide a platform for exploring the interactions between patients and the health system, and the links between TB and other social and economic factors (potential determinants of health). Population-based surveys of the prevalence of infection and disease, and occasionally of incidence, have been carried out since the 1950s. Annex 15 lists all the surveys of infection and disease that are known to WHO, including those that are in progress or planned.

Some countries have carried out surveys that effectively set baselines for monitoring progress towards the MDGs. China did prevalence surveys in 1990 (Ministry of Public Health, China, 1990) and 2000 (Ministry of Public Health, China, 2000). Indonesia's prevalence survey in 2004 (Ministry of Health, Indonesia, 2005) gave results that can be compared against surveys done in the early 1980s. Both sets of surveys showed significant reductions in prevalence. Cambodia carried out a well-executed prevalence survey in 2002 (Ministry of Health, Cambodia, 2005). India's TRC is closely and regularly monitoring changes in TB infection, incidence, prevalence, and deaths in the model DOTS project in Tamil Nadu in south India, and an expert group

has national TB prevalence survey in Eritrea conducted in 2005 collected sputum samples from all eligible individuals and used fluorescence microscopy to examine the samples (manuscript accepted for publication by Bull of WHO). National or subnational disease prevalence surveys are planned or under way in Armenia, Bangladesh, Myanmar (selected provinces), Tanzania, and Viet Nam. Some of these surveys have been funded with grants from the Global Fund to fight AIDS, Tuberculosis and Malaria.

1.3   Estimating the burden of TB disease

Data on the burden of TB disease are important for programme planners to determine resource requirements and to monitor the impact of TB control measures. The burden of TB must be estimated to measure progress towards the MDGs of halting the spread of TB and halving its prevalence and mortality from the disease by 2015. Epidemiological impact is measured in terms of prevalence and incidence, as estimated directly through prevalence surveys. Prevalence is the number of cases in the community at a given point in time, whereas incidence is the number of new cases during a defined period (usually one year). Prevalence directly determines risk of infection, which drives the course of the epidemic. Incidence is secondary to infection, following at a diminishing rate for many years after its onset, and, is therefore also an indicator of progress in controlling TB.

Incidence is the chief measure of progress towards elimination, which is the principal, long-term goal of TB control. Incidence in large populations (national or global) is best monitored through routine surveillance, provided the data obtained are of sufficient duration (to determine trends), of proven accuracy, and sufficiently comprehensive (to ensure that a high proportion of all cases are detected). When a high proportion of all countries have good surveillance, the trend in case notification will become a valid indicator of progress in programme implementation. Meanwhile, incidence estimates are fraught with difficulty and may not give accurate estimates of the level or trends of the disease.

As a short-term (less than five years) measure of the effect of TB control, incidence is less suitable than prevalence because it changes more slowly (so changes are statistically harder to measure), and there are fewer incident cases in any one year than prevalent cases at any one time before services become efficient enough to detect and treat the cases early. Consequently, in highly endemic countries, especially those with weak surveillance systems, the effect of TB control is better measured through periodic disease prevalence (cross-sectional) surveys.

Calculating incidence through prevalence surveys requires a way of detecting new cases that occur between surveys and is not considered a practical or feasible option. In principle, incidence may also be derived from prevalence and an estimate of the duration of disease (incidence = prevalence / average duration of disease). However, estimates of incidence derived from prevalence and duration of disease are generally highly uncertain.

Cost and logistic complexity are the main drawbacks of prevalence surveys. Two or more prevalence surveys can probably be done between now and 2015 in some—but not all—countries with a high burden of TB. Even in countries that can do repeated surveys, these are most useful if done at least every five years.

Because a variety of methods have been applied by different countries to measure prevalence, there is an urgent need for standardization. This document recommends methods and procedures for conducting a prevalence survey to estimate the prevalence of TB.

1.4   Deciding whether or not to do a TB prevalence survey

Countries with an estimated TB prevalence of more than 100 per 100 000 are encouraged to carry out a TB prevalence survey or a series of such surveys, as these are likely to be beneficial in assessing prevalence and trends, and in optimizing planning for TB control. In some countries, national prevalence can be estimated by calibrating the relation between risk of infection, as determined from tuberculin surveys, and prevalence, where both have been measured locally (e.g., in the model DOTS project in south India). However, the risk of infection is often hard to determine from tuberculin survey results if infection rates are low, sensitization to environmental mycobacteria is frequent, and BCG coverage is high, because of the limited specificity of the tuberculin test. Over the past five years, tuberculin surveys have been found to be useful in India but not in other settings like Afghanistan, Cambodia, and China. The use of standard cut-off points, or antimodes, gives results of doubtful validity because of the variable performance of tuberculin between surveys (due to variations in batch and field methods). Tuberculin surveys may be more generally useful in evaluating changes in the risk of infection over time, rather than the absolute risk. In addition, tuberculin surveys cannot be used to estimate the incidence of disease since there is no fixed relationship between incidence and annual risk of infection. A freely available generic protocol for tuberculin surveys in schools is at http://www.kncvtbc.nl.

Other population-based health survey platforms in existence (e.g., demographic and health surveys) have not been used to measure prevalence because the definitive diagnosis of TB requires bacteriology (sputum smear microscopy or culture), which is difficult to organize in the setting of such surveys.

The decision to carry out a prevalence survey in any country should be guided by a series of assessments of the following:

The estimated prevalence of bacteriologically confirmed TB— if lower than 100 per 100 000, then the required sample size for a prevalence survey may be excessively large. The table below shows sample sizes needed at various levels of estimated prevalence of bacteriologically confirmed TB (see chapter 4):

Prevalence per 100 000	Sample Size*	Cluster Size*
50	212 974	4259
100	106 434	2129
150	70 920	1418
200	53 164	1063
300	35 407	708
400	26 529	531

* Assumptions: 25% precision, design effect 1.3, 25% eligible cases missed, 50 clusters.

■ The quality of existing information on the burden and trends of TB disease.

■ The capacity of the national TB programme to translate survey results into policy.

■ The availability of prior TB prevalence surveys, with good-quality data that allow the assessment of trends in prevalence.

■ The commitment of the national TB programme to support a prevalence survey.

■ The anticipated level of participation of the population in the survey activities.

■ The human resource capacity to conduct a survey, the logistical feasibility of such a survey, and security for survey staff.

After such assessments, the prevalence survey should proceed in the following steps:

1. Identify investigators and partner agencies.

2. Develop a comprehensive survey protocol including a preliminary budget.

3. Pilot-test the survey methods and tools.

4. Amend the protocol on the basis of the experience from the pilot-testing.

5. Carry out procurement and implement the survey.

6. Monitor the survey.

7. Analyze and disseminate the results.

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2.   Survey Objectives

The statement of the objectives of a research project summarizes what is to be achieved by the study. Clear objectives will help to focus the study on essentials and avoid the collection of information that will not be used.

The general or primary objective of a study states in general terms what researchers expect to achieve by doing the study. The general objective of a tuberculosis (TB) prevalence survey can be expressed as follows:

To determine the prevalence of pulmonary TB at a defined point in time in a specified country

The general objective is broken down into smaller parts—specific or secondary objectives. Properly formulated, specific objectives will facilitate the definition of the research methods and will help to orient the collection, analysis, interpretation, and use of data.

Examples of specific objectives related to the general objective stated above are:

- To assess the prevalence of symptoms suggestive of TB.

- To assess the prevalence of radiological abnormalities suggestive of pulmonary TB.

- To assess the prevalence of smear-positive pulmonary TB.

- To assess the prevalence of culture-positive pulmonary TB.

Besides specific objectives that are directly linked to the general objective, others that state what will be done with the information obtained from the survey can also be included. Examples of these objectives are:

- To assess trends in TB prevalence.

- To assess the rate at which TB cases are detected by the TB programme (patient diagnostic rate [Borgdorff 2004]).

If the TB prevalence survey is combined with a tuberculin survey (Arnadottir et al. 1996) or if the results of a (recent) tuberculin survey are available, the following specific objectives can be included as well:

- To compare the prevalence of TB and the prevalence of tuberculous infection.

- To compare trends in prevalence of TB disease and infection.

- To explore the relationships between the prevalence of TB disease, TB infection, and notification rates.

A TB prevalence survey needs an extensive organizational structure. Given the amount of time and effort invested in a TB prevalence survey, it is prudent to use the opportunity to address other study questions in the survey population, whether related to TB or not. The additional studies can use all the individuals included in the TB prevalence survey or a sub-sample of this population. Whenever other studies are added, objectives should be formulated for each of them.

Examples of objectives for additional studies of health care–seeking behaviour are:

- To assess the health care–seeking behaviour of individuals reporting chest symptoms.

- To identify reasons for low case detection (low proportion of already known, under treatment, or notified cases among all cases detected during the survey).

- To assess health care-seeking behaviour among prevalent cases of pulmonary TB that are not being treated, compared with patients currently being treated for TB.

Examples of objectives for additional studies carried out to identify risk factors are:

- To assess the prevalence of HIV infection and risk factors for HIV in the sample studied in the prevalence survey.

- To assess the prevalence of risk factors for TB (e.g., smoking, diabetes, alcohol, malnutrition, crowding, indoor air pollution, silicosis, socioeconomic status) in the sample population.

- To determine the association between the risk factors above and TB cases detected by the survey.

Furthermore, if a prevalence survey is combined with a survey of patients registered for treatment in the TB programme, the demographic and socioeconomic profiles of those registered for treatment can be compared with the profiles of those not registered, to measure equity of access. Annex 13 details possible studies of potential socioeconomic determinants that can be added to a TB prevalence survey.

Additional studies should be included only if they do not compromise the ability of the main study to address the general objective. Before adding other studies, the capacity and capability of the research staff should be carefully assessed.

Both the general and the specific objectives can be limited to specific age groups or areas (e.g., urban/rural). If so, the objective should be formulated to make this explicit.

It must be clearly understood that the addition of other studies will have implications for the sample size required, particularly if these studies address associations with the TB patients detected in the survey. Before studies are added, such implications and the consequent added complexity and cost of the research must be carefully considered.

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3. Survey Design

The design of the study has a direct impact on survey planning and on sampling methods. Tuberculosis (TB) prevalence surveys usually follow a cross-sectional design, with the general population as the target population. Prevalence assessments are based on a mix of old and new cases with different durations of a disease, and do not provide specific information on new or incident cases.

Descriptive studies determine how often a disease occurs, what kinds of people it affects, and where and when it occurs. Such studies usually comprise observations of patients or subjects made at one point in time to examine particular characteristics—so-called cross-sectional, or "snapshot", studies. A descriptive study merely describes the occurrence of a disease in a population and makes no attempt to analyze the link between exposure and effect. A TB prevalence survey can be simply a descriptive study if it does not attempt to identify the determinants of the disease or to make a statistical analysis of associations of the disease with other factors.

On the other hand, the prevalence survey can also establish the association between a disease and the presence of a characteristic or exposition to a factor. Information collected on the presence or absence of a disease (TB) and the presence or absence of another factor (a potential determinant) can be used to analyze the association between the potential determinant and the disease by comparing the prevalence of the disease in those individuals with the potential determinant and in those without it.

Cluster sampling is commonly adopted in surveys for administrative and operational convenience, as simple random sampling of individuals is not practical in large populations. Certain geographical areas or population groups may be excluded from the sampling frame because of difficult terrain, poor security, or other reasons. In that case, their exclusion must be explicitly stated and conclusions drawn from the analysis tempered by this exclusion.

A TB prevalence survey is usually a national survey designed to provide an estimate of prevalence for the population of an entire country. All eligible people in the country are included in the sampling frame. Limiting the survey to a given area or a defined subset of the population has major implications for the conclusions that can be drawn from the survey and must therefore be considered carefully before such a decision is made. With few exceptions, it is recommended that prevalence surveys incorporate a representative sample of the national population so that conclusions can be drawn about the country as a whole.

The timing of the survey should be selected carefully. Most often, TB prevalence surveys are designed to estimate TB prevalence at one point in time, to obtain a measure of the current level of the epidemic. Seasonality may affect TB incidence and, to a lesser extent, TB prevalence. Ideally, the survey should last no longer than one year from the start of field activities.

Besides the geographical area to be covered by the survey, the characteristics of the study population need to be clearly specified. For example, most prevalence surveys are carried out to assess prevalence in the adult population aged 15 years and above. If this is the case, it must be clearly stated and taken into account in selecting the sampling frame.

3.1 Inclusion criteria

A census will most often have to be carried out to provide accurate information on the population in selected survey areas. Individuals who are eligible for inclusion in a TB prevalence survey are usually residents of the community who may be selected through their identification with a household. Consequently, issues such as migration and an understanding of who are members of a household are important considerations. If membership in a household is a clearly defined concept and migration is limited, being a member of a household can be used to define eligibility. Otherwise, definitions like "having slept in the household the night before the survey team visit" may be used. Demographic and health surveys typically use as an eligibility criterion either "all those who usually live in the house "or " all those who slept there the night before".

Since bacteriological positivity is rare in those below 15 years of age and collection of sputum samples difficult, the exclusion of this group will reduce the workload while having only a small effect on the prevalence estimate for the total population.

To increase the feasibility of data collection, institutionalized populations are sometimes excluded from the sampling frame. Those living in inaccessible or restricted areas are also often excluded from the sampling frame, as exposing the field teams to danger in areas where there is conflict or other incidents is regarded as inappropriate. This exclusion, which may bias the results of the survey, should be clearly stated in the report on the survey and explicitly considered when conclusions are drawn from the study. As much as possible, all groups in the population should be included in the sampling frame; any departure from this principle is a weakness of the study design.

Individuals can be included in the survey only if they have been fully informed of the objectives and procedures of the study and have freely given consent (oral or written). Individuals who cannot provide informed consent, perhaps because of psychiatric illness or language problems, cannot be included in the survey. The consent procedure must be carefully described and approved by an ethics review committee.

Examples of inclusion criteria are the following:

- all those who slept in the house the night before the survey team's visit;

- those who are at least 15 years old;

- those who have provided informed consent.

3.2 Exclusion criteria

Exclusion criteria mirror inclusion criteria. Temporary residents may be included depending on the selected criteria for inclusion. Nonresidents who are present in the selected area and are interested in undergoing examination are regarded as externals. Residents of the area who do not belong to any household in the community are regarded as homeless persons. A decision must be made whether or not to exclude from analysis some categories of patients such as externals or temporary residents screened for tuberculosis. Since homeless and internal migrants may have a higher burden of TB, they should be included in the assessment of prevalence in the community where they are staying. Screening all persons present at the time of the survey is likely to improve cooperation from the entire population in the selected area.

Examples of exclusion criteria are:

- all those who did not sleep in the house the night before the survey team visit;

- those who are less than 15 years old;

- those who are unable to provide informed consent or refuse to participate.

4. Sampling Methods

4.1 Sampling methodology

The prevalence of tuberculosis (TB) differs between countries, from below 10 per 100 000 in many established market economies to as high as 500–1000 per 100 000 in southern and east African countries. One needs a sample size of between 500 000 and 1 million in the established market economies, but only as few as 10 000 to 20 000 in southern and east Africa, to find at least 100 cases and estimate prevalence to within ±20% of its value.

It is important to optimize the design of the survey to get as much information as possible at the lowest cost in money and effort. To get a simple random sample one would start from a complete list of everyone in the country, choose the requisite number of people at random, and see how many of them have TB. But in practice, one is likely to have only an estimate of the population distribution across the country; the problem is to identify a sample that accurately represents the population of the country while minimizing costs and keeping the logistics manageable. For a representative sample, people from as many different places as possible should be included, but to minimize costs and keep the logistics manageable, it is better to include a number of people from each place that is sampled since much of the cost is associated with getting the survey team from one place to another. A better strategy, therefore, is to use a cluster design, in which a number of clusters in different places, and then a suitable number of people from each cluster, are chosen. The key to the survey design is deciding how many clusters and how many people in each cluster to choose so that the sample obtained is sufficiently large.

If the prevalence of TB is the same in all clusters, only the people from one cluster need to be sampled. In practice, however, prevalence will vary between clusters, depending on local conditions; the people who are chosen must therefore be representative of the general population as regards age, sex, social, and other circumstances and the clusters chosen must be representative of all possible clusters in the region of interest. Because prevalence is expected to vary from one place to another, enough clusters must be chosen to ensure that the variation between clusters averages out and does not greatly add to the overall uncertainty in the estimates.

One further decision has to be made. If, for example, the prevalence of TB is believed to be different in urban and rural settings or between northern and southern areas of the country, then a stratified cluster sample might be used to allow separate estimates of prevalence in urban and rural areas, or in the north and the south of the country. In that case the sample in each stratum must be large enough to give a sufficiently accurate measure of prevalence. The first task is to estimate the expected prevalence. WHO publishes annual estimates of the prevalence of TB in each country in the world.

If a previous survey was carried out, this, combined with an estimate of the probable changes since then, can be used to provide the initial estimate of prevalence. After that, the desired precision of the estimate of prevalence can be decided. A more precise estimate will require a larger sample size and cost more money. An initial estimate of the sample size and the cost of the survey can then be made. Typically, a prevalence survey will cost around $10–$20 per person sampled. The survey must be affordable. Depending on the sample size and the cost, more than one stratum or group of people can be surveyed: urban and rural, different geographical areas, perhaps men and women. If two or more strata are to be surveyed, then sample size should be calculated separately for each stratum. A distinction must be made between proportionate and disproportionate stratified sampling, depending on whether the sampling fraction differs between strata or not. The purpose of proportionate stratification is to ensure that the sample is correctly proportioned in each strata, to reduce standard errors for survey estimates. Proportionate stratification will be most efficient if prior information on tuberculosis prevalence suggests that prevalence may be concentrated in some areas that define specific strata. If dispoportionate sampling is used, some strata are over-sampled relative to others, with the main purpose to do separate analysis by sub-population, e.g. urban versus rural. To obtain unbiased estimates for a disproportionate stratified sample, the survey estimates have to be weighted. This is done by defining a weight per case, using a weight variable that equals the inverse of the sampling fraction used in the strata that the case belongs to. Proportionate and disproportionate stratification do not necessarily lead to an increas in precision of overall estimates.

Now consider just one stratum. A number of clusters will have to be identified, each corresponding to a well-defined unit such as a village, a town, or a district. These clusters are to be chosen from districts or possibly cities. Since the clusters should be representative of the whole population they should be chosen at random. However, when the average prevalence

over the whole country is worked out, clusters chosen from districts with many people will have to be given more weight than clusters chosen from small districts. The necessary weighting could, of course, be done after data collection, but it is more efficient, both statistically and logistically, to choose the sample to be self-weighting. All possible districts from which clusters may be drawn are therefore listed, with the help of the demographic data available, and then districts are chosen randomly from the list but in such a way that the probability of choosing a particular cluster is proportional to the size of the district. A decision must be made regarding the inclusion of confined populations within clusters, e.g. when a prison is included in the cluter area. For practical reasons and increased sampling efficiency, it may be decided to exclude such confined populations.

Finally, the number of clusters and the number of people in each cluster must be decided. The choice is between having more clusters and fewer people in each and having fewer clusters and more people in each, in such a way as to give the necessary overall sample size. Since setting up a new cluster is more expensive in time and money than adding people to an existing cluster, there should be as few clusters as possible. But if there is wide variation in the prevalence of TB between clusters, there should be as many clusters as possible to ensure that this variation averages out and does not add to the uncertainty of the estimate. The essence of good survey design is to find the optimal balance between the number and size of the clusters. This is discussed in the next section.

4.2   Sample size determination

Prevalence surveys have been carried out in many countries over many years. A survey that was particularly well designed and executed was recently completed in Cambodia, and it is used here to illustrate the general points raised and to show how sample sizes may be calculated.

The Cambodia prevalence survey was carried out in 2002 in parallel with the expansion of the DOTS programme. WHO had estimated in 1998 that for every 100 000 people in Cambodia 483 had sputum smear-positive TB, and the intention was to measure the prevalence in 2002 with 95% confidence limits equal to ±25% of the prevalence or less. If the number of people in the sample is taken to be n and the number with tuberculosis is p, then the mean prevalence of TB is

$$\mu = \frac{p}{n} \tag{1}$$

and the standard deviation of the estimate μ is given by the usual binomial expression

$$\sigma = \sqrt{\mu(1-\mu)/n} \tag{2}$$

Because TB is a rare condition, the mean prevalence μ is always very small. In the case of Cambodia the prevalence in the 1998 survey was μ = 483 / 100 000 = 0.00483. Since $(1 - \mu)$ 1, 95% confidence limits for the estimated values of μ are $\pm$ where = 1.96 so that

$$n = \frac{\mu}{\sigma^2} = \frac{1.96^2}{\mu\varepsilon^2} \qquad (3)$$

With μ = 0.00483 and = 0.25, equation gives a sample size n = 12 726.

When using a cluster sample one needs to think about how best to choose the clusters and also how the choice will affect the sample size. The problem, as noted above, is that in a survey the sample is taken from only a proportion of all possible clusters and the number of clusters needs to be kept as small as possible to minimize the costs and to simplify the logistics. However, if the prevalence of TB is different in different clusters and if a repeat survey with a different set of clusters may give a different answer, allowance must be made for this additional source of variation. If a previous survey was done, its results can be used in estimating the variation in prevalence between clusters; if not, one's knowledge of TB in the country can be used to make a best guess of the inter-cluster variation. If the prevalence of TB is taken to vary by $\pm \alpha$ times the actual prevalence (where α is the standard deviation, so that 1.96 α gives 95% confidence limits), then it can be shown that the variance of the estimated prevalence increases by a factor of D where

$$D = 1 + \alpha^2 m\mu \qquad (4)$$

D is called the "design effect" or variance inflation factor and m is the number of people in each cluster. D is big if the clusters vary considerably in the prevalence of TB so that α is big or if there are many people in each cluster and by implication relatively few clusters so that m is big or if the prevalence is high so that μ is big.

In the Cambodia survey there were 42 clusters so that $m \approx 12\ 726 / 42 = 304$ using an estimated prevalence of μ = 0.00483. If the variation between clusters were to be assumed, generously, to be about 80% of the average value so that the standard deviation is about 40% of the average value then $\alpha \approx 0.4$ and the design effect is

$$D = 1 + 0.4^2 \text{ x } 304 \text{ x } 0.00483 = 1.24 \qquad (5)$$

Because the design effect increases the variance by this amount, one must either accept the less-than-desired accuracy of the estimate or increase the sample size by this amount. Usually the latter is done; the sample size should therefore be about 12 726 $\times$ 1.24 = 15 780.

In a real field survey some people can always be expected not to show up or to drop out during the survey. In Cambodia it was assumed that as many as 25% of the people might not comply fully with the X-ray examination and the sputum collection. The sample size was therefore increased to 15 780/0.75 = 21 040. Finally, a decision was made to have X-rays of children who were at least 10 years old; the census data indicated that these were about 72% of the population, for a total population sample of 29 223, or about 30 000 people. With 42 clusters, the final sample size gave 700 people per cluster.

All of these estimates may be wrong to a greater or lesser degree; the important thing is always to err on the cautious side. If the sample size is too big it will cost more but a good result will be obtained; if the sample size is too small it will cost less but the estimate one gets may too inaccurate to be of any use.

The Cambodian survey therefore proceeded as follows:

- Cambodia has 11.4 million people living in 13 700 villages in 185 districts.

- A total of 42 districts (35 rural and 7 urban) were chosen randomly, with probability proportional to size (see Appendix 1 on page 217).

- One village was chosen at random from each district. There are 832 people per village on average. In villages with 680–760 people, all were included. If the number of people in the village exceeded 760, households were chosen at random until the number of people included reached 720. If the number in the village was less than 680, the people in the village immediately to the north were added and sampling continued.

- The work was planned to ensure that each 15-person survey team could complete the work in each cluster in one week. On days 1 and 2 a census was carried out and informed consent was sought; on days 3 to 6 interviews, X-rays, a tuberculin test, and sputum collection were done; and on day 7 the last sputum samples were collected and tuberculin indurations were read.

The point to be borne in mind is that the number of people sampled in each cluster, and so also the number of clusters, depends on the constraints imposed by the logistics of carrying out the survey.

Finally, the cost of the survey must be considered; it will depend not only on the size of the sample but also on the design and in particular the number of clusters. Detailed data on the costs of the Cambodia survey were not reported but some information was obtained from those who carried out the survey and is given in Table 4.2 below.

TABLE 4.2: Total and Marginal Costs Reported for the Cambodian Survey	
Item	**Cost ($)**
Capital investment	120 000
Consumables	60 000
Field operation	100 000
Training	30 000
Post-survey events	30 000
Others	60 000
Total costs[1]	400 000
Marginal costs[2]	
Adding a new cluster	1688
Adding a person to an existing cluster	2.5
Total (for 42 clusters and 29 000 people)*	143 000

[1] $220 000 from the World Bank, $150 000 from the Japan International Cooperation Agency (JICA), and $30 000 from the WHO Western Pacific Regional Office. Technical assistance was provided by JICA and WHO.

[2] The marginal costs were reported as being $2-$3, and of adding a cluster of 700–750 people about $3 000–$4 000.

It is important to note that capital investment, training, post-survey events, and other costs made up 60% of the total cost, while field operations and consumables made up 40%. Furthermore, adding a new cluster of 700 people would cost about $3400, while adding 700 people to an existing cluster would cost about $1800, or roughly half as much. If the intent is to double the sample size, adding more clusters will increase the total cost by 38% (to $543 000) but adding more people to existing clusters will increase the total cost by only 19% (to $476 000). The key design issue is to ensure that there are enough clusters to average out the variation between clusters but not so many as to make the costs prohibitive.

5. Testing Methods

5.1 Assessing symptoms with questionnaires

The questionnaire, an important measurement tool in a prevalence survey, is used to collect demographic data, information about tuberculosis (TB) symptoms, and other relevant information. To collect proper information, it is essential to ensure that information is collected in a standardized and unbiased manner. To ensure the quality of the information collected, the questionnaire must be carefully designed and the procedure for completing the questionnaire must be clearly described. The questionnaire should include a brief introductory statement indicating the objective of the questionnaire and the right of people to decline to participate or to answer specific questions. Furthermore, the confidentiality of the responses must be safeguarded.

Questionnaire design: personal information and confidentiality. Each person who participates in a prevalence survey is assigned an identification (ID) number. The ID number is used to label the questionnaire for that person. Personal information, such as name and address, are collected in a separate file. The link between personal information and the ID number should be carefully safeguarded and accessible only to authorized persons to ensure confidentiality.

Principles of questionnaire design. The key principle in designing a questionnaire is to ensure that the questionnaire is as clear, simple, and precise as possible.

The use of the questionnaire must have clear objectives. Questions should be included only if they collect information that addresses the objectives; irrelevant questions should be avoided. A short questionnaire can be completed within a reasonable time span, whereas people may lose patience and attention if the questionnaire is too long.

Questions should be simply worded and intelligible to the general population. They should be precise in meaning and should not be open to ambiguous interpretation. Wording that implies expectation of a particular answer should be avoided. The sequence of questions may substantially affect the quality of the response. The questionnaire should begin with easy and straightforward questions and keep complicated or embarrassing questions in its latter part. Previously standardized questions should also

come before others, and questions about symptoms before those about possible causes (for example, questions about respiratory symptoms should precede those about tobacco smoking). Questionnaires designed in a language that is not routinely used by the population should be translated into the local language. A translated questionnaire must be translated back into the original language and be checked by a different person who understands both languages to ensure that the meaning of the questions is properly understood.

Ease of data abstraction, entry, and analysis is a major issue in designing a questionnaire. A structured question with discrete answers is less ambiguous, more reliable, and much easier to enter into a database and analyze than an open-ended question. Procedures for completing the questionnaire are also crucial. The interviewer-administered questionnaire is recommended over self-completed questionnaires for prevalence surveys because self-completed questionnaires are more likely to be incomplete.

5.2   Chest radiography methods

In TB prevalence surveys, chest radiography is often used to screen individuals. Those with abnormalities in the chest X-ray are considered suspects. As the chest radiograph is a "medical and diagnostic" procedure in addition to being a measurement tool for the survey, it must be interpreted with both of these functions in mind.

5.2.1   Medical and diagnostic aspects of chest radiography

Promptly after the chest radiograph is obtained, a qualified individual must read the film to determine the presence of any abnormality that requires further investigation. The individual responsible at each location where chest radiography is being performed must be named and should have recognized qualifications to read the film. This individual must have written guidelines indicating the types of abnormalities sought and the plan of action to be taken when such abnormalities are found.

Whenever an abnormality is detected in a chest radiograph, the patient must be told and advised on further action. Before the patient is assisted further in seeking care, permission for such assistance must be obtained from the patient, even if it merely involves sharing the information from the chest radiograph with a health-care provider. Two types of action must be planned in this regard:

■ Immediate action. Certain abnormalities in a chest radiograph necessitate immediate action. An example of such an abnormality is the presence of a pneumothorax, pneumonia, or an abnormality that could be malignant. In such a case, the patient must be immediately advised of the necessity of seeking care and assisted in obtaining that care. Obviously, the written guidelines must contain information about the location where such care can be obtained at each of the study sites and the name of the responsible individual who will arrange for such care.

■ Necessary, but not urgent, investigation. The patient must be informed whenever any abnormality is detected in the chest radiograph. Where immediate action is not required, the designated individual who reads the film on the spot or the one who later reads the film for epidemiological purposes (if the abnormality is detected only at that point) must inform the patient about the abnormality and advise further action. Permission to notify a health-care provider, who will then assume the responsibility for further investigation and care, is sought from the patient. No action can be taken without specific permission from the patient to share information with other care providers. Once again, the written guidelines need to spell out exactly how such permission is to be obtained and a health-care provided notified at each study site.

5.2.2 Epidemiological aspects of chest radiography

No chest radiographic pattern is absolutely typical of tuberculosis, though certain configurations have traditionally been viewed as highly suggestive of tuberculosis. Chest radiography can undoubtedly be very helpful in localizing abnormalities in the lung, but to establish the etiology of such abnormalities further investigations are required; in the case of tuberculosis, bacteriological examination will confirm the diagnosis. Over-dependence on X-rays for diagnosis often overlooks the inherent limitations associated with their interpretation.

The observer error determines the reliability of X-ray diagnosis to a great extent. Besides this, it is difficult to assess the activity of the lesion and to determine the etiology on the basis of X-rays alone. Chest X-rays, being two-dimensional, conceal almost 20%–30% of the lung field because of overlapping structures and thus further limit interpretation. Hence, purely radiological criteria cannot give satisfactory evidence of tuberculosis in the individual patients without further investigation.

Despite the limitations described above, however, chest radiography is used as a survey tool in prevalence surveys.

Type of chest radiograph. For prevalence surveys, the full-size postero-anterior film is recommended. With each examination, the film must be correctly labelled with the survey number and must be of good quality.

X-ray reading. The film is ideally read by two independent readers. In case of discrepancy between the first two readers a third reading is obtained from an "umpire" reader.

5.2.3   Chest radiography equipment

In prevalence surveys, the X-ray unit is usually mounted onto a mobile van (vehicle) for radiological examination. Difficult terrain without passable roads may pose many problems for these units. In such cases, it may be possible to establish a centre to which all persons will be directed for X-ray. If some participants do not wish to take the time to have the examination, special arrangements must be made to transport these patients. Bringing back the films to the main centre for processing, arranging for independent reading, and preserving the films for future reference may also be difficult.

The use of chest X-rays is complicated by the high cost of the mobile chest X-ray equipment, logistical difficulties (accessibility of field sites by trucks, power supply, field robustness of the chest X-ray equipment, maintenance and repair), and the demands on human capacity, in particular the need for experienced clinicians in the field teams who can read chest X-rays.

Issues to consider when procuring chest X-ray equipment are the speed of operation (patient throughput and film development time), image quality, weight and size of equipment, power requirements, and safety (radiation dose). Three different types of chest X-ray systems are available: (1) mass miniature radiography; (2) conventional X-ray (with conventional or automated developing); and (3) digital X-ray (computed radiography, direct digital systems, or slot-scan systems).

Mass miniature radiography is no longer recommended since it has a large power requirement (three-phase, 30 kilowatt [kW] generator), gives significant radiation exposure to the patient, and produces low-quality images because of their small size. Fluoroscopy is not recommended for safety reasons. Furthermore, the lack of film complicates quality assurance.

Conventional chest X-ray systems with a conventional developer are relatively inexpensive ($30 000–$50 000); can be transported or built in

a small truck; are simple, robust, and easy to operate; and can often be maintained locally. Such systems use a condenser system running on a 3 kW generator. However, operation and the developing process are slow and the quality of film development is critical—a serious disadvantage in TB prevalence surveys. Furthermore, the developing process needs conditioned temperature (32°C).

Conventional systems with an automated developer have advantages over conventional systems with a conventional developer. The developing process is faster and technical errors are less critical. Small developers are available for less than $10 000. But these systems require calibration and maintenance, are still relatively slow, and have no instant quality check.

Conventional systems with a conventional developer or automated developer require transport of films for centralized rereading.

Digital X-ray systems are increasingly available for mobile use. They do away with the need for films, developing machines, and development solutions, and thus simplify the logistics. The X-ray images can be sent by Internet to a central place where they can be assessed by X-ray readers. In computed radiography, a phosphor plate is irradiated by a conventional chest X-ray system, and the phosphor image is converted into a digital image by a digital plate reader. Any conventional generator can be used, and the plates are reusable. There is no film developing and a quick quality check of the image is possible. Furthermore, the images are stored electronically and can be easily sent by Internet to a central place for rereading. Digital plate readers are relatively expensive ($30 000–$50 000), generally sensitive to dust and (probably) humidity, and large and must be transported by air-conditioned truck. But highly field-robust tabletop plate readers developed for military use have recently become available at reasonable cost. In completely digital systems the X-ray image is directly converted into a digital image by a transducer that takes the place of the image plate. There is no need for film developing or plate readers, and the image quality can be checked in real time. The transducers used are, however, expensive and highly sensitive to jarring. Mobile systems based on full-size transducers are therefore highly expensive (around $400 000) and vulnerable if transported over bumpy roads. Among other things, they require air suspension. The feasibility of using digital systems in prevalence surveys should still be assessed.

Slot-scan systems present an alternative that is increasingly being used in mobile X-ray. A small transducer moves in slots across the patient's chest along with a narrow X-ray beam and the image is computed from the digital images for each slot. These systems have the same advantages as those with full-size transducers but are less vulnerable. Image quality depends on speed of scanning. The fastest scanners produce the best images but are also expensive (up to $500 000). Slow scanners are less expensive. Slot-scan systems, particularly the moving parts of the X-ray generator and the transducer, are still relatively vulnerable and repair often requires specific expertise.

Most mobile digital systems use battery-operated X-ray generators and have relatively low power requirements (two-phase, 10 kW generator for the system and air conditioning).

An important factor in deciding which chest X-ray system should be procured is the availability of service contracts in the country.

5.3   Laboratory methods

Sputum specimens are collected from those defined as eligible by the protocol (all study subjects, "symptomatics" or TB suspects with an abnormal chest radiograph) and examined by smear microscopy, culture, and drug susceptibility testing (DST), depending on the protocol. To provide results for use in case management, smear microscopy should be done at a laboratory close to the survey sites. Sputum culture is better performed at an intermediate or central laboratory where competent workers, all the necessary equipment and materials, and adequate bio-safety facilities are available. DST should be carried out only at the national TB reference laboratory (NRL).

The detection efficiency of *Mycobacterium tuberculosis* (MTB) in clinical specimens varies greatly according to the quality, quantity, and freshness of the specimen (sputum), the sensitivity of the technique used, and the efficiency of the quality assurance programme. Sputum specimens of adequate volume and good quality must be collected, laboratory methods suitable for the survey environment must be used, and efficient quality assurance must be carried out to obtain accurate and reliable data on TB prevalence. The laboratory techniques should be carefully selected, with the survey design and local situation in mind. In general the use or inclusion of the standard procedures currently used in the national TB control programme would lend itself to future observation of trends and international comparability of the survey results.

Sputum examination: Laboratory procedures of sputum examination consist of smear microscopy, culture, identification of MTB complex, and drug susceptibility testing. The accuracy of those tests is a critical component of a valid prevalence survey, and benefits the TB patients detected. All tests should ideally be done at the same laboratory, such as the NRL, but the sputum samples must be transported quickly and safely from the survey sites and must stay fresh. This is of utmost importance for a valid culture examination.

Likewise, carrying out all tests at the NRL might delay the reporting of smear microscopy results for case management. The decision regarding which test is to be done at which laboratory must take into account the quick and safe transport of specimens, the availability of competent workers for culture (and DST) and of all the equipment, materials, and bio-safety requirements, and the choice of culture techniques.

Smear microscopy can be done at any laboratory in the network, but the choice of technique will determine where culture is performed. For example, the centrifugation method can be done only at the NRL. If the simple technique is to be used, culture can be carried out at an intermediate or even peripheral laboratory as long as it is equipped with a functional incubator, vortex mixer, and class-II safety cabinet. DST should also be performed only at the NRL.

Sputum smear microscopy. The standard smear microscopy technique most widely used is the carbol-fuchsin-stained direct-smear technique. If affordable, fluorochrome-stained direct-smear microscopy is highly recommended. Concentrated-smear microscopy is an option, but not normally recommended because technical efficiency may vary from laboratory to laboratory.

Sputum culture. Culture is more sensitive than smear microscopy in detecting MTB from sputum specimens, but efficiency varies according to the procedure used. The major factors that affect the efficiency of culture in isolating MTB from sputum specimens are: (1) quality and quantity of specimens, (2) time from sputum collection to processing for culture (freshness), (3) decontamination, and (4) processing of decontaminated specimens.

In general, the concentrated culture method (CC) using a centrifuge is more sensitive than the simple culture method (SC). However, because of its technical complexity CC requires skilled and motivated workers, better facilities, and more equipment and materials. It is therefore better carried out at the NRL (and not at the peripheral and intermediate levels), with

quick and safe transport of specimens. The technical complexity of this method, however, may increase the chance of cross-contamination, and efficiency differs between laboratories. Thus, the data obtained may not be as comparable as in other methods. SC, on the other hand, while less sensitive than CC in recovering MTB from sputum specimens, is technically simple enough to be performed at a laboratory with minimal equipment and materials, less cost, minimal chance of cross-contamination, and minimal variation in efficiency between laboratories in isolating MTB from sputum specimens. Thus, comparability between different locations or between different time intervals is better. SC has a clear advantage in a country where quick and safe transport of the survey sputum samples to the central laboratory is difficult or impossible.

