GENERIC GUIDELINES

FOR

THE ESTIMATION OF THE
ANNUAL RISK OF TUBERCULOUS INFECTION (ARTI)

WORLD HEALTH ORGANIZATION
Regional Office for South East Asia
New Delhi

January, 2006
ACKNOWLEDGEMENT

These guidelines were prepared by Dr V. Chadha, epidemiologist from the National Tuberculosis Institute, Bangalore, India through an Agreement for Performance of Work (APW SE/05/025811) with the World Health Organization.

The publication and distribution of this document has been made possible through assistance from Dr Erwin Cooreman, Medical Officer (TB), WHO/SEARO and Dr Nani Nair, Regional Adviser (TB), WHO/SEARO.
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<td>Annual Risk of Tuberculous Infection</td>
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<td>BCG</td>
<td>Bacille Calmette-Guérin</td>
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<td>C.I.</td>
<td>Confidence Interval (95% if not specified)</td>
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<td>DOTS</td>
<td>Internationally recommended strategy to fight tuberculosis</td>
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<td>EPI</td>
<td>Expanded Programme of Immunization</td>
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<td>HIV</td>
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<td>Union</td>
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<td>M. tuberculosis</td>
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<td>MDR-TB</td>
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<td>PPD</td>
<td>Purified Protein Derivative</td>
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<td>PPS</td>
<td>Population Proportional to Size</td>
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1. SUMMARY

Tuberculosis is a leading public health concern in all countries of the WHO South-East Asia Region. Successful implementation of DOTS* introduced from early 1990s is expected, on the one hand, to diminish the transmission of infection in the community. This will eventually lead to reducing the TB incidence. On the other hand, the HIV epidemic along with multidrug-resistant tuberculosis (MDR-TB) is likely to adversely affect on TB trends. Therefore, it is important to assess the epidemiological situation of TB in the community from time to time.

The Annual Risk of Tuberculous Infection (ARTI) is a sensitive indicator of the epidemiological situation of TB since it expresses the overall impact of incidence and duration of infectious cases in the community, efficiency of TB control programs as well as the environmental and social factors influencing transmission of infection. It is generally computed from the prevalence of infection estimated through cross-sectional tuberculin surveys in a representative sample of children. Compared to surveys for estimating prevalence and incidence of TB disease, tuberculin surveys are simpler to carry out, more cost-effective and operationally less cumbersome. Since the results of tuberculin surveys conducted among children indicate recent TB situation in the community, repeat tuberculin surveys are also used to assess the epidemiological trends of TB in the community.

The objectives of a tuberculin survey should be decided while taking into consideration the population of the country, the epidemiological setting and the available resources. It is suggested that national-level surveys may be planned in smaller countries, while in larger countries, it is best to plan separate surveys at sub-national levels (regional/state/provincial). It should also be decided whether the objective is to obtain a baseline estimate of the risk of infection or to study the trends in risk of infection. To study the trends, repeat tuberculin surveys may be carried out with an optimum interval of 5-7 years.

Tuberculin surveys for the ARTI estimation are preferably carried out among children below 10 years of age. The surveys may be carried out in schools when about 90% of the children in the selected age group are enrolled in school. Preferably, only first grade/class (or first & second grade) children who are usually between 6-7 years of age may be included in the study population. In communities with low school attendance, house-based surveys are preferred; in such settings, broader age groups (5-9 years or 0-9 years) are desirable for operational practicability.

In areas with high BCG immunization coverage (more than 70%), all children irrespective of their BCG status should be included in the study sample. In such case, the house-based surveys may preferably be conducted in 5-9 year age group. The younger age group is anyway excluded in school surveys. In communities of low coverage with BCG immunization, the survey may preferably be conducted among children without BCG scar. In such case the sample size would correspond to only the children without BCG scar. For repeat surveys, similar study populations in respect of age group and BCG scar should be selected.

It is recommended to use for tuberculin surveys two tuberculin units (TUs) of PPD RT 23 with tween 80. However, countries with experience of using one TU for tuberculin surveys in recent times may continue to use the same dose.

The sample size for a baseline tuberculin survey may be estimated using the following formula for obtaining the estimate of prevalence at 5% level of significance \( N = d (1.96)^2 \frac{(1-p)}{\varepsilon^2 P} \), where \( N \) is sample size, \( P \) the expected prevalence of infection, \( \varepsilon \) the relative precision and \( d \) the design effect. A precision of 10% and

* DOTS: internationally recommended strategy to fight tuberculosis
design effect of 3 is advocated. The estimated sample size is further increased by 20% to account for exclusions and dropouts. For repeat surveys, the sample size may be estimated using the following formula: \( N = 10.5 \frac{[P_1 (1-P_1) + P_2 (1-P_2)]}{(P_1-P_2)^2} \), where \( N \) is the sample size, value of \( P_1 \) is as estimated from the baseline survey and value of \( P_2 \) is estimated considering the expected change in the intervening period. The above formula applies to simple random sampling for an 90% chance of obtaining a difference at 5% level of significance. The estimated value of \( N \) is multiplied by the design effect for cluster sampling and further increased by 20%.

A Stratified two-stage cluster sampling is advocated for tuberculin surveys. Stratification may be adopted by rural-urban areas, by region or by topography. A sampling design for estimating ARTI for an entire country while considering rural-urban stratification, is illustrated in this document and may be suitably modified in other situations.

At first stage of sampling, districts are selected by population-proportional-to-size (PPS) method. The selection of districts should be carried out independently in individual strata. The number of districts is based on operational convenience and may be in the range of 5-25% of the total districts, in each stratum. It is best not to exclude any districts from the sampling frame, except for reasons of safety of field teams, accessibility or political reasons. The second stage of sampling involves the selection of clusters: schools in case of school-based surveys or villages/urban blocks in house-based surveys, by simple random sampling.

The estimated sample size is distributed into different strata in proportion to the population size. The sample size for individual strata is further divided equally between the selected districts. The number of clusters to be selected within individual districts depends upon the average number of children expected to be available in each cluster. In the selected clusters, all available children on the day of testing are included.

Repeat survey may preferably be carried out in the same districts as in the first survey. However, within individual districts, a fresh sample of clusters has to be selected by simple random sampling (SRS) method.

Ethical clearance by an ethical review committee should be obtained before initiating fieldwork to be carried out by trained teams.

In each district, a tentative action plan is prepared indicating the dates of planning, registration, testing and reading in each cluster.

A planning visit is undertaken to each cluster 2-3 days prior to the testing day to obtain necessary information and to solicit the support of school authorities and parents. In a school-based survey, consent of the school authorities is obtained after acquainting them with the purpose of the survey and characteristics of the tuberculin test. Printed information is provided to parents 2-3 days prior to testing. In case no objection is received from the parents or guardians by the testing day, consent may be assumed as given. In house-based surveys, parents/guardians are explained the purpose of the survey and nature of the test on the day of testing and their informed consent is obtained.

On the testing day, a child card is opened for each child present to record the identification particulars, age, BCG status and results of tuberculin testing and reading. A testing centre is temporarily set up at a suitable place in the cluster. Each child is administered with 0.1 ml of tuberculin intra-dermally on the mid-anterior aspect of the left forearm using a disposable tuberculin syringe. Children with a history of skin rash in the recent past, with high fever or receiving anti-TB treatment and whose parents/guardians did not agree for the test are excluded from tuberculin testing. The
reading of the tuberculin reaction is undertaken at about 72 hours after administration of the test. The maximum transverse diameter of the induration is recorded in millimeters.

For children having reactions of more than 10 mm, parents are enquired about the current health status of the child especially for presence of chest symptoms, loss/failure to gain weight, history of anti-TB treatment and history of contact with a known tuberculosis case. Children with any of these are referred to the nearest health centre for medical assistance.

Throughout the fieldwork, tuberculin vials are stored between 2-8 °C and carried to the field in vaccine carriers or thermos flasks. Maximum care is taken to protect tuberculin from heat and sunlight.

About 75-100 smear-positive cases of pulmonary TB should also be tuberculin tested with each batch of tuberculin used.

Children whose tuberculin tests were administered unsatisfactorily and outliers in terms of age are excluded at the time of analysis, from estimation of prevalence of infection.

Within a given stratum, data obtained in different districts are pooled together. Frequency distribution graphs should be generated separately for different strata.

If the survey was designed to estimate ARTI only among children without BCG scar, then the frequency distributions of reaction sizes and graphs are generated only for such children. Any of the following three types of graphs may be obtained; and the criteria for estimating prevalence of infection in each situation may be arrived as given below:

(i) A bi-modal graph showing two distinct modes and an easily identifiable anti-mode: reactions to the right of the anti-mode represent the sub-group of children infected with tubercle bacilli and reactions to the left represent those who are either uninfected or elicit non-specific tuberculin sensitivity. In this situation, the prevalence of infection is estimated considering all reactions greater than the anti-mode as specific for tuberculous infection. The estimation may also be undertaken by mirror–image technique as given below;

(ii) A bi-modal graph with two distinct modes but the anti-mode is not clearly identified: in such situation, the prevalence of infection is estimated by doubling the proportions of reactions larger than the second mode and adding to the frequency at the second mode. This is the mirror–image technique;

(iii) A graph in which, neither the anti-mode nor the mode on the right side of the distribution is clearly discernible: in such circumstances, the mode of reaction sizes observed among smear-positive cases is used for estimating the prevalence of infection by mirror–image technique.

If the survey was designed to estimate ARTI among children regardless of their BCG status, three frequency distributions are plotted: one for children without BCG scar, one for children with BCG scar and another for the combined group (overall study group). A clear anti-mode is usually not seen either among children with BCG scar or in the combined group. However, the second mode can usually be made out at the same reaction size in the three distributions. Therefore, the prevalence of infection for the overall study group is estimated using mirror–image technique. In case the second mode in the overall study group is not clearly discernible, the mode observed in children without BCG scar may be used for estimating prevalence in the overall study group. Otherwise, the mode observed in smear positive cases is used as above.
To reduce the influence of *digit preference* on the estimate of the prevalence of infection, smoothening of the data may be undertaken by calculating the expected frequency at each mm of induration as the average of five frequencies including two before and two after the induration of interest. Calculations of prevalence of infection are based on this new set of frequencies.

Using the anti-mode/mode decided as above and after smoothening of the data, the number of infected children is estimated separately for each stratum. The prevalence of infection is estimated on adding the number of infected children in different strata, dividing this number by the total number of children analyzed and multiplying with 100. The 95% confidence interval (CI) is estimated using appropriate formula. The ARTI is computed from the estimated prevalence applying the formula: \( R = 1-(1-P)^{1/a} \), where \( R \) is the annual risk of infection, \( P \) the estimated prevalence of infection and \( a \) the mean age of children.

For the study of trends in ARTI by repeat surveys, the average per annum change in ARTI may be computed from the expression: \( 1-(R_2 / R_1)^{1/T} \), where \( R_1 \) and \( R_2 \) are the estimates of ARTI at first and second surveys and \( T \) is the interval in years.

The survey results provide information on the epidemiological situation of TB. The estimated ARTI indicates the extent of transmission of tuberculous infection in the community, from infectious cases to the age group involved in the survey. The observed level of risk of infection in a community is the cumulative result of incidence of infectious cases, duration of their infectiousness and interaction between cases and susceptible individuals. Since the duration of infectiousness depends upon case finding and treatment, ARTI estimates also reflect on the efficiency of tuberculosis control programs.

A relationship had earlier been derived between ARTI and incidence of smear-positive TB cases. The Styblo rule stipulates that every one-percent of ARTI estimated in children corresponds to an incidence of 50 sputum smear-positive cases per 100,000 population. However, this relationship was derived from observational studies in the pre-chemotherapy (and pre-HIV) era; it should now be applied with caution.

The level of ARTI also determines the total pool of infected people in the community and gives an idea of the extent to which new cases are likely to emerge in the years to come.

A declining trend in ARTI observed from repeat tuberculin surveys reflects on the programme efficiency. An increasing trend points towards poor efficiency of control programmes and/or epidemiological factors such as an increase in HIV sero-prevalence in the community leading to a higher incidence of TB.