The final selection of the most appropriate culture method for a prevalence survey should take into account several factors: (1) where culture will be carried out, (2) the specimen transport system, (3) the routine culture method in use (if any), (4) available facilities and equipment, and (5) the skill and motivation of the laboratory workers. If safe and quick transport is available, the concentrated culture method using centrifugation of specimens decontaminated with 4% sodium hydroxide (NaOH) (see Section 13.2) can be chosen. But if quick and safe transport and a good cooling system in transit cannot be assured, cetylpyridinium chloride (CPC) decontamination is recommended. Otherwise, the simple culture method using decontamination with 4% NaOH should be the method of choice for the prevalence survey.

Rapid culture methods must be chosen with caution because they cannot provide quantitative results, can be used only with fresh specimens, require centrifugation, and are expensive.

For culture examination, sputum must be collected in a leak-proof, screw-capped bottle (universal glass or plastic bottles or Falcon centrifuge tubes that can resist over 3000 Gsf centrifugal force). Sputum should be decontaminated and centrifuged in the same container without being transferred to another container to minimize contamination and cross-contamination.

6. Screening Strategies

6.1   Procedures used in published surveys

In most published tuberculosis (TB) prevalence surveys (Van der Werf and Borgdorff 2007) the study population was first screened to identify individuals with high risk of pulmonary TB and to exclude those with extremely low risk of the disease. Sputum samples were collected only from those with relatively high risk, i.e., suspects.

Suspects were those with symptoms and abnormal chest X-rays, or with either of the two. After screening, the suspects identified were requested to provide one, two, or three sputum samples. Only spot specimens (samples provided on the spot) were collected in some surveys, only morning samples (samples produced immediately after waking up) in others, and both spot and morning samples in still other surveys.

In all published surveys, microscopy—either fluorescence microscopy or light microscopy after Ziehl-Neelsen staining—was used to examine the sputum samples. Both microscopic methods were applied in one survey. Most surveys also used culture techniques to examine sputum samples.

6.2   Screening strategies

6.2.1   Strategy 1

The identification of all smear - or culture-positive individuals can be assured only when sputum samples for microscopic examination and culture are collected from all eligible individuals. This strategy provides high-quality information but has been used in only a few small-scale studies (Den Boon et al., forthcoming; Gatner and Burkhardt 1980) because of the high cost involved and the significant demand for laboratory capacity. Besides the collection of sputum from all eligible individuals, a chest X-ray and the completion of a symptom questionnaire by all eligible individuals are recommended to provide more information and allow a comparison of the results of the survey with those of other surveys.

6.2.2   Strategy 2

The next best option is symptom screening and chest X-ray screening of all eligible individuals and the collection of sputum samples from these individuals for smear examination (Figure 6.1). The sputum of individuals

with symptoms, an abnormal chest X-ray, or a positive smear can then be tested with the use of a culture method. This approach is limited in that certain individuals with culture-positive pulmonary tuberculosis may not be identified through symptom assessment, chest X-ray examination, or sputum smear screening.

Figure 6.1: Screening Strategy in TB Prevalence Surveys
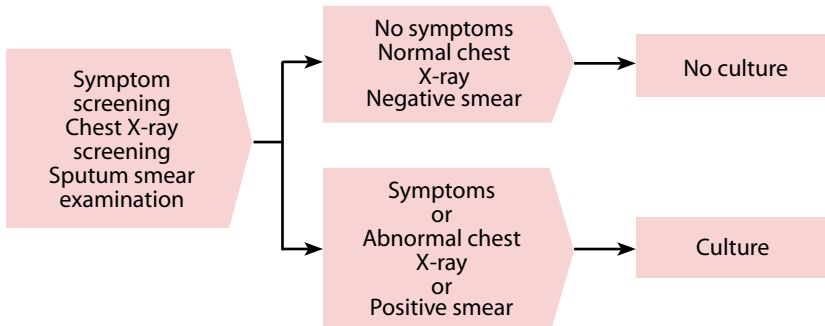
6.2.3   Strategy 3

If the screening method above is not feasible because of the large number of sputum samples that have to be examined by microscopy, symptom and chest X-ray screening (Figure 6.2) can be used. Those without abnormalities during screening are not considered tuberculous suspects and do not have to submit sputum samples. But individuals with abnormalities (symptoms or abnormal chest X-ray) are considered suspects and are asked to provide sputum specimens for smear microscopy and culture.

Figure 6.2: Alternative Screening Strategy in TB Prevalence Surveys
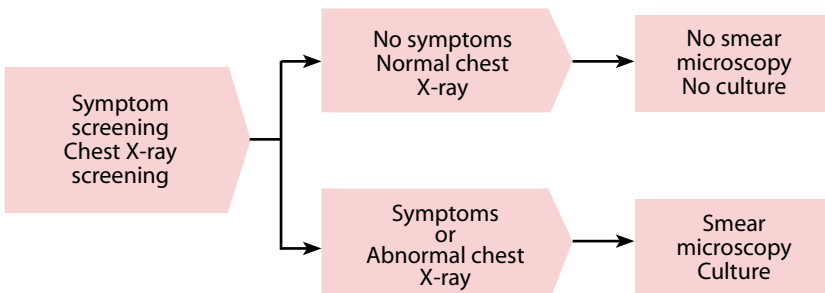
Further simplifying the screening strategy by using only symptom screening is not recommended because it will give a variable underestimation of the prevalence estimate.

6.2.4   Strategy 4

If neither chest X-ray nor culture is available, sputum samples for microscopic examination can instead be collected from all eligible individuals. This requires a fast method of sputum examination such as fluorescence microscopy, which has been shown to be more sensitive than light microscopy and to have equal specificity in diagnosing TB patients in the health system. Culture allows species identification, necessary to rule out non TB mycobacteria.

The table below shows the success of each strategy in identifying bacteriologically confirmed pulmonary tuberculosis.   The strategies are sorted in descending order of epidemiological information, logistical complications, and cost.

Screening Procedures for Identifying Bacteriologically Confirmed Pulmonary Tuberculosis			
Strategy	**Identified Cases**	**Missed Cases**	**Comments**
Strategy 1	all S(+), all C(+)	None	Very intensive lab and CXR requirements
Strategy 2	all S(+); most C(+)	S(−) C(+) sym(−) cxr(−)	Very intensive lab and CXR requirements
Strategy 3	most S(+); most C(+)	S(+) sym(−) cxr(−); S(−) C(+) sym(−) cxr(−)	Most common screening method
Strategy 4	all S(+)	S(−) C(+)	May be considered where infrastructure is very limited

C(+) = culture-positive, CXR = chest X-ray, CXR(−) = normal chest X-ray, S(+) = smear-positive, S(−) = smear-negative, sym(−) = no symptom

The advantage of screening populations for suspects is that it substantially reduces the number of individuals who are asked to provide sputum for microscopic examination (to ± 10% of the total surveyed population), thus allowing the research technicians to pay special attention to these individuals. The suspects can be taught the technique for producing a high-quality sputum sample, motivated to provide sputum, and traced if no samples are received. Having much fewer samples to examine also improves the quality of laboratory work.

The number of sputum samples collected from each individual will affect the prevalence estimate. Most TB control programmes require the examination of three sputum samples (spot-morning-spot) from each individual before a suspect can be regarded as smear-negative. Collecting three sputum samples from each individual in a TB prevalence survey will mean a substantial workload for the field teams and for the laboratory. Furthermore, most smear-positives will have been diagnosed by the first slide; examining a second slide will add some more cases, whereas examining a third will provide only a few more cases (Gopi 2004). Therefore, the examination of two sputum samples, rather than three, seems to be the better choice.

TB control programmes collect two spot samples and one morning sample. In TB prevalence surveys spot specimens may be easier to collect than morning specimens. But morning samples have often resulted in a higher positivity rate than spot samples. Thus, collecting one morning and one spot sample is advised. There is limited experience in the use of sputum induction methods in a prevalence survey. Collecting sputum samples from asymptomatics is generally difficult. A leaflet explaining how to collect sputum specimens must be prepared, to aid technicians in giving instructions to patients. Technicians should be trained or retrained in the basics of sputum collection, including how to produce a deep cough, how much sputum to collect, and how to visually assess sputum quality.

6.3 Symptom screening

The symptoms used for screening should be simple, unambiguous, and culturally appropriate. Questions on symptoms should be hierarchically ordered—the first question about the symptom should ask if the interviewee has the symptom, and the second, if the response is positive, for how long. Depending on the seriousness of the symptom, the period of recall over which the interviewee is prompted to determine the presence of the symptom should not be too far back, to minimize recall bias. Furthermore, to be able to compare the results of the survey with those of different surveys in the same country and with surveys in other countries, the same symptoms should be used for screening. Some symptoms and combinations of symptoms that have been used to identify suspects for screening in TB disease prevalence surveys are: (1) cough of at least three weeks; (2) cough of two weeks' duration or more, chest pain of one month's duration or more, fever of one month's duration or more or the coughing up of blood (haemoptysis) within the last six months; (3) chest symptoms; (4) persistent cough; (5) the coughing up of sputum or blood over the past month; and

(6) cough lasting for three weeks or more or sputum containing blood, or both (see Annex 6). For screening purposes, patients self-reporting with an HIV positive test result may be considered tuberculosis suspects.

Screening is intended to reduce the number of sputum samples that need to be examined while minimizing the number of missed TB cases. Therefore, the selected symptom should be tested before the survey to see whether it isolates a sufficiently small number of individuals (e.g., about 10% of the total surveyed population). An example of a symptom that can be used to identify suspects is a cough that lasts for at least three weeks.

6.4 Chest X-ray screening

No chest X-ray abnormality is sensitive and specific enough for a definite diagnosis of tuberculosis. Therefore, individuals with any abnormality on the chest X-ray should be considered suspects, and asked to provide sputum specimens for examination.

Theoretically, abnormalities on chest X-rays can be classified into: (1) those suggestive of TB; and (2) those not suggestive of TB. As shown in the table above, the sensitivity of the screening strategy in detecting a bacteriologically positive case decreases from Strategy 1 to Strategy 3. If sputum examination in Strategy 3 were to be restricted to people with abnormality suggestive of TB, the sensitivity in detecting bacteriologically positive cases would decrease further because it would leave out bacteriologically positive tuberculosis patients presenting with an abnormality not suggestive of TB. There is no international consensus concerning the type of abnormality that is or is not suggestive of TB.

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7. Measurements and case definitions

7.1   Epidemiological considerations

During the fieldwork for a tuberculosis (TB) prevalence survey, research technicians should strive to collect all information according to the protocol, as missing information complicates the interpretation of the results. Information may be left out because individuals refuse to participate or are not present during data collection, or because the diagnostic test results are inconclusive or the specimen may have been lost or unlabelled. As much as possible, all of these situations should not be allowed to happen. Refusal to participate can be reduced by clearly informing individuals and the community about the purpose of the survey, the benefits of participating, and the risks of not doing so. Those who are not present during data collection should be traced. Inconclusive test results can be prevented by training the persons performing the tests, using high-quality equipment, and carrying out good quality assurance.

Each measurement tool (questionnaire, chest radiograph, bacteriological examination) has its own characteristics such as sensitivity, specificity, and predictive values. These tools may be used for clinical or diagnostic purposes and for epidemiological measurement.

As stated in the previous chapter, eligible individuals should have a chest X-ray, complete a questionnaire, and undergo sputum examination. Alternatively, "suspects" may be identified for bacteriological examination through screening. Ultimately, therefore, there will be three pieces of evidence on each individual who is being considered: (1) symptoms, (2) chest X-ray, and (3) sputum examination results. On the basis of these three measurements, a classification system should be defined for the survey.   The possibilities are:

■   all three negative (not a case);

■   all three positive (definite case);

■   symptoms positive, X-ray positive, and bacteriology negative (possible case);

■   symptoms negative, X-ray positive, and bacteriology positive (definite case);

■   symptoms positive, X-ray negative, and bacteriology positive (definite case but unlikely);

■ symptoms positive but X-ray and bacteriology negative (not a case);

■ X-ray positive but symptoms and bacteriology negative (not a case)

■ bacteriology positive but symptoms and X-ray negative (definite case only if a second bacteriological test is positive).

Any follow-up that the individual will need will be either to have clinical evaluation for purposes of medical care based on the abnormalities detected during the survey or possibly to have a subsequent bacteriological examination if only one test is positive and all other tests are negative. In this scenario, only the last possibility would require further follow-up for epidemiological purposes and all except the first would require follow-up medical care.

Ideally, each eligible participant has each of the measurements proposed-questionnaire, X-ray, and bacteriological examination. If the follow-up differs between types of suspects, the follow-up may not be applied equally to all in the subset who are eligible for it and this may lead to bias.

To be able to assign an epidemiological outcome to an individual, that person may have to be traced for follow-up examinations. As soon as the result of the culture is available, the researchers should assess whether persons should be traced to obtain additional sputum samples. If only one smear is positive and there is no positive culture, the individual should be traced and additional sputum samples collected (see diagram). Also, if there is a positive culture and no positive smear and less than two sputum samples were examined, the person should be traced and asked to provide additional sputum samples to ensure that at least two sputum samples are examined. Furthermore, if chest X-ray was not obtained during screening, this must be done. In cases with missing information but no positive smear or culture results, additional data collection is normally not performed.

The flow diagram needs to be spelled out separately for medical care and for epidemiological follow-up. When pursuing epidemiological goals, follow-up should be undertaken only when a test result is unavailable. Medical follow-up is designed to ensure that every potential patient is given the necessary care. The steps for follow-up for medical care would then be as follows:

■ Urgent abnormality of X-ray: Take immediate action, get permission of patient to proceed, and initiate process of consultation with medical service.

■ Sputum smear-positive: Take second smear immediately, get permission of patient to proceed, and initiate process of consultation.

■ Any later abnormality on X-ray: Inform patient, get permission, initiate process of medical consultation.

■ Any later bacteriological positivity: Confirm results, inform patient, get permission, initiate medical consultation.

■ All others negative but symptoms from questionnaire: Inform patient, get permission, initiate consultation.

■ A separate course of action is needed if tests are not available:

■ what to do if the patient has no X-ray;

■ what to do if bacteriological examination is not complete (smear inadequate, culture contaminated, second specimen needed—one approach is to store part of one specimen for repeat examination in case a culture is contaminated);

■ what to do if questionnaire is incomplete;

■ how to assess patients under treatment, some of whom may be smear-positive but culture-negative and some of whom may be both smear- and culture-negative at the time of the survey.

Figure 7.1: Follow-Up Procedure to Ensure Correct Medical Care
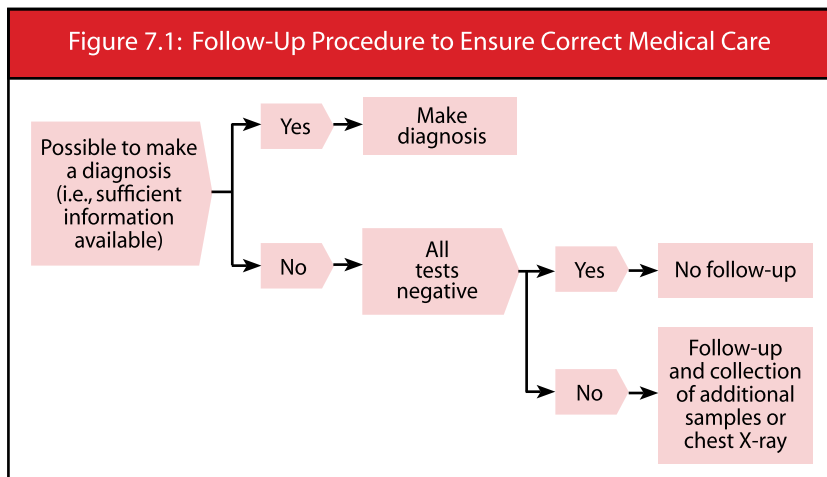
During the survey, individuals with symptoms and signs, individuals with abnormalities on the chest X-ray, and individuals with a positive smear or culture will be identified. Box 7.1 summarizes the clinical actions that should be taken, according to the findings of the survey.

The investigators must ensure that individuals identified by the survey to have abnormalities have access to health care. This can be done by including a clinician in the field team who can be consulted regarding such individuals and by having a written plan for referring identified individuals to the health-care services. Patients should be provided with free transportation or reimbursed for the costs of transportation to the health-care services. They should also receive health care free of charge or be reimbursed for the costs of the health care.

Box 7.1: Clinical Actions in TB Prevalence Survey

During the fieldwork

Symptoms not related to TB: Refer to a nearby health facility for examination and treatment by the medical doctor of the field team.

Chest X-ray abnormalities not related to TB: Refer to a nearby health facility for examination and treatment by the medical doctor of the field team or interpretation of the chest X-ray by the chest X-ray technician and prescription of treatment by an appropriate person.

After the fieldwork

Symptoms of TB but with negative smear and culture: Refer to a health facility for follow-up.

Diagnosis of TB: Trace and put on treatment as soon as possible.

Individuals identified with smear- or culture-positive TB should be put on anti-TB treatment as soon as possible. The investigators have an obligation to ensure that the identified case arrives at the health-care facility and receives appropriate treatment free of charge.

Where HIV testing is undertaken as part of the survey protocol, there should be appropriate care available free of charge for individuals identified with HIV infection, according to international guidelines.

If drug resistance testing is applied in the survey, those individuals identified to be drug-resistant should receive the appropriate treatment free of charge.

7.2   Case definitions

7.2.1   Measurement definitions

Three measurement tools used in the TB prevalence survey are crucial in determining whether an individual should be considered a case or not: smear microscopy, culture, and the chest X-ray.

A smear microscopy examination is positive if there is at least one acid-fast bacillus (AFB) in 100 immersion fields (see Box 7.2). A culture is considered positive if there are at least five colonies after the maximum incubation period for the applied culture method. The positive cultures should be tested for niacin production and nitrate reduction and susceptibility to paranitrobenzoic acid or another internationally accepted method should be applied in order to identify *M. tuberculosis.* A chest X-ray is positive if it contains radiographic abnormalities consistent with pulmonary tuberculosis. Section 7.3 describes the abnormalities consistent with pulmonary tuberculosis.

Box 7.2: Measurement Definitions of a Positive Test Result

AFB positive: At least one acid-fast bacilli in 100 immersion fields.

Culture positive: Culture with at least five colonies after the maximum incubation period for the applied culture method. The culture should test positive for niacin production and nitrate reduction and susceptibility to paranitrobenzoic acid in order to identify *M. tuberculosis.*

Chest X-ray positive: Radiographic abnormalities consistent with pulmonary tuberculosis (see Section 7.3).

Symptoms positive: Reported symptoms consistent with pulmonary tuberculosis (see Section 6.3).

Note: One to four colonies may be considered a positive culture result if the patient has TB-relevant symptoms or chest radiographs suggestive of TB, or both.

7.2.2 Case definitions

To be able to compare the outcomes of the survey with data from the TB programme the case definitions used in the survey should be similar to the definitions used in the TB programme. Furthermore, to be able to compare the results of different surveys and surveys in different countries it is necessary to formulate standard case definitions and to report separately

the number of smear-positives and culture-positives and the number with insufficient information to decide whether the individual is a case or not (see Annex 4).

Most country TB programmes use the definitions of WHO to define tuberculosis cases. Therefore, the use of these definitions as case definitions for TB prevalence surveys is recommended. In the WHO definitions three diagnostic tests are used to classify individuals with pulmonary TB: smear microscopy (AFB-positive or -negative), culture (culture-positive or -negative), and chest X-ray (abnormalities consistent with active pulmonary tuberculosis present or not). Cases are classified, through these tests, as smear-positive or smear-negative pulmonary tuberculosis, and as new or retreatment cases according to whether or not they have previously been treated for up to one month.

The main outcomes of a TB prevalence survey are the number of smear-positive pulmonary TB cases and the number of culture-positive pulmonary TB cases. A smear-positive TB case is defined as a case with at least two initial sputum smear examinations (direct-smear microscopy) AFB+, or one sputum examination AFB+ and radiographic abnormalities consistent with active pulmonary tuberculosis as determined by a clinician, or one sputum specimen AFB+ and culture-positive for *M. tuberculosis* (see Box 7.3). Furthermore, the number of culture-positive and bacteriologically confirmed pulmonary cases can be assessed. Smear-positive cases with non-tuberculous mycobacteria confirmed on culture should not be considered tuberculosis cases.

Box 7.3: Case Definitions for TB Prevalence Surveys

Smear-positive pulmonary tuberculosis case: At least two initial sputum smear examinations (direct-smear microscopy) AFB+, or one sputum examination AFB+ and radiographic abnormalities consistent with active pulmonary tuberculosis as determined by a clinician, or one sputum specimen AFB+ and culture positive for *M. tuberculosis.*

Culture-positive pulmonary tuberculosis case: At least one culture-positive for *M. tuberculosis.*

Bacteriologically confirmed pulmonary tuberculosis case: Smear-positive or culture-positive pulmonary tuberculosis case.

The identified smear-positive and culture-positive cases should be traced and classified as new cases, cases on treatment, relapse cases, default cases, or failure cases (Box 7.4).

Box 7.4: Types of TB Cases

New case: Patient who has never previously had treatment for tuberculosis or who has taken antituberculosis drug for less than a month.

Case on treatment: Patient who is currently being treated with anti-tuberculosis drugs.

Relapse case: Patient who was previously declared cured or who has completed treatment but with a new episode of bacteriologically positive (smear or culture) tuberculosis.

Default case: Patient whose treatment was interrupted for two consecutive months or more after at least one month of treatment.

Failure case: Patient who completed five months or more of treatment but who remained, or again became, bacteriologically positive (smear or culture).

Case detected outside the survey: Individual eligible for inclusion in the survey who is found with TB at a health-care facility (separately from the survey examinations) during or after the survey.

Note: The above categories are not mutually exclusive. Cases on treatment are considered TB cases until cured or treatment has been completed.

During and shortly after data collection for the survey, TB cases may be identified by the health services in the study population that were not identified in the survey. These TB cases should not be included in the results of the TB prevalence survey. They can be reported separately as cases detected in the survey population at the time of the survey but outside the survey examinations.

7.2.3 Follow-up examinations

If a person has been diagnosed with TB (and put on treatment) between the survey and the tracing for follow-up examinations, information should be collected from the health services that made the TB diagnosis. This information should be used to assess whether a person is a smear- or culture-positive pulmonary tuberculosis case.

7.3   Chest X-ray criteria

In a survey, the flow of testing is usually as follows:

■   Administer the questionnaire.

■   Carry out chest X-ray.

■   Obtain sputum specimen on the spot.

When the chest X-ray has been taken, it must be immediately evaluated for the presence of any abnormality. The types of abnormality present must then be separated into those that require urgent action (such as a pneumothorax or evidence suggesting cancer; (see Section 7.1) and those that require subsequent investigation. The participant should leave the study site only after the chest radiograph has been checked to ensure that it is of sufficient quality and that no abnormality requiring urgent action is present. Once the decision is made concerning the need for medical investigation, and any necessary investigation has been undertaken, the radiographs must be carefully sorted and prepared for standard reading for epidemiological purposes.

Standard reading of the chest radiograph for detection of tuberculosis used to be considered unreliable, according to a large international study (Nyboe 1968). But several recent publications—one evaluating the role of chest radiographs in diagnosis (van Cleeff et al. 2005) and the other evaluating its performance as an epidemiological tool (Den Boon et al. 2005)—have demonstrated quite acceptable levels of concordance among readers. These two systems classified the results of radiograph reading as follows:

■   presence or absence of any abnormality;

■   among those with abnormalities, consistency or inconsistency with
     tuberculosis.

One system further subdivided those abnormalities consistent with tuberculosis into those consistent or highly consistent with tuberculosis. The other system classified abnormalities consistent with tuberculosis into those of parenchyma, pleura, and central structures, with decreasing levels of concordance in reading.

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8. Social Determinants and Risk Factors

Measures of socioeconomic status (SES) and exposure to various risk factors for tuberculosis (TB) may be included in a TB prevalence survey for two main reasons:

■ To establish prevalence in different socioeconomic groups and to assess equity of access to services provided within the national TB programme. Comparing the SES profile of people with TB identified in a prevalence survey with the SES profile of people registered for treatment in the national TB programme provides a measurement of equity of access. Such comparison would require a concurrent survey of patients in the programme. For a programme with equitable access, the SES profile of TB cases identified in the community would be similar to the SES profile of those diagnosed and treated in the programme. Annex 13 provides advice on how to measure SES.

■ To estimate prevalence of exposure to specific TB risk factors in the population, and to determine association between such risk factors and TB disease. In order to understand the reasons for change in TB prevalence over time, it is important to know the change in the exposure of the population to risk factors for TB, such as HIV, malnutrition, smoking, diabetes, indoor air pollution, crowding, and silicosis. Repeated TB prevalence surveys provide a platform for monitoring the change in prevalence over time of such risk factors.

The evidence base for a causal link between these and other risk factors is varied. Furthermore, the relative risk of TB associated with some of these factors is not well established, and is likely to vary across populations because of effect modification related to a varying mix of risk factor exposure. A TB prevalence survey can be used as a platform for analytical studies of the relationship between various risk factors and TB disease.

The measurement of exposure to risk factors requires additional questions in the questionnaire, and may also involve clinical investigations, e.g., for HIV, nutritional status, and diabetes. Annex 13 provides advice on how to measure selected risk factors.

It is important to stress that in a prevalence survey one will typically identify not many more than a hundred TB patients, making it difficult to assess associations with sufficient power in case of weak association or rare exposure. However, the power may be sufficient when the assumed relative risk is above 2 and the prevalence of exposure is more than 10%. Annex 13 provides sample-size estimates for different assumptions of relative risk and prevalence of exposure.

If the objective is only to determine the prevalence of exposure to one or several risk factors in the population, it will not generally be necessary to perform these investigations in the entire population. A random sub-sample of people from each cluster will generally provide a sample of sufficient size. Similarly, for the analysis of associations, the control group would not need to include all non-TB cases since the statistical power is normally saturated at around five controls per case. A "nested case control" approach would be particularly valuable for expensive and complicated procedures such as diagnosis of diabetes and HIV, and biochemical assessment of nutritional status.

In any event, the primary objective of the prevalence survey must not be compromised by collecting too much additional information. Any additional information should be collected only after careful consideration of the impact on the survey, the importance of the data, and the likelihood that it will provide useful information for interpreting the results of the survey or future trends in prevalence.

9.   Ethics

9.1   Principles

The general conduct of biomedical studies is guided by statements of internationally recognized principles of human rights, including the Nuremberg Code and the World Medical Association's Declaration of Helsinki (Grodin 1992), revised in 2000. The first principle of the Code was the centrality of the voluntary participation of subjects with their informed consent. The Declaration built on the Nuremberg Code, adding a distinction between therapeutic and non-therapeutic research, a call for institutional review mechanisms, and a provision for family members to consent for the subject when the subject could not give consent. The revised Declaration issued by the World Medical Association in 2000 (Declaration of Helsinki 2000) reflected the deepening appreciation of the many elements included in fully informed consent. It made clear the critical importance of ethical review by a committee independent of the researcher. Empirical investigations have sparked concerns about the extent to which researchers adhere to the multiple aspects of consent (WHO 2004). These investigations repeatedly demonstrate that, although subjects involved in studies have apparently consented, as reflected in signed forms, many subjects, especially those involved in research sponsored by wealthy countries and conducted in less-developed host countries, do not fully appreciate the nature of the projects with which they will be involved, their right not to participate, or their right to withdraw when they so decide.

These principles were emphasized further in 2002 when the Council for International Organizations of Medical Sciences (CIOMS) published a revision of its International ethical guidelines for biomedical research involving human subjects (CIOMS 2002). Given the increased concern about the exploitation of research populations in less-developed countries by investigators from sponsoring wealthy countries, the CIOMS guidelines gave sustained attention to the steps necessary to prevent exploitation and to ensure culturally sensitive informed consent. Further, the guidelines underscore the obligation of investigators to protect the confidentiality of the information they obtained from research participants.

Ethical issues often arise as a result of conflict among competing sets of values, such as, in the field of public health, the conflict between the rights of individuals and the needs of communities. The purpose of ethical review is to consider the features of a proposed study in the light

of ethical principles, so as to ensure that investigators have anticipated and satisfactorily resolved possible ethical objections, and to assess their responses to ethical issues raised by the study. Not all ethical principles weigh equally. A health survey may be assessed as ethical even if a usual ethical expectation, such as confidentiality of data, has not been comprehensively met, provided the potential benefits clearly outweigh the risks and the investigators give assurances of minimizing risks. It may even be unethical to reject such a study, if its rejection would deny a community the benefits it offers. The challenge of ethical review is to make assessments that take into account potential risks and benefits, and to reach decisions on which members of ethical review committees may reasonably differ. Ethical review committees should safeguard the rights, safety, and well-being of all study subjects or surveyed individuals, with special attention to vulnerable subjects (WHO 2002), and should be guided by the following four principles. Survey participants should:

- be protected from any harm related to testing procedures and stigma associated with tuberculosis (TB);

- participate in the benefits of the survey, and have access to free treatment services if diagnosed with TB;

- be informed of the procedures and risks; and

- freely choose whether or not to participate in the study, and not in any way be coerced into TB testing by promises of benefits such as free treatment.

9.2   Confirmation of review by an ethics review committee

The proposed survey must undergo review by appropriate ethics review committees. The review and ethical approval for conducting the survey is a requirement. The survey funding agency should obtain from the ethics review committee a statement that the survey is organized and operates according to applicable laws and regulations. Documented approval should also be obtained.

Ethics review committees may be created under the aegis of national or local health administrations, national medical research councils, or other nationally representative health-care bodies. The authority of committees operating on a local basis may be confined to one institution or extend to all biomedical studies undertaken in a defined political jurisdiction. However committees are created, and however their jurisdiction is defined,

they should establish working rules—regarding, for instance, frequency of meetings, a quorum of members, decision-making procedures, and review of decisions—and they should issue such rules to prospective investigators.

9.2.1 Composition of the ethics review committee

Local review committees act as a panel of investigators' peers, and their composition should be such as can ensure adequate review of the study proposals referred to them. Their membership should include epidemiologists, other health practitioners, and lay persons qualified to represent a range of community, cultural, and moral values. These committees should have diverse composition and include representatives of any populations specially targeted for study. The members should change periodically to prevent individuals from becoming unduly influential, and to widen the network involved in ethical review. Independence from the investigators is maintained by precluding any member with a direct interest in a proposal from participating in its assessment.

The community to be studied should be represented in the ethical review process. This is consistent with respect for the culture, dignity, and self-reliance of the community, and with the aim of achieving full understanding of the study among community members. Lack of formal education should not disqualify community members from joining in constructive discussion on issues relating to the study and the application of its findings.

9.2.2 Balancing personal and social perspectives

In performing reviews, ethical review committees will consider both personal and social perspectives. While, at the personal level, it is essential to ensure individual informed and free consent, such consent alone may not be sufficient to render a study ethical if the individual's community finds the study objectionable.

9.2.3 Assuring scientific soundness

The primary functions of ethical review are to protect human subjects against risks of harm or wrong, and to facilitate beneficial studies. Scientific review and ethical review cannot be considered separately: a study that is scientifically unsound is unethical in exposing subjects to risk or inconvenience and achieving no benefit in knowledge. Normally, therefore, ethical review committees consider both scientific and ethical aspects. An ethical review committee may refer technical aspects of scientific review

to a scientifically qualified person or committee, but will reach its own decision, on the basis of such qualified advice, and scientific soundness. If a review committee is satisfied that a proposal is scientifically sound, it will then consider whether any risk to the subject is justified by the expected benefit, and whether the proposal is satisfactory with regard to informed consent and other ethical requirements.

9.2.4 Externally sponsored studies

Externally sponsored studies are studies undertaken in a host country but initiated, financed, and sometimes wholly or partly carried out by an external international or national agency, with the collaboration or agreement of the authorities of the host country. Such a study implies two ethical obligations: (1) the initiating agency should submit the study protocol to ethical review, in which the ethical standards should be no less exacting than they would be for a study carried out in the initiating country; and (2) the ethical review committee in the host country should satisfy itself that the proposed study meets its own ethical requirements.

It is in the interest of the host country to require that proposals initiated and financed externally be submitted for ethical approval in the initiating country, and for endorsement by a responsible authority of the same country, such as a health administration, a research council, or an academy of medicine or science. Investigators must comply with the ethical rules of the funding country and the host country. Therefore, they must be prepared to submit study proposals to ethical review committees in each country. Alternatively, there may be agreement to the decision of a single or joint ethical review committee. Moreover, if an international agency sponsors a study, its own ethical review requirements must be satisfied.

9.2.5 Information to be provided by investigators

Whatever the pattern of the procedure of ethical review, the investigator must submit a detailed survey protocol comprising:

■ a clear statement of the objectives, having regard to the present state of knowledge, and a justification for undertaking the investigation;

■ a precise description of all proposed procedures and interventions, including intended treatments for all forms of diagnosed TB;

■ a statistical plan indicating the number of subjects to be involved;

■ the criteria determining admission and withdrawal of individual subjects, including full details of the procedure for obtaining informed consent;

■ evidence that the investigator is properly qualified and experienced, or, when necessary, works under a competent supervisor, and that the investigator has access to adequate facilities for the safe and efficient conduct of the survey;

■ a description of proposed means of protecting confidentiality during the processing and publication of survey results;

■ a reference to any other ethical considerations that may be involved, indicating that the provisions of the Declaration of Helsinki will be respected;

■ a plan for case management, including the provision of free TB treatment for cases found with TB (smear-negative, culture-positive patients may not have access to free treatment through the national TB programme), and detailing case management procedures for non-TB conditions diagnosed during the survey; and

■ an insurance policy for the survey, whether or not there is a legal requirement for one.

9.3   Informed consent

Each potential survey participant must be adequately informed of the aims, methods, and sources of funding of the survey, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study, and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the survey or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the investigator should then obtain the subject's freely given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

A well-designed consent form should include a description of the screening and diagnostic procedures and the implications thereof, in the respondents' own language, easy enough for all participants to understand. The purpose of the informed consent is to inform individuals of all the procedures and any potential risks involved and to allow them to decide freely whether or not to participate in the survey. For participants to be truly informed, they must understand the implications of the consent. However, ascertaining whether the individual really understands the implications of consent is difficult. One option is for survey staff to pose questions to the individuals; another would be to allow the survey participants to pose questions to

the interviewers. Allowing individuals to ask questions will help clarify the process and could increase the response rate. Key elements in an informed consent form include:

■ an explanation of the purpose of the survey;

■ a description of the procedures;

■ an explanation of the risks and benefits;

■ a description of how anonymity and/or confidentiality will be protected;

■ an opportunity for the participants to ask questions;

■ a statement that participation is voluntary and refusal will not affect any potential benefits.

9.4   Confidentiality

Investigators should make arrangements for protecting the confidentiality of such data by, for example, omitting information that might lead to the identification of individual subjects, or limiting access to the data, or by other means. It is customary in epidemiological surveys to aggregate numbers so that individual identities are obscured. Where group confidentiality cannot be maintained or is violated, the investigators should take steps to maintain or restore a group's good name and status. Information obtained about subjects is generally divisible into unlinked and linked information. Typically, in prevalence surveys of TB disease, information will be linked to subjects.

Linked information may be:

■ anonymous, when the information cannot be linked to the person to whom it refers except by a code or other means known only to that person, and the investigator cannot know the identity of the person; or

■ nominative, when the information is linked to the person by means of personal identification, usually the name.

Identifying information should be discarded when consolidating data for purposes of statistical analysis. Identifiable personal data will not be used when a study can be done without personal identification. When personal identifiers remain on records used for a survey, investigators should explain to review committees why this is necessary and how confidentiality will be protected. If, with the consent of individual subjects, investigators link different sets of data regarding individuals, they normally preserve confidentiality by aggregating individual data into tables or diagrams. In government service the obligation to protect confidentiality is frequently reinforced by the practice of swearing employees to secrecy.

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10. Survey Organization

Figure 10.1: Prevalence Survey Organization

```
                    ┌────────────┐        ┌────────────┐
                    │  Steering  │────────│    Data    │
                    │  Committee │        │ Monitoring │
                    │            │        │   Centre   │
                    └────────────┘        └────────────┘
                           │                     │
┌────────────┐      ┌────────────┐        ┌────────────┐
│ Technical  │      │   Survey   │        │  Monitors  │
│  Advisory  │──────│ Coordinator│        │            │
│   Group    │      │            │        └────────────┘
└────────────┘      └────────────┘
                           │
┌────────────┐            │
│  Medical   │────────────┤
│  Director  │            │
└────────────┘            │
              ┌───────────┴──────────────┐
        ┌──────────┐              ┌──────────┐
        │ Central  │              │  Field   │
        └──────────┘              └──────────┘
        │    │    │    │          │        │
     ┌─────┐┌────┐┌──────┐┌────────┐ ┌──────┐┌──────┐
     │ Lab ││CXR ││ Data ││Logistics│ │ Team ││ Team │
     │     ││    ││Manager││        │ │Leader││Leader│
     └─────┘└────┘└──────┘└────────┘ └──────┘└──────┘
```
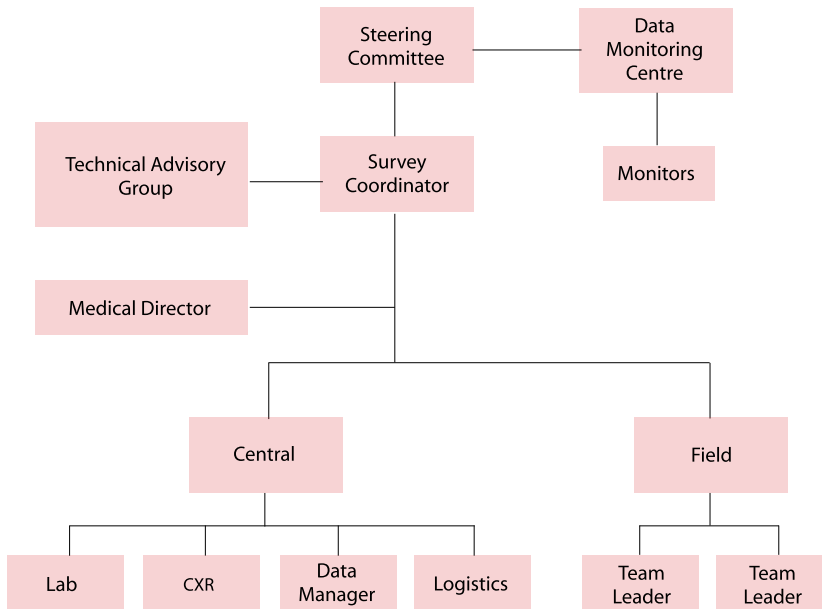
CXR = chest X-ray

10.1 Steering committee

A survey steering committee (Figure 10.1) should be established to support, direct, and monitor procedures and activities to ensure the successful implementation of the survey and dissemination of its results.