The observed estimates of ARTI and its trends should stimulate a review of the existing TB control policies as well as appropriate actions for further strengthening of control activities.
2. BACKGROUND AND RATIONALE

Tuberculosis (TB) is a leading public health concern in all countries of the WHO South-East Asia Region. Every year, about three million new TB cases occur in the Region, thereby contributing one third of the global incidence. Approximately 600,000 people die from TB every year. To control TB, the DOTS strategy was introduced from early 1990s and has been expanded in a phased manner. By 2004, about 90% of the population in the region lived in areas where DOTS services are available. Treatment success rates in the order of 80-85% among new pulmonary smear-positive cases (PTB) have been accomplished consistently in most countries. A further improvement in case detection is required to achieve the 70% target. Successful implementation of DOTS is expected to diminish the transmission of infection in the community by shortening the duration of infectiousness of sputum smear-positive PTB cases. A reduction in the pool of infected individuals is expected to eventually reduce the TB incidence and mortality. There are also about six million people in the Region living with HIV infection, 40-50% of whom may be infected with tubercle bacilli. This, along with high levels of multidrug resistance in previously treated TB cases, is likely to impact adversely on TB trends. Therefore, it is important to assess the epidemiological situation of TB in the community from time to time.

The Annual Risk of Tuberculous Infection (ARTI) is defined as the average probability of a group of individuals acquiring new tuberculous infection in the course of one year. It is a sensitive indicator of the epidemiological situation of TB and its trends. It expresses the overall impact of incidence and average duration of infectious cases in the community, efficiency of TB control programmes as well as the environmental and social factors influencing transmission of the infection. It is generally computed from the prevalence of infection estimated through well planned and carefully conducted cross-sectional tuberculin surveys in a representative sample of children. Compared to surveys for estimating the prevalence of TB disease, tuberculin surveys are simpler to carry out, cost-effective and operationally less cumbersome. Disease surveys are more difficult to undertake due to the high cost and requirement of skilled staff. The problem gets compounded when disease surveys are repeated to estimate TB incidence. Further, it is hard to generate data on mortality due to TB in communities where accurate death certification systems are not in place.

Since tuberculin surveys to estimate ARTI are generally conducted among children, the results indicate recent TB situation in the community. The ARTI is also sensitive to changes in the TB situation in epidemiological terms. One of the aims of TB control programmes is to decrease the transmission of the infection in the community. Hence, it is important to know whether the risk of infection is decreasing or not and, if it is decreasing, what is the rate of decline per year. Therefore, repeat tuberculin surveys are also used to assess the impact of TB control programmes and epidemiological trends of TB in the community.

The following guidelines have been formulated to enable any country or agency to plan, implement and analyze tuberculin surveys for estimating ARTI. Care has been taken to make the entire undertaking practical while being scientifically accurate.

3. OBJECTIVES

While deciding upon the objectives of a tuberculin survey, the following important factors should be taken into account:

a. Population size of the country
b. Epidemiological setting
c. Baseline or repeat survey
Available resources

It has to be decided beforehand whether a single ARTI estimate for an entire country will be adequate or separate estimates will be required for different regions in the country\(^b\). Similarly, separate surveys may be planned for different strata with pronounced epidemiological differences in TB situation (e.g. rural/urban). It is suggested that national-level surveys may be planned in smaller countries. In larger countries, it is best to plan separate surveys at sub-national levels (regional/state/provincial).

It should also be decided whether the objective is to obtain a baseline ARTI estimate or to study the ARTI trends. To study the trends, repeat tuberculin surveys may be carried out with an optimum interval of 5-7 years.

The study population should be clearly stated in the Objectives (see next section).

The final decision on the objectives depends on the requirements of the investigators and available resources.

4. STUDY POPULATION

Tuberculin surveys for estimation of ARTI are preferably carried out among children below 10 years, since the estimates obtained in this age group reflect on relatively recent disease situation and its trends. Moreover, the prevalence of infection with environmental mycobacteria is lower in this age group. In older age groups, a higher prevalence of environmental mycobacteria may interfere with the interpretation of the survey results\(^4,5\). Children are also a more accessible population. To choose the study population, three decisions are required to be taken:

i. whether the survey should be carried out in schools or it should be house-based

ii. whether children with a BCG scar can also be included or the ARTI should be estimated exclusively among children without BCG scar

iii. what should be the age of children to be included for the survey

**School enrolment**

School-based surveys are operationally more convenient than house-based surveys. Preferably only first grade/class (or first and second grade) children who are usually between 6-7 years old may be included in the study population as an increasing number of students may drop out of school in higher grades. Therefore, tuberculin surveys may be carried out in schools when about 90% of the children in the selected age group are enrolled. The survey may be conducted in kindergartens in countries where the proportion of children attending kindergartens is high.

In communities with low school attendance, house-based surveys are preferred since children not attending schools may be at a different risk of infection. However, a broader age group (5-9 years or 0-9 years) is desirable for operational practicability and cost-effectiveness.

\(^b\) If independent estimates are required, the sample size is estimated separately for each region/stratum and the surveys in each region/stratum are carried out independently from each other. A uniform protocol should be followed though.
**BCG immunization policy and coverage**

Tuberculin surveys to estimate ARTI have traditionally been conducted among children not immunized with BCG. However, it has now become operationally difficult to obtain an adequate sample of non-immunized children due to the high immunization coverage. In most countries of the South East Asia region, the BCG vaccine is given at birth without re-vaccination. Even though a majority of immunized children elicit low levels of tuberculin sensitivity⁶,⁷,⁸, inclusion of such children in the 0-4 year age group for tuberculin surveys may interfere with the interpretation of the survey results⁹. However, in the 5-9 year age group, inclusion of BCG immunized children have not been found to significantly influence the survey results⁶,⁸. Therefore, in areas with a high BCG immunization coverage (i.e. more than 70%), all children, irrespective of their BCG status, should be included in the study sample. In such case, the house-based surveys may be conducted in the 5-9 year age group. The younger age group is anyway excluded in school surveys. Inclusion of BCG immunized children not only eliminates concerns about the study sample being representative but also makes the survey cheaper and less time consuming.

In countries where re-immunization with BCG is practised at the time of school entrance, re-immunization may be deferred in areas selected for the survey till the survey has been completed.

In communities with a low BCG immunization coverage, the survey may preferably be conducted among children without BCG scar. In such case, the sample size would correspond to only the children without BCG scar. However, all children in the selected age group encountered during the registration process should be tuberculin tested in a given setting, irrespective of BCG scar status. Children with BCG scar are excluded at the time of analysis for estimating ARTI.

For repeat surveys, a similar study population in respect of age group and BCG scar status should be selected.

5. **MATERIAL AND METHODS**

5.1 Tuberculin

The tuberculin product, its dose, antigenicity, number of batches to be procured and maintenance of cold chain are important considerations for ensuring reliable results of a tuberculin survey.

Two tuberculin units (TUs) of PPD RT23 with tween 80 is recommended for tuberculin surveys⁶,¹⁰. However, countries with experience of using 1 TU for tuberculin surveys may continue to use the same dose¹¹-¹³.

The entire quantity of tuberculin should be procured from the same laboratory in the minimum possible number of batches. This should be done through proper planning of the survey. This is because the antigenicity and stability of PPD have been found to vary from laboratory to laboratory and even from batch to batch in the same laboratory¹⁴. The ready-for-use solutions of PPD RT23 may be procured from Statens Serum Institute, Copenhagen (SSI) or from the designated country laboratory. In some countries, PPD RT 23 with tween 80 received as dry powder from SSI is diluted in an isotonic buffer solution in a specific laboratory. PPD solutions are usually supplied as 2-ml vials, from which about 8-10 tuberculin injections can be administered.
About 75-100 sputum smear-positive cases of PTB should be tuberculin tested with each batch of PPD prior to its use in the survey. The frequency distribution of reaction sizes among the tested cases is plotted as a histogram for locating the mode. The sensitivity of the test is estimated at different cut-off levels. The modes of tuberculin reaction size in smear-positive cases have generally been found to vary between 16-20 mm and sensitivity of about 90% at 10-mm demarcation and about 75-85% at 14 mm has been observed in different studies. In the event of a notable decline in the mode of reaction sizes or sensitivity of the test with a particular batch of PPD, it should be discarded and a fresh batch ordered.

The guidelines for maintaining the cold chain during supply and transportation of tuberculin vials are given in section 5.4 (Planning & organization).

5.2. Estimation of sample size

The sample size estimation depends upon whether it is a baseline survey or a repeat survey.

5.2.1 Sample size estimation for a baseline survey

The following factors are taken into account to estimate the sample size (or the number of children to be investigated) for a baseline tuberculin survey: expected prevalence of infection, precision and design effect.

The prevalence of infection (P) observed during any earlier survey in a similar age group as included in the present survey might be considered as the expected prevalence provided no change is expected to have taken place in the intervening period. If a change is expected, then P may be estimated accordingly. In case no earlier survey has been carried out, the prevalence of infection observed in other areas with similar characteristics may be substituted for the value of P.

The precision denotes the likely difference of prevalence in study sample from the true prevalence in the study population that the investigator is prepared to accept. The bigger the sample size, the higher the precision. However, while determining the sample size, it is essential to strike a balance between precision and operational feasibility including cost. It is fair to obtain an estimate of the prevalence of infection in the study sample within 10% (relative precision) of the true prevalence. Wherever constraints of resources exist, the precision may be reduced to 15%.

The design effect greatly facilitates to carry out tuberculin testing in defined clusters (groups of children) rather than in randomly selected samples, which is operationally too difficult. Since this affects the precision, the sample size is increased by an appropriate factor called as design effect (d), in order to obtain the prevalence estimate as precise as with simple random sampling. The value of d may be estimated from the data of an earlier tuberculin survey of similar sampling design, as the ratio of cluster sample variance to the variance as if it was a simple random sample. If such data are not available, it is appropriate to take the value of design effect as '3' for tuberculin surveys using two-stage sampling for selection of clusters.

The formula used for estimating sample size is:

$$N = \frac{d (1.96)^2 (1-P)}{\varepsilon^2 P}$$

where $N$ is the estimated sample size, $d$ the design effect, $P$ the expected prevalence of infection expressed in fraction and $\varepsilon$ the relative precision. The above formula
provides the sample size for obtaining the estimate of prevalence at 5% level of significance. For example, a sample size of 9732 would be required for an expected prevalence of 7% considering a relative precision of 15% and a design effect of 3. The estimated sample size is further increased by 20% to arrive at the number of children that are to be registered during the survey. This addition is made to account for exclusions, drop outs and errors that occur during tuberculin testing or reading.

5.2.2 Sample size estimation for repeat surveys

For repeat surveys, the sample size may be estimated using the following formula\(^\text{10}\):

\[
N = 10.5 \left[ P_1 (1-P_1) + P_2 (1-P_2) \right] / (P_1-P_2)^2
\]

where \(N\) is the estimated sample size, value of \(P_1\) is the expected prevalence as estimated from the baseline survey and \(P_2\) as estimated considering the expected rate of change in the intervening period. In developing countries with improved TB control programs, 5% per annum decline in the risk of infection may be considered for this purpose.

The above formula applies to simple random sampling for 90% chance of obtaining a difference at 5% level of significance. The value 10.5 represents the squared sum of \(z\) values for two-tailed \(\alpha\) error and one-tailed \(\beta\) error. The estimated value of \(N\) is multiplied by the design effect for cluster sampling. For example, if the prevalence of infection in the first survey was 7% \((P_1)\), the expected prevalence of infection after 7 years would be 4.5% \((P_2)\), assuming a 5% per annum decline in the risk of infection. Substituting the values of \(P_1\) and \(P_2\) in the above formula and considering a design effect of 3, the estimated sample size for the repeat survey after an interval of 7 years is 8867. This is further increased by 20% to account for exclusions and possible drop outs as in the baseline survey.

5.3 Sampling technique

The purpose of sampling is to draw a representative sample of the population without any bias.

5.3.1 Stratified two-stage cluster sampling: rationale

For the design of tuberculin surveys proposed hereunder, the term *cluster* means a school in case of school-based surveys and a village or urban block in case of house-based surveys.

Stratification is intended to divide the population into distinct strata if there is a likelihood of significant difference between the strata as far the epidemiological situation of TB is concerned. Such stratification ensures a better representation of the study sample. However, the number of strata should be kept small. It has been observed in some countries that the TB situation might be significantly different between rural and urban areas. Therefore, rural–urban stratification might be desirable for tuberculin surveys. Large cities may be considered as a third stratum as per the country’s situation. Alternately, stratification may be adopted by region, e.g. northern, southern, eastern and western parts of a country; or by topography, e.g. mountainous, hilly or plain areas. However, it is best to limit stratification to only one stage of sampling, in order to keep the design simple. For example, if there is likely to be a difference in TB situation by region as well as by rural–urban areas, it is desirable to plan independent surveys in each of the regions and take up rural–urban stratification within each region.
The sampling design illustrated hereunder is for estimating ARTI for a country as a whole while considering rural–urban stratification. It may be suitably modified in other situations.