The steering committee has primary responsibility for designing the study, maintaining the quality of study conduct, and writing the final study report. It comprises investigators, possibly other experts not otherwise involved in the trial, and, often, representatives of stakeholders such as the public health service, local institutions, or the funding agency.

10.2 Contract research organization

The sponsor is a person or an organization responsible for conducting the survey. The chief or head of a health organization or division under the health ministry of a country responsible for tuberculosis services can be a sponsor. Conducting a survey may be one of the various tasks of the tuberculosis (TB) control programme in the country.

The sponsor may entrust the responsibility for conducting the survey to another organization such as a research institute or university research group. For this, the sponsor needs to transfer the duties and functions of the survey either partly or fully. It is better to transfer the entire responsibility of conducting the survey to an organization that is capable of conducting the survey by itself. The organization should implement the quality assurance and quality control measures in the survey. These quality checks should be implemented at every stage of the survey, beginning with planning. These checks are implemented to ensure good-quality data is generated from the survey. The protocol of the survey should be designed in order to collect reliable data free of bias and discrepancies. The contract research organization should be able to do this successfully.

10.3   Investigator's qualifications and agreements

Qualifications and agreements are two important prerequisites for an investigator of the survey. The investigator should be adequately qualified and should have experience in conducting surveys. The investigator and the associated institution must have the necessary infrastructure required for the survey. Adequate staff must be available to carry out the tasks outlined in the protocol. To ensure this, it may be necessary to recruit the staff required according to the protocol for the survey, if they are not already available in the institution. The training of the staff is very important and the investigator should make arrangements for it, either in the institution or in another organization or centre that has resources for the training. In the latter case, the kind of training required must be made very clear to the institution conducting training. There should be a schedule giving details of the training, including the starting day and the concluding day. The training needs to be given systematically to all staff members, and they should be assessed before being declared suitable for the post to which they have been recruited. This is required for all staff including, for example, the team leader, the census taker, the coordinator, the X-ray technician, the secretary to the coordinator, the sputum collector, and all field assistants.

The X-ray readers are also given training in reading the films independently and should be assessed against a standard reader. The intra- and inter-reading variations should be minimized to the greatest possible extent. The method of assessing the X-ray reader is given in Chapter 13.

10.4   Staff organization

A large-scale survey requires an organizational framework that covers all managerial and advisory levels in preparation, execution, and reporting. Each level (and each individual) has its own terms of reference and responsibilities, which should be clearly described.

This paragraph describes the managerial and advisory levels that are to be considered, with their respective terms of reference. These can be adapted to local circumstances; what is of importance is that all tasks and responsibilities listed are covered in the organogram. Representation in the steering committee is detailed in Section 10.8.

Steering committee/Principal investigator. The steering committee has final responsibility for protocol, funding, data collection, data management and analysis, and dissemination of the results. If the funding agency requires that a person be assigned the role of principal investigator, the latter should be a member of the steering committee.

The steering committee may call for applications from contract research organizations, which in turn may develop a survey protocol including budget requirements.

The steering committee should be advised by a technical advisory group (TAG) and delegate tasks and responsibilities regarding the day-to-day management of the survey to the survey coordinator.

Survey coordinator/Coordinating team. The survey coordinator has day-to-day responsibility for the execution of the survey. In large countries a coordinating team, consisting of a central coordinator and several regional coordinators for administrative subdivisions, may be needed. The coordinator/coordinating team will:

■   prepare the field manual/standard operating procedures (SOPs);

■   plan the fieldwork;

■   be responsible for editing and production of the study materials;

■   arrange training and pilot-testing;

■   supervise the fieldwork;

■   supervise data management;

■   prepare monitoring reports; and

■   report, upon request or without request, any major problems in preparation, execution, or data management of the survey.

The coordinator/coordinating team should be answerable to the steering committee. This is best achieved through regular (e.g., monthly) reporting of progress, based on reports from the field teams, from field supervision/monitoring, from the laboratory, from the data management unit, and from additional data collection units (e.g., X-ray rereading) if applicable. In addition, the survey coordinator should notify the steering committee in time of major problems in preparation, execution, or data management of the survey.

The coordinator/coordinating team should receive technical support from the TAG.

Technical advisory group. The TAG advises the steering committee, and assists the survey coordinator/coordinating team, on technical issues regarding the preparation and execution of the survey, and the management, analysis, and reporting of the data.

The TAG will:

■ advise on the survey protocol;

■ produce the technical parts of the field manual/SOPs;

■ advise on the procurement of equipment and supplies;

■ advise on the design, pretesting, and production of study materials;

■ provide technical assistance in training and pilot-testing;

■ monitor data collection and quality control;

■ advise on data management and analysis; and

■ advise on the reporting of results.

The TAG should include technical experts in all the areas relevant to study design, data collection, and data management and analysis, such as:

■ census methodology;

■ interviewing;

■ laboratory;

■ X-ray (technical and reading), if included;

■ tuberculin testing and reading, if included;

■ data management; and

■ data analysis.

TAG members should also be available on an ad hoc basis for technical advice to the steering committee and the survey coordinator/coordinating team.

Field team leaders. Each field team should have a field team leader and a deputy field team leader.

The field team leaders have direct responsibility for the implementation of the fieldwork. They will:

■   visit the selected clusters before the fieldwork;

■   lead the field team;

■   be responsible for logistics and organization during the fieldwork;

■   coordinate the day-to-day fieldwork;

■   communicate with local, district, and provincial authorities on issues regarding the fieldwork;

■   be responsible for the completion of the field report; and

■   report, upon request or without request, any problems in implementing the survey protocol in the field.

The deputy field team leaders should assist the field team leader and replace the latter when required.

A list of required qualifications for the field team leaders should be prepared. These generally include experience with surveys, professional accountability, managerial skills, and availability for fieldwork.

The team leaders are answerable to the survey coordinator/coordinating team. This is best achieved through field reports that summarize data collection, data quality, and problems encountered in each cluster. In addition, the field team leaders should promptly notify the survey coordinator/coordinating team of any problems they encounter in implementing the survey protocol in the field.

In order to make clear what is expected of each field team member, the field manual should include detailed terms of reference for the other field team members as well. These depend on the design of the survey, the flow of data collection, and specific design tasks.

Data manager. A central data manager with day-to-day responsibility for the management and entry of the survey data should be appointed.

The data manager will:

■ lead the data management unit;

■ coordinate all steps in data management;

■ prepare data entry screens and data files;

■ be responsible for the validation of double-entered data files;

■ check validated data files regularly for systematic errors (cleaning);

■ be responsible for completion of regular data management reports; and

■ report, upon request or without request, any problems encountered in data management.

The data manager is answerable to the survey coordinator/coordinating team. This is best achieved through regular data management reports that summarize progress in data entry, data validation, and cleaning. In addition, the data manager should promptly notify the survey coordinator/coordinating team of any problems encountered in data management.

In order to make clear what is expected of each member of the data management unit the field manual should include detailed terms of reference for these as well.

Data monitoring committee. The data monitoring committee (DMC) advises the steering committee on issues relating to survey implementation, data quality and validity, and safety of survey subjects.

It will:

■ review the progress of survey implementation;

■ check whether data collection and management are conducted according to protocol;

■ review data quality; and

■ review issues related to the safety of survey subjects.

The DMC should meet regularly. Reviews are best based on reports from the field teams, the laboratory, the data management unit, and additional data collection units (e.g., X-ray rereading) if applicable. Another important source of information for the DMC is the reports from field supervision (or monitoring) visits that should be made to each cluster during the fieldwork.

Medical director. The medical director, who is in charge of medical decisions, can be called by field teams, and may overrule the teams' decisions on case management, for the benefit of patients.

Field teams. The number of field teams to be deployed for data collection will depend on several considerations including the survey design, the number of field clusters, the time needed to complete the fieldwork, and the availability of staff.

Each field team should have fixed and flexible components. The fixed component is established at the central level and is the same for the various clusters. The flexible component is established at the local (regional/provincial and district) level and differs between clusters. The flexible component allows adaptation to local circumstances (e.g., local staff will be most effective in tracing subjects for sputum collection), while the fixed component guarantees standardized survey procedures across clusters.

Fixed parts of teams can rotate, i.e., for each team needed in the field at any given point in time, two teams can be hired and trained so that they take part intermittently in the fieldwork.

The fixed part of a field team should cover at least the following field activities:

■   team management;

■   liaison with local authorities;

■   census;

■   registration for screening or for further investigation (i.e., as TB suspect);

■   interviewing;

■   instruction for sputum production;

■   checking of data for completeness and discrepancies; and

■   transport of field staff.

These other activities, if included in the fieldwork, will also be among the functions of the field team:

■   X-ray taking;

■   X-ray reading;

■   microscopic smear examination;

■   tuberculin test administration; and

■   tuberculin test reading.

These functions can be combined in groups. For the average cluster size of around 1500 adults with a fieldwork duration of two weeks, the fixed part of a field team generally consists of:

■ one field team leader (and a deputy from the staff);

■ census/registration/interviewing group (2 staff);

■ laboratory group (1–2 staff, depending on whether microscopy is done in the field);

■ X-ray group, if included (2 staff—one technician and one reader);

■ tuberculin group, if included (2 staff); and

■ transport group (2–3 drivers).

The flexible part of a field team could cover at least the following field activities:

■ information of the cluster population;

■ site preparation (e.g., field lab, X-ray unit, rooms for interviewing);

■ catering for field staff;

■ assistance with the census;

■ organization of flow of subjects;

■ tracing of survey subjects and TB suspects;

■ collection of sputum specimens;

■ preparation of sputum specimens for transport;

■ assistance with smear microscopy;

■ sputum transport;

■ feedback of positive laboratory results; and

■ checking of district and other TB registers for patients currently under treatment.

An important additional function of the flexible part of the team is to involve the local community and local health workers. For this purpose, including the following in the flexible part of the field team should be considered:

■ one or more staff of the district TB team;

■ one or more laboratory technicians from the provincial/regional or district TB team;

- one or more community health workers;

- one or more staff of the community/district population department;

- one or more staff of the district health team involved in health one or more drivers, to transport disabled survey subjects, among one or more staff of the community/district education department, if tuberculin testing is included (tuberculin testing may be most easily organized in schools).

When deciding on the composition and size of the field teams, it is important, in particular for remote clusters, to take into account the number of vehicles that are available and the number of drivers needed.

10.5   Management of laboratory specimens

10.5.1 Sputum collection

Collection of sputum specimens of adequate volume and good quality is of utmost importance in the prevalence survey. The freshness of sputum specimens is critical in the recovery by culture of *M. tuberculosis* from clinical specimens without contamination or failure of growth. In this regard, it is essential to establish safe and rapid transport of specimens to the laboratory where they are to be examined. The selection of the culture laboratory should take into account the transit time in order to preserve the freshness of the specimens.

Individuals from whom sputum specimens are to be collected. Sputum specimens should be collected from all individuals above 15 years of age or from a subset of "suspects", according to the protocol. A responsible member of the survey team must:

- explain the reason why sputum examination is necessary;

- explain how to collect a sputum sample, preferably with a pictorial leaflet;

- confirm the ID on the survey card and register;

- label the suspect's name, ID, and the date of collection on the sputum container;

- demonstrate how to open and close the screw cap;

- show how much sputum should be collected; and

- explain to the suspect the importance of sputum quality and proper amount in light of the need for accurate diagnosis.

Number of samples and mode of sputum collection. Two consecutive morning sputum specimens, or a morning and a spot sample, should be collected from a suspect. The first sputum should be collected on the spot. The sputum sample should contain a minimum of 3ml, and should be collected in a leak-proof container with a screw cap. The individual should be instructed to do the utmost to collect a good quality and adequate amount of sputum.

Place of sputum collection at the survey site (spot specimen). Sputum from subjects who come to the survey site should be collected at a place in the open air near the headquarters of the prevalence survey team, or outside the home of the subject during the visit. If collection is undertaken at the survey site, the place should be arranged beforehand to ensure confidentiality.

Reception of sputum specimen. Sputum specimens that the subject brings from home should be checked for proper labelling and for any contamination outside the container. If contamination is found, the container should be cleaned on the outside with a cotton ball soaked in 70% alcohol after the screw cap is tightly sealed, and labelled again if the label is not clear. Each sputum container should be placed in a vinyl bag with a leak-proof zipper together with two layers of cleansing tissue. The container, with cap tightly sealed, should then be placed in a box with ice packs or leak-proof vinyl bags with ice cubes.

Waste disposal. All possible contaminated materials and infectious waste should be collected in vinyl bags and burned, incinerated, or autoclaved at the nearest laboratory.

Materials for sputum collection. The following materials are needed for sputum collection and handling: leak-proof sputum containers (preferably a McCartney bottle) with screw cap; cotton balls soaked in 70% alcohol, in a short wide-mouth glass jar with screw cap; vinyl gloves; cleansing tissue; styrofoam cooler box for storing sputum specimens; ice packs; vinyl bags for contaminated wastes; laboratory gowns; folding tables (1 set); folding chairs(3 sets); a wall tent, if necessary; forms and leaflets; pens; portable lighting devices; a vehicle for transporting specimens and for use in visiting or picking up sputum samples from the suspects' homes; and carry-on bags.

10.5.2 Transport of sputum specimens

A survey team member should transport specimens to the laboratory as soon as possible, in a cooler with ice packs. If ice is used instead, it should be put in vinyl bags to prevent water from coming in contact with the sputum specimens. The specimens with Cetyl pyridinium chloride (CPC), however, should not be kept in a cool ambient temperature.

Transport time should not exceed two days. Ideally, specimens should be processed for microscopy and culture at the same time in one laboratory. Processing all cultures in a central laboratory may delay diagnosis and patient care and may result in increased contamination. A decision has to be made whether to process the specimens in decentralized laboratories or at a central laboratory, given the logistical constraints.

Specimens arriving at the laboratory should immediately be stored in a refrigerator, after first being checked to see if any leakage has occurred and if the labelling is clear. If a specimen has leaked or contaminated others, disinfectant should be applied in a safety cabinet (class I or II). After 2–3 hours, the decontaminated specimens should be salvaged carefully. The outside of the salvaged sputum containers should be sterilized with cotton balls soaked in 70% alcohol and then labelled clearly again. If contamination into other specimens is suspected, all the specimens affected should be discarded into a vinyl bag and autoclaved or incinerated. The field survey team should be informed so that all the contaminated sputum specimens can be re-collected.

11. Field Operations

11.1 Mobilization

The success of the fieldwork during a tuberculosis (TB) prevalence survey absolutely requires close cooperation with the communities where the survey is being conducted. Close cooperation is possible only when the project is supported by stakeholders in the public health services, when community leaders are consulted by the research team, and when community members are properly informed about the objectives and the conduct of the survey.

11.1.1 Commitment to the survey

Before any field activity can start, there must be approval and commitment from all the parties involved to implement a tuberculosis (TB) prevalence survey. This process involves three major steps:

■ organizing a stakeholders meeting;

■ communicating with local authorities; and

■ communicating with communities.

The main aim of a stakeholders meeting is to make an inventory of the parties (persons or institutions) that are involved in the survey and to assess the specific interests of each party in the conduct of the survey. The preferred result of a stakeholders meeting is agreement on the rationale and the objectives of the survey, and the express commitment of the parties involved to the design and the implementation of the survey. The stakeholders can include, for example:

■ ministry of health;

■ national TB control programme;

■ research institutes; and

■ funding agencies.

There are extensive guidelines on how to perform a stakeholder's analysis (Schmeer 1999).

With respect to a national TB prevalence survey, full support for the survey from the ministry of health (MOH) is vital. This support should be communicated to all relevant authorities at the administrative levels that are involved in the implementation of the survey, such as provinces, districts,

and local communities. One has to consider carefully which channels to use for this communication. There are two obvious routes:

- The MOH directly informs provincial and district health authorities and asks for cooperation with the research team; or

- The national TB control programme uses its decentralized infrastructure to inform the local authorities.

Whichever route is chosen clearly depends on the country, but in all instances the goal must be that the research team gets the full cooperation from the relevant authorities within the communities where the survey is being conducted.

The local communities where the survey is being implemented should be visited before the survey. The objectives of this pre-survey visit are to:

- explain the objectives and procedures of the survey;

- confirm the commitment of the community to implementing the survey;

- obtain the consent of the community; and

- assess the situation for the research team.

This visit is vital in establishing a good working relationship between the community leaders and the research team. It is therefore important that the right persons within a community are visited and that the representative(s) of the research team have seniority and authority.

11.1.2 Participation of the community

The fact that local authorities and community leaders support the survey does not guarantee that the community members will cooperate with the research team. There are several things that can be done to improve community participation:

- Provide adequate information to the community. Explicitly define:
  - all target groups in the community that should be informed;
  - the message to be conveyed;
  - the means of conveying the message; and
  - the timing of providing the information.

- Make sure that the field activities are as minimally intrusive as possible.

■ Discuss with the community leaders which target groups need to be approached. Possible groups are district and local health-care providers (governmental, nongovernmental, private), community-based organizations, and community leaders (such as teachers). These discussions can best be done during the pre-survey visit.

The message should be simple and to the point. How the message is phrased should be carefully considered. Using words like "poverty" or "HIV" might scare people away. Neutral phrasing and simple wording is best—for example: "We are visiting the community to find out how often lung diseases affect people in the country." This phrasing will lead into a description and justification of the use of the tests (such as chest X-rays) that will be used during the survey. The essential parts of the message are:

■ the objective(s) of the study;

■ the voluntary aspect of participation, including the right of refusal to participate in any aspect of the survey;

■ an explanation of the methods to be used (X-ray, questionnaires, microscopy);

■ the benefits (early detection and treatment) and risks (physical: X-ray; personal: confidentiality) to the participant;

■ a clear description of the process that will be followed if any abnormality (TB or other lung disease) is detected; and

■ reassurance that the study results will not be used for any other purposes without specific permission from the participant.

There are many different ways in which these messages can be conveyed to community members, such as community meetings or role-playing. The research team should discuss with the community leader which method is most appropriate for the community. Having a focal person from within the community taking part in conveying the message will strengthen the trust of the community members in the research team.

The community is best informed shortly before the actual survey. This task can be done by a team that precedes the actual field team by just a short period (several weeks at most).

Informing the community at the time of the much earlier pre-survey visit will have some drawbacks. First, the team will not have the benefit of the advice of the community leader regarding target audiences and the means of conveying the message. Second, by the time the actual survey is implemented in the community the messages may have been forgotten.

When the time between information and implementation is expected to be long, the research team has to find a way to reinforce the message conveyed to the community. In Cambodia, an innovative approach was chosen. Each household was given a small notebook. On the front and back cover were photos of procedures in the survey, while the first few pages contained information on the survey rationale, benefits, and risks. The rest of the notebook was empty and could be used by individuals or households for their own purposes. Given the usefulness of the notebook, it is less likely to be thrown away after reading, as is often the case with leaflets.

The field activities have to be designed to minimize the inconvenience to community members to ensure their cooperation. The most appropriate way involves a careful trade-off between the need for certain activities and the convenience of the participants.

Issues to consider in carrying out the survey activities are:

- timing;
- location; and
- frequency.

Participants should not be unnecessarily interrupted in their daily work. The working hours of the research team should be tailored to the activities of the community, and not the other way around. This is especially crucial in urban settings.

As many activities as possible should be performed at the house of the participants. Field team members could collect sputum at the participants' house rather than having the participants bring the samples to a central field site. But other activities, such as X-ray, cannot be done this way.

Participants should not have to return several times to a field site or be visited several days in a row.

11.2   Manuals

The TB prevalence survey will be based on the study protocol. For the proper implementation of the survey, two other manuals are needed: a field manual and a supervision manual.

11.2.1 Field manual

The field manual describes in detail the procedures for all activities performed in the field. There can never be too much detail in the description because

there is often no opportunity in the field to contact the survey coordinator quickly when the field manual gives no answer to a problem encountered. Issues that should be dealt with in the field manual are:

■   job descriptions for each member of the staff involved in field activities;

■   step-by-step instructions for the tasks involved in a particular field activity;

■   day-to-day description of activities performed by the field team; and

■   all contact information for key persons and institutions cooperating in the survey.

Writing a field manual is made easier when representatives of the different groups within the research team sit together and discuss the flow of activities. Doing so forestalls conflict within the field station with respect to manpower or timing of the various activities that must be carried out.

11.2.2 Supervision manual

The supervision manual describes the lines of supervision and the responsibilities of the supervisors at each level of the survey. These responsibilities depend highly on how the survey and the research team are organized. Issues that should be included in the manual are:

■   a description of the "line of command" in the survey (preferably by flow-chart);

■   job descriptions for each member of the survey team with a supervisory task;

■   the steps involved in particular supervisory tasks, including

-   what exactly is to be supervised,
-   what tools are to be used for supervision,
-   what actions are to be taken when supervision identifies a problem,
-   when the supervision is to be carried out, and
-   how often the supervision is to be carried out; and

■   all contact information for persons and institutions cooperating in the survey.

Careful attention needs to be given to the description of the tasks of the overall coordinator of the survey. The supervision manual should state when and how often the survey coordinator will monitor the performance

of the research team, which tools are going to be used for this purpose, and which actions are going to be undertaken when performance falls below the expected standard.

11.3   Training

A TB prevalence survey involves a wide range of activities that have to be conducted in a short period of time at a specific location. Such activities can be completed only when everybody involved is competent to carry out the tasks assigned and has a clear overview of the contribution of particular tasks to the survey as a whole. Proper training therefore involves not only technical issues related to the task but also issues related to survey implementation.

11.3.1 Technical training

Technical training refers to training in the specialized activities that have to be carried out during the survey. Typical specialized activities during the survey are:

- taking a census;

- doing an interview;

- taking and interpreting X-rays;

- collecting sputum;

- carrying out bacteriological examination;

- taking blood samples;

- performing tuberculin testing; and

- managing data at the central level.

The training needs for these activities must be identified. Identification will involve the following steps:

- List all the activities that are to be performed in the survey.

- List all the steps needed to perform the activity.

- Identify the persons who will perform the activity.

With the list of steps and the names of personnel that have to receive technical training, designing a training plan and a time-frame for the technical training becomes straightforward.

Three pitfalls should be avoided in this regard:

■ Never assume that an activity needs no explaining and no additional training.

■ Do not exclude persons working at a central laboratory or X-ray department from training on the assumption that they are experts.

■ Do not train too few persons for any activity.

Most technical activities in the survey will be similar to routine activities. However, the different conditions under which the activities must be performed in a survey underscores the need for, and the importance of, (re)training persons working in routine practice.

A TB prevalence survey takes a considerable amount of time. Staff members who fall ill or resign have to be replaced at short notice. Therefore, adequate numbers of staff have to be trained for each activity from the very start.

11.3.2 Field training

Field training refers to survey-specific activities. These include

■ setting up the field station;

■ managing the flow of participants and study forms within the field station;

■ tracing survey participants;

■ transporting samples;

■ recording of data collected; and

■ performing quality control procedures on collected data.

The inventory of the training needs for these activities follows the same steps described above for the technical training. The task is made easier if there is a flow-chart of all "stations" corresponding to the field activities where specimens are collected or data are generated. From such a flow-chart it becomes clear who is to work where and who should be responsible for a certain activity within the survey as a whole.

11.3.3 Timing and frequency of training

The timing of the training sessions is a trade-off between having enough time to ensure thorough training and having training sufficiently close to the actual start of the survey.

The technical training involves retraining persons working in routine health care, and training new personnel recruited specifically for the survey. Existing training modules for this will often suffice and will allow the training to be planned independently of the start of the TB prevalence survey.

The field training involves activities that are not part of routine care. Training sessions should be designed for this. Since there is no natural environment for applying these activities, the training should be planned as close to the start of the survey as possible (not more than a few weeks before the survey).

The technical training can be organized separately from the field training. However, the "technical personnel" must be part of the field training as well because the organization of the field site, the record keeping, and the quality control measures should be clear to everybody involved in the field activities.

Some of the activities are performed by the flexible part of the research team (see Chapter 10.3). This implies that the training for these activities should be repeated several times during the survey. All efforts should be made to ensure that the timing does not extend more than one month between the end of the training for the flexible part of the research team and the start of the survey in the clusters where the persons from the flexible part are to work.

For the activities performed by the fixed part of the research team, training also needs to be repeated to ensure that adequate expertise is maintained. As a rule of thumb, the persons from the fixed part of the research group must go through a refresher course at least once every six months.

11.4   Model training and mobilization schedule

Time Before Start of Survey	Activity	Remarks
6 months	Completion of draft field manual	
5 months	First pre-survey visit	To obtain community consent
		To assess the field sites
4 months	Revision of field manual	Based on field site assessments
	Technical training sessions	
2 months	First field training sessions	At easy-to-reach locations for each area
1.5 months	Second field training sessions	In community settings
		To pre-test the survey
4 weeks	Completion of final version of field manual	Based on training sessions
3 weeks	Second pre-survey visit	To elicit community information
	Pilot field operations, if needed	
2 weeks	Preparatory workshop	To discuss final field operations
	Kick-off ceremony	
START OF SURVEY		

References

Schmeer K. Stakeholder analysis guidelines. In: *Policy toolkit for strengthening health sector reforms.* Bethesda, Maryland, Abt. Associates, Inc., 1999.

12. Monitoring

Monitoring is done to verify that:

■ the rights and well-being of survey participants are protected;

■ the reported data are accurate, complete, and verifiable against the source documents; and

■ the survey is being conducted according to the approved protocol and applicable regulations.

Monitors should be appropriately trained and should have the scientific and/or clinical knowledge needed to monitor the survey adequately. The monitors' qualifications should be documented.

The determination of the extent and nature of monitoring should be based on considerations such as objectives, survey design, complexity, and size. Pre-survey assessments of clusters, laboratory and chest X-ray facilities, and the central data management unit should be carried out and documented.

There is a need for systematic on-site monitoring during the survey. External assistance may be sought to ensure that the survey is conducted according to the approved protocol and best practice.

Monitoring activities include quality assurance (see Chapter 13) and must be undertaken with these considerations in mind:

■ how the monitoring is organized—the establishment, role, and responsibilities of a data monitoring center (see Chapter 10);

■ the role and responsibilities of monitors (including the completeness and accuracy of case report forms, all aspects of quality assurance, and issues that arise during the fieldwork);

■ field reports, which are essential, since monitoring through field supervision can provide only a snapshot of the situation during the fieldwork in each cluster (see Annex 4);

■ the exact nature of reporting systems for quality assurance and monitoring that are used in laboratories, the data management unit, and X-ray rereading; and

■ early monitoring results in the first few weeks of the survey, which will help in identifying and addressing problems unforeseen during the field pilots.

13. Quality assurance

Quality assurance is a process that must be applied to all aspects of research and in particular to any measurement undertaken within a research study. It is aimed at providing the evidence necessary to inspire confidence in the quality of the product produced (in this case, the measurements used in the research). The quality assurance activities are quality control, quality assessment, and quality improvement. Quality control involves specific tasks and procedures undertaken to ensure the highest quality, including standardization of procedures and definitions, training, and supervision. Quality assessment is the measurement of the level of quality (for example, the comparison of one set of measurements against a "gold standard" of measurements), and quality improvement is the process of using the information gained through assessment to improve the quality of the measurements. Setting standards for agreement in measurements and excluding or re-educating those whose measurements do not achieve sufficient agreement with the standard are quality improvement concerns.

13.1 Quality assurance of chest X-ray assessment

The best and longest-established system of classification of chest radiographs for use in international comparisons is the International Labour Office (ILO) international classification of radiographs of pneumoconiosis, which can be found at http://www.cdc.gov/niosh/topics/chestradiography/breader.html. The guidelines published in 1980 indicate that "This Classification provides a means for recording systematically the radiographic abnormalities". The system is used for epidemiological research, for surveillance, and for clinical purposes. The ILO system is presented here for the purpose of illustrating standardization of reading.

The system was developed to describe and record the appearance of films in terms of light and shadow, as opposed to interpreting these appearances, as is more usual in clinical practice. Skill in interpreting radiographic appearances is dependent on the clinical experience of readers, and the accuracy of the interpretation is limited by the wide variety of manifestations of diseases.

Of vital importance in ensuring quality and reliability in reading radiographs is the principle of reading only films of standard size that offer a postero-anterior view, are correctly positioned (without excluding any part of the lung field), and are of a defined degree of technical quality (neither too dark nor too light; no movement while the film was taken). The degree of

concordance of readings is highly dependent on the quality of the films. The principle of refusing to read poor-quality films is extremely important. There are four methods of ensuring the reliability and quality of radiograph reading.

■ **Definitions.** Selected variables to be recorded concerning the radiograph are defined precisely and limited in number. In the ILO system, definitions for each of the six main variables occupy seven pages of the written guidelines and are specified in detail. Definitions that are descriptive and not interpretive must be established. For example, shadows are termed "opacities" and not "infiltrates" or "nodules". The text providing these definitions should always be available while X-rays are being read.

■ **Standardization.** As part of the process of developing the exercise and of training individuals to become expert readers, it is important to prepare a set of "standard films". This should be done for each of the main variables that are to be evaluated. For example, in the ILO system, the "extent" of large opacities is defined as

- A = greatest diameter exceeding 10 millimeters (mm) and up to and including 50 mm, or several opacities, each greater than 10 mm and the sum of whose greatest diameters does not exceed 50 mm;

- B = one or more opacities larger or more numerous than those in category A whose combined area does not exceed the equivalent of the right upper lobe; and

- C = one or more opacities whose combined area exceeds the equivalent of the right upper lobe.

Three "standard" films are provided in the ILO guidelines to illustrate each of these situations. To determine which category the film corresponds to, the two "standard" films to which the chest X-ray being read most closely corresponds are placed on either side of the film being read and the appropriate category is selected and recorded. To ensure quality of reading and a high degree of reliability (concordance) among readers, a set of standard films is essential.

■ **Training.** When the definitions have been precisely developed and a set of standard films has been selected, training must be provided so that those who will read the films can gain experience and skill. The training should, in addition to making use of standard films, also provide one set of films that illustrates the characteristics to be read that can be used for the training, and a second set that can be used for evaluating each of the trainees to determine the degree of concordance.

■ **Accreditation.** A key principle in the quality assurance of chest radiograph interpretation (as in other measurements such as reading the tuberculin skin test) is accreditation. This is done to evaluate the degree of concordance of the reader with an "expert" in assessing a set of films. In the ILO system, 125 films are read over six hours and scored in a standard fashion. The set of films encompasses the various major variables being evaluated, and the degree of concordance (agreement) required is preset before the examination. As a result of achieving a higher score than required, the reader is "accredited" and allowed to participate as an expert (accredited) reader in the evaluation of chest radiographs. Accreditation has a time limit and must be re-established after the time limit has passed.

13.2   Assessment of X-ray readers

The X-ray reader needs to be trained to reading the films. The standard reader has to give initial training to the X-ray trainee reader. This includes explaining to the trainee reader the purpose of reading the films, the abnormalities and the different classifications (such as lung pathology other than tuberculosis, inactive tuberculosis, possibly active, and probably active tuberculosis) and how they are to be coded on the X-ray result sheet and the abnormality marked in the lung figure, with the help of the manual for X-ray reading. The trainee reader goes through the film, tries to identify the existing abnormalities, and then compares the reading with that of the standard reader, continuing this exercise with subsequent films to gain familiarity with reading the films. The trainee reader may view the films and discuss any discrepancy in coding the results with the standard reader.

The trainee reader should read an adequate number of films during the training period of about two weeks without the help of the standard reader to gain confidence in reading the films independently before undergoing assessment.

13.2.1 Pre-assessment

The trainee reader reads independently without the help of the standard reader, using the guidelines for the classification of the films. A statistical comparison is then made between the trainee's readings and the standard reader's to determine the degree of concordance between the two readings. If the performance is satisfactory then the trainee reader can go for a final assessment. In case of disagreement with the standard reader, the trainee reader should identify the area(s) needing more attention, and the standard reader assists the trainee in improving the latter's skill in reading the films correctly without missing any abnormality.

13.2.2 Final assessment

The trainee reader reads another set of films independently and gives the readings to the statistician. The statistician once again computes the concordance between the readings of the standard reader and the trainee reader as follows:

- **Crude agreement.** A tabulation of the two readings is prepared and the diagonal readings where both agree give a crude estimate of agreement between the two. The crude agreement needs to be corrected for the expected agreement due to chance. For this, expected cell frequencies are calculated for the discordant cells and the weighted Kappa statistic is computed (see below). The tabulation can be condensed in a dichotomous classification as follows.

Trainee Reader	Standard Reader		Total
	E	NE	
E	a	b	a+b
NE	c	d	c+d
Total	a+c	b+d	a+b+c+d(=N)

Table 13.1: Cross-Tabulation of Results of X-ray Readings for Statistical Analysis of Concordance

E = abnormality on X-ray reading, NE = no abnormality on X-ray reading

The extent of agreement between the two readings may be expressed by the following:

- **Sensitivity = a/ (a+c):** the degree to which the trainee is able to identify the abnormalities identified by the standard reader (the proportion of the abnormalities identified by the standard reader that are picked up by the trainee—expected to be 80%–90%).

- **Specificity = d/ (b+d):** the proportion of those without abnormalities identified by the standard reader that are picked up by the trainee reader (expected to be 90%–95%).

- **Over-diagnosis = b/(a+b):** the proportion with abnormalities identified by the trainee reader that are really not abnormalities (should be < 20%).

- **Under-diagnosis = c/ (c+d):** the proportion without abnormalities identified by the trainee reader that are really abnormalities (should be < 5%).

- **Kappa statistics = $(P_0 - P_c)/ (1 - P_c)$:** the extent of agreement between the two readers after adjusting for expected agreement due to chance, where P_0 is the crude agreement and P_c is the agreement corrected for chance agreement ($K \geq 0.6$). If the agreement is within the limits given within parentheses against each measure above, the trainee reader may be certified as an expert reader. Those trainees who do not achieve a sufficient level of agreement need further training and assessment until they achieve a sufficient level.

Table 13.2: An Example of the X-ray Reading Code Used in the South India Prevalance Surveys

0.0   Normal (not to be recorded by readers)

(11) (11)   Lost, not read

0.(11)   Technically inadequate

1.   Extra-respiratory

       1.1.   Cardiac
       1.2.   Vascular
       1.3.   Bony abnormalities (e.g., scoliosis)

2.   Respiratory, definitely extra-pulmonary

       2.1.   Very dense spot or spots in hilar region (calcification)
       2.2.   Obliterated costo-phrenic angle and/or pleural scar and / or pleural calcifications
       2.3.   Evidence of chest surgery
       2.4.   Enlarged mediastinal and/or hilar glands
       2.5.   Basal-parietal opacity, indicative of pleurisy with effusion (in any area)
       2.6.   Pneumothorax or hydro-pneumothorax
       2.7.   Special pathology not specified above

Opacity or opacities in lung fields (3–9)

3.   Very dense and very well demarcated (e.g., calcifications)

4.   Dense and well demarcated (e.g., fibrosis)

5.   Special patterns

       5.1.   Uniformly dense, round opacity, single or multiple (e.g., cyst)

       5.2.   Atelectasis

       5.3.   Opacity

       5.4.   Less dense opacity combined with cardiac abnormality (6 and 1.1 both present in one person)

6.   Less dense opacity, or less well demarcated (e.g., infiltration)

7.   Ill-demarcated or doubtful cavity

8.   Well-demarcated cavity or cavities, each less than 4 cm (less than 6mm on the small film)

9.   At least one well-demarcated cavity more than 4 cm (more than 6mm on the small film)

In case of multiple lesions, only the most serious is recorded. For readings 3, 4, 6, 7, 8, and 9 above, a second digit from Table 13.3 is used:

Table 13.3: Method of Recording Multiple Lesions from Chest X-ray Standard Readings			
	Location		
Total Extent of Opacities	**Single Opacity**	**More Opacities in One Lung Only**	**Both Lungs**
Less than one sq cm (1.5sq mm on the small film) or linear bands	1	2	3
Less than one-sixth of total area of lung fields	4	5	6
More than one-sixth of total area of lung fields	7	8	9
Small spots, widely disseminated in both lungs	-	-	0

sq cm = square centimetre, sq mm = square millimeter

For a first-digit reading of 1–9, one of the four categories listed below (A, B, C and D) is used to complete the code (except for calcifications):

■   A - lung pathology other than tuberculosis;

■   B - tuberculosis, inactive;

■   C - tuberculosis, possibly active; or

■   D - tuberculosis, probably active.

For example, 6.4C is the code for less dense single opacity (6) with less than one-sixth of the total area of lung fields (6.4) with possibly tuberculosis (C).

13.3 Laboratory

The Laboratory should be equipped with facilities for the receipt and processing of specimens for bacteriological results. The quality and quantity of the sputum specimens collected should be maintained and arrangements should be made to transport the specimens in refrigerated conditions to the laboratory without delay. The technician who processes the specimens should also be trained to follow the guidelines laid down for making the smear and preparing the media for culture. The technician also

needs training in reading the smear and performing culture including drug susceptibility test and recording the results in the relevant documents.

Provision should be made for retraining during the survey to ensure that staff follow procedures correctly and understand their duties and responsibilities.

13.3.1 Smear microscopy

Quality control. In every series of staining smears prepared from samples obtained in the prevalence survey, one unstained known-positive (2+) and two unstained known-negative smears should be included as internal quality controls for staining. Two negative smears should be stained again after reading during the next staining series, up to three times in order to rule out false negatives due to contamination of negative smears by environmental mycobacteria during the primary staining. This blinded rechecking after re-staining serves as an external quality assessment to validate the smear microscopy data of the prevalence survey. All survey smears should be kept in the slide boxes after the reading.

All positive smears should be reconfirmed by another microscopist in the same laboratory at the time of smear examination.