5.3.2 First stage of sampling

The first stage of sampling involves selection of districts or equivalent. The districts are selected by population proportional to size (PPS) method. The selection of districts should be carried out independently in individual strata. As a result, a different set of districts may be selected for rural and urban strata. An example of selecting districts by PPS sampling is given in Annex 1.

The number of districts to be selected within a stratum should neither be too small nor too large. Similarly, the number of children to be investigated in each district should not be too large. Thus, the number of districts is based on operational convenience and sample size and may be in the range of 5-25% of the total districts, in each of the stratum.

It is best not to exclude any districts from the sampling frame. However, in some circumstances, it may be necessary to exclude certain districts for reasons of safety of field teams, accessibility or political reasons. Any areas to be excluded should be decided before sampling and should be recorded in the survey report, since populations in such districts may be at different risks of infection.

5.3.3 Second stage of sampling

The second stage of sampling involves the selection of clusters within individual districts: schools in case of school-based surveys or villages/urban blocks in house-based surveys. Simple random sampling (SRS) is preferable since populations of children in individual clusters are often unknown. As an example, for selection of rural schools in a particular district, all primary schools in rural areas of a district are listed in a serial order and a sample selected by SRS.

To decide on the number of clusters to be selected in individual districts, refer to the following section on allocation of sample size.

5.3.4 Allocation of sample size

The estimated sample size is distributed into different strata in proportion to population size. The sample size for individual strata is further divided equally between the selected districts.

The number of clusters to be selected within individual districts depends upon the average number of children expected to be available in each cluster. Therefore, the number of clusters may vary from district to district. For example, the number of schools to be selected in a district in a rural stratum would be estimated from the sample size allocated to the district by the average number of children expected to be available during registration and tuberculin testing.

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* Towns and cities are often delineated into blocks. If not, urban areas in selected districts may be divided into blocks for the purpose of the survey in a way that approximately 100 children in the age group chosen for the survey reside in each block.
In the selected clusters, all children present on the day of testing are included. No sampling is recommended within the clusters\textsuperscript{d}.

The suggested sampling design is also presented in Figure 1.

\textbf{5.3.5 Sampling for repeat survey}

For the study of trends, repeat surveys may preferably be carried out in the same districts as in the first survey. However, within individual districts, a fresh sample of clusters has to be selected by SRS in view of the different number of clusters that should be selected due to a change in sample size and also because new clusters might have been added in the intervening period.

\textbf{5.4 Planning and organization}

The survey should be planned and conducted under the technical and managerial support of a central coordinating institution having experience in field studies. The coordinating centre has the overall responsibility of planning and organizing the survey.

\textbf{5.4.1 Collection of data}

The following information needs to be collected to decide on study population, sample size and to undertake sampling:

\checkmark from the education department: proportion of children enrolled in the first grade of primary schools;

\textsuperscript{d} In case of school surveys, all children in first grade are included, since it may not be easy to convince the school authorities to take a sample of children. Similarly, if all available children in a given village are not included, eliciting community participation shall be difficult, especially during the repeat survey.
from the office of the Expanded Programme of Immunization (EPI): data on BCG coverage and age at time of immunization; it is desirable, though, to carry out a small-scale survey in the chosen study population to get an approximate figure on the proportion of children with BCG scar;

from reports or publication in literature: previous data on prevalence of infection;

from the census office: population of all districts;

for school-based survey, from the education department: complete list of schools by location (rural/urban) in the selected districts, with the average number of children attending the first grade;

for house-based survey, from the census office or district administration: list of villages/urban blocks in the selected districts, with average population of the children in the age group chosen for the survey.

5.4.2 Preparation of protocol and work instructions

A protocol has to be prepared on the lines as suggested in this document. It should include the objectives, the study population, the estimated sample size, the sampling technique, dose and type of tuberculin and the skills of tuberculin testers and readers to be employed for fieldwork.

The work instructions given in Annex 2 may be modified according to local needs. The field teams are provided with copies of the work instructions to be followed during the field work; as well as with guidelines regarding when to suspect TB in children for their referral to the nearest health centre for medical assistance.

5.4.3 Information to concerned authorities

All relevant authorities (health department, education department, district administration) at national and regional/state/provincial levels and in the selected districts should be informed of the purpose of the survey and field procedures to be undertaken. The authorities in the selected districts have to be intimated well in advance about the time schedule and provided with a copy of the protocol. Their support in organizing the field work should be sought. They should be informed on the logistic requirements, including vehicles, accommodation for field teams, refrigerator for storing PPD vials, district maps showing location of areas and local health personnel for field support.

5.4.4 Procurement and supply management

Major procurement should be done centrally. The following materials need to be procured:

- Tuberculin vials and disposable tuberculin syringes: Supply order must be given well in advance with clear instructions about the numbers and time when required. This is to ensure the usage within the expiry period. The quantity required would depend upon the sample size and requirement for training. It has been the experience that about 8-10 injections can be administered from a 2-ml vial.

- Vaccine carriers for transportation of tuberculin, from EPI authorities.
✓ Printed study forms. Forms for information to general population/parents of children should be printed in the local language. Examples of all forms are provided in Annex 3.

✓ Drugs required for the management of children suspected to be suffering from TB and referred by field teams, in selected districts.

The supplies are delivered to the selected districts prior to starting the field work. A complete list of materials and equipment required for the survey is given in Annex 5.

The cold chain has to be maintained during supply and transportation of tuberculin vials from the point of manufacture to the point of usage in the field. During storage, PPD vials should be refrigerated at 2-8 ºC and not allowed to freeze. The vials may preferably be airlifted to the survey districts wherever facilities are available. Vaccine carriers should be used to transport tuberculin vials, which must be used within the expiry period as specified. During the field work, maximum care should be taken to protect the tuberculin from heat and sunlight and vials once opened must be used on the same day or at latest on the following day.

One field team is required for each of the strata. If the entire country is divided into two strata, then two teams are recruited. If independent surveys are planned in each region, then separate teams are recruited for each region and stratum. A complete list of required staff is given in Annex 5.

The field workers are trained intensively on all tasks involved in the survey, especially tuberculin testing and reading. They are evaluated before deploying in the survey. This is to maintain standardized levels of working procedures considering the sensitive nature of tuberculin test. An example of a training protocol for this purpose is given in Annex 4.

5.4.5 Ethical clearance

Ethical clearance for the survey should be obtained from a designated ethical review committee in conformity with the laws and regulations of the country.