External quality assessment. Blinded rechecking of the survey smears should be undertaken with a sample of slides selected by non-laboratory staff using the lot quality assurance sampling (LQAS) technique, (Association of Public Health Laboratories 2002). All sample smears are examined before and after re-staining. If there are fewer than two low-grade false results and no high-grade false result, smear microscopy data of the survey are declared valid.

13.3.2 Sputum culture

To validate the sputum culture data in the prevalence survey, the results of quality assurance (QA) must be provided. If QA is implemented for the routine culture service, it can be applied to the survey culture performance.

Evaluation of quality of newly prepared culture media. The quality of newly prepared media is evaluated in the following two ways:

■ Physical evaluation and sterility tests. Color, bubbles, consistency, and firmness of newly prepared media should be evaluated macroscopically. Firmness can be tested by striking on the palm two to three times.

Sterility of newly prepared media is tested by incubating at 35°C–37°C for three days and counting contaminated media.

■ Mycobacteria growth tests

Materials:

- *M. tuberculosis* H37Rv (or BCG) suspension in skim milk (with known viable count) kept at –70°C
- *M. fortuitum* suspension in skim milk (with known viable count) kept at –70°C
- Media prepared (L-J medium or acid-buffered medium)
- Cryotubes (2 ml)

Preparations:

- *M. tuberculosis* H37Rv (or bacillus Calmette-Guérin [BCG]) suspension: - Homogenize colonies of two- to three-week-old *M. tuberculosis* H37Rv (or BCG) in L-J media in a bottle with glass beads and a few drops of sterile water and then dilute to ensure that the suspension has a turbidity equivalent to McFarland number 1 (about 1 mg/ml). Add 1 ml of suspension to 100 ml of skim milk and aseptically dispense 1.5 ml volume into a cryotube (2 ml) and keep in a –70°C freezer. Select three frozen bacterial suspensions in skim milk and thaw in order to count viable bacilli. Dilute the thawed suspension with sterile DW 10 times (0.01 mg/ml) and 100 times (0.001 mg/ml). Inoculate 0.1 ml of each dilution and spread on the surface of five L-J or Ogawa media slants before incubation. After four weeks' incubation at 35°C–37°C, count the number of colonies and calculate the average colony-forming units per ml of frozen bacterial suspension in skim milk.
- *M. fortuitum* suspension: Suspend the *M. fortuitum* culture in sterile saline and adjust its turbidity to McFarland number 1. Add one ml of suspension to 100 ml of skim milk and dispense into cryotubes before freezing. Determine the viability count in the manner previously described for BCG suspension.
- Select 10 newly prepared media and label clearly.

Procedure:

- Dilute *M. tuberculosis* H37Rv (or BCG) and *M. fortuitum* suspensions in order to obtain 10–50 colonies per slope (in 0.1 ml of inoculum) on the basis of the average viability count observed in the frozen suspension.

- Inoculate 0.1 ml of each of bacterial suspensions onto five newly prepared media and incubate at 35°C–37°C.
- Observe *M. fortuitum* growth one week after incubation and *M. tuberculosis* H37Rv (or BCG) growth, four weeks after incubation. If the average count falls within one standard deviation of the known average count and the same growth speed, the quality of the newly prepared media is acceptable. If the viability count of *M. fortuitum* is acceptable the media can be used immediately, but the final validation decision should be based on the viability count of *M. tuberculosis* H37Rv (or BCG).

Evaluation of decontamination and processing of specimens

■ Transfer 1 ml of *M. tuberculosis* H37Rv (or BCG) suspension (in skim milk) into a sputum container and include in the middle of every series of culture examinations with sputum samples from the prevalence survey.

■ Add 1 ml of 4% NaOH into a container with 1 ml of bacterial suspension.

■ Make viability counts of bacteria after six weeks' incubation. Calculate the reduction in viability count on the basis of the average viability count in skim milk untreated with decontaminant. More than 70% killing of bacteria could be regarded as too harsh decontamination.

Analysis of *M. tuberculosis* recovery efficiency. After the completion of culture using sputum specimens from the prevalence survey, the contamination rate of inoculated media and smear-positive, culture-positive rate of the diagnostic specimens should be analyzed and recorded. *M. tuberculosis* recovery analysis (smear positive culture positives) should be made with sputum specimens collected from patients who are not currently under anti-tuberculosis treatment.

13.4   Other components (questionnaires)

The investigator should have adequate procedures for receiving documents from the field, reviewing the documents for completeness and clarity, and computerizing the data. There should be adequate staff for datamanagement and analysis.

13.4.1 Training, pilot-testing, and revision

To ensure the quality of information collected, training and clear instruction are essential. The number of interviewers should be kept to a minimum to reduce the magnitude of interpersonal variation. Interviewers must be trained to administer questionnaires in a standardized manner. Clear instructions must be given on how to proceed, how to ask questions with careful wording, and how to gain cooperation from the interviewees. The interaction between interviewer and interviewee may affect the quality of information. Interviewers should have the capacity to engage people in a positive and polite way.

If translation into local languages is required, back-translation may be useful to assess the quality of translation.

Pilot-testing provides an opportunity to identify the constraints of a questionnaire. Trained interviewers should be commissioned to perform pilot-testing. The wording of questions, their sequence, and the structure and overall length of questionnaires should be improved on the basis of the findings of the pilot-testing.

13.4.2 Questionnaire administration

Questionnaires should be administered only by trained interviewers. Participants should be well informed of the purpose of interview, their right to refuse to participate, and the way they should answer specific questions. Confidentiality should be assured to encourage participants to answer embarrassing questions frankly.

13.4.3 Storage and backup of questionnaires

After an interview, questionnaires should be collected, placed in a secure location, and transported to the data collection centre according to the written procedure. Backup copies of questionnaires are essential to prevent losing valuable information.

Reference

Association of Public Health Laboratories. *External quality assessment of AFB smear microscopy.* Washington, 2002

14. Safety

Attention must be paid to safety in every component of a survey. This includes safety of travel, security of location, prevention of illness, as well as specific actions to minimize risk from any testing procedure. Accordingly, every survey should have written procedures that spell out these issues and how they are to be handled, as well as addressing themselves to the testing procedures. The test procedures that require particular attention are chest radiography and bacteriological examination.

14.1 Safety of chest radiography

Any radiological equipment that is to be used for survey purposes must meet the most stringent safety requirements set out in both the country where the survey is undertaken and the country of origin of any experts carrying out the survey. Accordingly, the regulations in the relevant countries must be obtained and, before the equipment is used, it must be checked to ensure that it meets the specifications spelled out in the regulations and has been properly maintained such that it operates safely.

Numerous guidelines on the safety of radiological examinations are available. For example, advice to patients from the American College of Radiology and Radiological Society of North America can be obtained from http://www.radiologyinfo.org/. Regulations of various jurisdictions are also available—for example, http://www.e-laws.gov.on.ca/DBLaws/Regs/English/900861_e.htm.

Even when equipment is of good quality and has been maintained well and when procedures are followed correctly, the person being examined will receive some radiation. This exposure should be minimized as much as possible. The amount of radiation resulting from a chest radiograph is estimated to be equivalent to 10 days of natural background radiation when the equipment is properly operated.

Specific measures must be taken to minimize radiation during survey examinations including:

■ checking that the equipment meets specifications;

■ maintaining the equipment properly;

■ focusing the radiation precisely on the field to be examined;

- directing the radiation field towards nearby spaces where people neither live nor work;

- using the minimum dose required to obtain an adequate test;

- providing protective devices to reduce radiation of workers operating the machines and any vulnerable person, including women of child-bearing age; and

- excluding pregnant women from chest X-ray examination but allowing them to participate in the other parts of the survey (questionnaire on symptoms and sputum examination).

14.2   Safety of bacteriological examinations

In order to prevent production of and exposure to aerosols containing live *M. tuberculosis*, all tuberculosis (TB) laboratory examinations should be carried out at a laboratory with appropriate safety facilities and equipment, and safety procedures followed by every worker.

Sputum collection can generate numerous infective aerosols and thus must take place in an open space or in a well-ventilated room. Technicians should avoid exposure to aerosols during sputum collection. They should stand at a distance from and not in front of the patient if collection is in an open space, or stay in another room if collection is done in a closed setting (in which case adequate environmental measures for infection control, including ventilation systems, should be in place). If such precautions are taken, technicians do not have to wear N95 masks.

Unless live cultured organisms are handled, there is no measurable risk of infection to the worker. Sputum smear microscopy poses minimal risk of TB infection to the workers, but smears should be made in a bio-safety class II cabinet to protect sputum from contaminants in the air because they are to be processed for culture at a later time. The laboratory must be cleaned with an appropriate disinfectant after work has been carried out.

Sputum must be processed for culture in a safety cabinet to protect not only workers from TB infection but also specimen and culture media from contamination during decontamination and inoculation. Subculturing, identification, or DST must be carried out only in a laboratory equipped with at least a class II safety cabinet and a well-controlled air-flow system with efficient and safe HEPA air filtration .

All the laboratory materials that might have been contaminated with MTB should be incinerated or autoclaved before being discarded or cleaned for reuse.

15. Documents and data management

15.1 Essential documents

All essential documents pertaining to the prevalence survey should be maintained and kept until the survey final report is completed and approved. Essential documents include:

- signed protocol and amendments, if any;

- information given to survey participants (informed consent form and any other written information);

- financial reports of the survey;

- signed agreements between involved parties, e.g., between investigator and sponsoring agency or contract research organizations, including access to data, reports, and publications;

- dated, documented approval or favourable opinion of institutional review board or independent ethics committee;

- checklists to identify and document the required steps for each of the various survey activities (e.g., investigator selection, approvals and clearances, monitoring, reports);

- signature logs and other forms documenting who completed which activities when and in what sequence;

- signed informed consent forms;

- signed and completed questionnaires for each scheduled study visit to capture all of the necessary data collected from and reported for each subject, including documentation of questionnaire corrections;

- chest X-rays and laboratory tests, e.g., established quality control and/ or external quality assessment for sputum microscopy, culture, and chest X-ray;

- instructions for handling biological samples, e.g., sputum samples and cultures;

- reports of monitoring visits; and

- progress reports, annual reports, and final survey report.

15.2   Data management

Data management is aimed at producing high-quality data on individual characteristics and aggregated indicators such as tuberculosis (TB) prevalence (KNCV 2006). 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, analyzing, 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 such documents;

■   coding/terminology for patient characteristics and medical history (data dictionaries);

■   data entry and data processing (including laboratory and chest X-ray data);

■   database closure;

■   database validation;

■   secure, efficient, and accessible data storage; and

■   data quality assessment (i.e., reliability of data) and quality assurance;

Databases should be managed from a central location, and a database manager should be appointed to take charge of the process.

A plan documenting appropriate data management systems should be developed. The investigator must take responsibility for implementing such systems to ensure that the integrity of survey data is preserved. The data management plan describes the procedures and processes for creating accurate, complete, verifiable data with source documents (primary data) 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.

15.2.1 Organizational aspects of data management

It is essential to establish a data management unit headed by an experienced central data manager for the coordination, management, and entry of the survey data, with the following responsibilities:

- ensuring uniformity of data entry and validation;

- monitoring data flow and processes;

- reporting problems to the survey coordinator and the survey committee; and

- improving the efficiency of tasks allocated to staff, equipment, and technical assistance.

Source documents (registers and forms including questionnaires) should be sent to the data management unit after photocopying at field level. The survey coordinator can decide to split data management activities over regional units covering one or several clusters, and the central data management unit. The central data manager retains overall responsibility for data management.

Staffing and responsibilities. The **central data manager** is a member of the study team and has day-to-day responsibility for survey data entry and management.

Recommended terms of reference for the data manager should include:

- participating in the study design;

- coordinating all steps in data management at central and regional levels;

- advising the survey coordinator on data management issues;

- supervising the implementation of electronic systems for data entry, validation, and backup, according to documented specifications;

- validating double-entered data files and correcting data entry errors;

- checking validated data files regularly for systematic errors and inconsistencies;

- defining the roles and responsibilities of staff involved in data management activities;

- ensuring that electronic data files are properly stored and backed up; and

- reporting each quarter to the survey coordinator on the progress of data management and on the completeness and quality of the data.

A **deputy central data manager** should be appointed to assist or replace the central data manager when needed.

Other data management staff may include staff for sorting and filing raw data at central level, data entry clerks, and regional data manager(s) to cover data management needs over a number of clusters and to transmit documents and data to the central data management unit.

Data management register. The central data management unit will keep a register that contains cluster information on whether and when

■   registers and forms from the field were received;

■   additional forms (e.g., laboratory forms, X-ray rereading forms) were received;

■   forms and registers were entered in the computer and by whom;

■   specific data files were validated and by whom; and

■   validated data files were modified and by whom.

Progress reports. The data manager must produce a written progress report periodically (e.g., every three months), to summarize progress in data management processes, to document the quality of data, and to describe problems and solutions. Progress reports are a basis for discussions and decisions in the coordinating committee, and for technical recommendations by technical assistance partners.

15.2.2 Procedures

The following documents should be considered:

■   **Census register for adults.** One for each cluster. The census register for adults contains one record for each surveyed adult. This is the main data source on adults. The census register is to be used in the field, photocopied at the regional level, and sent to the central data management unit for data entry.

■   **Census register for children.** One for each cluster. The census register for children contains one record for each surveyed child, similar to that for adults.

■   **Suspect register.**   One for each cluster. The suspect register contains one record for each TB suspect (individual who requires full investigation for TB) and should include complete documentation for suspects, including chest X-ray readings. This register is the main data source for TB suspects and should be linked to the census register for

adults using household identifiers. This register is to be used in the field, photocopied at the regional level, and sent to the central data management unit for data entry.

■ **Questionnaires.** Questionnaires are administered by the field investigators, they should be photocopied at the regional level and then sent to the central data management unit for data entry.

■ **Laboratory forms.** Laboratory forms should be used at regional level, then photocopied and sent to the central data management unit for data entry.

■ **Chest X-ray forms.** Chest X-ray forms are filled out at the chest X-ray rereading units. They should be used at the regional level, then photocopied and sent to the central data management unit for data entry.

■ Other forms and registers not to be used for data entry into the survey database:

   - **Data management register.** Serves to monitor the completeness of registers and forms from various sources. To be kept by the data manager.

   - **Monitoring report forms.** Serve to monitor various aspects of data quality and completeness. To be kept by the survey coordinator.

   - **Specimen dispatch form.** Kept at the laboratory, specimen dispatch forms contain transport details of sputum specimen batches from clusters to the laboratory.

Data monitoring. Data monitoring should take place as close to data collection in the field as possible, so that memory is still fresh and surveyed individuals can still be approached to check any errors or discrepancies. Initial data monitoring should be done before the completion of fieldwork in each cluster. The field team leader should ensure that all registers and forms are checked and completed or updated as necessary. All remarks and corrections by the field team leader should be clearly documented. Registers and forms should be checked for completeness and consistency before the completion of the fieldwork in each cluster. If they are found to have missing data or inconsistencies, field team members or study subjects should be approached to provide clarifications. The registers and forms should then be completed or updated. Changes made at this stage should be done in such a way that previous as well as new information remains

legible. The date of each modification and the initials of the person who made the change should be indicated.

Data transfer and filing. At the field/regional level, the completed or updated data should be photocopied and sent to the data management unit. Original registers and forms should be sent, and photocopies should remain at the regional level. The field/regional data manager should keep records of transport details. When copies of source documents (copies of forms and registers) arrive at the data management unit, they will be entered in the data management register, sorted, and filed using personal identification codes. The coordinating data manager should oversee this process and make sure that required documents are made available for data entry. The data management register should be checked regularly to see which source documents have not yet been received.

Data entry and backup. Data entry should be done by designated data entry clerks (see section on software, Chapter 16). Data entry systems should use electronic signatures:

■ To ensure that individuals have the authority to proceed with data entry, the data entry system should be designed so that individuals need to enter electronic signatures, such as combined identification codes/passwords, at the start of any data entry session.

■ To ensure that entries are attributable, each entry to an electronic record, including any change, should be made under the electronic signature of the individual making that entry. However, this does not necessarily mean a separate electronic signature for each entry or change. For example, a single electronic signature may cover multiple entries or changes. The printed name of the individual who enters data should be displayed on the data entry screen throughout the data entry session. This is intended to preclude the possibility of a different individual inadvertently entering data under someone else's name. If the name displayed on the screen during a data entry session is not that of the person entering the data, then that individual should log on under his or her own name before continuing.

■ Individuals should work only under their own passwords or other access keys and should not share these with others. Individuals should not log on to the system in order to provide another person access to the system.

■ Passwords or other access keys should be changed at established intervals.

■ When someone leaves a workstation, that person should log off the system. Failing this, automatic logoff may be appropriate for long idle periods. For short periods of inactivity, there should be some kind of automatic protection against unauthorized data entry. An example could be an automatic screen saver that prevents data entry until a password is entered.

Data entry should be a continuous process to prevent large numbers of forms and questionnaires from piling up. All data should be double-entered to identify and correct non-systematic entry errors. After an individual record has been entered by one clerk, another clerk will enter it again into a separate database or table with the same structure, for later validation. If a value is encountered for which the right entry in the source document file is not legible, the data manager will decide how to correct the value. The data manager will keep a list of such decisions to ensure consistency.

Once entered into a computer, electronic data should be checked for errors and extreme values, and all inconsistencies should be corrected, so that data files accurately reflect the values written in forms and questionnaires. When needed, requests for clarifications may be generated and sent back to the field teams to try to correct data errors. Frequency tables can be prepared for all variables to check for extreme values. Variables related to each other can be cross-tabulated to check for inconsistencies. Distributions and scatter plots of variables should be prepared and examined. Decisions have to be made on whether and how to impute missing data. If data are transformed during processing, it should always be possible to compare the original data and observations with the processed data. A mechanism should be in place to document changes in the database, keeping a record of past and new values where data are corrected, along with dates when the changes were made. Alternatively, updated data files can be stored under different names (e.g., by appending the current date to the file name when saving the work at the end of the day) while former files are kept so that changes can be tracked if necessary.

Backup data should be stored in a safe place in a separate room (in case of fire). Data should be backed up at the end of the day on each day that data files are entered.

Data validation. The data manager should validate double-entered data files using a validation programme or script. Details of the procedure should be documented. Discrepancies should be checked against the raw data and updated in the validated file.

The data manager should clean double-entered files according to a documented procedure, which should include checking for duplicate identification codes. A record should be kept of errors found and the steps taken to address them. Data validation should be done regularly until all the data have been entered and processed and the final survey data set has been validated.

Analysis and reports. Progress analysis should be done systematically, e.g., every three months, to monitor data collection by:

- providing additional checking of the quality of the data;

- showing whether all items needed for the analysis are captured by the data collection procedures; and

- showing whether assumptions with regard to expected numbers of suspects and TB patients are correct.

Details of the interim analyses should be described in a standard operating procedure.

Confidentiality. Registers that allow linkage of personal identifier numbers with names of individuals (or any other information that permits an individual to be identified) should be kept under lock at the data management unit, under the supervision of the data manager. Analyses and reports must not contain the names of surveyed individuals.

Reference

KNCV Tuberculosis Foundation. Data Management Plan. October 2006.

16. Data analysis

Surveys never go precisely according to plan. If the prevalence is much less than anticipated, the sample size will be too small and the estimate of the prevalence will be much less precise than was intended. If the estimated variation among clusters is too low, the estimated design effect will be too high and the estimated prevalence will be less precise than was intended. The survey will be designed to sample different districts with probability proportional to the size of the district. But if the census data are inaccurate the population estimates may not be correct and this could contribute a further source of error. It is unlikely that all of the people in a particular sampling unit will actually take part in the survey and if those that do not take part are at higher or lower risk of having disease than those that do take part this too may bias the results. All of these factors must be considered when analysing the data.

16.1 Preliminary analysis

The data analysis is best described with reference to an example and will use data from the 2002 Cambodian survey given in the table below. It is important to note that a complete analysis would use the individual-level data, as was done in the actual survey. Stata (http://www.statacorp.com) has an excellent set of procedures for doing this (using the "survey" or "svy" set of commands), but other software packages, including SAS and SPSS, can also be used.

16.1.1 Prevalence of sputum smear-positive tuberculosis (TB) in Cambodia

The survey data in the table are aggregated over each cluster so that the effects of age or gender, for example, cannot be explored but the key concepts involved in the analysis can be illustrated.

The final survey included 22 160 adults in 42 clusters (column 6 in table 16.1) and of these 81 had sputum smear-positive TB. The prevalence, P_{S+}, is therefore 81/22 160 = 0.00366, or 366 / 100 000 adults. The size of the clusters did not vary greatly (range 469 to 578) and each cluster will initially be treated here as though its size were equal to the average cluster size of 528. A histogram of the frequency distribution of sputum smear positive cases in the 42 clusters is plotted in Figure 16.1. If the true prevalence were the same in all clusters, then the distribution of the number of cases could be expected to follow a Poisson distribution with a mean of

1.93 (the mean prevalence per cluster), which it does quite precisely. The design effect is expected to be small.

If there were no clustering, the variance, σ^2, of the estimated prevalence, μ, would be given by equation (2) *(see page 18)* so that

$$n\sigma^2 = \mu\,(1-\mu) \qquad\qquad (6)$$

and $n\sigma^2 = 0.00364$. With $n = 22\,160$ (the number of adults that attended the survey) the 95% confidence limits for the prevalence (1.96×) are ±79/100 000 adults.

Cluster	Urban/ Rural	All Ages		10 or Older		S+	S-C+	C-	C+
		Eligible	Attended	Eligible	Attended				
1	U	739	729	494	484	2	4	6	6
2	R	729	710	510	494	0	4	15	4
3	R	750	721	560	538	2	3	4	5
4	R	782	775	547	540	6	15	13	21
5	R	720	712	505	497	3	10	6	13
6	R	749	716	516	484	4	0	3	4
7	R	732	714	578	560	0	3	6	3
8	R	765	762	539	537	4	7	8	11
9	R	722	705	534	520	3	11	11	14
10	R	709	695	492	481	1	5	10	6
11	U	737	720	593	577	2	6	12	8
12	R	738	721	546	529	4	8	5	12
13	U	742	718	591	569	0	5	11	5
14	R	757	727	601	578	2	5	1	7
15	R	802	777	574	552	2	4	2	6
16	U	726	719	523	517	1	0	2	1
17	R	721	700	559	539	2	8	7	10
18	R	713	700	573	561	3	4	12	7
19	R	701	688	509	498	0	6	4	6
20	R	731	718	507	496	0	1	1	1
21	R	719	710	534	525	2	4	8	6
22	R	716	686	490	469	0	2	4	2
23	R	718	676	517	478	2	3	4	5
24	R	720	704	541	530	1	3	9	4
25	R	734	724	521	513	1	12	3	13
26	R	728	719	524	515	0	6	4	6

Table 16.1   Data, by Cluster, for the Cambodia Survey

Cluster	Urban/ Rural	All Ages		10 or Older		S+	S-C+	C-	C+
		Eligible	Attended	Eligible	Attended				
27	R	738	726	540	528	2	3	10	5
28	R	727	713	579	565	1	4	10	5
29	R	740	719	534	519	3	0	4	3
30	R	740	735	564	559	4	7	19	11
31	R	750	713	565	558	0	4	9	4
32	R	758	744	498	488	2	1	0	3
33	R	754	742	557	547	3	3	11	6
34	R	720	703	537	523	4	3	7	7
35	R	713	729	558	546	2	8	10	10
36	U	749	731	561	546	5	3	6	8
37	R	782	773	587	578	2	6	9	8
38	R	739	728	533	522	1	2	3	3
39	U	747	595	650	509	0	3	3	3
40	U	750	569	651	481	2	2	1	4
41	R	740	730	554	544	2	0	24	2
42	R	773	706	632	566	1	2	9	3

Clusters were in urban or rural districts. The table gives the number of people of all ages and of adults who were eligible for and attended the survey, and the number in each cluster who were positive and negative with regard to sputum smear, culture, and X-ray.

The variance can also be calculated from the prevalence within each cluster, thereby including the effect of the variation among the clusters. Let μ_C be the average prevalence of sputum smear-positive patients per cluster (the number of sputum smear-positive patients divided by the number of clusters) and let n_i be the number of sputum smear-positive patients in cluster i. Then

$$n\sigma_D^2 = \frac{\sum_{i=1}^{l} (n_i - \mu_c)^2}{(l-1)m} \qquad (7)$$

where l is the number of clusters and m is the size of each cluster. For the Cambodian data $\mu_C = 81/42 = 1.93$, and $n\sigma_D^2 = 0.00429$.

The design effect is then

$$D = \frac{\sigma_D^2}{\sigma^2} \qquad (8)$$

giving $D = 1.18$. The design effect can be used to correct the estimated error in the estimate so that $\pm 79 \times 01.18 = \pm 86 / 100\,000$ adults, giving a mean prevalence of $366 \pm 87 / 100\,000$ adults, or 366 ($279–453$) / $100\,000$ adults.

Figure 16.1 : Frequency Distribution of the Number of Smear-Positive TB Cases in the 42 Clusters
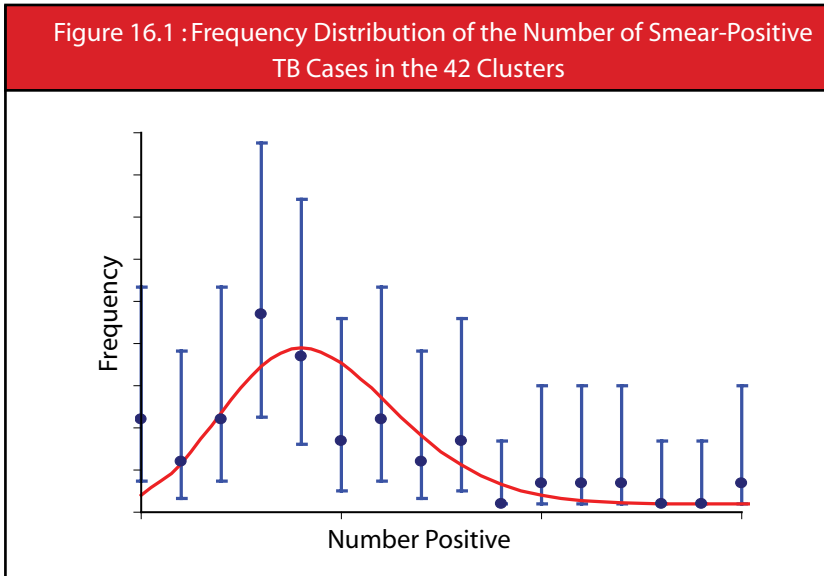
Note: The fitted line gives the Poisson expectation with a mean value of 4.52 in each cluster. Error bars are 95% confidence limits for the observed numbers.

Variation in the design effect. The estimate of $n\sigma_D^2$ is much less precise than the estimate of $n\sigma^2$. Furthermore, after suitable scaling will follow a X^2 distribution with $l - 1$ degrees of freedom so that 95% confidence limits on the design effect are achieved, and will be approximately

$$\pm\, 1.96 \sqrt{\frac{2}{l-1}}\, D$$

For smear-positive TB patients in Cambodia, the design effect then is $D = 1.18 \pm 0.51$ so that the difference from 1 is not significant[1], and it may also be noted that the estimated design effect of 1.25 (equation 5, page 19) is within the range of the measured value.

Poisson errors. In the calculation of the standard deviation of the observed prevalence and the correction to allow for the design effect, the Poisson distribution of counts with a normal distribution has effectively been approximated. To get a better estimate of the confidence limits, the exact Poisson limits can be calculated, such that 366 (285−464) / 100 000, allowing for the design effect. The confidence limits are no longer symmetrical but are still close to the previous values.

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[1] The true value of the design effect must always be greater than 1, but the value estimated from a particular set of data can be less than 1.

Logistic regression. To simplify these calculations and to illustrate the principles underlying these calculations, the average cluster size for each cluster has been used to estimate the design effect. A more precise estimate of the mean prevalence and the design effect can be obtained with the use of a statistical package such as Stata (http://www.statacorp.com – see Appendix 2 of Annex 14). With access to the original data, the appropriate Stata command would be the "survey" command. However, Stata can also be used to do the analysis on grouped data using the "blogit" command (see Appendix 2 of Annex 14). Using Stata and the grouped data in the table above gives a mean prevalence of 366 (291–464) / 100 000 and a design effect of 1.12.

This differs only slightly from the estimate of the mean and the confidence limits using exact Poisson errors of 367 (285–464) / 100 000. If the survey had not been carried out so carefully and the number of people sampled in each cluster had differed more widely, allowing for the sizes of the different clusters would have had a greater effect on the estimated mean prevalence and on the confidence limits.

Adult and population prevalence. These calculations give the prevalence of sputum smear-positive TB among adults. The aim now is to calculate for prevalence in the whole population. The ratio (from the table above) of the number of eligible people of all ages to the number of adults that attended the survey is 1.40 so that the best estimate of the prevalence in the whole population is 270 (214–341) / 100 000.

Correcting for the response rate. The Cambodian survey was very well done and 96% of all eligible adults attended the survey. The attendance, or "response", rate was 74% and 78% in two clusters; in all others it was above 90%, with an average of 96%—much better than the anticipated rate of 75%. Nevertheless, the prevalence should be corrected for the variation in the response rate by cluster. If e_i is the number of eligible adults in cluster i, a_i the number that attended the survey, and p_i the number of sputum smear-positives, then the sputum smear-positive prevalence, corrected for the response rate is

$$P_{SS+} = \frac{\sum \dfrac{P_i}{a_i} \times e_i}{\sum e_i} \qquad (9)$$

For the Cambodian data this reduces P_{SS+} from 366 (291–464) to 363 (289–460) / 100 000 adults and from 270 (214–341) to 269 (2113–340) / 100 000 population. For surveys with a lower and more variable response rate the correction for the response rate could, of course, be much greater.

Correcting for sampling bias. Because the response rate in the Cambodia survey was so high, it is unlikely that those who responded were at significantly different risk for TB as compared with those who did not. However, in surveys in which the response rate is significantly lower, this should be checked and the data corrected accordingly if necessary. To do this the risk factors for TB such as age and sex would be checked, and the distribution of such variables among those who took part in the survey would be compared with the distribution among those who were eligible but did not take part. If those that did not take part differed significantly in age or sex distribution from those that did, for example, the impact that this difference might have on the estimates of prevalence should be considered.

16.1.2 Prevalence of smear-negative, culture-positive TB in Cambodia

The analysis is repeated for those recorded as being smear-negative but culture positive (table). The data deviate significantly from the fitted Poisson distribution ($p = 0.0003$) as a result of the number of clusters with 0, 12, and 15 patients (Figure 16.2). For these data the design effect is 2.45 (1.63–3.68) and the between-cluster variability is 0.56 (0.37–0.77). The observed prevalence is 857 ± 190 / 100 000.

Figure 16.2: Frequency Distribution of the Number of Clusters with 0, 1, 2 … Smear-Negative, Culture-Positive People
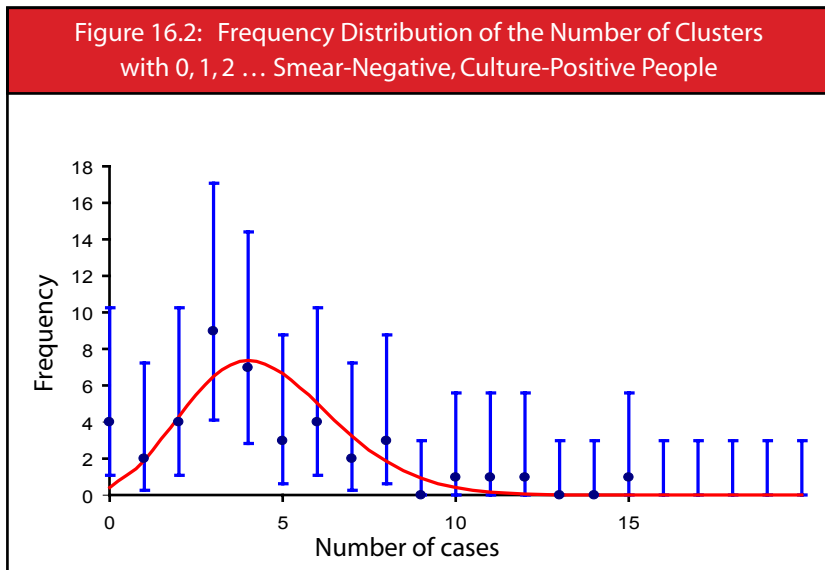
Note: Error bars are for the observed, not the fitted, values.

16.2   Software

Depending on available resources, various options for data entry can be considered, from single-user data entry programs, such as EpiData (http://www.epidata.dk), to more complex solutions using relational database systems either installed on client machines or accessible in a local area network, or even Internet-based if remote data entry is planned. Statistical analysis should be carried out using a reputable statistical package that features specific facilities for survey data and creates detailed logs of commands used to carry out an analysis. Logs of commands document the analysis and ensure its replicability. Analysis logs should be dated and kept along with the final validated data set.

Recommended statistical packages include, among several others, the reasonably priced Stata (http://www.statacorp.com) and the freely available R (http://www.r-project.org).

References

Ministry of Health, Cambodia. National TB Prevalence Survey, 2002. National Center for Tuberculosis and Leprosy Control, Phnom Penh, 2005; 77.

World Health Organization, *Global Tuberculosis Control: Surveillance, Planning, Financing*. 2006, World Health Organization: Geneva.

17. Reporting the results of the prevalence survey

To ensure that data of different surveys are comparable, it is important to apply the same methodology in designing a survey and to use the same reporting format. Standardized reporting captures the overall picture of each survey and allows further analysis of the trend of tuberculosis in the population. Standardized methodology in survey design and standardized reporting are also essential if prevalence surveys carried out in different populations are to be compared. This chapter describes the format of a core report of a prevalence survey, to which further details of results may be added.

17.1 Population

Some residents in the selected area may not be eligible to participate in a prevalence survey. Among these ineligibles are registered residents who have long since moved to other areas. It is useful to compare the estimated population with the eligible population, by age and sex. It is also important to report the number of persons excluded and the reasons for their exclusion.

On the other hand, people who are eligible may not participate in the survey. They may be unavailable or may refuse to participate. As the tuberculosis epidemic usually varies by age and sex, it is essential to compare, among those who are eligible, those who participate and those who do not, by age and sex. Table 17.1 is the format recommended for reporting this information if cluster sampling is the sampling design. If the study population has a high proportion of people aged 65 or more, an age group 65–74 and ≥75 years can be added to the table.

17.2 Completeness of measurements

Four measurements are used in a prevalence survey, namely, questionnaire, sputum smear, sputum culture, and chest X-ray. Among those who participate in the survey, some may fail to have all four examinations. A cross-tabulation of the number of people with each of the four examinations provides an overview of the completeness of the examinations, which can be summarized as shown in Table 17.2. The table shows, among other things, the number of those with sputum smear examination who also completed the questionnaire and underwent chest X-ray and sputum culture.

Completeness of examinations and screening strategy used in a prevalence survey. Various screening strategies can be used in a prevalence survey. The manner in which information on the completeness of examinations is presented depends on the strategy used.

Table 17.1: Comparison between Eligible Persons Who Participate in the Survey and Those Who Do Not, by Sex, Age, and Cluster					
Item	No. of Eligible Persons	No. of Participants in the survey	Proportion of All Participants	No. of Non-participants	
				Unavailable	Refused
TOTAL					
MALE					
Age group (years old)					
15–24					
25–34					
35–44					
45–54					
55–64					
≥65					
FEMALE					
Age group (years old)					
15–24					
25–34					
35–44					
45–54					
55–64					
≥65					
Cluster					
Cluster 1					
Cluster 2					
Cluster 3					
Cluster…					

- **Screening strategy 1**: Questionnaire, chest X-ray, sputum smear, and sputum culture for all eligible persons

  As all four examinations are applied to all eligible persons in this strategy, the completeness of examinations can be presented as in Table 17.2. The number of people with at least one examination—which should be the number of eligible persons who participate in the survey—

should be indicated. Furthermore, as two sputum examinations are recommended, it is important to report the number of persons with one sputum examination (for smear and for culture) and the number with two.

Table 17.2: Cross-Tabulation of the Number of People with Questionnaire, Sputum Smear, Sputum Culture, and Chest X-ray				
Examination	**Smear**	**Questionnaire**	**Chest X-ray**	**Culture**
Smear	NA			
Questionnaire		NA		
Chest X-ray			NA	
Culture				NA

NA = not applicable

- ■ **Screening strategy 2**: Questionnaire for all, chest X-ray for all, smear for all, and culture for those who are smear-positive and/or questionnaire-positive and/or chest X-ray-positive

  As three examinations (questionnaire, chest X-ray and sputum smear) are applied to all eligible persons to identify suspects, and sputum cultures are performed only for those who are positive on any of the three examinations, the completeness of examination can be summarized as in Table 17.3. It is essential to report, among those who are positive on any of the three examinations, how many have had sputum culture. As examination of two sputum specimens is recommended, it is important to report the number of persons with one sputum examination (for smear and for culture) and the number with two. Persons with any smear-positive should be classified as smear-positive and need to have sputum culture.

- ■ **Screening strategy 3:** Questionnaire and chest X-ray for all to identify suspects, followed by sputum smear and culture if specific symptoms are present and/or chest X-ray shows abnormalities.

  As both questionnaire and chest X-ray are used as screening tools to identify suspects for sputum examination, it is important to ensure that all eligible people have both examinations. However, some people may have one examination but not the other. If a substantial proportion of people fail to have both examinations, the estimates of prevalence may be biased. Therefore, the number of people with one examination and the number with both examinations need to be presented, as in Table 17.4.

In this strategy, people with specific symptoms and/or chest X-ray abnormalities need sputum smear examinations and culture for *M. tuberculosis.* The completeness of smear and culture examinations among those who are positive on the questionnaire and/or the chest X-ray can be presented as in Table 17.5. As two sputum examinations are recommended, it is important to report the number of persons with one sputum examined (for smear and for culture) and the number with two.

Frequency of symptoms identified by questionnaire and of chest X-ray abnormalities can be reported in further detail. Chest X-rays can be classified according to the presence or absence of any abnormality; those with abnormalities can be classified according to consistency or inconsistency with tuberculosis.

Table 17.3: Completeness of Sputum Smear, Chest X-ray, Questionnaire, and Culture Examinations					
				No. of Positives with Sputum Culture	
Item	Total	Negative	Positive	One Culture	Two Culture
Any examination					
Sputum smear					
Number of specimens					
One specimen					
Two specimens					
Sex					
Male					
Female					
Age group (years)					
15–24					
25–34					
35–44					
45–54					
55–64					
≥ 64					
Questionnaire					
Sex					
Male					
Female					

Item	Total	Negative	Positive	No. of Positives with Sputum Culture	
				One Culture	Two Culture
Age group (years)					
15–24					
25–34					
35–44					
45–54					
55–64					
≥ 64					
Chest X-ray					
Sex					
Male					
Female					
Age group (years)					
15-24					
25-34					
35-44					
45-54					
55-64					
≥ 64					

Table 17.4: Completeness of Chest X-ray and Questionnaire Examinations to Identify Suspects				
Chest X-ray				
Questionnaire	**Total**	**Positive**	**Negative**	**Not Done**
Total*				
Positive				
Negative				
Not done				0 [†]

* Total number of people with at least one examination, either chest X-ray or questionnaire.

[†] None of the patients must be without both the chest X-ray and the questionnaire.

Examination	Total	Negative	Positive	No. of Positives with Sputum Examination	
Table 17.5: Completeness of Smear and Culture Examinations, among Questionnaire- and/or Chest X-ray-Positives					
				One Examination	**Two Examination**
Any examination					
Questionnaire					
Sex					
Male					
Female					
Age group (years)					
15–24					
25–34					
35–44					
45–54					
55–64					
≥65					
Chest X-ray					
Sex					
Male					
Female					
Age group (years)					
15-24					
25-34					
35-44					
45-54					
55-64					
≥65					

Furthermore, while two sputum smears and cultures are the standard procedure in a prevalence survey, some may fail to submit the required number of sputum specimens. Thus, it is essential to present the number of people with one sputum specimen examined and the number with two, by smear and culture. Further details can be added to Table 17.6 to indicate the results of smear examination, in the following permutations: two positive; one positive and one negative; one positive and one not done; two negative; one negative and one not done; two not done. It would be useful as well to report smear-positivity grade (scanty, 1+, 2+, 3+) of positive smears and culture-positivity grade of specimens with positive culture.

17.3   Reporting sputum smear and culture examination results

In an ideal situation, people who are eligible for sputum examinations in a prevalence survey would have two specimens for smear and culture.

In the real world, sputum smear or culture may fail to be done because of administrative errors (sputum smear examination performed but not culture, sputum culture performed but not smear, specimens mislabelled, errors made in recording, etc.,) or technical errors (contamination of culture). A patient may have results of smear but not culture, or have results of culture but not smear. Furthermore, a culture positive for mycobacteria needs species identification to differentiate between *M. tuberculosis* and nontuberculous mycobacteria. Species identification may not be performed for all positive cultures. Thus, there are three potential results for sputum smear examinations (smear-positive, smear-negative, and smear not done) and six for sputum culture (culture-positive for *M. tuberculosis,* culture positive for nontuberculous mycobacteria, culture-positive but species identification not tested, culture-negative, culture contaminated, and culture not done). Cross-tabulation of three potential results of smear and six potential results of culture gives 18 combinations of smear and culture results (Table 17.6). Always reporting the results of sputum examinations as in Table 17.6 is recommended.   If all people eligible for sputum examination in a prevalence survey have two specimens of sputum examined for both smear and culture, there will be two possibilities for smear (positive or negative) and four possibilities for culture (culture-positive for *M. tuberculosis*, culture-positive for nontuberculous mycobacteria, culture-positive but species identification not tested, and culture-negative). If species identification is done for all positive cultures, the possible culture results will be reduced to three (culture-positive for *M. tuberculosis*, culture-positive for nontuberculous mycobacteria, and culture-negative). In this case, patients who are culture-positive for nontuberculous mycobacteria would not be classified as a tuberculosis case, and the rest of the patients would be classified into four categories: smear-positive, culture-positive; smear-negative, culture-positive; smear-positive, culture-negative; and smear-negative, culture-negative.

Table 17.6: Standard Reporting Format for Bacteriological Examination of Sputum in a Prevalence Survey						
Culture						
Smear	Positive			Negative	Contaminated	Not Done
	NTM	MTB	Not Speciated			
Positive						
Negative						
Not Done						

MTB = Mycobacterium tuberculosis, NTM = nontuberculous mycobacteria

17.4 Tuberculosis cases and estimation of prevalence

The number of tuberculosis cases identified needs to be presented by sputum results and by sex, age, tuberculosis treatment history, and cluster. Table 17.7 can be used to present the smear positive patients identified, and Table 17.8 culture-positive patients.

It is also important to report the results of all four measurements (symptoms, chest X-ray, sputum smear, sputum culture) in detail, as in Table 17.9.

From the information reported above, it is then possible to estimate the prevalence of tuberculosis, by age, sex, and sputum results, taking into account response rate and other factors. In surveys using stratified sampling, separate estimates of the prevalence of different strata can be reported.

Table 17.7: Smear-Positive Tuberculosis Case Identified in a Prevalence Survey						
Item	No. of Persons Examined	No. of Smear Positives*	Smear Positive Tuberculosis			
			No. of Culture Positives	No. of Culture Negatives	No. of Contaminated Culture	No. with Culture not Done
Total						
Male						
Age group (years)						
15-24						
25-34						
35-44						
45-54						
55-64						
≥65						

Continuation of Table 17.7

Item	No. of Persons Examined	No. of Smear Positives*	Smear Positive Tuberculosis			
			No. of Culture Positives	No. of Culture Negatives	No. of Contaminated Culture	No. with Culture not Done
Female						
Age group (years)						
15-24						
25-34						
35-44						
45-54						
55-64						
≥ 65						
Type of Person						
No History of TB						
Under treatment						
Previously treated						
Symptoms						
Cough						
No Cough						
Chest X-ray						
Any abnormality						
No abnormality						
Cluster						
Cluster 1						
Cluster 2						
Cluster 3						
Etc						

Excluding nontuberculous mycobacteria osis Identified in Prevalence Survey

Table 17.8: Culture-Positive Tuberculosis Identified in Prevalence Survey					
Item	No. of Persons Examined	Number Culture positive for *M. Tuberculosis*	Culture-Positive *M. Tuberculosis*		
			Smear Positive	Smear Negative	Smear Not Done
Total					
Male					
Age group (years)					
15-24					
25-34					
35-44					
45-54					
55-64					
≥65					
Female					
Age group (years)					
15-24					
25-34					
35-44					
45-54					
55-64					
≥65					
Type of Person					
No History of TB					
Under treatment					
Previously treated					
Symptoms					
Cough					
No Cough					
Chest X-ray					
Any abnormality					
No abnormality					
Cluster					
Cluster 1					
Cluster 2					
Cluster 3					
Etc					

Results of Screening	Culture Positive		Culture Negative	
	Smear Positive	Smear Negative	Smear Positive	Smear Negative
Symptom-positive				
Chest X-ray-positive				
Chest X-ray-negative				
Symptom-negative				
Chest X-ray-positive				
Chest X-ray-negative				

Table 17.9: Results of Symptom Screening, Chest X-ray, Sputum Smear, and Sputum Culture Examinations

18. Budget

Just a decade ago, disease prevalence survey was only a dream for most developing countries. However, incremental funding for tuberculosis (TB) control and international technical assistance have changed things.

Budgeting for a prevalence survey is basically very similar to budgeting for regular activities. There are three stages along the timeline: survey preparation, the survey itself, and post-survey activities.

■ Before the survey

    – procurement
    – training

■ During the survey: field operation and central management including quality assurance

■ After the survey

    – data entry and analysis
    – dissemination of results

The size of the budget is influenced more by the screening strategy selected than by the size of the survey: three vans, each with a digital X-ray unit, may cost more than $1 million, while three portable X-ray might be purchased for $100 000. The availability of enough cars to support field activities is also a key issue in preparing a budget.

It is necessary to pay attention to the following budget items, because they are likely to be neglected, or the workload not properly estimated:

■ security arrangements;

■ information, education, and communication needs, including those required to obtain informed consent;

■ central laboratory work;

■ treatment arrangements, directly observed treatment (DOT), for detected cases especially in remote clusters without access to the national TB programme;

■ data entry and management;

■ dissemination of results; and

■ technical assistance.

Estimating the quantity of consumables such as X-ray films and sputum cups is often well understood as this is a part of routine procurement within the national tuberculosis programme. However, it is often necessary to purchase a different type consumable from that used in routine work (for example, the sputum container with screw cap for transportation). Careful consultation in the preparation stage is therefore essential.

Example: *Cambodia survey with 42 clusters of 30 000 subjects with screening by direct X-ray*

Total cost: $550 000 (staff salaries excluded)

Capital Investment: $120 000

Consumables: $60 000

Training: $30 000

Field Operation: $120 000

Other Local Cost (including central unit operation): $60 000

Post-survey Events: $30 000

Second-Hand X-ray van (donation): $50 000 equivalent

Technical Assistance (6 person-months): $80 000

Marginal cost:

$3000 – $4000 per cluster with 700 – 750 participants

($4 – $6 per participant)

$2 – $3 to add a participant to an existing cluster

The table below shows essential budget items for a prevalence survey.

Table 18.1: Budget Outline for a Prevalence Survey				
Item	**Unit Cost**	**Units**	**Total**	**Comments**
SALARIES				
Key Investigators				
Field staff				
Temporary staff				
PREPARATORY WORK				
Planning workshop		1		meeting
Sensitizing workshop		1		meeting
Protocol development		1		TA
Pre-visit to each cluster		No. of Cluster		per diem and fuel

Item	Unit Cost	Units	Total	Comments
CAPITAL INVESTMENT				
X-ray units including accessories		No. of operations +1		with one backup unit
Generators		No. of operations		field use
Desktop computers		3		for central management
Laptop computers and portable printers		No. of Teams		for field management
Incubators (laboratory)				expansion of lab capacity if necessary
OTHER PROCUREMENT				
X-ray film		No. of samples x1.3		
Developers and fixers		No. of samples/101		
Sputum containers		No. of samples x0.3x4		
Iceboxes and ice packs				sputum transportation
Vinyl bags				
Tents, sheets, rope				
Portable desks				
Portable chairs				
Water tanks				
Stationery				
Laboratory consumables				for smear and culture
Memory tips				
T-shirts and caps for staff				
T-shirts and caps for field volunteers				different color
Incentives for participants				
First-aid kits				
PRINTING				
Manuals				
Logbooks and forms				
IEC documents				
Regular reports				

Item	Unit Cost	Units	Total	Comments
TRAINING				
Census team		5 days		
X-ray team		5 days		
Laboratory team		5 days		
Comprehensive training session		3 days		
Field pilot-testing		5 days		
FIELD OPERATION				
Per diem (overnight)		days x no. of staff x no. of clusters		
Local staff/Volunteer honorariums or lunch allowance		no. of clusters		
Security fees		no. of clusters		
Car rental				
Fuel (car)				
Fuel(generator)				
Rental of survey site		no. of clusters		
Local purchases (ice, water, etc.)		no. of clusters		
Telecommunications				
Ferry, highway, parking fees, etc.				
TREATMENT				
Specially arranged treatment of detected TB cases in difficult areas				
CENTRAL OPERATION				
Steering/Technical committee				regular meeting
Supervision (per diem and fuel)				
X-ray reading (honorariums if necessary)				
Laboratory (examination fees or overtime)				
Data entry				salary or piecework
Communications				

Item	Unit Cost	Units	Total	Comments
MIDTERM REVIEW AND RETREAT				travel and per diem
Consensus workshop				
Dissemination workshop				
Publication				
International conference				
TECHNICAL ASSISTANCE				
Protocol development				
Field management				
Laboratory				

Annexes

Annex 1: Patient Consent Form

All study subjects must voluntarily give consent to participate in the research. They must affirm that they have read and/or understood an explanation of the nature and purpose of the study, what is expected of them, and what participation may involve for them.

When the potential subjects are children (under 16 years of age or as legally defined locally) the consent of the parents or guardians must be obtained in line with local custom and practice. Any competent child who refuses to participate, regardless of parental or guardian consent, should be excluded.

It is often desirable also to obtain the consent and collaboration of local civil leaders and health-care providers, on whom the successful conduct of the study frequently depends.

Obtaining consent

There are two possible ways of obtaining consent:

■   Each participant reads the information sheet, asks any questions he/she wishes, and, if content to participate, signs the consent form. This form should be countersigned by a named witness who is not related to the participant.

■   The information sheet is read to the participant. If the participant agrees orally to participate he/she signs or marks his/her agreement, which must be witnessed and countersigned. This procedure normally applies only where the research is being undertaken in illiterate populations.

Any deviation from either option must be explained and justified by the applicants. Details of any alternative procedures proposed must be given.

The consent form

The consent form should include the following information and statements:

- Project title

- Name of principal investigator and contact details

- "I have read the information sheet concerning this study (or have been given a clear oral account)"

- "My questions concerning this study have been answered to my satisfaction by………………………………..."

- "I now understand what will be required of me and what will or may happen to me if I take part"

- "I understand that I may withdraw from this study at any time without giving a reason and without my withdrawal affecting my usual care and treatment"

- "On these terms I agree to take part in the study" or "I agree as parent or legal guardian of……………………………… to his/her participation in the study"

Signed………………………………………………Date………………………

Print name………………………………………………(state if parent/guardian)

Witnessed by………………………………………..Date………………………

Print name………………………………………………………………………..

Annex 2: Sample Staff Organization from South India Prevalence Surveys

Survey organization chart

There should be a flow-chart giving the details of the survey activities right from the planning visit. A sketch map of the sampling unit is another document for the survey. The responsibility of the census taker in registering the participants and taking the census—including symptom inquiry, chest X-ray examination, sputum collection, and dispatch of the documents to the centre, where it is scrutinized by the person in charge of the documents—also needs to be shown on the flow-chart. The various activities as understood by everybody in the team should be indicated very clearly (the flow-chart will be drawn after getting the input of the members).

Constitution of field team

The team should be formed before the survey begins. The team members and the responsibility to be assigned to each one should be discussed and finalized.

The following are details of the team members' roles and responsibilities.

Supervisor

He/She is responsible for the overall survey and is available in the field throughout the days of survey. He/She supervises the team members in the performance of their duties. In case of any deviation or discrepancy in the data collection procedure, he/she explains what is being done wrong and corrects the procedure. The supervisor is responsible for quality checks during the survey. For this purpose he/she independently elicits the status of symptoms from a 5%–10% random sample of those already interviewed and compares the findings with those already gathered by the regular census taker. In case of any deviation he/she takes immediate corrective action.

Team leader

The survey team leader, usually a senior staff with baseline experience in all fieldwork activities, is responsible for the day-to-day work of the team. He/She goes around the village, divides the area into convenient blocks on the basis of the sketch map drawn for the village, and assigns blocks of households to the census takers and technicians. The survey team leader checks all the relevant entries made in the symptom questionnaire and X-ray particulars such as the date of X-ray and the roll numbers and exposure lists prepared by the X-ray technicians for chest X-rays at the centre. After the symptom inquiry at the participants' doorstep, he/she directs the participants to the centre, where X-rays are taken by the mobile X-ray unit. The survey team leader also supervises the X-ray technicians.

In the absence of the supervisor, the survey team leader has overall responsibility for the survey.

X-ray technician

The X-ray team normally has one or two X-ray technicians, each one with an attender and driver. The X-ray technician takes X-rays of the survey participants and is responsible for the quality and accuracy of the chest radiographs and other documents and for the regular maintenance of the equipment. He/She keeps the X-ray unit ready and operates the X-ray equipment according to the guidelines. The X-ray technician and the marshall see to it that the persons lining up for X-rays have the completed questionnaire with their identification particulars. After the X-ray, the X-ray roll number and the date of the X-ray are copied onto the individual cards with relevant entries in the exposure list. After the film is developed in the processing unit it is sent to the place where it is read by two independent readers.

Census taker

The census taker or enumerator, being an important person constantly in close touch with the study population, must always be patient, good-humoured, and persuasive, firm and resolute when needed, but never bullying. He/She should know enough to answer the routine queries of the population in a convincingly easy manner. His/Her handwriting should be neat and legible, and he/she should be careful to avoid omissions.

The major task of a census taker is to register systematically the entire population under study. Before that, he/she goes round the village or block with the survey team leader, to get an idea of the spread of the village and to be able to register, number, and map households in a definite order. Moving from house to house, the census taker usually contacts and interview the head of the family. Before entering the details on the individual cards, the census taker should get a clear idea of the number of persons residing in the house. So as not to miss any registration, he/she should ask probing questions, particularly about relatives, household helpers, and visitors. Whenever possible, the census taker seeks the help of an important person in the locality, who may not necessarily be the village headman. Individual cards should be filled systematically in chronological order, from the head of the family to the wife, children, brothers, sisters, father, mother, relatives, and household help.

Coordinator

The coordinator (card controller) is responsible for the participants directed by the census taker to the centre where the X-ray unit is installed. He/She checks the identification particulars on the individual cards of the participants, elicits symptom status if this has not already been elicited by the census taker at their residence, and then directs the participants with their individual cards one by one in an orderly way to the X-ray marshall. The coordinator keeps a list of participants who do not turn up for X-ray and asks the survey team leader to direct those participants to the centre for X-ray. The coordinator arranges to have the individual cards with all identification particulars duplicated for sputum collection and handed over to the sputum team. At the end of each day the coordinator tallies the number of participants X-rayed against the individual cards and keeps the individual cards of participants who have not yet been X-rayed, for further action the next day.

Sputum team members

The sputum team consists of a sputum team leader, a sputum collector, and an attendant. The sputum team leader is responsible for the quality and quantity of the sputum specimens. He/She keeps the sputum-eligible cards prepared each day by the X-ray team and arranges the cards in the order of priority and the place of sputum collection. Two specimens (one spot and one early-morning specimen) are collected by the sputum collector from the eligible participants. The attendant helps the sputum collector in handing out the specimen bottles with the necessary instructions for

collecting the specimens, and enters on the sputum-eligible cards the specimen bottle numbers, the date of collection, and the type of specimen ("spot" or "overnight"). At the end of the day the attendant makes an ordered list of the bottle numbers on a separate sheet and cross-checks these against the entries made on the individual cards. The specimens collected are kept in the thermocol box with ice pieces and sent with the relevant smear sheets to the laboratory on the same day.

The same team collects the overnight specimens the next day, and the relevant entries are made as usual. If any person is absent during sputum collection the proper reason code for the failure to collect the specimens is entered on the card. The completed sputum-eligible cards are checked and dispatched to the centre for further action. The sputum team also collects sputum specimens to replace the ones that were contaminated while processing for culture.

In addition to the above team members, there will be other attendants, attenders, and drivers depending on the size of the area to be covered, the number of teams, and the assistance required for each team.

Annex 3: Sample Terms of Reference for Field Team Members

Team functions are grouped. Also see the chapter "Survey Organization".

Field team leader

- Leads the first pre-survey visit;

- Is responsible for logistics and organization of the fieldwork;

- Coordinates the day-to-day fieldwork;

- Communicates with local, district, and provincial authorities on issues regarding the fieldwork;

- Is responsible for the completion of the field report.

Census and interview group

- Visits all households in the clusters, explains aims the of the survey, mobilizes participation in the survey, fills in the census registers for adults and children and the first part of the screening questionnaire;

- Interviews each adult to screen for TB treatment in the previous two years and for coughing, using the screening questionnaire;

- Interviews all suspects for details, using the suspect questionnaire;

- Keeps the adult's census register to monitor data collection and fills in the first part of the suspect register;

- Assists in tracing patients for sputum collection and children for tuberculin testing.

X-ray group

- Sets up the X-ray unit and central examination site;

- X-rays all eligible adults;

- Enters the identifier on the digital film, or the film number on the back of the screening questionnaire;

- Develops films and stores digital images;

- Reads all films on the same day or the next day and records the results on the back of the screening questionnaire.

Tuberculin group

- Assists in the census;

- Keeps the children's census register to monitor data collection;

- Prepares the (school) lists for children to be tested;

- Tests all eligible children on the census list who report for testing;

- Traces non-participants, motivates the parents to have their children participate in the testing, and carries out the test if possible;

- Reads all children tested and examines BCG vaccination scars;

- Records all results on the tuberculin form;

- Cooperates with the census and interview group in interviews and defaulter tracing.

Laboratory group

- Sets up the laboratory (district unit);

- Instructs suspects in sputum production;

- Keeps the suspect register to monitor data collection and fills in the second part;

- Collects sputum samples from all TB suspects;

- Examines sputum samples for acid-fast bacilli;

- Prepares sputum samples and arranges for their transport to the reference laboratories;

- Traces non-participants and motivates them to submit sputum samples;

- Records the results on laboratory forms.

Annex 4: Suggested Items for the Field Report

From: Protocol of Vietnam Prevalence Survey (2006)

On the last day in the cluster the field team leader prepares a field report on the basis of the information in the census and suspect registers and from the field team groups. The report is made available to the local authorities and the technical committee.

The information includes the following:

- Name of village, date of fieldwork, and name of survey team leader;

- Number of adults on the population list and in the survey census;

- Number of adults who participated in the survey;

- Number of adults who had X-rays taken;

- Number of abnormal X-rays;

- Number of adults who have been coughing for more than two weeks;

- Number of suspects;

- Number of adults who had sputum examination and number of smears taken;

- Number of specimens with mucoid, mucopurulent, or bloody sputum, versus specimens with saliva only;

- Number of specimens sent for culture;

- Number of smear-positive suspects;

- Number of children on the population list who took part in the survey census;

- Results of repeat interviews.

Annex 5: Census Form (Household Registry)

Address: _____

SR No.	Survey No.	Name	Age	Date of Birth	Sex M	Sex F	Relation to Household Head	Occupation	Duration Lived* in Area	Eligibility	Remarks (Reason for Absence)	Attendance
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												

*If not permanent/registered resident.

Note: When there are 12 people or more in a family, use another sheet (page 2) and fill from row 2 (write "12").

Number of eligible subjects in this household: _____

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Annex 6: Individual Survey Card

Interviewer: _____

1. Individual Code Number: ☐☐☐☐☐ -- ☐☐☐☐☐ -- ☐☐☐☐☐

2. Name: _____

3. Sex: ☐ Female ☐ Male

4. Age: _____ Check if estimated ☐

5. Occupation: _____ Category code _____

6. Do you have symptoms? If yes, how long have you had these symptoms?

	No	Yes	Duration days	weeks	months
6.1 Cough	☐	☐	____	____	____
6.2 Sputum	☐	☐	____	____	____
6.3 Blood-stained sputum	☐	☐	____	____	____
6.4 Chest pain	☐	☐	____	____	____
6.5 Body weight loss	☐	☐	____	____	____
6.6 Fatigue, malaise	☐	☐	____	____	____
6.7 Fever	☐	☐	____	____	____
6.8 Other ( )	☐	☐	____	____	____
6.9 TB suspect, by symptom(s)	☐	☐	____	____	____

7. Current TB treatment: 7-1 ☐ No ☐ Yes

    (Start [month] [year]; TB No.   )

    ☐ Public hospital/NTP   ☐ Health center/NTP
    ☐ Other hospitals   ☐ GP
    ☐ Pharmacy   ☐ NGOs
    ☐ Traditional healers   ☐ Other

8. Past history of TB treatment: ☐ No ☐ Yes

    (Start [month] [year]; TB No.   )

    ☐ Public hospital/NT   ☐ Health center/NTP
    ☐ Other hospitals   ☐ GP
    ☐ Pharmacy   ☐ NGOs
    ☐ Traditional   ☐ Healers
    ☐ Other

9. Behaviour regarding symptom(s)/Yes for 6.9 –TB suspect, only:

    ☐ Not applicable   ☐ Not recognized as illness
    ☐ Ignored   ☐ Self-treatment
    ☐ Consulted

9.2 (If care was sought) Place of consultation
(can choose more than one):

☐ Public hospital/NTP   ☐ Health center/NTP
☐ Other hospitals   ☐ GP
☐ Pharmacy   ☐ NGOs
☐ Traditional healers   ☐ Community health worker
☐ Other

9.3 Examination:

1   Sputum   ☐ No   ☐ Yes
2. X-ray   ☐ No   ☐ Yes

10.   X-ray

10.1 ☐ Requested
☐ Exempted (reason: _____)
☐ Rejected

10.2 Result of field screening:

☐ Normal   ☐ Active TB
☐ TB suspect   ☐ Healed TB
☐ Heart disease
☐ Other lung disease (active)
☐ Other findings in lung (inactive)
☐ Other _____
☐ NA _____

10.3 Sputum request, by X-ray result: ☐ No   ☐ Yes

10.4 Result of central reading

☐ Normal   ☐ Active TB
☐ TB suspect   ☐ Healed TB
☐ Heart disease
☐ Other lung disease (active)
☐ Other findings in lung (inactive)
☐ Other
☐ NA

11.   Sputum examination

11.1 ☐ Not requested   ☐ Requested

11.2 Smear:

11.2.1.1   SP1:
☐ Not collected   ☐ Negative
☐ Positive   ☐ NA

11.2.1.2   If positive
☐ 3+   ☐ 2+   ☐ 1+
☐ Scanty 3 or more   ☐ Scanty, < 3

11.2.2.1 SP2:

☐ Not collected ☐ Negative

☐ Positive ☐ NA

11.2.2.2 If positive:

☐ 3+ ☐ 2+ ☐ 1+

☐ Scanty 3 or more ☐ Scanty, <3

11.3   Culture (AFB):

11.3.1.1 SP1:

☐ NA ☐ Negative

☐ <5 colonies ☐ Positive

☐ Contaminated

11.3.1.2 If positive

☐ 4+ ☐ 3+

☐ 2+ ☐ 1+

☐ colonies (5 or more)

11.3.2.1 SP2:

☐ NA ☐ Negative

☐ Positive ☐ Contaminated

11.3.2.2 If positive:

☐ 4+ ☐ 3+

☐ 2+ ☐ 1+

☐ colonies (5 or more)

11.4   Identification:

☐ NA ☐ TB

☐ Non-TB ☐ Pending

12.   Final Diagnosis

12.1. TB:

☐ No

☐ Smear-positive TB

☐ Smear-negative/Culture-positive TB

☐ Bacteriologically negative, active TB suggested

☐ TB suspect

☐ Healed TB

12.2. Other findings:

☐ No abnormality

☐ Other lung disease (active)_____

☐ Other finding (inactive) _____

☐ Heart disease

☐ Other site_____

Remarks:

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Instructions for Filling Out Individual Survey Sheet (example from Cambodia survey)

I. Friendly greetings and brief explanation of what you will do

Hello. How are you?

My name is XXX. Today I will ask some questions about your health. Your very very honest answers are important. All examinations provided here are free of charge. And if our doctors find out that you have TB, we guarantee free treatment. Do you agree to participate?

QUESTION NO.	WHAT TO ASK	WHAT TO DO	COMMENTS
II. Confirmation of census data			
	First, may I verify your information, to make sure our list is correct or not?		
1	May I see your survey number on your survey ID?	*Confirm survey no.*	*Those who cannot read may bring a family member's survey ID. You must verify that he/she is the right person.*
2	What is your name?		
3	Are you Mr. (Madam) ------?		*Ask only when sex is unclear.*
4	How old are you? or, Can you tell me when you were born/your birthday?	*Check a box when age given is an estimate*	*Older people may not know their exact age. Get a best guess/estimate by asking around.*
5	What do you do?		*The categories used in a country demographic health survey (DHS) can be used if necessary.*
			When an answer to a question above is different from the information obtained in the census, confirm the answer and correct the information from the census. *When you are not sure if you are interviewing the right person or not, and are doubtful about the person's eligibility, report the matter to your team leader immediately.*

QUESTION NO.	WHAT TO ASK	WHAT TO DO	COMMENTS
III. Identification of TB-related symptoms and duration			
6	Do you have any health concerns? What is your problem?	*If some concerns are mentioned, check the appropriate box(es)*	*This question gives the participant time to consider his/ her health status before you ask about the presence of symptoms. This open question also makes the participant feel that you are listening. Even when the answer is "Not at all", go to 6-1.*
	I will ask about some important symptoms, one by one:		
6-1	Do you have cough? If yes, how long have you had it? When did you start coughing?	*If duration is less than two weeks, express duration in days;*	*If the interviewee coughs during the interview despite answering "No", you should ask, "You are coughing, aren't you? When did that cough start?"*
6-2	Ask in the same way as 6-1	*if two weeks to eight weeks, in weeks;*	*The most critical points in the survey: Does the interviewee meet the criteria for sputum examination? the NTP criteria for TB suspects? Use a calendar if necessary.*
to	"Do you have ____?"	*if two months or more, in months;*	
6-8	"How long have you had it?""When did it start?"	*if a year to two years, in one year and months;*	
		if more than two years, in years.	
6-9	(this is not a question for the interviewee) Choose according to definition		*"Yes" if the interviewee has been coughing for 14 days or more or has blood in sputum. When X-ray is not taken, add three or more Yes answers.*
IV. Identification of current and past TB treatment history			
7-1	Are you being treated for TB right now? (If "yes") When did the treatment start? Do you have a TB patient's ID? (continue to 7-2)		*Those receiving TB treatment should have their sputum examined. A local health worker may help you get proper information.*

QUESTION NO.	WHAT TO ASK	WHAT TO DO	COMMENTS
7-2	Where do you get your TB medicine?	*Identify the responsible TB treatment facility.*	*The purpose of this question is not to know the type of DOTS but to identify the primary responsible medical facility that should treat the TB.*
8-1	Have you been treated for TB before? (If "yes") When were you diagnosed? Do you have any record anywhere?	*If interviewee has been treated more than once, record all events.*	*People with a history of TB should have their sputum examined regardless of whether or not they show symptoms, especially when chest X-ray has not been taken.*
8-2	Where did you get your TB medicine? *(ask about the last event if the interviewee has been treated twice or more)*		*The purpose of this question is not to know the type of DOTS but to identify the primary responsible medical facility that should treat the TB.* *A local health worker may help you get proper information.*
V. Identification of behaviour towards symptoms: only when Yes in 6-9			
9-1	You have the symptom(s) above *(mention them)*. Have you done anything about it/them? Have you seen any health personnel? (If "no") a Why not? Don't you think you are ill? Don't you want treatment? Would you rather deal with your symptoms yourself? *(If "yes", continue toà 9-2)*	*Choose "Not Applicable (NA)" if interviewee has already received TB treatment.*	*This section is optional. These questions can be asked in a post-survey interview when TB patients detected by the survey receive treatment.*
9-2	Where did you go for consultation? Did you visit a hospital? a health center? a GP? --------?	*Check box(es). Several boxes can be checked.*	
9-3	Did you have a sputum examination there? How about a chest X-ray?	*Y/N*	

QUESTION NO.	WHAT TO ASK	WHAT TO DO	COMMENTS
10-1	The next section is X-ray. X-ray is a very safe and established medical examination even for pregnant women. But healthy pregnant women, especially in their early-middle stage of pregnancy, and people who have had an X-ray recently can be exempted from the examination. Do you have any concerns about having an X-ray? (For ladies only) Are you pregnant? If so, when are you due?	Pregnant women without any TB-related symptoms should be exempted from X-ray.	*When a participant refuses to have an X-ray taken despite not meeting the exemption criteria, ask the team leader to decide.* *If a participant exempted from X-ray is coughing, sputum should be collected.* *Standard criteria for exemption from X-ray exam should be developed by the country expert.*
10-2 10-3	*(for X-ray section)* *X-ray results should be recorded by the X-ray section at the survey site*		
10-4	*(data management section)*	*Copy report from central reading team.*	
VII. Laboratory examination			
11-1	*(for responsible laboratory staff)*	*Decide if a sputum exam is needed or not.*	*Only a team leader and/or a physician can decide on the basis of criteria other than the screening criteria (e.g. a pneumothorax case who needs urgent medical intervention can be exempted from sputum exam). But known smear-positive TB patients under treatment must have their current bacteriological status evaluated.*
11-2 to 11-4	*(for central data management section)*	*Copy reports from the laboratory.*	
VIII. Final Diagnosis			
12	*(for central committee)*	*TB: Check only one Other findings: one or more boxes can be checked*	*The central committee should examine all participants with smear-positive results including those with only a single scanty slide and/or with culture-positive results.*

Annex 7 : Post-Survey Questionnaire (optional)

Date _____

Dr _____
    District TB Coordinator
    _____ District

Survey Individual Report and Post-Survey Questionnaire

Survey Number: _____

Name: _____ Sex: _____ Age: _____

Address: _____

In the National TB Prevalence Survey conducted on   /   /2007, the person above was diagnosed as having active TB as follows:

☐ Smear-positive
☐ Smear-negative/Culture-positive
☐ Smear-negative/Culture pending/X-ray suggestive
☐ Smear negative/Culture negative/X-ray suggested

Please arrange medical intervention. The post-survey questionnaire on the next page should be completed and submitted with the next regular quarterly report. Your cooperation is highly appreciated.

Thank you very much for your active participation in the National TB Prevalence Survey.

Dr
NTP Manager

Post-Survey Questionnaire (optional)

Survey Number: _____

Name: _____Sex:____ Age:____(Date of Birth)____/____/____

Address:_____

If any of the above information is wrong please correct it. (Do not change the survey number.)

Please check the following box if this patient is living outside the designated survey area and was NOT ELIGIBLE to participate in the survey.

☐ This person was NOT an eligible survey participant.

Sputum Results of Patients Already Under Treatment

Sp1: (☐ 3+   ☐ 2+   ☐ 1+   ☐ Scanty ____ [no.]   ☐ Negative)

Sp2: (☐ 3+   ☐ 2+   ☐ 1+   ☐ Scanty ____ [no.]   ☐ Negative)

Sp3: (☐ 3+   ☐ 2+   ☐ 1+   ☐ Scanty ____ [no.]   ☐ Negative)

1. Type and duration of symptom(s) before the survey

	No	Yes	days	weeks	months
			Duration		
1.1 Cough	☐	☐	____	____	____
1.2 Sputum	☐	☐	____	____	____
1.3 Blood-stained sputum	☐	☐	____	____	____
1.4 Chest pain	☐	☐	____	____	____
1.5 Body weight loss	☐	☐	____	____	____
1.6 Fatigue, malaise	☐	☐	____	____	____
1.7 Fever	☐	☐	____	____	____
1.8 Other ( )	☐	☐	____	____	____

2. Were you taking treatment for tuberculosis at the time of the survey?

☐ No   ☐ Yes
                       (Start   [month],   [year]; TB No. _____)

Responsible facility:

☐ Public hospital/NTP   ☐ Health center/NTP

☐ Other hospitals   ☐ GP

☐ Pharmacy   ☐ NGOs

☐ Traditional Healers   ☐ Other_____

Type of TB:

☐ S(+)Pul   ☐ S(−)Pul   ☐ Unknown Pul

☐ Extra pul   ☐ Latent infection   ☐ Unknown

3. Past history of TB treatment:

☐ No   ☐ Yes (Year; TB No. _____)

Responsible facility:

☐ Public hospital/NTP   ☐ Health center/NTP

☐ Other hospitals   ☐ GP

☐ Pharmacy   ☐ NGOs

☐ Traditional Healers   ☐ Other_____

Type of TB:

☐ S(+)Pul   ☐ S(−)Pul

☐ Unknown pul   ☐ Extra pul

☐ Latent infection   ☐ Unknown

4. Behavior in response to symptom(s):

4.1 What action did he/she take before the survey?

☐ No symptom   ☐ Not recognized as illness

☐ Ignored   ☐ Self-treatment ☐ Sought care

4.2: *(If care was sought)* **Place of consultation**; write number (1, 2, 3, etc.), according to the chronological sequence of visits, and ask when taking the date of the survey day as day 0.

First symptom appeared	☐ days	☐ weeks	☐ months ago
Public hospital/NTP	☐ days	☐ weeks	☐ months ago
Health center/NTP	☐ days	☐ weeks	☐ months ago
Other hospitals	☐ days	☐ weeks	☐ months ago
GP	☐ days	☐ weeks	☐ months ago
Pharmacy	☐ days	☐ weeks	☐ months ago
NGOs	☐ days	☐ weeks	☐ months ago
Traditional healers	☐ days	☐ weeks	☐ months ago
Community health worker	☐ days	☐ weeks	☐ months ago
Other _____	☐ days	☐ weeks	☐ months ago

4.3: Did he/she receive any TB examination before the survey?

1. Sputum   (☐ No   ☐ Yes)
2. X-ray   (☐ No   ☐ Yes)

4.4: *(Question for the interviewer)* Why was the patient not diagnosed before the survey? What prevented such diagnosis?

Remarks

_____

_____

_____

_____

_____

_____

Date: _____   Filled out by: _____

Annex 8: Smear Microscopy

Smear preparation

After confirming the identification number of the sputum specimen, wipe the outside of the sputum container with a cotton ball soaked in 70% alcohol without erasing the label. Next, carefully open the container to pick and transfer a mucopurulent specimen with a flame sterilized loop onto a clean and correctly marked slide glass. Make an appropriate size and thickness of smear and cover the container tightly with the screw cap. After completing the smear preparation, remove and flame-sterilize the specimen on a loop. Keep the remaining sputum specimens in a refrigerator until they are processed for culture, or transported to another laboratory for culture and drug sensitivity testing.

Smear fixation

Completely dry smears in the racks at room temperature or on the slide warmer, then fix over a Bunsen burner flame or an alcohol lamp.

Ziehl-Neelsen staining and microscopy

Staining
Place fixed smear slides on staining rods in a sink and apply carbol-fuchsin (preferably 1.0% fuchsin) over the slides by flooding the entire surface. Warm over the flame (with an alcohol soaked cotton plug at the end of iron bar or wire) until vapor appears. Staining should take place for at least 5 minutes. Pour off stain and rinse gently with clean water. Drain water carefully using a forceps.

Decolorizing
Flood the stained and washed slides with 3% acid-alcohol (or 25% sulfuric acid) and decolorize for up to 3 minutes. Drain the decolorizing reagent and rinse with clean water. Drain water before proceeding to the next step.

Counterstaining
Flood the decolorized slide with 0.1% methylene blue and stain for about 30 seconds. Drain the stain and rinse with clean water.

Transfer the drained slides to a rack for drying. Prepare the slide for reading under a microscope only after the slide is completely dried and cleaned. Clean the other side if unclean because of stains.