5.4.6 Pilot Study

A pilot study is carried out under the watchful eye of the central office to streamline the field procedures and to test the study forms. Each constituted field team should carry out a pilot study with strict adherence to the work instructions. It should be conducted in 2-3 clusters different from those selected for the main survey, in each of the strata.

5.4.7 Advance Planning visit to Districts

A team leader with a letter of introduction should make an advance visit to each survey district to oversee the arrangements and apprise personally all relevant authorities. The team leader will seek cooperation for support in field work, managing children referred by field teams and for any practical problems that may be encountered during the field work. Further details are given under work instructions in Annex 2.

5.4.8 Time frame

A period of 3-4 months should be set aside for initial planning—collection of necessary data, writing protocol and work instructions, sampling of clusters, recruitment of staff,
procurement of supplies and for obtaining ethical clearance. Another two months would be required for training the field teams and conducting the pilot study.

The time required for the field work depends on the sample size, the average number of children available in each cluster and local conditions such as distances and transportation. Each team may plan to complete one cluster per day during the field work. In larger clusters, up to 100 children can be tested each day. The time required in individual districts may be estimated accordingly while giving an additional week for initial planning and settling down and for rounding off the camp. School holidays and examination days, major festivals and difficulties in travelling during rainy season or winter should be kept in mind while preparing the time schedule for field work. It should be attempted to complete the entire field work over the period of one year.

Data entry should be undertaken simultaneously while allowing extra 2-3 months for completion. The data analysis and report writing would require three more months.

Therefore, the total period required for the entire survey will be approximately two years.

5.5 Field procedures

The field work comprises of four major activities:

1. planning of field activities;
2. registration of eligible children;
3. tuberculin testing; and
4. reading of tuberculin reactions.

The field work is carried out by well trained field teams. Each cluster is visited three times on different days: (i) planning on the first visit; (ii) registration and testing during the second visit; and (iii) reading on third visit.

5.5.1 Planning of field activities

On arrival in each district, a tentative district work plan (Annex 3, Form 1) is prepared indicating the dates of planning, registration and testing and reading in each cluster. The fieldwork should be planned in a way that the selected clusters are covered in a contiguous fashion. Field work for house-based surveys should be undertaken in the late afternoon or early evening hours when most children are back from school. However, testing and reading should be done under adequate light.

The district health administration is requested to issue a circular to the centres providing health services in the selected clusters urging them to depute the area health workers to the respective clusters on the days of testing and reading. In case of school-based surveys, the education department is also requested to issue a circular letter to the heads of schools for their cooperation with the field teams. The team leader, in consultation with district health authorities, should decide the health centres where to refer children suspected of tuberculosis. A circular to this effect may also be issued by the district administration.

The team members should carry with them the district maps for locating clusters, copies of the circulars issued by district authorities and the work instructions for field teams.
A planning visit is undertaken to each cluster 2-3 days prior to the testing day. In school-based surveys, the support and consent of the school head is solicited during the visit. The pamphlets containing information to parents is sent through children (Annex 3, Form 3A). For house-based surveys, a rough sketch of the cluster is prepared in consultation with local people showing all lanes and by-lanes with the approximate number of houses in each. The support of the local health centre, opinion leaders and social workers is solicited. For guidance of the testing and reading teams, a planning sheet (Annex 3, Form 2) is prepared outlining the distance and travel route to the cluster along with names of heads of school/community leaders to be contacted during fieldwork.

5.5.2 Registration of eligible children

In school-based surveys, a list of children enrolled in the first grade is obtained from the attendance register and entered in the school survey registration form (Annex 3, Form 4A).

For registration of eligible children in house-based surveys, the enumerator visits every household starting from one end of the first lane in the cluster and covering all houses in a contiguous manner. All households in the cluster irrespective of the presence of eligible children are numbered serially.

In each household, parents/guardians are explained the purpose of the survey and that the test is given in the skin using a disposable syringe. They are informed that the test is safe though there may be slight irritation at the test site. They are further told that in case the child is found to be infected with TB bacilli and there are other reasons for suspicion of TB, then the child shall be further investigated free of cost at the designated health center and treated appropriately (Annex 3, Form 3B). The eligible children in each household along with their age in completed years are entered in the household registration form (Annex 3, Form 4B). The parents/guardians are guided to bring all eligible children to the temporary testing centre set up in the cluster.

At the testing centre a child card (Annex 3, Form 5) is opened for each child to record identification particulars, age, BCG scar status and results of tuberculin testing and reading. Date of birth or age is obtained from parents/guardians and in case of doubt verified with documents where available. Care must be taken to record the age as accurate as possible.

5.5.3 Tuberculin testing

A testing centre is temporarily set up under shade at a suitable place in the cluster.

In house-based surveys, informed consent is obtained from the parent/guardian before administering the test. In school-based surveys, information to parents has already been sent during the planning day through children. Consent is assumed to have been given in case no objection is received from the parent.

Children with any of the following conditions should be excluded from tuberculin testing: severe malnutrition, history of skin rash in the recent past, high fever, known immunocompromised condition, presently on anti-TB treatment or refusal by parents/guardians.

The co-tester inspects the upper third of both arms for a pea sized hypopigmented shiny scar produced by BCG immunization. The presence or absence of a
BCG scar is recorded in the child card. If a scar is present but does not have the characteristics of a BCG scar, it is recorded as doubtful.

The tester administers exactly 0.1 ml of tuberculin intra-dermally (Mantoux technique) on the mid-anterior aspect of the left forearm. The left forearm is chosen by convention to avoid error in locating the test site during reading. However, in case of injury or scar on left forearm, the test is given on right forearm and a note is made in remarks column of child card. Similarly, the anterior side is preferred since in people with dark complexion, the test site may be difficult to locate if given on dorsal side.

The injections are given with 1-ml disposable tuberculin syringes with graduations of one-tenth of a mm, fitted with a 26-gauge needle of 1 cm length and 20° bevel. The needlepoint is inserted with the bevel upward in the superficial layer of the skin of the forearm while the skin is slightly stretched in the direction of the needle. The syringe is held by the barrel; the plunger should not be touched until the needlepoint has been satisfactorily inserted. A volume of 0.1 ml is slowly injected and the finger is removed from the end of the plunger before the needle is withdrawn.

The co-tester observes each test and records the test as satisfactory if it raises a flat pale wheal with clearly visible pits and well demarcated borders. It is recorded as unsatisfactory in case of leakage or if it is a subcutaneous injection as shown by a less anaemic dome-shaped papule rather than a flat pale wheal.

5.5.4 Reading of tuberculin reactions

The reading of tuberculin reaction is undertaken about 72 hours after administration of the test. All efforts should be made to read the reactions at about 72 hours and only in case of utmost exigency it may be undertaken at any other time between 48-72 hours.

In house-based surveys, reading is undertaken by visiting children in their houses.

While reading the reactions, the BCG scar status of the child should not be known to the reader. Therefore, all child cards should be in possession of the co-reader who verifies the identity of each tested child before the reader reads the reactions.

The reader identifies the margins of induration by carefully palpating the edges of the reaction. The induration may be easily recognizable when firm and well circumscribed or it may be a soft ill-defined swelling in which case its margins must be identified very carefully. The maximum transverse diameter of the induration is then measured in millimeters, using a transparent ruler. A small ruler of 10-15 cm length and calibrated in mm should be used for this purpose. Care is taken not to measure the erythema. The reader also examines the test site for presence of bullae, vesicles, necrosis or lymphangitis. The reader dictates his observations to co-reader who records them on the child card.

5.5.5 Maintaining standard of field work

An important aspect of the survey is to maintain high levels in carrying out all tasks as per the protocol and work instructions, without any deviation.

All efforts should be made to test-read 80-90% of the registered children.

During festivals, school attendance may be low on the day of testing. In such case, another day of testing may be planned. Similarly, if the proportion of tested
children available for reading is low, then home visits may be undertaken to supplement the coverage.

Sometimes, a particular cluster may not be approachable for some reason. In such circumstance, it may be substituted by the nearest cluster (school/village/urban block) in either direction.

The team leader should ensure satisfactory and smooth conduct of the field work and verify all forms for completeness and correctness.

The principal investigator and co-investigators should closely supervise the fieldwork.

5.5.6 Referral of children

For children having reactions ≥ 10 mm, parents are enquired about the current health status of the child especially for presence of chest symptoms, loss/failure to gain weight, history of anti-TB treatment and history of contact with a known case of TB. Children with any of these are referred to the designated health centre for further investigations and medical assistance.

5.5.7 District summary report

A cluster summary (Annex 3, Form 7) is prepared on completion of fieldwork in each cluster. These are consolidated into a district summary (Annex 3, Form 8). In case the total number of children registered in the district is below the target, the required number of additional clusters are selected by SRS.

5.5.8 Dispatch of study forms

All forms are filled in duplicate and originals are dispatched to the central office.

5.5.9 Storage of tuberculin

The tuberculin vials are stored between 2-8°C and carried to the field in vaccine carriers or thermos flasks. Maximum care is taken to protect tuberculin from heat and sunlight. The vials once opened should be used on the same day or latest by next day.

5.5.10 Tuberculin testing of smear-positive cases

About 75-100 smear-positive cases of pulmonary TB should be tuberculin tested with each batch of tuberculin used. This may be done at central level and the data may be entered in a single form (Annex 3, Form 9). The known HIV-positive cases should be excluded for this purpose.

5.6 Ethical Considerations

In school-based surveys, consent of the school authorities should be obtained after acquainting them with the purpose of the survey and characteristics of the tuberculin test. A meeting with the parents may be arranged to acquaint them with the purpose of the survey, nature of tuberculin test and to obtain their informed consent. Alternately, printed information (Annex 3, Form 3A) may be provided to parents 2-3 days before
testing. In case no objection is received from the parents/guardians by the testing day, consent may be assumed as given.

In house-based surveys, parents/guardians should be explained the purpose of the survey and the nature of the test through inter-personal interaction (Annex 3, Form 3B). Their informed consent is obtained before administering the test.

No child is forced to participate.

All reagents and procedures used in the survey have been used widely and serious adverse reactions have been reported to be extremely rare at the rate of about one per million doses dispensed\textsuperscript{21}. However, in case of any such eventuality children should be rushed to designated medical facility.

Children with suspicion of tuberculosis should be referred to the designated health center for further investigations and treatment if required.

5.7 Analysis

The data are entered twice into the computer and validated to avoid any errors.

Children whose tuberculin tests were administered unsatisfactorily and outliers in terms of age are excluded at the time of analysis.

5.7.1 Setting the criteria for estimation of prevalence of infection

The criteria for estimating the prevalence of infection are determined from the frequency distributions of reaction sizes.

Within a given stratum, data obtained in different districts are pooled together. Frequency distribution graphs should be generated separately for different strata, with reaction size on X-axis and percentage of reactors on Y-axis.

If the survey was designed to estimate ARTI only among children without BCG scar, than frequency distributions of reaction sizes and graphs are generated only for such children. Three types of graphs may be obtained; the methodology for estimating prevalence of infection in each situation is described below\textsuperscript{22}

\begin{enumerate}
\item The graph illustrated in Figure 2 is a bi-modal graph showing two distinct modes and an easily identifiable anti-mode. The reactions to the right of the anti-mode represent the subgroup of children who are infected with tubercle bacilli. The reactions to the left of the anti-mode represent those who are either uninfected or elicit non-specific tuberculin sensitivity due to infection with environmental mycobacteria. The two subgroups are clearly demarcated at the anti-mode. Such graphs are observed in areas with low prevalence of non-specific tuberculin sensitivity. In this situation, the prevalence of infection is estimated considering all reactions greater than the anti-mode as specific for tuberculous infection. The estimation may also be undertaken by mirror–image technique as further explained. 

\textbf{Fig 2: Frequency of reaction sizes among children}

\textit{Bi-modal distribution with anti-mode}
\end{enumerate}
(ii) The graph shown in Figure 3 is also a bi-modal graph with two distinct modes but the anti-mode cannot be clearly identified. Distributions of this kind are observed in areas with a moderate prevalence of non-specific sensitivity, which obscures a clear separation of reactions due to infection with tubercle bacilli from others. In these situations, the prevalence of infection is estimated using mirror–image technique. In this technique, the proportions of the reactions larger than the second mode is doubled and added to the frequency at the second mode\textsuperscript{22,23}. The rationale of this approach is that tuberculin reactions due to infection with tubercle bacilli are distributed normally around the mode.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{bi-modal_distribution.png}
\caption{Frequency of reaction sizes among children
Bi-modal distribution without clearly identified anti-mode}
\end{figure}

(iii) In the third type of graph (Figure 4), neither the anti-mode nor the mode on the right side of the distribution are clearly distinguishable. Such distributions are obtained in areas with a very high prevalence of non-specific sensitivity and/or a low prevalence of infection with tubercle bacilli. In these circumstances, the mode of reaction sizes observed among smear-positive cases is used for estimating the prevalence of infection by mirror–image technique. The rationale of this approach is that the position and shape of tuberculin reactions due to tuberculous infections has been found to be similar to distribution of
tuberculous reactions in sputum smear-positive TB cases\textsuperscript{4,15-17,24,25}. The frequency distribution of tuberculin reaction sizes in such cases is invariably uni-modal. An example is given in Figure 5. The mode in these cases has been found to vary between 16-20 mm in different countries and is usually the same or thereabout as the mode of tuberculous reactions in children\textsuperscript{4,15-17}.