Microscopy

Before reading stained smears, check the microscope to see if there is any physical functional or mechanical defect in the optical outfit or objective/ ocular lens. See to it that every outfit in the light path is functioning. After placing a stained slide on the stage, focus a clean microscopic field under the low-power objective lens with a coarse knob and adjust the light intensity to the most comfortable level using the lamp intensity and diaphragm. After finding the clearest field with the fine-focus knob, apply a drop of immersion oil over the stained smear and focus the field. Fit the right eyepiece to the right eye by turning the fine-focus knob and then the left eyepiece using the diopter adjustment.

Examine at least 100 microscopic fields by moving from one end of the smear, if the smear is negative, to 1+. An appropriate examination of 100 fields, even by a skilled microscopist, takes at least five minutes. To prevent possible carry-over, wipe off immersion oil from the lens with a soft tissue only after examining a positive smear.

Recording and reporting

Record the microscopy results on the individual cards and logbook quantitatively by standardized grading. Report the results to the local TB center through the survey team leader for the immediate registration of the cases found for treatment.

After completing microscopy, keep all slides in slide boxes with a layer of soft tissues to absorb immersion oil dripping from the slides.

Fluorescence microscopy (FM)

Staining

Stain fixed smears with fluorochrome (0.1% auramine O in 3% phenol solution) (Annex 12) for at least 15 minutes. Rinse stained slides with clean water (chlorine-free) and drain it before decolorizing.

Decolorizing

Decolorize stained slides with 3% acid-alcohol for two minutes and then rinse with clean water. Drain water before proceeding to the next step.

Counterstaining

Counterstain the stained slides with 0.5% potassium permanganate (Annex 12) or 0.01% acridine orange in 0.01% sodium phosphate (Annex 12) or others (for example, blue ink). Rinse the slides with clean water and then drain.

Microscopy

After they are completely dry, read the stained slides under a fluorescence microscope and record and report the results quantitatively. It is advisable to read fluorochrome stained smears within 24 hours after staining, always keeping the slides away from light (especially ultraviolet light).

Reporting

Smear results should be reported to the local TB center through the survey team leader for prompt case-management.

Reporting of smear microscopy results				
Report	No. of AFB (Z-N microscopy, 1000x)	Fluorescence Microscopy Magnification		
		250x	450x	630x
0 or "—"	None/100 fields	0	0	0
1–9/100	1–9/100 fields	← Divide observed count by 10***	← Divide observed count by 4***	← Divide observed count by 2***
1+	10–99/100 fields			
2+	1–10/field*			
3+	>10/field**			

* The results should be recorded after at least 50 microscopic fields are scanned.
** The results should be recorded after at least 20 microscopic fields are scanned.
*** AFB counts found under fluorescence microscope should be divided by this number and then adapted to the Z-N microscopy count for reporting and recording.

Annex 9: Sputum Culture

Petroff's sodium hydroxide (NaOH) method using centrifuge

Materials

■ 4% NaOH

■ 0.067M phosphate-buffer (PB), pH 6.8, or sterile distilled water (DW)

■ Löwenstein-Jensen (L-J) media (see formula in Annex 12)

■ Sputum specimens in the container that can be centrifuged directly

■ Disinfectant in a jar or 2000 ml flask with funnel

■ Cotton balls soaked in 70% alcohol in a stainless jar with lid

■ Safety cabinet (class II)

■ Centrifuge with a windshield and swing bucket rotor

■ Vortex mixer

■ Bunsen burner or alcohol lamp

■ Incubators

■ Refrigerators

■ Slant racks

■ Culture bottle racks

■ Autoclave

Preparations

■ 4% NaOH: Dissolve 40 grams of NaOH in 1000 ml of DW and sterilize by autoclaving.

■ 0.067M PB: Dissolve 9.47 grams of disodium phosphate (Na_2HPO_4) in 1000 ml of DW (stock A) and dissolve 9.07 grams of monopotassium phosphate (KH_2PO_4) in 1000 ml of DW (stock B). Mix stock A with stock B in 1:1 ratio and sterilize by autoclaving.

■ Label all media.

Procedure

■ In the safety cabinet (class II), clean the outside of the sputum container with cotton balls soaked in 70% alcohol if necessary, after confirming the identification (ID) number and name of the TB suspect.

■ Open the screw cap carefully and add an approximately equal volume of 4% NaOH to the sputum sample if the specimen looks fresh and not too thick. After tightly recapping the bottle, vortex the NaOH containing sputum for about 1 minute until the sample is completely homogenized. Leave the sample for 15–20 minutes to decontaminate at room temperature. Dilute the decontaminated sputum with at least five volumes of phosphate buffer before centrifugation. If the sputum is thick or not fresh, it must be decontaminated with two volumes of 4% NaOH.

■ Centrifuge the diluted sputum sample at at least 3000xg for 15–20 minutes, and pour off the supernatant into a jar with disinfectant.

■ Inoculate the sediment onto two slopes of L-J medium with a volume of 0.1– 0.2 ml (or 2–4 drops). Confirm the labelling of the culture media before inoculation. Spread the inoculum over the entire surface of the medium in a horizontal position in the slant rack. Leave the tubes slightly open during incubation for 2–3 days at 37°C in order to evaporate excess water. Keep the remaining sediments in a refrigerator.

■ After checking for any evaporation of excess liquid (without drying), tightly cap the inoculated media and incubate for up to eight weeks before discarding as a negative (no growth) culture.

■ Examine all cultures one week after incubation in order to detect rapidly growing mycobacteria. This will rule out slow-growing mycobacteria, including *Mycobacterium tuberculosis* complex. Record any growth quantitatively with a description of colony morphology. Thereafter, examine weekly if possible or, if not, after 3–4 weeks of incubation and eight weeks before discarding the media as no-growth (negative).

■ Record and report the results quantitatively according to the grading recommended by WHO and the International Union against TB and Lung Disease (see 8.4).

Note: If inoculated medium is contaminated during the three days of incubation, mix the remaining sediment kept in the refrigerator with an equal volume of 5% oxalic acid if contaminated with bacteria, or with 4% NaOH if contaminated with fungi. Vortex and decontaminate for 20 minutes. Dilute the decontaminated specimen with PB or DW and then

centrifuge. Inoculate the sediment onto L-J media and then process by the same methods described above.

■ After completing the reading of the cultures, autoclave all culture bottles and discard unless further testing or subculture is planned.

Cetylpyridium chloride (CPC) method using centrifuge

Materials

■ 1% cetylpyridinium chloride (CPC)

■ 2% sodium chloride (NaCl)

■ Sterile saline

Preparations

■ CPC digestant-decontaminant: Dissolve 20 grams of NaCl in 1000 ml of sterile distilled water and add 10 grams of CPC. Although it does not need to be autoclaved for sterilization, do not keep the solution in a refrigerator because the CPC will re-crystallize at a low ambient temperature. Dispense 100 ml of CPC solution in an amber colored bottle with a screw cap for distribution to the field survey teams.

■ Sterilize saline by autoclaving.

Procedure

■ Collect each sputum specimen in a container with a leak-proof screw cap. After cleaning the outside of the container with an alcohol-soaked cotton ball without erasing the label, add an equal volume of CPC decontaminant at the nearest laboratory equipped with a safety cabinet (class I or II). CPC-containing specimens undergoing liquefaction and decontamination should be kept in a box without cooling.

Note: Cooling re-crystallizes CPC! Once re-crystallized it cannot protect specimens from contamination, and when centrifuged the crystals will enter the sediment and suppress MTB growth in the culture medium.

■ Sputum specimens in CPC should be transported within one week after collection to the culture laboratory (preferably National Refernce Laboratory), where a safety cabinet and centrifuge are available.

■ After confirming the labelling, dilute the specimen in sterile saline and centrifuge at least 3000xg for 15–20 minutes. Pour off the supernatant and resuspend the sediment in sterile saline before centrifuging again to thoroughly wash out the CPC.

■ Inoculate the sediment onto L-J media as described above, and then incubate. Examine the culture media and record and report the results using the same method described above.

■ If inoculate media are contaminated, process the left-over sediment with 5% oxalic acid or 4% NaOH as described above.

■ After completing the reading of the cultures, autoclave all culture bottles and discard unless further testing or subculture is planned.

■ Simple culture method with NaOH decontamination

Materials
■ 4% sodium hydroxide
■ Kudoh-modified Ogawa medium

Preparations
■ Dissolve 40 grams of NaOH in distilled water and sterilize by autoclaving.
■ Label all media.

Procedure
■ After checking the ID number on all labels, add an equal volume of 4% NaOH to the fresh sputum specimen and vortex to homogenize for approximately one minute. Decontaminate the homogenized sputum for approximately 15–20 minutes at room temperature.

■ Inoculate 0.1 ml (or 2 drops) of decontaminated sputum and spread on the surface of the acid-buffered medium and incubate at 37°C in a horizontal position with a slightly opened cap as described above.

■ When excess liquid has evaporated (after 2–3 days), close the screw cap of the culture media tightly and continue the incubation.

■ Examine the culture media weekly as described above and record and report the results quantitatively.

■ After completing the reading, autoclave all culture bottles and discard unless further testing or subculture is planned.

Reporting of culture results	
Reading	**Report**
No growth	Negative or "—"
1–19 colonies	Actual count
20–100 colonies	1+
100–200 colonies	2+
200–500 colonies (difficult to count, but discrete colonies)	3+
>500 colonies (confluent growth)	4+
Contaminated	Contaminated or "C"

Identification

MTB must be differentiated from other mycobacterial isolates. Species identification of nontuberculosis mycobacteria (NTM) should be limited to isolates with clinical significance. Except for morphologically distinct NTM (also identified by their growth rate), all mycobacterial isolates should be subcultured for storage and other tests, such as susceptibility testing against paranitrobenzoic acid (PNB) and thiophen-2-carboxylic acid hydrazine (TCH) (if pyruvic acid medium is included to isolate *M. bovis*), to identify MTB complex. The isolates that show susceptibility to 500 $\mu l/ml$ of PNB are identified as MTB complex. If any culture shows resistance to PNB but its growth rate and morphology are consistent with those of MTB, it has to be subjected to niacin and nitrate reduction tests. *M. tuberculosis* always shows a positive reaction. These biochemical tests should also be performed for the isolates found to be susceptible to both PNB and TCH (5 $\mu l/ml$) to identify *M. bovis*, which will show a negative reaction to both niacin and nitrate reduction tests.

Susceptibility testing against para-nitrobenzoic acid (PNB)

Materials

- 500 $\mu l / ml$ PNB medium

- Drug-free L-J media

Preparations

- PNB medium: Dissolve 250 mg of PNB in 20 ml of dimethylsulfoxide (DMSO) and add it to 1000 ml of L-J medium before dispensing. Inspissate at 85ºC for 45 minutes at 80% humidity.

- Label all media correctly.

Procedure

- Take about 2 mg (2 mm3) of colonies from the primary cultures aged within two weeks after their growth appears, using a wire loop (wire diameter 0.7 mm) with 3 mm internal diameter. Discharge the growth taken into 0.4 ml of DW in a screw-capped bijou bottle (7 ml) with sixglass beads (3 mm in diameter) and shake on a mechanical shaker for one minute. Add 2 ml of sterile DW.

- Adjust the turbidity of bacterial suspension to McFarland number 1. Inoculate a loopful of suspension onto two drug-free and one PNB (and one TCH) media. Using a 0.4 mm nichrome wire loop (external diameter 3 mm), plant approximately 0.01 ml (i.e., 0.01 mg) of bacterial suspension. After 4–6 weeks of incubation at 35ºC–37ºC, read the growth. No growth or less than 20 colonies is read as susceptible while 20 or more colonies is read as resistant.

Niacin tests

Materials

- Reagent-impregnated paper strip for the detection of niacin (commercially available from Difco and Remel)

- Sterile, sealable test tubes with screw cap (12–13 x 75 mm)

Controls

- Extract from *M. tuberculosis* H37Rv culture as a positive control

- Extract from *M. avium* complex as a negative control

Procedure

- Add 1.0 ml of sterile DW to the test culture grown in egg-based medium and leave it in a horizontal position, covering the entire surface of the culture for 15 minutes to facilitate the extraction of niacin.

- Transfer 0.6 ml of the culture extract into a test tube.

- Insert the strip with the identification end up and immediately seal the

tube. Leave the tube in an upright position at room temperature for 15–20 minutes with occasional agitating without tilting.

■ Observe the color of the liquid (not strip) in the bottom of the tube against a white background.

■ After opening the cap discard the reaction tubes into alkaline disinfectant.

Results and interpretation

■ Positive: development of yellow color in extract.

■ Negative: no color development.

Nitrate reduction tests

Materials

■ Sodium nitrate, $NaNO_3$

■ Monopotassium phosphate, KH_2PO_4

■ Disodium phosphate, $Na_2HPO_4.12H_2O$

■ Hydrochloric acid HCl

■ Sulfanilamide

■ N-naphthylethylenediamine dihydrochloride

■ Distilled water

■ Zinc dust

■ Screw-cap test tubes, 16 x 125 mm

■ Water bath, 37°C

Preparations

■ Substrate (0.01 M $NaNO_3$ in 0.022M phosphate buffer, pH 7.0): Dissolve 0.085 g $NaNO_3$, 0.117 g KH_2PO_4, and 0.485 g $Na_2HPO_4.12H_2O$ in 100 ml of DW and sterilize by autoclaving.

■ Reagent 1: Carefully add 50 ml of concentrated HCl to 50 ml of cold DW.

■ Reagent 2: Dissolve 0.2 g of sulfanilamide in 100 ml of DW.

■ Reagent 3: Dissolve 0.1 g of N-naphthylethylenediamine dihydrochloride in 100 ml of DW.

■ Store the substrate and reagents in the dark at 5°C. Discard the reagents if the color changes or a precipitate forms.

Controls

■ Positive: *M. tuberculosis* H37Rv

■ Negative: *M. bovis* (BCG)

■ Negative: reagents without organisms

Procedure

■ Add 0.2 ml of DW into a screw-capped tube.

■ Transfer two spadefuls of the growth from a 4-week-old culture on egg-based medium into a tube and emulsify it.

■ Add 2 ml of the substrate ($NaNO_3$) to the tube and shake by hand, and then incubate it upright for 2 hours in a 37°C water bath.

■ Remove the tube from the water bath and add one drop of reagent 1.

■ Add two drops of reagent 2.

■ Add two drops of reagent 3.

■ Examine immediately for a pink-to-red color.

Results and interpretation

■ Positive: range from pale pink to deep red (can be graded with the use of the color standards, but preparation not described)

■ Negative: no color. Add a small amount of zinc powder to all negative tubes. If nitrate is still there, it will be catalytically reduced by the zinc, and a red color will develop, indicating a true negative. If no color develops in the presence of zinc the original reaction was positive, but the nitrite was reduced beyond nitrite. Repeat the test in this case to confirm the observation.

Annex 10: HIV Testing in Population-Based TB Prevalence Surveys

Introduction

Historically, national-level human immunodeficiency virus (HIV) seroprevalence estimates in resource-limited settings have been modeled using HIV prevalence among pregnant women, but not among disease-specific groups. Population-based surveys with HIV testing are becoming a standard approach for obtaining additional information for measuring HIV prevalence levels in the general population. These prevalence estimates, in conjunction with antenatal (ANC) sentinel surveillance data and other population-based surveys, can be used to improve national HIV estimates. In the past few years, population-based surveys, such as the demographic and health survey (DHS), have incorporated HIV testing to give the general population seroprevalence. Generally, survey participants are requested to consent to HIV testing but are told that they will not receive their test results. Often, they are given vouchers and referred to a voluntary counseling and testing (VCT) site if they are interested in knowing their HIV status. Before the availability of antiretroviral therapy in resource-limited settings, there was little impetus for people to know their status since HIV care and treatment was not available to them. Now that treatment for HIV/AIDS is becoming more widely available, the need for testing has increased and the imperative to test for HIV in national-level surveys is stronger.

According to UNAIDS/WHO, in the past few years the number of population-based surveys including HIV information has increased (WHO and UNAIDS 2003a). These include population based TB prevalence surveys, where TB patients have been tested, although TB suspects could have been tested as well. Early surveys were designed for unlinked anonymous testing (UAT), where the results could not be traced to individuals (Nelson et al. 2005, Decosas and Boillot 2005). As antiretroviral therapy becomes more widely available, UAT is increasingly becoming an untenable surveillance strategy, given the need to return results to clients so they can have HIV care and treatment. WHO/UNAIDS has published, and in doing so have set, an international standard of care, calling for all patients with TB to be tested for HIV and receive the results of their HIV test (WHO and UNAIDS 2004b). Since the early 1990s surveillance monitoring of TB has been based

on the WHO Directly Observed Short Course (DOTS) strategy, a strategy that been very useful in tracking TB prevalence globally (WHO) [5]. The World Health Organization has recently published guidelines that will include the recording of HIV information of TB cases and suspects in the TB register (WHO 2006). Because routine HIV information will, theoretically, arrive with TB information up the same DOTS reporting chain, routine TB/HIV surveillance will be made possible in the not too distant future. Routine TB/HIV surveillance is likely to supplant other methods used in the past to track the epidemic in TB patients. Estimates of HIV prevalence among TB patients in the interim will, however, require adjunct surveillance strategies such as sentinel surveillance and/or periodic surveys to calibrate routine TB/HIV surveillance activities (WHO and UNAIDS 2003a, WHO and UNAIDS 2003b).

UNAIDS and WHO have laid out the strengths of including HIV testing in population-based surveys. First of all, in generalized epidemics, population-based surveys can provide representative estimates of HIV prevalence for the general population, as well as for different subgroups. Second, the results of the population-based surveys can be used to adjust the results from sentinel surveillance systems. Third, population-based surveys provide an opportunity to link HIV status with socio-behavioral information, which is not often collected. And lastly, population-based surveys include those living in rural areas, an underrepresented group in HIV surveys, as many surveys are performed in urban, maternal/child health, or antenatal health clinics.

HIV testing in population-based surveys also has its weaknesses, however. In population-based surveys, sampling from households may not adequately represent high-risk and mobile populations. In low-grade or concentrated epidemics, population-based surveys therefore underestimate HIV prevalence. In addition, non-response can bias population-based estimates of HIV. Logistically, population-based surveys are difficult and expensive to conduct, and therefore cannot be conducted frequently. These surveys are not the optimal method to use to follow trends.

Another weakness is that the measured HIV prevalence in prevalent TB cases may differ from HIV prevalence in newly notified cases. As this matter has not been adequately studied (few surveys so far have included HIV testing), it is not known if HIV prevalence would be higher or the same. This question is actually an important one to be asked in the context of a TB prevalence survey, and a good reason to include testing. If HIV prevalence

is higher than previously estimated, this suggests that prevalent TB cases that are infected with HIV could have a higher mortality as they are dying before they can be reported. They would need faster identification and care — a strong argument for active case finding for TB. This could inform TB/HIV policy and collaborative activities in the country.

There are several specific reasons for performing HIV testing in TB patients during a national survey. First, TB patients are a sentinel population in determining the rate of HIV infection in the country. In some countries, up to 60%–70% of reported TB cases are infected with HIV, providing a sometimes accessible and defined population in which to determine HIV seroprevalence.

Second, such testing can serve to expand HIV testing in TB patients, and to estimate the number of patients potentially eligible for antiretrovirals (ARVs) during the survey period. In the 2004 Interim Policy on Collaborative TB/HIV Activities, the WHO STOP TB Department and the Department of HIV/AIDS recommended surveillance of HIV prevalence among TB patients. Surveillance is essential to inform programme planning and implementation. The chosen surveillance method (periodic/special surveys, sentinel surveys, and data from routine counselling and testing) will depend on the underlying HIV epidemic state, the overall TB situation, and the availability of resources. The recommendations currently are as follows: (1) there should be HIV surveillance among TB patients irrespective of national prevalence rates; (2) countries with unknown rates among TB patients should conduct a periodic or sentinel survey; and (3) in countries with a generalized epidemic, HIV counseling and testing for all TB patients should form the basis of surveillance. HIV counseling and testing is a prerequisite for HIV care and treatment for TB patients, and can contribute to HIV prevention.

Third, testing can foster information sharing between programs. The TB and HIV/AIDS programs in many countries need joint strategic planning to collaborate successfully and systematically. Generating epidemiologic data for planning is one crucial collaborative activity that can be accomplished. This can be used to improve the HIV testing infrastructure, to modify forms and registers, or to potentially link the surveillance systems. The HIV/AIDS programme may be better financed than the TB programme, or vice versa, to accomplish this activity.

Fourth, testing can increase knowledge of TB/HIV epidemiology. In some countries little epidemiological data may be available to the national TB programme. Including HIV testing in TB surveys provides an opportunity to further the knowledge of the TB/HIV situation unique to that setting. It can inform the programme about trends over time and enable monitoring of the impact of changing HIV seroprevalence on TB incidence and a better understanding of the association between HIV and TB drug resistance. Lastly, TB and HIV epidemiologic data help in national programme targeting of resources, and planning of interventions. It can then assist in evaluating the impact of those interventions, including acceptance of HIV testing, use of services, and trends in TB/HIV incidence.

Guidelines for including HIV testing in population-based surveys were published by UNAIDS/WHO in 2005 (UNAIDS and WHO 2005). This section will highlight some of the important issues to weigh when considering adding HIV testing to a population-based TB prevalence survey.

Planning a population-based survey that measures HIV infection in prevalent TB cases

A TB prevalence survey can be different from other sentinel and population-based surveys, in that blood is not routinely collected. Remnant specimens are often not available for use for testing biomarkers. In addition, pre-test counselling is not always available in the field for participants, thus posing logistical and budgetary challenges for field teams.

Countries conducting national population-based TB prevalence surveys must decide whether or not to include children in their study. If so, they also need to decide whether to include children in the HIV testing. This decision will depend on the local epidemiology of TB/HIV in the country. Although some countries have high rates of HIV infection in TB patients in children aged 5–14 years, most countries do not. Including children under 15 years old may be too logistically difficult and not cost-effective. Including children aged 18 months or younger also requires technologies that identify HIV antigens or viral nucleic acid, as the HIV antibody tests can be positive through the presence of maternal antibodies.

Given the expense of population-based TB prevalence surveys, if a country decides to include HIV prevalence it should also aim to collect information on social, behavioral, and biomedical characteristics of participants. Surveys can collect information related to an individual's participation in HIV testing,

demographic characteristics, health status, exposure to HIV programmes, and HIV-related knowledge, attitudes, and behavior. A full list of HIV-related socio-behavioral data can be found in the UNAIDS/WHO guidelines for conducting HIV prevalence surveys.

Returning test results. One of the most important issues to discuss when planning HIV testing during a TB prevalence survey is whether HIV results will be returned to the survey participants, and how this will be done. If test results are not made available, then options for alternative testing should be made available. Current international standard practice, given the widespread availability of ARVs, is to return the test results. The ministry of health should determine when results will be returned, and which testing strategy to use. If test results are not returned, an exemption from this practice should be obtained from the ministry of health.

The advantages of returning HIV results to participants at the time of the survey are: (1) participants can potentially know their status right away; (2) counselling is provided to everyone who receives the test; (3) couples have the opportunity to learn their status at the same time; (4) behavior change could come about; (5) individuals can be directed to services; and (6) the VCT process can promote VCT and routine testing.

The disadvantages of returning HIV results to participants at the time of the survey are: (1) confidentiality in the field may be a problem; (2) ensuring privacy when returning results may be difficult; (3) ensuring accuracy may be difficult; (4) the additional time required may disrupt the survey process; (5) larger survey teams require laboratory technicians, counsellors, and equipment; (6) ensuring proper counselling and follow-up with HIV-positive people can be logistically challenging in the field; (7) in low-prevalence settings, effectiveness may be low; (8) stigma may be attached to testing. The options for returning HIV test results include returning the result to the participant's home, doing rapid tests at home, issuing vouchers for free VCT at fixed sites or mobile VCT venues in concurrence with the survey time period, or performing home-based VCT. Each of these methods must be accessible to the participant. The options for informing respondents about their HIV status are summarized below:

■ Result of dried blood spot–based HIV test is returned at home or elsewhere after the fieldwork (issues: possibility of a mix-up in results, change in HIV status, difficulties in finding survey respondents, confidentiality, cost).

■ Rapid test is conducted at home after the interview (issues: low participation rates, confidentiality, logistics, increase in survey duration, training, bio-safety, privacy).

■ Dried blood spot is collected and rapid test is conducted at home by counsellors (issues: logistics, survey duration, privacy, confidentiality).

■ VCT is done at home or at other alternative locations (issues: coordination, logistics, cost).

■ Participants are referred to existing VCT sites (and survey data are used to identify areas of high infection, and funds that might have been spent for returning test results will be spent instead on additional VCT services).

■ If the screening test done during the survey is not the diagnostic test returned to the patient, then that test result (usually rapid test) can be used without the need for a confirmatory test. If the test taken during the survey is the same test to be returned to the patient, then a confirmatory test needs to be performed and returned to the patient.

■ A consultation on population-based surveys and HIV testing in May 2006, sponsored by the US Office of the Global AIDS Coordinator, issued the following concluding statement (Office of the Global AIDS Coordinator and Global AIDS Program, CDC, 2006):

■ The purpose of population-based surveys is to provide data for programme planning. The moral obligation of the survey implementers, if HIV testing is part of the survey, is to provide eligible respondents the opportunity to know their HIV status. This may include a range of VCT activities, from providing vouchers at fixed or mobile sites for VCT, vouchers for transport, referrals to free VCT, a list of VCT sites, home-based testing and referrals to care. This approach is consistent with the current practice of the Demographic and Health Survey plus (DHS+). The approach will depend on the country context. The specific approach will be developed in country depending on the local situation. We recommend that country teams, as part of their survey planning, conduct an analysis and review of the following considerations:

■ HIV prevalence levels and stage of the epidemic to justify a large scale population based survey

■ VCT access, uptake, quality assurance, testing acceptability

■ Feasibility and logistics

- Confidentiality and privacy concerns

- Testing issues for minors

- National VCT and routine testing policy or guidelines

- Experiences of other countries' testing strategies

- National ethical committees

- Cost

It must be remembered that while the purpose of both a demographic and health survey and TB survey is surveillance, unlike a demographic survey, as an international standard of care, HIV information is understood to be necessary for the management of the patient. According to the UNAIDS/ WHO Policy Statement on HIV testing: "Diagnostic HIV testing is indicated whenever a person shows signs or symptoms that are consistent with HIV-related disease or AIDS to aid clinical diagnosis and management. This includes HIV testing for all tuberculosis patients as part of their routine management" (WHO and UNAIDS 2004). This recommendation could be seen as setting limits on what can be done with HIV testing in TB prevalence surveys.

Types of HIV testing in prevalence surveys

In the population-based approach to HIV testing, household members are asked to provide a blood sample; they are not given results but referred to VCT facilities. The dilemma is that we want to know the HIV status of persons participating in the surveys, but the surveys were never designed to provide HIV testing and results. When assessing the ethics of HIV testing in population-based surveys versus diagnostic testing, the debate centers on the purpose of the test. In population-based surveys, the results are intended to benefit populations, whereas in diagnostic testing the benefits are intended for the individual. If these two purposes can be separated in the conduct of the survey, then there should be no ethical dilemma. In testing TB patients for HIV, these two purposes are ethically more difficult to separate for the reasons stated above (diagnostic HIV testing is the standard of care for TB patients). If a country has a poor DOTS programme with poor coverage, then one could arguably say that the rights of the community are favoured over the rights of the individual. For population-based surveys, the 2004 WHO ethical guidelines state that there must be a consent process, persons must have the right to decline, they must know the risks

and benefits, and VCT must be available. UNAIDS states that the participant should benefit from the research and must have access to VCT, and positive test results must be confirmed before being provided to individuals. In anonymous surveys, identifiers are not collected, so it is impossible, even from the start of the survey, to link any test result to any individual patient. In anonymized surveys, identifiers are collected but then removed later. This can happen at the point of collection, or at a point further down the chain such as at a laboratory. If this process is to be followed, individual names should never be kept in the same database together with data. One file with the linking numbers can be kept in a safe place, and then destroyed at the proper time before analysis. All confidential identifying information must be kept confidential in this process. Most surveys use linked data— the test data and specimen can be traced to an individual patient through a code number or even a name. Informed consent is needed for linked data, with risks and benefits clearly explained. Oral consent is now recognized to be valid, as long as the participation of the individual is voluntary, and not coerced. This is compared with HIV testing in TB patients for diagnostic purposes, which is considered the international standard of care.

The different types of HIV testing in population-based surveys are seen in the table. As noted at the beginning of this annex, UAT has been commonly used in HIV sentinel serosurveys of pregnant women and other groups (WHO and UNAIDS 2003a). However, with increased access to ARVs, UAT has become less common. UAT (without consent) in TB patients must pass a higher ethical threshold as HIV care packages become more available. For TB patients, linked anonymous HIV testing with consent is the norm. In most TB studies, blood is not routinely collected for HIV testing; hence, this would be a new activity for the study team. In addition, HIV tests can be run on sputum specimens, or on remnant specimens, although these are thought to deliver lower sensitivity and specificity compared with serum. HIV testing on sputum is not recommended where HIV prevalence is < 10%.

Methodological issues

Whom to test

A primary question to consider, once the decision has been made to conduct HIV testing during a TB prevalence survey, is whom to test. Testing could occur among several different subgroups, including suspects, cases, or even contacts of cases. Most countries have now instituted HIV testing of TB cases, but may not have a policy of testing TB suspects. In settings of a generalized epidemic, the opportunity to test suspects and to allow them access to care if found positive is a potential benefit of the survey.

Decisions have to be made about the type of specimens to collect and the type of tests to run on those specimens. HIV tests exist for blood, saliva, and urine. Tests can be either rapid tests that return results within minutes, or laboratory-based testing that takes several days to weeks to return a test. Oral fluid and urine tests may appear appealing because the specimens are easier to collect for both participant and field worker, and pose less risk, but these tests are not currently recommended because they lack the advantages of blood-based methods (which distinguish between HIV-1 and HIV-2, and detect viral subtypes). Also, testing on these specimens cannot be confirmed by a second test.

For population-based TB prevalence survey purposes, where testing is being done at a central laboratory, the most preferred method is to collect a dried blood spot—where a small amount of blood from a finger-prick is collected onto filter paper. Dried blood spots are easy to collect and transport, and after drying these samples can be kept for 30 days before testing.

HIV testing strategies have been proposed by UNAIDS and WHO to maximize the sensitivity and specificity of HIV testing. The number and types of tests depend on the reason for the test (surveillance, screening, or diagnosis), the cost, and the level of prevalence in the country. For surveillance, specificity does not need to be close to 100%, whereas for diagnosis 100% specificity is recommended. UNAIDS and WHO recommend a second confirmatory test that detects different HIV antigens from those in the first test in cases where the first test is positive. The general WHO and UNAIDS recommendation for countries is to use ELISA and/or rapid testing strategies for HIV antibody detection as well as for confirmatory testing. The Western blot technique is not recommended. Testing is best performed in country, to allow capacity building in regional laboratories.

Oral secretions vs. sputum

Oraquick ADVANCE ® lateral flow test is a new and potentially useful rapid test that can be used in population-based TB prevalence surveys, as it is approved for use on gingival secretions by the US Food and Drug Administration. The test has demonstrated high sensitivity and specificity in the field, and could potentially be used on sputum specimens. It is relatively simple, rapid, and inexpensive. In one study in Botswana, ADVANCE ® was used on the sputum of adult TB patients who were prospectively enrolled into a cohort study evaluating TB diagnostic modalities. Compared with serum ELISA, sensitivity was 97% and specificity was 98%, although both of these measures decreased with time (each day) (Talbot et al. 2003). However, when the same test was used in Tanzania, specificity declined from 93% on the first day to 65% on the seventh day (Mfinanga and Cobelens 2005). There are currently no international recommendations for the use of Oraquick or other rapid-testing methods on patient sputum specimens.

Ethical and human rights issues

There are four key principles to consider when including HIV testing of human subjects in a survey: (1) protection from harm; (2) participation in the benefits of the research; (3) information about the procedures and risks; and (4) free choice to participate or not, without coercion. There are extensive reviews and reports on the general ethics of research and HIV research (Office of Human Research Protections, US Department of Health and Human Services, 2004; WHO and UNAIDS 2004a). The Belmont Report (on respect for persons, justice, and beneficence) and the WHO Ethical Guidelines for consideration in second generation surveillance also provide helpful guidance when considering the ethics of including HIV in surveys (WHO and UNAIDS 2004a).

The potential positive effects of providing test results to participants include empowerment of individuals, contribution to their use of HIV prevention measures and access to treatment, and a possible community benefit through individual behaviour change. The potential negative effects of HIV testing in these surveys include increased cost (and also therefore potentially fewer surveys), logistical complexity from additional laboratory testing, quality control of the HIV test, the logistics of providing counselling, increased bias in the test, the possibility of having no access to care and treatment after a positive test, diversion of funds from more permanent voluntary counselling and testing services, and the implications

of returning false-negative and false-positive results. The possible negative effects may outweigh the benefits.

There are also ethical issues to consider for the different options for returning HIV test results, as seen below:

■ Is the goal to get an accurate measure of seroprevalence, or to offer testing and provide results to as many as possible?

■ What level of benefit is acceptable?

■ How far should the programme go to provide treatment?

■ Is there an obligation to link to treatment services?

■ Could rapid testing at home introduce harm by breaching confidentiality or privacy?

■ Does one provide the benefit of testing to all household members? How should one approach contacts of TB cases that are identified?

■ Are some people more likely to know their status than others because of some strategies for providing access to testing during a population-based survey?

■ Should resources be invested in behaviour change/reinforcement for those who test negative?

■ Should there be a large investment in VCT in a low-prevalence country?

To guarantee that in-country ethical principles are adhered to, the procedures for proposed study protocols must be reviewed by the appropriate ethical review board(s). These boards should always include the national ethical review committee (or institutional review board) in the country where the survey will be conducted, but might also include international/foreign ethical review bodies if organizations outside the country of the survey are significantly involved in the design and/or conduct of the survey. If so, the ethical standards of the countries involved should be upheld. An important issue in testing for HIV is whether informed consent is required in the country where the survey is conducted. Is consent always necessary or practical in population based surveys that in some fields may be seen as epidemiological research, and in others as routine public health surveillance?

Informed consent should first be obtained for participation in the TB prevalence survey, and then separately for the provision of biological samples to be tested for HIV. A certain proportion of the sampled population may consent to participate in providing a sputum sample, but may not want to receive HIV testing.

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HIV Testing Methods			
Test Type	**Components**	**Advantages**	**Disadvantages**
Unlinked anonymous testing (UAT) without informed consent	No personal identifiers obtained; unlinked

Specimens collected for other purposes

No counselling required | Avoids selection and participation bias

Consistency makes data comparable

Often used for antenatal sentinel surveillance | No informed consent

Limited demographic information (age, education, occupation, residence, gravidity, parity)

Participants must have access to HIV testing and counselling programmes |
| UAT with informed consent | Testing of unlinked specimens solely for surveillance purposes

Coded specimens | Informed consent required

No personal identifiers or names required

No counselling required

Participants must have access to HIV testing and counselling programmes | Participation bias

Training time |

Test Type	Components	Advantages	Disadvantages
Linked anonymous testing with informed consent	Testing of samples linked to the person by code Collected primarily for surveillance	Informed consent and pre- and post-test counselling required No personal identifiers or names obtained Coded specimens Code given to patient so that only he/she may obtain the results	Participation bias Training time
Linked confidential testing with informed consent	Test of samples linked to the person by name Collected for clinical care and surveillance Personal identifiers or names obtained Coded specimens; linked to personal identifying information	Ethical obligation to return results to patient	Participation bias Training time Reporting time for results

Annex 11: Drug Susceptibility Testing in Population Based TB Prevalence Surveys

Introduction

In 1994 the World Health Organization and the International Union against Tuberculosis and Lung Disease launched the Global Project on Anti-tuberculosis Drug Resistance (Aziz et al. 2006). To date, three reports have been published (and a fourth is to be published in 2007) including data from 90 countries either through continuous surveillance of all TB cases or through periodic cross-sectional surveys (WHO 2005). Patients are categorized according to treatment history either as new cases or previously treated cases. The methods and results of these surveys have been described elsewhere. Despite the expansion in surveillance coverage for drug-resistance in new and previously treated cases, however, data on drug resistance are still lacking for more than 100 countries.

Positive cultures from a TB prevalence survey could be tested for drug susceptibility using standard solid or liquid-culture media. With appropriate selection criteria, this would provide an estimate of the proportion of cases with resistance to isoniazid, rifampicin, or both. Drug susceptibility testing can also be done using second-line drugs.

Issues to consider

Advantages of including drug susceptibility testing

If the country or region conducting a TB prevalence survey does not have data from a representative drug resistance survey, including drug susceptibility testing (DST) in a TB prevalence survey can give a rough estimate of the prevalence of resistance. This estimate can be used in the future to determine sample size requirements for a true drug resistance survey and arrive at a more precise estimate. *M. tuberculosis* isolates from the prevalence survey may be fairly representative of the population, but the numbers are likely to be too small to provide precise estimates of the prevalence of drug resistance. Therefore drug susceptibility testing of isolates from a TB prevalence survey should not substitute for a true drug resistance survey.

There may be specific research objectives that warrant drug susceptibility testing. For example, one assumption could be that prevalent TB cases have on average a longer duration of disease, because of longer diagnostic delays, which could be related to the bacterial fitness of *M. tuberculosis* isolates. A programme might want to see if these isolates have different resistance patterns from cases presenting to clinical services. In addition, determining the frequency of isoniazid resistance can also be a first step towards implementing isoniazid preventive treatment programmes.

Disadvantages of including drug susceptibility testing

Obtaining representative data on anti-tuberculosis drug resistance in a country relies on testing a statistically valid representative sample of patients in the country/region, determining their prior treatment for TB with a high degree of reliability, and testing a sufficient number of patients to provide the desired precision of +/- 1-2% (WHO 2005). The three main data collection methods for estimating drug resistance are routine DST testing, cluster sampling, and special drug resistance surveys (WHO website). Overall, in a prevalence survey the number of prevalent cases found are likely to be small, and the resulting estimates of drug resistance may be subject to bias and have limited precision. Conducting drug susceptibility testing is also expensive, especially if liquid culture methods are used. Ethical issues

Lastly, and most importantly, patients with isolates found to have resistance to isoniazid and rifampin should be entitled to proper treatment of multidrug resistant TB with the best second-line drugs available in the country. In fact, one could argue that there is an ethical responsibility that DST results should be given back to patients and their providers, and access to second-line drug regimens should be provided in case a patient is found to have MDR TB. There are examples of some countries applying to the Green Light Committee for access to concessionally priced second-line drugs, precisely to treat MDR TB patients identified during a drug-resistance survey.

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WHO website. http://www.who.int/tb/surveillanceworkshop/status_analysis/dr_obtaining_susceptibility_data.htm

Drug susceptibility testing

If the country decides to conduct drug susceptibility tests for *M. tuberculosis* isolates from patients screened from the sample population of the prevalence survey, it is desirable to use one of the DST methods recommended by WHO and IUATLD. Of the recommended DST procedures, the proportion method is most commonly used in many laboratories. It consists in calculating the proportion of resistant bacilli present in test strains. Poor reliability of test results often stems from failure to incorporate an accurate amount of potent drug into culture medium, and to control the inoculum size. Other factors influence the reliability of DST results such as quality of medium (moisture, aeration space, pH, etc.), incubation temperature, and time for reading the results.

Drug testing should be decided carefully. In general it is recommended to test susceptibility against first line drugs, isoniazid (INH), rifampicin (RMP), dihydrostreptomycin (DHSM), and ethambutol (EMB). If the country wants to know about XDR TB, kanamycin (KM) and ofloxacin (OFX) can also be included.

Materials

- Drug-free and drug-containing media (L-J)

- Bijou bottles (7 ml)

- Glass beads (3 mm in diameter)

- Nichrome wire (22 SWG, diameter 0.7 mm) loops with a 3 mm internal diameter

- Nichrome wire (27 SWG, diameter 0.4 mm) loops with a 3 mm external diameter

- Bunsen burner or alcohol lamp

- Slant racks

- Culture bottle racks

- McFarland turbidity standard No. 1

- Steel baskets

- Plastic buckets

- Disposable plastic tubes with and without snap caps (13 x 100 mm)

- Pipettes, 1 ml, 5 ml, 10 ml

- Pipette fillers

- Vinyl bags

- Safety cabinet (class II) in an airflow-controlled room (negative pressure)

- Equipment and materials for culture media preparation (see Annex 12: Culture media preparation)

- Anti-tuberculosis drugs

- Vortex mixer

Preparations

- Drug-free and drug-containing media (L-J)

Drug Concentrations To Be Tested with M. tuberculosis Clinical Isolates and Drug-Susceptible Control Strain, H37Rv, in Löwenstein-Jensen Medium						
Test Drugs	**Solvent**	**Dilution**	**H37Rv (mg/L)**			**Test Strains (mg/L)**
			1st	**2nd**	**3rd**	
Isoniazid (INH)	Sterile DW	Sterile DW	0.025	0.05	0.1	0.2
Rifampicin (RMP)	DMSO	Sterile DW	2.5	5.0	10.0	40.0
Dihydro-streptomycin (DSM)	Sterile DW	Sterile DW	0.5	1.0	2.0	4.0
Ethambutol (EMB)	Sterile DW	Sterile DW	0.125	0.25	0.5	2.0
Kanamycin (KM)	Sterile DW	Sterile DW	5.0	10.0	20.0	30.0
Ofloxacin (OFX)	0.1N NaOH	Sterile DW	0.5	1.0	2.0	2.0

DW= distilled water, DMSO= dimethylsulfoxide, NaOH = sodium hydroxide.

Para-nitrobenzoic acid susceptibility testing should be included in order to rule out NTM. All drug powders purchased should be kept in a vacuum desiccator with silica gel (desiccant) in a refrigerator.

■ Incorporation of anti-tuberculosis drugs into the medium

- Potency calculation of drug = assay purity x active fraction x (1 – water content). For example, if dihydrostreptomycin sulfate (purchased from a commercial source) showed 100% purity by HPLC, 80% active fraction, and no water content (0%), then its potency is 1 x 0.8 x 1 = 0.8.

- Solvent (sterile DW for DHSM) volume calculation = weight (mg) x potency (/mg) / desired concentration of stock solution. If the amount weighed, using analytical balance, is 123 mg in a clean screw-capped glass container and the desired concentration of stock solution is 400 / , then the required solvent is 123 x 800 / 400 = 246 ml. So, 123 mg should be dissolved in 246 ml of DW.

- Add 1 ml of DHSM stock solution to 100 ml of L-J medium before inspissation, and then dispense over 5 ml into a tube or a universal bottle leaving more than three volumes of airspace.

- Inspissate media at 85°C for 50 minutes. Drug media must be tested for sterility by incubating at 35°C for 48 hours and then kept in a refrigerator, but they should be used within 4 weeks.

- All other drug media should also be prepared the same way as mentioned above, with the exception of RMP, which must be dissolved in DMSO, and OFX, which must be dissolved in 0.1N NaOH.

- Bacillary dispersion bijou bottles with 6 glass beads and 0.4 ml DW

■ Plastic bucket with disinfectant

■ Alcohol sand flask

■ MacFarland turbidity standard no. 1

Add 0.1 ml of 1% barium chloride (BaCl2) to 9.9 ml of 1% sulfuric acid (H2SO4) and dispense in bijou bottles.
Label all media correctly.

Procedure

■ Using a 22 SWG loop, take a representative sample (2–5 mg) of the growth (from no more than 4-week-old culture) (with caution not to take medium) and discharge it into a bacillary-dispersing bijou bottle. Shake the bottle on a vortex mixer for 30 seconds and le it stand for 5–10 minutes. Carefully add 2–5 ml of DW to the bottle and shake again after the cap is securely sealed. Adjust the turbidity to that of McFarland no. 1. The suspension should contain approximately 1 mg/ml of TB bacilli.

■ Make a 10-times serial dilution of bacterial suspension in order to obtain 10-3 and 10-5 mg/ml suspensions. Make a 10-times dilution by discharging 0.5 ml of bacterial suspension into 4.5 ml of sterile DW.

■ Inoculate each dilution of bacterial suspension (10-3 and 10-5 mg/ml) onto two slopes of each drug medium and two slopes of drug-free medium using a volume of 0.1 ml. Spread the inoculum on the surface of the medium and place it in a horizontal position in the slant rack. Incubate the inoculated media at 35°C–37°C.

■ Count the number of colonies grown on drug-free and drug-containing media and calculate the proportion of resistant organisms grown at the critical concentration of drug. If the proportion is 1% or more, the test result is interpreted as resistant; if less than 1%, then susceptible.

■ If the growth of control media is not profuse at a 4-week reading, incubation should be extended up to six weeks. The number of colonies grown with the highest dilution of inoculums should be countable in order to calculate drug resistant proportion. If colony counts with the heaviest inoculums (10-3 mg/ml) are less than 20 in drug free media, a reliable interpretation is possible for resistant strains, but not possible for susceptible strains. Thus, the test should be repeated with the latter strains.

Quality assurance of DST

Quality control

Drug concentrations incorporated into medium. Inoculate 10-3 and 10-5 mg/ml of H37Rv suspensions onto two slopes each of the first, second, and third concentrations in the table above. Even if the range of minimal inhibitory concentration (MIC) of H37Rv to anti-tuberculosis drugs varies greatly depending on inoculums sizes, approximate MIC of INH is 0.05 mg/L and RMP, 5.0 mg/L; DSM, 2.0 mg/L; EMB, 0.5 mg/L; KM, 20 mg/ml; and OFX, 1 mg/L.

If H37Rv is resistant to the first concentrations but susceptible to the second and/or third concentrations, the results of the test strains are considered valid.

If H37Rv is susceptible (no growth at all) to the first concentration or resistant to the third concentration, the test results cannot be regarded valid and the tests should be repeated.

As internal resistant controls, strains with known resistant pattern can be tested to the critical concentrations of test drugs.

External quality assessment

DST must be performed only after the proficiency has been approved by one of the supranational TB reference laboratories (SRL).

Annex 12: Laboratory Reagents and Media

Staining reagents

All staining reagents are stored in screw-capped bottles (preferably 500 ml) at room temperature. Each bottle should be carefully labelled with the name of the reagent as well as the preparation and expiry dates.

Ziehl-Neelsen (Z-N) staining

Well-prepared Z-N staining reagents will keep for at least six months to one year at room temperature.

Carbol-fuchsin

- Add 50 grams of phenol in the flask with 100 ml of denatured ethanol or methanol and then dissolve by shaking.

- Add 3 (or 10) grams of basic fuchsin into the flask and shake to dissolve dye. If necessary, add 100 ml of distilled water. The mixture should be shaken with occasional slight heating until the dye crystals are completely dissolved.

  Note: If fuchsin content does not exceed 85%, the amount to be added has to be corrected by dividing the prescribed amount by the decimal equivalent of the dye content.

- Only after fuchsin is completely dissolved, add distilled water to make a total amount of 1000 ml (850 or 750 ml) and filter in order to remove undissolved dye crystals.

Decolorizing agent

Either 3% acid-alcohol or 25% sulfuric acid can be used for decolorizing.

- 3% acid-alcohol: Carefully and slowly add 30 ml of concentrated hydrochloric acid in the flask with 970 ml of 95% ethanol (always add acid to alcohol, not vice versa). If too much heat is generated, cool with tap water. Store in an amber bottle.

- 25% sulfuric acid: Carefully and slowly add 250 ml of concentrated sulfuric acid into the flask containing 750 ml of distilled water, directing the flow of acid gently along the inner side of the flask. If too much heat is generated, cool with tap water. Store in an amber bottle.

Counterstain: 0.1% methylene blue

Add 1 gram of methylene blue powder to the flask containing 500 ml of distilled water and then dissolve by shaking. When the dye is completely dissolved, add 500 ml of distilled water to make a total amount of 1000 ml and then mix again.

Fluorochrome staining

Fluorochrome staining reagents should be stored in tightly stoppered amber bottles for three months.

Flourochrome stain: 0.1% auramine O in 3 % phenol
- Dissolve 1 gram of auramine O crystals in 100 ml of 95% ethanol (technical grade) (A).

  Note: Direct contact with auramine O powder or solution must be avoided because it has been shown to be a carcinogen.

- Dissolve 30 grams of phenol in the flask containing 870 ml of distilled water by warming (B).

- Mix 100 ml of auramine O solution (A) with 900 ml of phenol solution (B) and store in a screw-capped amber bottle away from heat and light.

Decolorizing agent: 0.5% acid-alcohol

Carefully and slowly add 5 ml of concentrated hydrochloric acid in the flask containing 1000 ml of 70% ethanol (technical grade).

Counterstains
- 0.5% potassium permanganate: Dissolve 5 grams of potassium permanganate ($KMnO_4$) in 1000 ml of distilled water.

- 0.01% acridine orange: Dissolve 0.1 gram of anhydrous sodium phosphate dibasic in 1000 ml of distilled water and then add and dissolve 0.1 gram of acridine orange in it.

Quality control of freshly prepared stains

The quality of newly prepared staining reagents must be checked with known unstained AFB positive and negative smears before use during routine examination. Positive smears should show bright red–colored AFB in an expected count, and negative smears should not show any AFB or AFB artifacts.

Media preparation

The formulas for three different culture media will be introduced here. Their use will vary according to their purpose.

Materials

■ Mixer

■ Dispenser

■ Heater

■ Stirrer, magnetic

■ Refrigerators with freezer

■ Steel slant racks

■ Inspissator

■ Incubator

■ Autoclave

■ Various glass or plastic ware items (beakers, flasks, funnels, measuring cylinders)

■ Culture bottles

■ Various chemicals for media preparation (see formula)

■ Fresh eggs (not more than one week old) from hens not fed with antibiotic-containing feed

Preparations

Formulas for Culture Media		
Chemicals	Löwenstein-Jensen Medium (IUTM)	Modified Ogawa Medium
Mineral salt solution		
L-asparagine	6.0 g	
Sodium glutamate		5.0 g
Monopotassium phosphate (KH$_2$PO$_4$), anhydrous	4.0 g	20.0 g
Magnesium sulphate (MgSO$_4$.7H$_2$O)	0.4 g	
Magnesium citrate	1.0 g	1.0 g
Glycerol*	20 ml	40 ml
Distilled water	1000 ml	1000 ml
Whole fresh eggs		
Fresh egg homogenate	1600 ml	2000 ml
Malachite green solution		
Malachite green solution	50 ml**	40 ml***

* If the survey includes the isolation of M. bovis, glycerol should be replaced with sodium pyruvate, 1.2% in mineral salt solution.

** 1% weight/100ml aqueous malachite green solution

*** 2% weight/100ml aqueous malachite green solution

- **Mineral salt solution:** Dissolve mineral salts in distilled water by heating the mixture, and then add glycerol. Autoclave at 121°C for 30 minutes to sterilize. Cool to room temperature.

- **Malachite green (MG) solution:** Add 10 grams (1%) or 20 grams (2%) of malachite green to 1000 ml of sterile DW and dissolve in the incubator for 1–2 hours. Always use freshly prepared MG solution.

  Note: Use only commercially available malachite green that has passed antimycobacterial activity testing.

■ **Homogenized whole eggs:** Soak fresh hens' eggs in plain alkaline soap for 30 minutes and clean by scrubbing thoroughly with a brush in warm water. Rinse eggs thoroughly under running water and soak them in 70% ethanol for 15 minutes. Crack the eggs one by one into a sterile beaker and check freshness before combining in a sterile blender for homogenization.

■ **Preparation of medium:** Mix mineral salt solution, fresh malachite green solution, and fresh egg homogenate in the ratio shown in the table above, and dispense as required by the type of culture bottles, leaving at least three volumes of airspace. Place media bottles in the slant racks and inspissate for 50 minutes at 85°C.

Sterility tests of newly prepared media should be done by incubating in an incubator at 35°C–37°C for 2–3 days. Put media with no contamination into vinyl bags and store in a refrigerator. Media can be stored for several weeks, but should be used only if not dried and not contaminated.

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Annex 13: Study of Risk Factors

Socioeconomic status

Socioeconomic status may be measured as income, occupation, assets, or some combination of these, and the most appropriate measure may vary among and within countries. Data on income, purchasing power, and expenditure may be relatively easy to collect and quantify (Khan and Hotchkiss 2006). Furthermore, income or purchasing power determines a household's ability to acquire food, shelter, and other necessities, while income and expenditure may determine the nutritional status of children and access to education and health care.

Poverty is commonly measured in relation to the poverty line, defined as the lowest level of income or expenditure needed to achieve an adequate standard of living. The poverty line may be defined as a proportion of median income, in which case it is measured relative to the society in which the person lives, or it may be fixed in US dollars for a given year, typically US$1/day, in which case it gives a measure of absolute poverty and can be used to make comparisons between countries.

Measuring income and expenditure can be expensive and time-consuming. Even the poorest households in low-income countries may have many sources of income, including home production, gifts, benefits, and payments in kind, and as many different kinds of expenditure. People may be reluctant to provide such information, and recall bias can be substantial. To address these problems and to try to ensure consistency and comparibility, the World Bank has developed living standard measurement surveys (LSMS), which provide a standard methodology and a benchmark for the measurement of income and expenditure in low-income countries. Other standard surveys are the Social Dimensions of Adjustment Integrated and Priority Surveys for the African region (www4.worldbank.org/afr/poverty/default.cfm), the Demographic Health Survey (www.measuredhs.com/) and the Core Welfare Indicator Questionnaire (www4.worldbank.org/afr/stats/cwiq.cfm) (Zeller 2004). In most countries that have conducted living standard measurement surveys, complete data sets can be obtained at relatively low cost (see http://www.worldbank.org/lsms for details).

The standard survey questionnaires mentioned above are generally too detailed to be used in a TB prevalence survey, and it will generally be necessary to ask a more limited set of questions that can easily be answered and that are likely to be associated with socioeconomic status as measured using one of the more detailed survey questionnaires. Possible indicators include the sex of the household head, the number of children under five years of age, household assets and amenities such as the material used to construct the roof or walls, the source of drinking water, and ownership of a television or motorbike.

Many developing countries have conducted demographic and health surveys (DHSs) using "asset scores" based on items that are easy to identify to measure socioeconomic status. (Information on household assets that best predict socioeconomic status and DHS data and asset score sets are available for many countries, especially in Africa http://www.measuredhs. com).

When selecting and developing questionnaires to assess socioeconomic status for use in TB prevalence surveys, it is important to bear in mind that:

■ Questions should be country-specific. Although international asset lists have been proposed, the contribution of particular assets to a household's socioeconomic status can be substantial.

■ If a previous survey of socioeconomic status is used in the design of a questionnaire, the questions used may no longer be appropriate.

■ Different questions may be needed in urban and rural areas. For example, the source of drinking water or the type of cooking fuel may be more strongly associated with poverty in urban than in rural areas.

■ Ownership of various assets can be ascertained most easily, efficiently, and accurately when visiting houses while taking the census for the survey. Most items can be seen and only few questions need to be asked.

■ Validated questionnaires, in particular those designed to determine assets, may appear to be rather long, but this is mainly because many are multiple-choice items with many response options, and each response option has been assigned an individual weight. The total number of items is typically between 9 and 17, but these can usually be answered rapidly.

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Smoking

Exposure to tobacco smoke increases a person's risk of developing TB (Hassmiller 2007) and should be included in TB prevalence surveys where smoking is common. While there is no globally accepted, standard survey protocol for estimating tobacco use or exposure to tobacco smoke, many surveys have been done (www.who.int/infobase). Many countries now carry out "STEPS" surveys, under the guidance of WHO and these cover a range of potential risk factors that include not only smoking but also diabetes, malnutrition, body-mass index, and several others (http://www.who.int/chp/steps/manual/en/index.html).

To estimate the increased burden of TB attributable to tobacco, one needs to estimate the prevalence of active and passive smoking. The questions in the table below are intended to be consistent with those in the Global Adult Tobacco Survey.

Active smoking
Active smoking is associated with being infected, developing disease, and dying from TB (Hassmiller 2007; Bates et al. 2007; Lin, Ezzati, and Murray 2007) and these associations remain when controlling for age, sex, socioeconomic status, alcohol consumption, or HIV status. The causal nature of this association is supported by the observed dose-response relationships (Hassmiller 2007). Questions 1 to 4 in the table below are intended to determine a person's current and past smoking status. Questions 5 and 6 are intended to estimate the duration and amount of smoking. The limited available evidence suggests that the risks do not depend greatly on the particular product that is smoked (Gajalakshmi et al. 2003).

Passive smoking
There is some evidence that passive smoking increases the risk of TB disease (Alcaide et al. 1996, Altet et al. 1996, Ariyothai et al. 2004, Tipayamongkholgul et al. 2005). Children may be particularly sensitive to environmental tobacco smoke (Hassmiller 2007) but this may be compounded by the level of exposure. Question 7 is intended to estimate the extent of exposure to other people's smoke.

Use of smokeless tobacco
Tobacco may be used in ways other than smoking and these are also associated with TB mortality although the relative risk is less (Gupta et al. 2005). Question 8 is intended to establish the use of tobacco in ways that do not involve smoking.

Assessment of Tobacco Exposure, Modified from the Global Adult Tobacco Survey

I am going to ask you some questions about your exposure to tobacco. Unless I say otherwise, I am asking about smoking tobacco, including (fill in with locally appropriate examples such as cigarettes, bidis, cigars, pipes).

1. Do you currently smoke tobacco daily, less than daily, or not at all?

    Daily   _____   SKIP TO QUESTION 5
    Less than daily   _____   SKIP TO QUESTION 2
    Not at all   _____   SKIP TO QUESTION 3

2. Did you smoke tobacco daily in the past?

    Yes   _____   SKIP TO QUESTION 5
    No   _____   SKIP TO QUESTION 6

3. In the past, did you smoke tobacco daily, less than daily, or not at all?
    Note: If respondent smoked "daily" and "less than daily" in the past, he/she should respond, "Daily".

    Daily   _____
    Less than daily   _____
    Not at all   _____   SKIP TO QUESTION 7

4. How long has it been since you last smoked daily?

    _____ Years OR
    _____ Months   SKIP TO QUESTION 8

5. How old were you when you first started smoking tobacco?

    _____ Years old

6. On average, how many cigarettes (bidis, etc., depending on what is most typically smoked in the population) do you currently smoke on days that you smoke?

    Note: If respondent says he/she smokes, but less than once per day, leave the field blank and check the box to the right. If the respondent reports consumption in packs or cartons, probe to find out how many cigarettes are in each and calculate total the number.

    _____ per day, or
    _____ mark here if less than 1/day but more than 0

7. In the past week, approximately how many times have you been exposed to the tobacco smoke of others at home, work, or in public places (where exposure is for a minimum of five consecutive minutes each time)?

Not at all	_____
A few times a day on some days	_____
Many times a day on some days	_____
A few times a day on most days	_____
Many times a day on most days	_____

8. Do you currently use smokeless tobacco (including, for example, spit or chewing tobacco) daily, less than daily, or not at all?

Daily	_____
Less than daily	_____
Not at all	_____

It is possible to validate self-reported exposure to tobacco smoke by measuring the concentration of nicotine and/or cotinine (its major metabolite) in saliva or urine (Bramer and Kallungal 2003, Gourlain and Galliot-Guilley 2005, Man et al. 2006). This can be done both to eliminate biases in self-reported data arising from the stigma that is associated with smoking, especially among women, and also to obtain better estimates of the dose of exposure. There are many different kinds of tobacco product which are used in many different ways (Mackay, Eriksen, and Shafey 2006) and exposure may involve more than one kind of exposure. If the responses to the questionnaires can be validated using chemical measures this will strengthen the study.

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Malnutrition

Malnutrition is a classic risk factor for TB. The evidence suggests that it is associated with increased risk of progress from infection to disease, but it is uncertain if malnutrition also increases risk of infection (Cegielsky and McMurray 2004).

Malnutrition is a very wide concept that includes a range of different types of micro- and macronutrient deficiencies. The way in which different types of malnutrition influence the risk of TB needs further study.

Anthropometric measurements indicating general nutrition status—for example, weight for height indices such as body-mass index, upper mid-arm circumference, and skin-fold thickness—have been associated with risk of TB. However, the strength of the association is not well established for different levels of general nutrition status.

Obtaining the weight and height measurement of study subjects enables an estimate of prevalence of low weight for height (usually defined as a body-mass index of <18.5 kg/m2). Practical advice on how to measure weight and height under household survey conditions are provided in WHO's "STEPwise approach to surveillance" (STEPS) (WHO 2003). For details see section 4 of the STEPS user manual (http://www.who.int/chp/steps/Part3_Section4.pdf). If the aim is only to obtain an estimate of the prevalence of this risk factor, weight and height can be measured in a subsample only.

It is also possible to study the association between different types of malnutrition and TB within a prevalance survey. However, establishing a causal relationship based on cross-sectional data is not possible, since tuberculosis leads to malnutrition. In order to study the effect of malnutrition on the risk of TB, one would need to ascertain the nutrition status before the disease occurred. This can be done in principle, for example, by asking about weight in the past, but this measure is likely to be imprecise. Alternatively, an indirect measurement of history of malnutrition can be obtained by asking questions about food security in the past. One example of an instrument for this is the Household Food Insecurity Access Scale (Coetes, Swindale, and Bilinsky 2006) (http://www.foodsec.org/News/tr/nut/hfias.pdf).

To obtain information about micro- and macronutrient deficiencies, it is normally necessary to do blood tests, and hence this is not feasible in most prevalence surveys. However, studies in a subsample may be possible.

Advice on how to measure vitamin and mineral status is provided, for example, by the US Centers for Disease Control and Prevention (US CDC 2006).

Indoor air pollution from solid fuel use

In developing countries and in some middle-income countries solid fuel is used for cooking, boiling water, and heating. Solid fuels such as coal, charcoal, wood, dung, and crop residues generate high levels of pollutants when burned in open fires or inefficient traditional stoves, including fine particles, carbon monoxide, and poly-aromatic hydrocarbons, especially if the ventilation is poor. Indoor air pollution that results from burning solid fuels is a significant risk factor for childhood pneumonia, chronic obstructive pulmonary disease, and lung cancer (WHO 2006, Bruce et al. 2006).

Few epidemiological studies have been carried out in developing countries to establish the increased risk of TB resulting from indoor air pollution. The authors of a recent meta-analysis concluded that the available evidence is suggestive but not conclusive, with a relative risk for TB of 1.5 (95% confidence interval: 1.0–2.4) in adults over 15 years of age (WHO 2004). TB prevalence surveys, perhaps with nested case-control studies, may provide better evidence on the relationship between indoor air pollution and TB infection and progression to active disease.

Exposure to indoor air pollution may be estimated using household surveys. These data can be supplemented with measurements of the concentrations of small particles of carbon monoxide in houses and still better data on exposure can be obtained using personal monitoring equipment. Such direct measurements are unlikely to be feasible in most prevalence surveys, but further information can be found in WHO (forthcoming).

The extent of exposure to indoor air pollution due to combustion of solid fuel is determined by:

- **Type of fuel used for cooking and other household energy needs:** Burning crop residues or dung tends to be more polluting than burning wood, charcoal and coal are less polluting than wood, whereas kerosene and liquefied petroleum gas are the least polluting of all.

- **Type of stove:** A closed stove with a combustion chamber burns fuel more cleanly and efficiently than an open fire or open stove. A suitable chimney or hood will move much of the pollution outside.

- **Cooking location:** Different people in the same household will have different levels of exposure depending on the time they spend in different more or less polluted parts of the home and the extent to which they do the cooking (or are kept close to the fire during cooking in the case of small children). Cooking in a room where people sleep will increase exposure; cooking in a separate room or building or even outdoors will decrease exposure.

- **Ventilation:** Well-ventilated or semi-open structures, as are often found in tropical climates, disperse the smoke more efficiently than do the poorly ventilated, well insulated houses often found in cold climates.

The proportion of people that use solid fuels, an indicator for assessing progress towards achieving the Millennium Development Goals, is the measure of exposure used in most epidemiological studies of indoor air pollution and health and has been used to assess the burden of disease due to indoor air pollution from solid fuel use (Rehfuess et al. 2006, WHO 2006, WHO 2007). On its own this is a crude proxy indicator of exposure and should, if possible, be supported by information on the type of stove being used, the place where cooking is done, and, where appropriate, information on heating.

Four questions concerning the main fuel used for cooking, the type and ventilation of stove, the place where the cooking is done and the overall ventilation, provide basic information on household cooking practices. Three further questions may be used to provide information on heating needs, the type of heating fuel used, and in what kind of heating stove.

The questions presented here (see chart) or modifications thereof have been used as part of several nationally representative household surveys, including the World Health Survey (WHO), the Demographic and Health Surveys (ORCMacro/USAID), and the Multiple Indicator Cluster Survey (UNICEF). They have been shown to work well in a variety of geographical and cultural settings, but the questionnaire should always be adapted to reflect local conditions. Different types of fuel that are used in particular places may need to be included, different categories of stoves with suitable graphics should reflect local practice, and questions on heating may not be needed in hot climates.

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| 1. WHAT TYPE OF FUEL DOES YOUR HOUSEHOLD MAINLY USE FOR COOKING? | Electricity | 01 | 01⇨Q5 |
	Liquefied petroleum gas (LPG)	02	02⇨Q5
	Natural gas	03	03⇨Q5
	Biogas	04	04⇨Q5
	Kerosene	05	05⇨Q5
	Coal/Lignite	06	
	Charcoal	07	
	Wood	08	
	Straw/Shrubs/Grass	09	
	Animal dung	10	
	Agricultural crop residue	11	
	No food cooked in household	12	12⇨Q5
	Other (specify)	96	
2. WHAT TYPE OF STOVE IS USUALLY USED FOR COOKING? Probe for type.	Open fire	01	
	Surrounded fire	02	
	Improved single-pot stove	03	
	Improved multiple-pot stove	04	
	Griddle stove	05	
	Other (specify)	96	
2A. IS SMOKE REMOVED BY A CHIMNEY OR HOOD?	Chimney	01	
	Hood	02	02⇨Q3
	Neither	03	03⇨Q3
2B. WHEN WAS THE CHIMNEY LAST CLEANED?	Less than 1 month ago	01	
	From 1 to 3 months ago	02	
	More than 3 months ago	03	
	Never	04	
	Don't know	96	

3. IS THE COOKING USUALLY DONE IN THE INDOOR LIVING SPACE, IN A SEPARATE KITCHEN/ BUILDING, OR OUTDOORS?	In a room used for living/ sleeping	01	
	In a separate room used as kitchen	02	
	In a separate building used as kitchen	03	
	Outdoors	04	
	Others	96	
4. WHAT TYPE OF VENTILATION IS PRESENT WHERE THE STOVE IS USED?	Closed room	01	
	Room with eave spaces	02	
	Room with open windows/ door	03	
	Room with three or fewer walls	04	
	Other (specify)	96	
5. DO YOU HEAT YOUR HOUSE WHEN IT IS COLD?	Yes	01	
	No	02	02⇨NEXT S.
6. WHAT KIND OF FUEL DO YOU MAINLY USE FOR HEATING?	Electricity	01	01⇨NEXT S.
	LPG	02	
	Natural gas	03	
	Biogas	04	
	Kerosene	05	
	Coal/Lignite	06	
	Charcoal	07	
	Wood	08	
	Straw/Shrubs/Grass	09	
	Animal dung	10	
	Agricultural crop residue	11	
	Other (specify)	96	

7. WHAT TYPE OF STOVE IS USUALLY USED FOR COOKING?	Open fire	01	
	Surrounded fire	02	
	Improved single-pot stove	03	
	Improved multiple-pot stove	04	
	Griddle stove	05	
	Other *(specify)*	96	

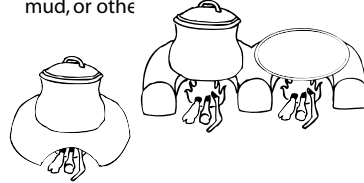## 2. What type of stove is usually used for cooking?

1. Open fire

- unprotected fire
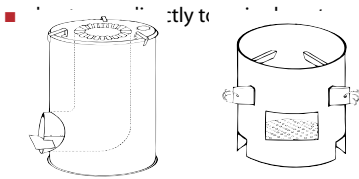- pot or griddle is supported with rocks, mud or other mater
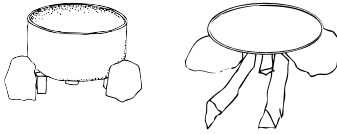
2. Surrounded fire

- fire is partially or completely surrounded
- pot or griddle is supported with rocks, mud, or othe

3. Improved single-pot stove

- fire is completely surrounded
- open pot hole
- pot may be sunk into the stove
- to improve combustion, fuel is placed on grate or inside combustion chamber
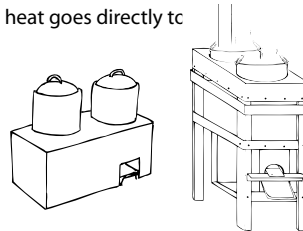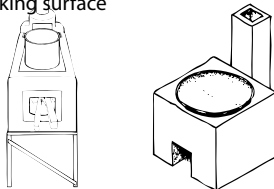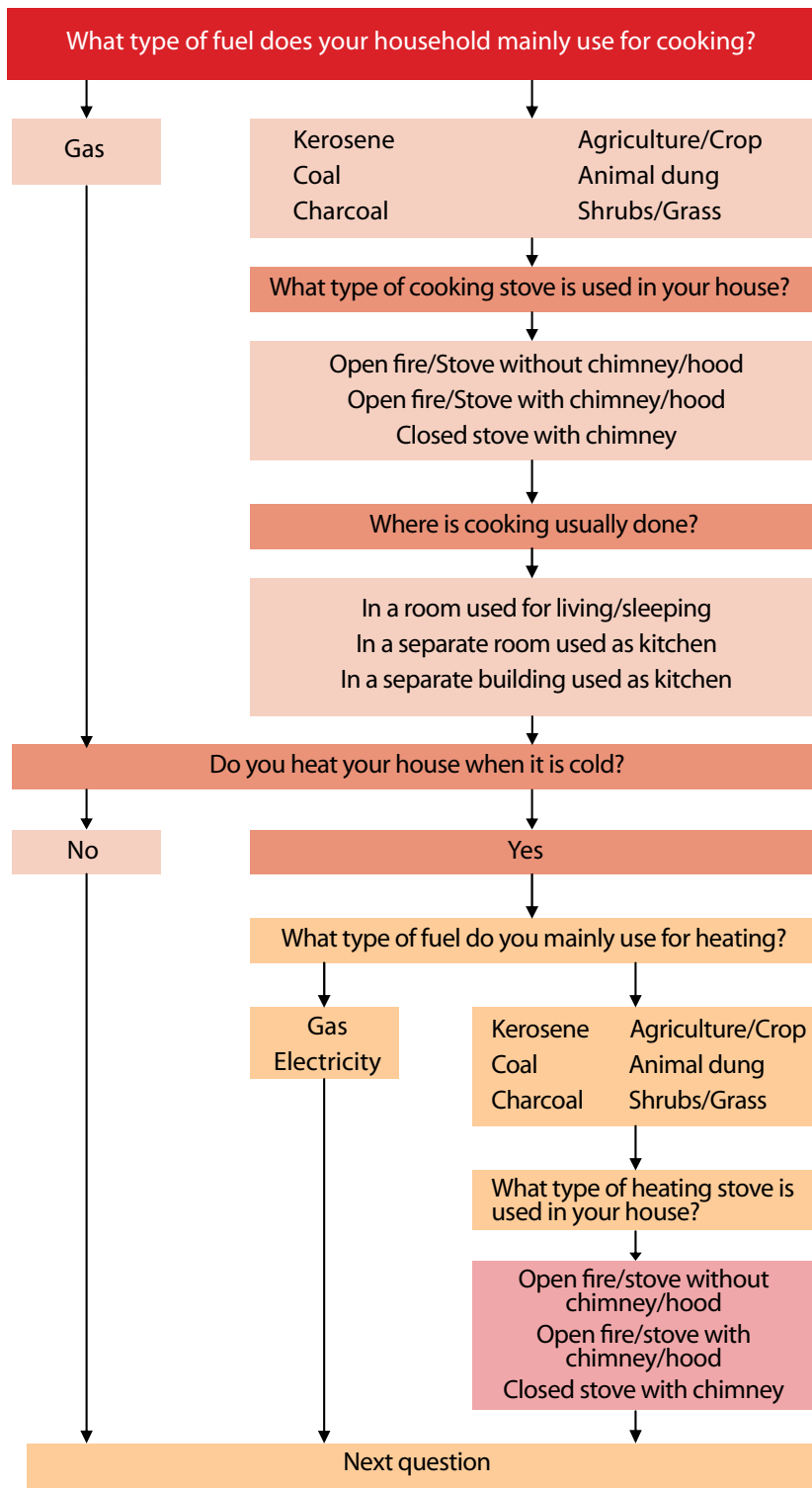- :tly t

4. Improved multiple-pot stove

- fire is completely surrounded
- open pot hole
- pot may be sunk into the stove
- to improve combustion, fuel is placed on grate or inside combustion chamber
- heat goes directly tc

5. Griddle stove

- fire is completely surrounded
- pot is placed on top of a metal or clay cooking surface

What type of fuel does your household mainly use for cooking?

Gas

Kerosene
Coal
Charcoal

Agriculture/Crop
Animal dung
Shrubs/Grass

What type of cooking stove is used in your house?

Open fire/Stove without chimney/hood
Open fire/Stove with chimney/hood
Closed stove with chimney

Where is cooking usually done?

In a room used for living/sleeping
In a separate room used as kitchen
In a separate building used as kitchen

Do you heat your house when it is cold?

No

Yes

What type of fuel do you mainly use for heating?

Gas
Electricity

Kerosene
Coal
Charcoal

Agriculture/Crop
Animal dung
Shrubs/Grass

What type of heating stove is used in your house?

Open fire/stove without chimney/hood
Open fire/stove with chimney/hood
Closed stove with chimney

Next question

Alcohol

An association between alcohol abuse and higher risk of TB infection and higher risk of TB disease has been shown in several studies. This may be related to specific social mixing patterns for people abusing alcohol as well as the compounding effects of the socioeconomic conditions often associated with alcohol abuse. It has not been well established that high level of alcohol intake in and by itself is a risk factor for infection or progress to disease, though some evidence exists for a biological explanation for such causal link.

Below are suggested questions for a three-item set of questions about alcohol consumption that can be used to establish drinking status, volume of consumption, and volume of high-risk consumption (WHO 2006).

Drinking status can be ascertained from Q.1, with abstainers defined as those who reported never drinking any alcoholic beverage in the past year and drinkers defined as all others. A crude measure of volume can be calculated as a product of the overall frequency of drinking (days per year as estimated from the midpoints of the frequency categories in Q.1) x the number of drinks usually consumed (Q.2) x the assumed ethanol content of a standard drink, A. In the absence of any pre-existing information on the consumption distribution, an estimate of six drinks may be used as the assumed quantity consumed on days of drinking 5+ drinks. An assumption of five drinks on each of those days would represent the most conservative possible estimate, and countries with patterns of very heavy consumption may choose a number considerably higher than six drinks.

Using the techniques described above, the volume of consumption on high-risk days can be estimated as (frequency of drinking 5+ drinks x assumed number of drinks on days when 5+ drinks are consumed x assumed ethanol content of a standard drink). The volume of alcohol in excess of the daily thresholds for high-risk consumption will be subtract from this: (frequency of drinking 5+ drinks x 60 g). In both cases the proportion of total consumption that is high-risk then needs to be calculated.

Module with minimum required items (three questions)

1. In the past year, how often did you drink an alcoholic beverage, for instance, beer, coolers, wine, spirits or fermented cider? (Show respondent card containing response categories or read categories aloud.)

☐ Every day
☐ Nearly every day
☐ 3 to 4 times a week
☐ 1 to 2 times a week
☐ 2 to 3 times a month
☐ Once a month
☐ 7 to 11 times in the past year
☐ 4 to 6 times in the past year
☐ 2 or 3 times in the past year
☐ Once in the past year
☐ Never drank any alcoholic beverage in past year
☐ (Skip remaining alcohol Q.)•
☐ Never in my life (Skip remaining alcohol Q.)

2. How many drinks did you USUALLY have on days when you drank alcoholic beverages in the past year? By "drink", I mean the equivalent of a 33 cl glass, can, or bottle of beer or cooler, a 20 cl glass of wine, or 4 cl of spirits, not counting any mixer, water, or ice.

☐ Number of drinks

3. In the past year, how often did you drink five or more drinks of any alcoholic beverage or combination of beverages in a single day? (Show respondent card containing response categories or read categories aloud.)

☐ Every day
☐ Nearly every day
☐ 3 to 4 times a week
☐ 1 to 2 times a week
☐ 2 to 3 times a month
☐ Once a month
☐ 7 to 11 times in the past year
☐ 4 to 6 times in the past year
☐ 2 or 3 times in the past year
☐ Once in the past year
☐ Never drank five or more drinks in past year

Crowding

Crowding is associated with the risk of infection. However, the strength of the association in relation to different levels of crowing is not well established. Unfortunately, there is still no widely accepted definition of crowding, with regard to disease risk, that is applicable across different geographical and cultural settings. Demographic and health surveys use two simple questions to determine crowding:

■ How many people are in the household?

■ How many bedrooms are in the house?

From these variables, the number of people per room can be calculated. The definition of "crowding" varies from >2 per room to >3 per room.
Another often-used measure is average floor area per household member, which can be calculated if a question about the total household floor area is added. However, there is no well established cut-off point for this parameter.

Considerably more work needs to be done to refine the definition of crowding and until this is done questions concerning crowding may also be based on knowledge of the local conditions and circumstances.
In order to assess ventilation, a question about number of windows (that can be opened) in the household, may be used.

Diabetes

Diabetes (type I and II) increases the risk of TB and the risk of poor treatment outcomes of TB patients (Kim et al. 1995, Ponce-De Leon et al. 2004, Restrepo et al. 2006, Morsy et al. 2003).[1-4] Intermediate hyperglycaemia (impaired fasting glucose or IFG and impaired glucose tolerance or) may also increase the risk of TB (Mugusi et al. 1990)[5] but the evidence is weak (Stevenson et al., forthcoming).

It is often hard to know if diabetes is type I or type II. It is often assumed in epidemiological studies that diabetes diagnosed before the age of 30 and requiring insulin within 1 year of diagnosis is type I; all other diabetes is type II.

Questions about previously diagnosed diabetes and any ongoing treatment with anti-diabetic medicines can easily be added to a prevalence survey instrument. People who have been diagnosed with diabetes by a doctor

but are not on medication may still have diabetes. It shoud be noted that in many populations the true prevalence of diabetes is more than double the diagnosed prevalence, as diabetics are unaware of their condition (Aspray et al. 2000, Ramachandran 2005)7,8 especially if they have intermediate hyperglycaemia.

Ideally, people should be tested for diabetes by measuring their blood glucose concentration after fasting overnight (fasting capillary blood glucose or FCG) or 2 hours after receiving 75 g of glucose orally (oral glucose tolerance test, or OGTT). However, this involves substantial additional costs and logistic requirements. The OGTT is more sensitive than the FCG, which depends on the persons adhering to the fasting. If OGTT is not available FCG may be used for epidemiological purposes but has a sensitivity of only about 70% (Decode Study Group 1999, Qiao and Nakagami 2000).

The WHO criteria for measuring diabetes are given in the table below.

WHO Criteria for Diagnosing Diabetes	
Diabetes Type/Test	WHO Diagnostic Criteria, 1999
Diabetes Fasting glucose 2-h glucose	≥ 7.0 mmol/l **or** ≥ 11.1 mmol/l
IGT Fasting glucose 2-h glucose	< 7.0 mmol / l **and** ≥ 7.8 and < 11.1 mmol/l
IFG Fasting glucose 2-h glucose	6.1 to 6.9 mmol/l **and** < 11.1 mmol/l

Source: WHO (2006)

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Silicosis

Silicosis is a fibrotic lung disease caused by inhalation of microscopic crystalline silica particles that stimulate scarring and inflammation (Mossman and Churg 1998).1 It is a strong risk factor for TB, unlike other occupational lung diseases such as asbestosis and coal worker's lung disease. Although silica is globally abundant, exposure to concentrations high enough to cause silicosis is rare outside of the workplace. Chronic silicosis typically develops after 10 years or more of exposure (although an accelerated form may present after 5 years), but clearance of silica particles from the lungs is extremely slow and so radiological abnormalities may progress and/ or become visible for the first time many years after leaving a dusty job (Hnizdo and Sluis 1993, Trapido et al. 1998, White et al. 2001). Because of this it is important to enquire about past as well as current employment whenever radiological silicosis is suspected.

Radiological appearance of chronic silicosis

The hallmark of chronic silicosis (Figure 1) is the development of discrete fibrous nodules that are detectable as small rounded opacities on the chest radiograph and are usually 1 to 3mm, but can be up to 10mm in diameter. These are symmetrically distributed, and first appear in the upper lung zones. Lymph nodes nodules may cause visible hilar enlargement. Conglomerates of nodules (more than 1 cm in diameter) can take shape, and are classified as a form of progressive massive fibrosis. In a small percentage of cases, nodules are accompanied by so-called "eggshell calcification" of hilar lymph nodes, visible on the chest radiograph as rings of calcification up to 2 mm thick and 1 to several cm in diameter.

The International Labour Office (ILO) has produced a widely used system for grading silicosis from chest radiographs based on the number of visible nodules, and provides reference chest radiographs to guide interpretation (Mossman and Churg 1998).1 Radiographs are graded into nodule profusion categories between 0 (none) and 3 (numerous nodules: vascular markings obscured). Inter-rater agreement is poor, and formal grading should ideally be attempted only by certified "B" readers who have undergone training in the radiological classification of pneumoconiosis.

Tuberculosilicosis

Silicosis is a strong risk factor for TB. Workers with radiological silicosis have TB incidence rates several times those of fellow employees with normal chest radiographs, and up to 30 times those of adults in the general population (Mossman and Churg 1998, Rieder 1999). Patients with both HIV infection and silicosis are at particularly high risk of TB (Corbett et al. 2000).6 TB-complicating silicosis is most often pulmonary and presents with typical TB symptoms of prolonged cough, weight loss and fever. Silicosis complicated by TB typically results in a complex radiographic appearance of asymmetrical tuberculous consolidation and fibrosis on a background of silicotic nodules (Figure 2). Both silicosis itself and post-tuberculous fibrosis predispose to nontuberculous mycobacterial disease, which is clinically identical to TB (Corbett et al. 1999).

## When to diagnose silicosis in a TB prevalence survey

Any participant with a suggestive X-ray appearance (see above and Figures 1 and 2) should be asked about their complete employment history. Workers at high risk of silicosis include foundry workers, miners in both formal and informal operations, stoneworkers, gemstone workers, grinders, tunnellers, and workers in the construction and ceramics industry (Rees and Murray 2007). Sandblasting (using sand with pressurized air to clean metal surfaces or walls) is a particularly high-risk activity. Hard rock dust (e.g. granite, gemstones) and sandstone are high risk, especially when mechanical tools or explosives have been used for stone working or breaking. Softer rocks and stones (e.g., soapstone and clay) are unlikely to generate high concentrations of silica particles of respirable size.

The diagnosis of silicosis should be restricted to individuals who have both strongly suggestive radiological changes and a prolonged period of employment in an at-risk occupation. Most patients with uncomplicated silicosis are asymptomatic. Patients with prominent symptoms may have silicosis complicated by TB or another reason for their abnormal X-ray, such as cancer.

Figure 1: Extensive Silicotic Nodules throughout the Lung Field in a Gold Miner

Figure 2: Right Apical Tuberculosis Complication Silicosis
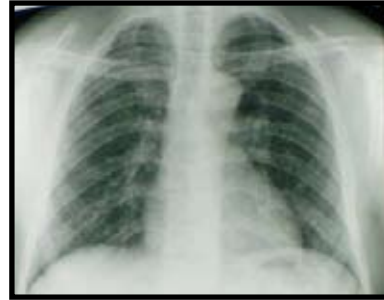
Note: Silicotic nodules are visible in the zones unaffected by TB.

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Appendix: Sample size in case-control studies

We wish to calculate the sample size needed for case-control studies if we are investigating risk factors for TB as part of TB prevalence surveys. We proceed as follows.
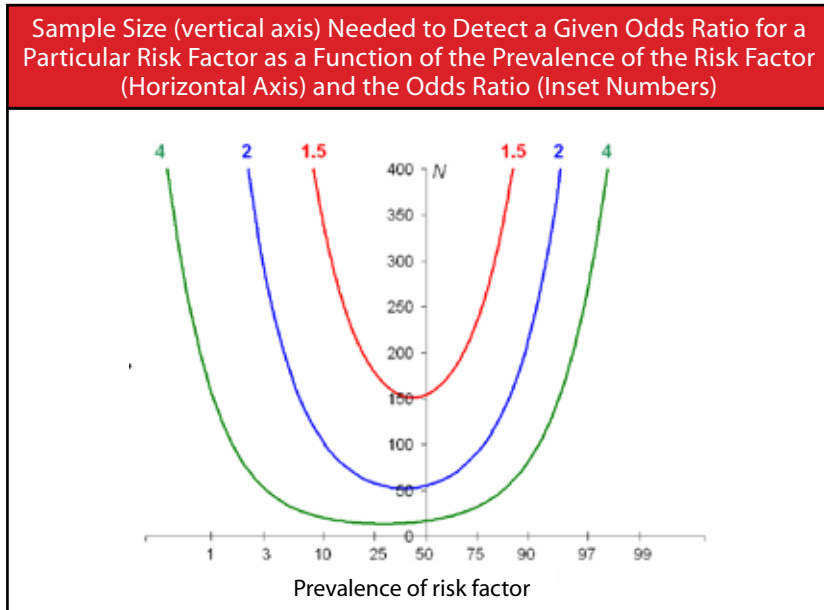
Let the prevalence and the odds of the relevant risk factor in people without TB be p and π, respectively, the odds of the relevant risk factor in people with TB be r and ρ, respectively, and the odds-ratio be Ω. Then

$$\frac{q}{1-q} = \rho = \pi\Omega = \frac{p}{1-p}\,\Omega$$

Given p and Ω we can then calculate ρ from which

$$q = \frac{\rho}{1+\rho}$$

We then calculate the sample size needed to detect a prevalence q of the risk factor in TB patients against the null hypothesis that the value is p with a significance level of 5% and a power of 80%. We assume that the number of controls is much greater than the number of cases so that the errors are entirely due to the limited number of cases. The results are given in the figure.

Sample Size (vertical axis) Needed to Detect a Given Odds Ratio for a Particular Risk Factor as a Function of the Prevalence of the Risk Factor (Horizontal Axis) and the Odds Ratio (Inset Numbers)

What is rather interesting is that if the prevalence of the risk factor is about 10% and the odds-ratio is 4 then we only need about 20 cases. If the odds ratio is 2 we need about 100 cases and if it is 1.5 then we need about 370 cases. If the risk factor is fairly common and we are only interested in odds ratios greater than 2 we should be able to pick up the effect in most surveys (provided there are at least 100 cases).

Annex 14: Sample Size, Design Effect, and Optimal Sampling

Measurement variation

If there are no differences among the clusters then the observed prevalence, μ, is just the number of TB cases, c, divided by the number of people in the sample, n, so that

$$\hat{\mu} = \frac{c}{n} \qquad (10)$$

The expected value of is the true mean μ with variance of μ is which we will write:

$$\hat{\mu} \sim \left(\mu, \frac{\mu \left(1 - \mu\right)}{n} \right) \qquad (11)$$

If we estimate the variance from the data ignoring the effect of clustering, then

$$\hat{V}\left(\mu\right) = \frac{\hat{\mu}\left(\hat{\mu} - 1\right)}{n - 1}$$

We now wish to consider what happens when we sample data from a set of clusters, within each of which the prevalence of TB differs.
We will assume that:

- There are l clusters (labelled i=1 to l);

- In each cluster there are m people (labelled j=1 *to m*);

- The total sample size is n (labelled k=1 to n) so that $n=lm$

  and $k=m(i-1) + j$.

And $y_{ij} = y_k = 1$ if person k has TB and 0 if he or she does not.

We now imagine that in the set of all possible clusters each cluster has a different true prevalence so that the mean prevalence across all the clusters is μ, as before, and the variance among all the clusters is v. The essential point is simply that if we repeat the survey, but choose different clusters, we will get a different estimate of the overall mean and it is this additional source of variation that we need to take into account.

With this notation, the probability that the person i in cluster j has TB is μ_i. We will write the binomial variance associated with examining this person as $\phi_i = \mu_i(1-\mu_i)$. For person j in cluster i we have

$$y_{ij} = \mu_i + \varepsilon_{ij} \tag{12}$$

so that if $\varepsilon_{ij} = 1-\mu_i$ if $y_{ij} = 1$ and $\varepsilon_{ij} = -\mu_i$ if $y_{ij} = 0$. Formally,

$$\mu_i \sim \{\mu, \nu\} \tag{13}$$

$$\varepsilon_{ij} \sim \left[0, \mu_i(1-\mu_i)\right] \equiv (0, \phi_i) \tag{14}$$

If we write $\overline{y}$ for the overall mean then

$$n\overline{y} = m\sum_{i=1}^{l}\mu_i + \sum_{i=1}^{l}\sum_{j=1}^{m}\phi_i \tag{15}$$

So that

$$V(n\overline{y}) = m^2 l\nu + m\sum_{i=1}^{l}\phi_i \tag{16}$$

Since the expected value of ϕ_i is $\phi = \mu(1-\mu)$ we have

$$E\left[nV(\overline{y})\right] = m^2 l\nu + n\phi \tag{17}$$

And we get

$$V(\overline{y}) = \frac{m\nu}{n} + \frac{\phi}{n} \tag{18}$$

We will now let the ratio of the between cluster variance to the within-cluster variance be

$$\rho = \frac{\nu}{\phi}$$

And write equation 18 as

$$V(\overline{y}) = \frac{\phi}{n}\left(1 + \frac{m\nu}{\phi}\right) = \frac{\phi}{n}(1 + m\rho) \tag{19}$$

The first term on the right-hand-side of equation 19 is the expression for the variance if there is no variation among clusters and the second term in brackets is called the design-effect D. D is big if either the number of

people per cluster is big or if the between-cluster variance ρ is much greater than the within-cluster variance ϕ.

We can further simplify equation 19 for the special case where the prevalence, μ, is small (so that $1 - \mu \to 1$ and we have Poisson errors). We will also define the variability, α, of the cluster prevalence as the standard deviation divided by the mean so that $\alpha = \sqrt{v}/\mu$.
In this case $\phi = \mu$, $v = (\alpha\mu)^2$

$$D = 1 + m\alpha^2\mu \tag{20}$$

Equation 20 makes it clear that the design effect D depends on the particular survey design that is used through the value of m while the variability α and the mean prevalence μ are independent of the survey that is carried out and depend only on the natural history of TB in a particular place. If we wish to use the results of one study to inform the design of another then it is α and μ that we should estimate and not D. (For further discussion see McCullagh and Nelder [1997].

For a particular survey we may want to estimate the design effect from the results of the survey. Our best estimate of ϕ/n is $\hat{\phi}/(n-1)$ is so that equation 19 becomes

$$V(\bar{y}) = \frac{\hat{\phi}}{n-1}\left(1 + \frac{mv(n-1)}{\hat{\phi}n}\right) = \frac{\hat{\phi}}{n-1}\left(1 + \frac{v(m-1)}{\hat{\phi}}\right) \tag{21}$$

And equation 20 becomes

$$D = 1 + (m-1)\alpha^2\hat{\mu} \tag{22}$$

We can also write, from equation 18, that

$$D = \frac{SS_T^2}{SS_W^2} = 1 + \frac{SS_B^2}{SS_W^2} \tag{23}$$

where SS_B and SS_W are the between- and within groups sums of squares. The *design effect* or *the variance inflation factor (D)* tells us by how much the variance, estimated without considering the effect of clustering, needs to be increased to obtain the correct variance. Similarly, to obtain a given precision in our measured prevalence we have to increase the sample size by an amount D.

Predicting the design effect

When we design our survey we need to know how big the sample should be and how this depends on the design effect. To illustrate this, suppose that we have an expected (mean) prevalence μ and that the prevalence among clusters is between 0.5μ and 1.5μ. For this uniform distribution the variance is $\mu^2/12$. The design effect is then

$$D = 1 + \frac{m\mu}{12} \qquad (24)$$

Suppose that we have 100 clusters, with 1 200 people in each cluster so that the total number of people is 120 000 and that the prevalence is 100/100 000. Then the design effect is 1.1. If the prevalence among clusters varied from 0 to 2μ then the design effect is 1.4.

Minimizing the cost

The important thing is to consider the design effect as something that we can vary by choosing different designs, and we wish to find the design that allows us to measure the prevalence, with a certain precision, as cheaply as possible. To determine the optimal sample size we therefore proceed as follows. First of all, we need to know the expected prevalence, μ, and the precision, ε, with which we want to measure it, where

$$\varepsilon = \frac{1.96\sigma_\mu}{\mu}$$

so that the 95% confidence limit of our estimate will be $\mu \pm \varepsilon\mu$.
Then, without allowing for the design effect the required sample size would be

$$n = \frac{1.96^2}{\mu\varepsilon^2} \qquad (25)$$

We then proceed as follows. We first make an estimate of the variation of μ between clusters either from our experience of TB or from other surveys. Suppose that the standard deviation of μ arising from the variation among clusters is $\alpha\mu$. We have already estimated μ; now we also need to specify

α, the precision with which we wish to measure it. Then we can write the design effect

$$D = 1 + \alpha^2 m\mu \qquad (26)$$

Then from our first estimate of n we can calculate the required sample size, allowing for the design effect, for any given value of m and hence the number of clusters that we need. In order to determine the cluster size that gives both the desired precision and the minimum cost per patient we finally need to know the cost of starting a new cluster compared with the cost of adding one patient to an existing cluster. If we let this ratio be ρ then the cost is

$$C = \frac{1.96}{\mu\varepsilon^2}\left(1 + \alpha^2 m\mu\right) + l\rho \qquad (27)$$

or

$$C = \frac{1.96^2}{\mu\varepsilon^2}\left[\left(1 + \alpha^2 m\mu\right) + \frac{\rho}{m}\right] \qquad (28)$$

And minimizing C by varying m gives

$$m^* = \sqrt{\frac{\rho}{\alpha^2\mu}} \qquad (29)$$

or

$$l^* = \frac{1.96^2}{\varepsilon^2}\sqrt{\frac{\alpha^2}{\rho\mu}} \qquad (30)$$

We note, in particular, that the optimal number of people per cluster is independent of the precision, ε, with which we wish to measure the prevalence. As expected we should increase the number of clusters (and decrease the number of people per cluster) if the variability among clusters, α increases, or if the prevalence, μ, or the cost per cluster decreases.
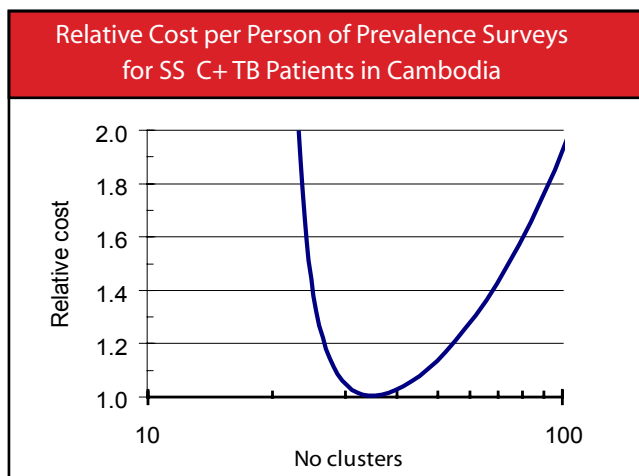
These calculations are illustrated in the figure above.   For example, using the data for SS - C+ TB cases in the Cambodia survey:

■   The prevalence is 857 per 100 000;

■   We wish to measure this with 95% confidence limits equal to 25% of the true value;

■   We will assume that the prevalence varies among the clusters with a standard deviation of about 60% of the mean value;

■   The cost of adding one new cluster is 750 times the cost of adding one person to an existing cluster.

Then the optimal number of clusters is 34, the optimal number of people per cluster is 538, the optimal sample size is 18k, and the design effect is 2.54. In the actual survey there were 42 clusters, 528 people per cluster (on average), and a total sample size of 22k adults, close to the optimum design. To decide how close to the optimal design we need to be we note that to measure the prevalence with the desired accuracy without increasing the cost by more than 20% then the number of clusters should not be less than 26 or more than 52.

Appendix 1: Choosing clusters from districts with probability proportional to size

We use Cambodia to illustrate the procedure. Cambodia has 185 districts from which we wish to choose 42 clusters. To choose districts with probability proportional to size we simply list all the districts in a random order and then calculate the cumulative population of the districts. The total population of Cambodia is about 11 million people. We then use a computer to generate a random number between 1 and 11 million and choose the district for which the cumulative population includes this number. We then repeat this 42 times. For a more detailed description see the paper by Nagelekerke et al. (2000).

Appendix 2: Design effect with grouped data

If you have access to the individual data you can use the (survey) command in Stata to calculate the mean prevalence allowing for different strata and for the effect of clustering and it will give you the design effect directly. If you have access only to the aggregated data, for example, the number of people that are positive you can use the blogit command in Stata to determine the design effect. Run the following commands in Stata on the data in the table:

> **blogit *pp* adat, cluster(cluster)**
> **blogit *pp* adat**

where the variable *adat* is the number of those over the age of 10 who attended the survey (column 6 in the table) and the variable *pp* is the number of these that were smear- and culture positive (column 7 in the table). The output will include a value for "coefficient" and for the "robust standard error" for each of the blogit commands. The values are -5.608 ± 0.1198 and 5.608 ± 0.1131. Because (blogit) does a logistic regression the mean value is $e^{-5.608} = 0.00367$ or 367/100k, and the design effect is the ratio of the variances of the two estimates or $(0.1198/0.1131)^2 = 1.12$. Both the mean and the design effect differ slightly from the values obtained above 364/100k and 1.18 because the Stata command has allowed for the differences in the size of each cluster.

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Annex 15: TB Prevalence Surveys Recorded in the WHO Database

Source: References for Tables A3.1 and A3.2 in Global TB Report 2007

National surveys	Disease
Bangladesh	1964 (Ministry of Health, Bangladesh, 1967), 1987 (Ministry of Health, Bangladesh, 1989)
Cambodia	2002 (Ministry of Health, Cambodia, 2003
China	1979 (Ministry of Public Health, China, 1979), 1984 (Ministry of Health, China, 1985), 1990 (Ministry of Public Health, China, 1990), 2000 (Ministry of Public Health, China, 2000)
Eritrea	2005 (Ministry of Health, Eritrea, 2006)
Gambia	1960 (WHO 1960)
Ghana	1957 (WHO 1958)
Indonesia	2004 (Ministry of Health, Indonesia, 2005; Soemantri, forthcoming)
Iraq	1970 (Ministry of Health, Iraq, 1996)
Japan	1953 (Omura et al. 1962, Yamaguchi et al. 1955), 1958 (Omura et al. 1962; Ministry of Health and Welfare, Japan, 1958), 1963 (Wakamatsu 1964), 1968 (Murunaka 1968)
Kenya	1948 (Haynes 1951), 1958 (Roelsgaard and Nyboe 1961)
Liberia	1959 (WHO 1961)
Libyan	Arab Jamahiriya 1976 (WHO 1961, Leowski 1993)
Malaysia	2003 (Dye 2004)
Mauritius	1958 (WHO 1959)
Myanmar	2006 (Kluge 2006)
Netherlands	1970 (Styblo, Broekmans, and Borgdorff 1996)
Nigeria	1957 (WHO 1957)
Pakistan	1959 (Dolin 1997), 1987 (Dolin 1997)

National surveys	Disease
Philippines	1981 (National Institute of Tuberculosis, Philippines, 1984; Department of Health, Philippines, 1992), 1997 (Tupasi et al. 1999; Department of Health, Philippines, 1997; Tupasi et al. 2000)
Rep. of Korea	1965 (Ministry of Health, Republic of Korea, 1965), 1970 (Ministry of Health, Republic of Korea, 1970), 1975 (Ministry of Health, Republic of Korea, 1976), 1980 (Ministry of Health, Republic of Korea, 1981), 1985 (Ministry of Health, Republic of Korea, 1986), 1990 (Ministry of Health, Republic of Korea, 1991); Hong et al. 1993), 1995 (Ministry of Health, Republic of Korea, 1996; Hong et al. 1998)
Samoa	1975 (Leprologist 1977)
Sierra Leone	1958 (WHO 1959)
Somalia	1956 (WHO 1956, Munim et al. 2006)
Sri Lanka	1970 (WHO 1971)
Uganda	1958 (WHO 1959)
Viet Nam	2006

Subnational surveys	Disease
Afghanistan	1982 (Aneja 1982)
Bangladesh	1995 (Chowdhury et al. 1997), 2001(Hamid Salim et al. 2004), 2002 (Zaman et al. 2005, Zaman et al. 2003), 2006 (Zaman et al. 2006)
Botswana	1981 (Fourie et al. 1980, Fourie and Knoetze 1986, Maganu 1981), 1995 (Fourie et al. 1980, Fourie and Knoetze 1986, Maganu 1981)
Brunei Darussalam	1985 (Fu 1987)
China	1957 (Quo 1959), 1959 (Penington 1959)
Cambodia	1981 (Ministry of Health, Cambodia, 2005), 1982 (Ministry of Health, Cambodia, 2005), 1983 (Ministry of Health, Cambodia, 2005), 1984 (Ministry of Health, Cambodia, 2005), 1985 (Ministry of Health, Cambodia, 2005), 1988 (Ministry of Health, Cambodia, 2005; Zuluaga et al. 1992), 1995 (Ministry of Health, Cambodia, 2005), 1998 (Ministry of Health, Cambodia, 2005; Norval, Roustit, and San 2004)
Colombia	1988 (Zuluaga et al. 1992)
Cyprus	1963 (Geser 1964, Elias et al. 1995)

Subnational surveys	Disease
Ethiopia	2001 (Demissie et al. 2002)
India	1948–1993 (numerous surveys) (Chakraborty 1996)
Indonesia	1979 (Ministry of Health, Indonesia, 1995), 1983–1993 (Ministry of Health, Indonesia, 1995), 1994 (Ministry of Health, Indonesia, 1995)
Iraq	1961 (WHO 1962)
Japan	1954 (Omura et al. 1962, Yamaguchi 1955), 1964 (Konishi 1964)
Kenya	1958 (WHO and UNICEF 1959, WHO and UNICEF 1960), 2006
Liberia	1959 (WHO 1961)
Malawi	1960 (Ministry of Health, Malawi, 1997)
Malaysia	1970 (WHO 1971)
Mozambique	1961 (WHO 1962)
Myanmar	1972 (WHO 1972), 1989 (Sudre 1992), 1990 (Sudre 1992), 1991 (Sudre 1992), 1994 (WHO 2003)
Nepal	1965 (WHO 1994), 1976 (Shimao 1986), 1994 (Sharma and S.I. 1996)
Nigeria	1957 (WHO 1958), 1973 (Pust, Onejeme, and Okafor 1974)
Pakistan	1962 (WHO 1962)
South Africa	1972–1985 (WHO 1996)
Spain	1991 (de March-Ayuela 1994)
Syrian Arab Republic	1960 (WHO 1962)
Thailand	1962 (Payanandara 1993), 1970 (Payanandara 1993), 1977 (Payanandara 1993), 1983 (Payanandara 1993), 1987 (Payanandara 1993), 1991 (Payanandara 1993)
Tunisia	1957, 1961 (WHO 1961)
Turkey	1971 (Atlamaz 1971)
Uganda	2000
UR Tanzania	1958 (WHO 1958)
Viet Nam	1961 (Broekmans 1989)
Zambia 1980	(Watts 1983)

National surveys	infection
Afghanistan	1978 (Aneja 1982), 1982 (Aneja 1982)
Algeria	1949, 1966, 1980 (Amrane 1996), 1985 (Amrane 1996,Chaulet et al. 1990)
Argentina	1979 (The tuberculosis situation in Argentina, 1983)
Bahrain	1969 (Fernandes and Mahmoud 1986), 1981 (Fernandes and Mahmoud 1986, Khan 1982), 1985 (Fernandes and Mahmoud 1986), yearly 1988–1994 (Ministry of Health, Bahrain, 1997)
Bangladesh	1964 (Ministry of Health, Bangladesh, 1967)
Benin	1987 (Gninafon et al. 1995), 1994 (Trebucq and Lambregts-van Weezenbee 1995)
Botswana	1956 (Fourie et al. 1980), 1981 (Fourie and Knoetze 1986, Maganu 1981)
Cambodia	2002 (Ministry of Health, Cambodia, 2005)
China,	Hong Kong SAR 1999 (Leung et al. 2005)
China	1970 (Ministry of Public Health, China, 1979), 1979 (Ministry of Public Health, China, 1979), 1984 (Ministry of Health, China, 1985), 1990 (Ministry of Public Health, China, 1990), 2000 (Ministry of Public Health, China, 2000)
Cyprus	1955 (Geser 1964)
Djibouti	1994 (Eilers and Borgdorff 2004), 2001 (Eilers and Borgdorff 2004)
Egypt	1951 (El Ibiary et al. 1999), 1996 (El Ibiary et al. 1999)
Ethiopia	1954 (Azbite 1992), 1989 (Azbite 1992)
Gambia	1958 (WHO 1960)
Ghana	1957 (WHO 1958)
Greece	yearly 1981–1991 (Bouros et al. 1995)
India	2000 (Chadha et al. 2005)
Indonesia	2004 (Ministry of Health, Indonesia, 2005)
Iraq	1995 (Ministry of Health, Iraq, 1996)
Japan	1953 (Omura et al. 1962, Yamaguchi et al. 1955), 1958 (Omura et al. 1962; Ministry of Health and Welfare, Japan, 1958), 1963 (Wakamatsu 1964), 1968 (Murunaka 1968)
Jordan	1986 (Styblo 1991), 1990 (Styblo 1991)
Kenya	1958 (Roelsgaard and Nyboe 1961), 1986 (Bosman et al. 1991, Odhiambo et al. 1999), 1995 (Odhiambo et al. 1999)

National surveys	infection
Lao PDR	1995 (Vangvichit et al. 1995)
Lesotho	1956 (Fourie et al. 1980), 1981 (Fourie and Knoetze 1986)
Libyan Arab Jamahiriya	1976 (Leowski 1993)
Madagascar	1991 (Champetier de Ribes et al. 1997)
Malawi	1994 (Salaniponi et al. 2004)
Mauritius	1956 (WHO 1959), 1958 (WHO 1959, WHO 1959)
Mexico	1961 (Ministry of Health, Mexico, 1995)
Myanmar	1972 (Sudre 1992)
Nepal	2006 (Pushpa et al. 2006)
Netherlands	yearly 1956–1979 (Sutherland et al. 1983), 1989 (Bleiker 1991)
Pakistan	1987 (Dolin 1997)
Philippines	1981 (National Institute of Tuberculosis of the Philippines 1984; Department of Health, Philippines, 1992), 1997 (Tupasi et al. 1999, Tupasi et al. 2000)
Rep. of Korea	every 5 years 1965–1995 (Hong et al. 1993, Hong et al. 1998)
Samoa	1975 (Leprologist WRCT 1977)
Somalia	1956 (WHO 1956), 2006 (Munim et al. 2006)
Sudan	1976 (Ministry of Health, Sudan, 1976), 1986 (Ministry of Health, Sudan, 1986)
Thailand	1980 (Daramas, Konjanart, and Sunakorn 1980)
Tunisia	1959, 1986 (WHO 1986)
Uganda	1958 (WHO 1959), 1970 (Stott et al. 1973), 1989 (Migliori et al. 1994)
UR Tanzania	1985 (Tanzania Tuberculin Survey Collaboration 2001), 1990 (Tanzania Tuberculin Survey Collaboration 2001), 1995 (Tanzania Tuberculin Survey Collaboration 2001), 2002 (Egwaga et al. 2003)
Yemen	1991 (Ministry of Public Health, Yemen, 1991)

[There was a national tuberculin survey in Sudan in the 1950s]

Subnational surveys	infection
Afghanistan	1985 (Spinaci et al. 1989), 1989 (Spinaci et al. 1989, Cordola et al. 1990)
Algeria	1938 (Amrane 1996, Chaulet et al. 1990), 1948 (Amrane 1996, Chaulet et al. 1990), 1958 (Amrane 1996, Chaulet et al. 1990), 1968 (Amrane 1996, Chaulet et al. 1990), 1976 (Amrane 1996, Chaulet et al. 1990), 1981 (Amrane 1996, Chaulet et al. 1990)
Angola	1991 (Ministry of Health, Angola, 1991)
Bhutan	1991 (WHO 1996)
Botswana	1989 (Maganu et al. 1989)
Brazil	1970 (WHO 1994), 1973 (WHO 1994), 1979 (WHO 1994), 1983 (WHO 1994), 1986 (WHO 1994), 1988 (WHO 1994), 1990 (WHO 1994)
Burundi	1982 (Sudre 1991)
Cambodia	1955, 1968, 1981, 1995 (Ministry of Health, Cambodia, 2005; Norval, Roustit, and San 2004)
Cameroon	1984 (Delolme et al. 1984)
Central African Republic	1988 (Sarda et al. 1993)
Colombia	1970–1998 (de la Pava, Salguero, and Alzate 2002)
Cyprus	1963 (Geser 1964), 1995 (Elias et al. 1995)
Czech Republic	1961, 2001
France	1990 (Schwoebel, Hubert, and Grosset 1994)
Gabon	1987 (Ministry of Health, Gabon, 1990)
Guinea	1989 (Sow et al. 1990)
India, Bangalore	1962 (National Tuberculosis Institute, Bangalore, 1974), 1963 (Chakraborty et al. 1982), 1965 (Chakraborty et al. 1982), 1967 (Chakraborty et al. 1982), 1977 (Chakraborty et al. 1992, Chakraborty et al. 1979)
India, Chingleput	1969 (National Tuberculosis Institute, Bangalore, 1974), 1979 (Mayurnath et al. 1991), 1984 (Mayurnath et al. 1991)
India, other	1948–1993 (Chakraborty 1996)
Indonesia	1952–1965 (Ministry of Health, Indonesia, 1995), 2005 (WHO 2007), 2006 (WHO 2007)
Iran (Islamic Republic of)	1946, 1952, 1963, 1972, 1983, 1990 (Chaulet 1990, Dolin 1998)
Iraq	1989 (Ministry of Health, Iraq, 1996)
Italy	1997 (D'Amelio et al. 2000)

Subnational surveys	infection
Japan	1954 (Omura et al. 1962, Yamaguchi 1955), 1964 (Konishi 1964), 1992 (Rahman et al. 2001)
Jordan	1949, 1970, 1976, 1982 (Styblo 1991)
Kenya	1974 (Paul et al. 1975), 2006
Kuwait	1962, 1972–1981, 1991, 1993–1997 (WHO 1998)
Lebanon	1994
Lesotho	1962, 1992
Libyan Arab Jamahiriya	1954 (WHO 1961), 1959 (WHO 1961), 1971 (Leowski 1993)
Morocco	1994 (WHO 2002)
Mozambique	1961 (WHO 1962), 1987, 1988
Myanmar	1991 (Sudre 1992)
Nepal	1947 (Sharma and Smith 1996), 1962 (Sharma and Smith 1996), 1963 (Sharma and Smith 1996), 1965 (Sharma and Smith 1996), 1966 (Sharma and Smith 1996), 1973 (Sharma and Smith 1996), 1974 (Sharma and Smith 1996), 1976 (Sharma and Smith 1996), 1979 (Sharma and Smith 1996), 1980 (Sharma and Smith 1996) 1988 (Sharma and Smith 1996), 1989 (Sharma and Smith 1996), 1990 (Sharma and Smith 1996), 1991 (Sharma and Smith 1996), 1992 (Sharma and Smith 1996), 1993 (Sharma and Smith 1996), 1994 (Sharma and S.I. 1996)
Oman	1995
Pakistan	1992 (Dolin 1997), 1994 (Dolin 1997)
Peru	1981 (WHO and Pan American Health Organization 1994), 1982 (WHO and Pan American Health Organization 1994), 1987 (WHO and Pan American Health Organization 1994), 1993 (WHO and Pan American Health Organization 1994)
Philippines	1992 (Department of Health, Philippines, 1992)
Saudi Arabia	1988 (Kaleta 1988)
Sierra Leone	1958 (WHO 1959)
Somalia	1986 (Peltola et al. 1994)
South Africa	1972–1985 (Fourie et al. 1980), 1988 (WHO 1996, Berman et al. 1992)
Syrian Arab Republic	1960 (International Tuberculosis Surveillance Center 1984), 1978 (International Tuberculosis Surveillance Center 1984), 1983 (International Tuberculosis Surveillance Center 1984), 1992

Subnational surveys	infection
Togo	1978, 1986, 1988 (Ministry of Health and Population, Togo, 1995)
Tunisia	1980 (WHO 1986)
Turkey	1994 (Yorulmaz et al. 2002)
Uganda	1971 (Migliori et al. 1994), 1987 (Migliori et al. 1994)
UR Tanzania	1958 (WHO 1958), 1988–1992 (Tanzania Tuberculin Survey Collaboration 2001), 1993–1998 (Tanzania Tuberculin Survey Collaboration 2001), 2000 (Egwaga et al. 2003
USA	1997 (Daniel and Debanne 1997)
Viet Nam	1955 (Broekmans 1989, Huong et al. 2006), 1961 (WHO 1963), 1986 (Broekmans 1989), 1990 (Huong et al. 2006), 1991 (Huong et al. 2006), 1996 (Huong et al. 2006)
Zambia	1980 (Watts 1983)

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Assessing tuberculosis prevalence through population-based surveys

Errata

Page 19 – several symbols are missing. The top of the page should read:

Because TB is a rare condition, the mean prevalence μ is always very small. In the case of Cambodia the prevalence in the 1998 survey was $\mu = 483 / 100{,}000 = 0.00483$. Since $(1 - \mu) \approx 1$, 95% confidence limits for the estimated values of μ are $\pm \varepsilon$ where $\varepsilon = 1.96\sigma$ so that

$$n = \frac{\mu}{\sigma^2} = \frac{1.96^2}{\mu\varepsilon^2} \tag{1}$$

With $\mu = 0.00483$ and $\varepsilon = 0.25$, equation 1 gives a sample size $n = 12726$.