If the survey was designed to estimate ARTI among children regardless of their BCG scar status, three frequency distributions are plotted as follows: one for children without BCG scar, one for children with BCG scar and another for the combined group of children. A clear anti-mode is usually not seen either among children with BCG scar or in the combined group, due to higher proportion of intermediate sized reactions. However, the second mode can usually be recognized at the same reaction size in the three distributions. Therefore, the prevalence of infection for the overall study group is estimated using \textit{mirror–image} technique. In case the second mode is not clearly distinguishable, the same mode as observed in children without BCG scar may be used for estimating prevalence in the entire study group. Otherwise, the mode observed in smear-positive cases is used as above. The rationale is that the influence
of BCG-induced tuberculin sensitivity on estimate of prevalence of infection is neutralized if the estimation is done using mirror–image technique. This has been demonstrated in some studies.

5.7.2 Smoothening of data

Some readers have propensity to measure reactions in even numbers and some others may have preference for digits ending with zero or 5. This phenomenon is termed as digit preference. In spite of proper training in reading of tuberculin reactions, some amount of digit preference may still be present. To reduce its influence on the estimate of prevalence of infection, smoothening of the data may be undertaken. This involves calculating the expected frequency at each mm of induration as the average of five frequencies including two before and two after the induration of interest. Further calculations of the prevalence of infection should be based on this new frequency distribution.

5.7.3 Estimating prevalence

Using the anti-mode/mode decided as above and after smoothening of the data, the number of infected children is estimated separately for each stratum. It may be noted that the anti-mode/mode may at times vary to some extent between different strata.

The prevalence of infection is estimated on adding the number of infected children in different strata, dividing this number by the total number of children analyzed and multiplying with 100.

The 95% confidence interval (CI) of the estimate is obtained using the following formula.

\[
CI = p \pm 1.96 \sqrt{\frac{\sum \sum (P_u - P_s)^2}{k(k-s)}}
\]

where \( p \) is the estimated prevalence in the entire sample; \( P_u \) the proportion infected in cluster \( i \) of stratum \( s \); \( P_s \) is the proportion infected in stratum \( s \); \( k \) is the total number of clusters; and \( s \) is the number of strata.

5.7.4 Sensitivity analysis

In case the prevalence of infection has been estimated by cut-off point (anti-mode) method, it is desirable to vary the cut-off point by 1 to 2 mm on either side. The results may be expressed as a range of estimates. A similar analysis is not advisable for mirror–image technique since the estimates may change to a remarkable extent on moving even by 1 mm to either side, due to the doubling effect.

5.7.5 Estimating Annual risk of infection (ARTI)

The estimated prevalence of infection represents the cumulative risk of infection experienced by the study sample from birth to the time of survey. Thus, ARTI is computed from the estimated prevalence applying the following formula.

* Since the sample size is allocated to different strata in proportion to their population size, no weights are given to stratum estimates.
where \( R \) is the annual risk of infection, \( P \) the estimated prevalence of infection and \( a \) the mean age of children. The mean age is calculated after adding 0.5 years to the age in completed years. Since \( P \) is expressed as a fraction in the above formula, multiply the value of estimated \( R \) by 100 to obtain the annual risk in percentage.

As stated above, the estimated ARTI is the average of the annual risks of infection experienced by the study sample from birth to the time of survey. However, this risk may not have been constant over the period. Thus, it is assumed that for a decreasing or increasing risk of infection, the estimated ARTI would correspond most closely to the mid-point of the average lives of the individuals included in the study sample. This mid-point is estimated on dividing the mean age by 2 and subtracting from the year of the survey. For example, if the survey were to be conducted in the year 2006 among school children 6 years of age, the estimated ARTI would correspond to the year 2003.

### 5.7.6 Estimating trends in risk of infection

The average per annum change in ARTI may be computed from the following expression:

\[
1 - (R_2 / R_1)^{1/T}
\]

where \( R_1 \) and \( R_2 \) are the ARTI estimates at the first and second surveys and \( T \) is the interval in years between the two surveys.

Note that a mixture analysis of tuberculin survey data may be carried out if the required statistical expertise is available.

### 6. INTERPRETATION OF ARTI ESTIMATES

The survey results provide information on the epidemiological situation of TB. The estimated ARTI indicates the extent of transmission of tuberculosis infection in the community, from infectious cases to the age group involved in the survey.

The observed level of risk of infection in a community is the cumulative result of incidence of infectious cases, duration of their infectiousness and interaction between cases and susceptible individuals. Since the duration of the infectiousness depends upon case finding and treatment, ARTI estimates also reflect on the efficiency of tuberculosis control programmes.

A relationship had earlier been derived between the annual risk of tuberculosis infection and the incidence of smear-positive PTB, according to which every one percent of ARTI estimated in children corresponds to an incidence of 50 sputum smear-positive cases of PTB per 100,000 population. However, this relationship was derived from observational studies in the pre-chemotherapy (and pre-HIV) era and thus should be applied with caution. For example, the mean duration of infectiousness of the cases is reduced in a well-performing DOTS programme and thus a larger number of incident cases would be needed to infect one percent of the population. Similarly, in situations where a significant proportion of TB cases is attributed to HIV, a higher case fatality among such cases reduces the average duration of infectiousness. However, with an increase in urbanization, population density and mobility, there is an
increased opportunity of transmitting infection that has an opposite effect on the above ratio.

The level of ARTI also determines the total pool of infected people in the community. Therefore, ARTI estimates give an idea of the extent to which new cases are likely to emerge in the years to come.

While interpreting results, it should be kept in mind that ARTI estimated among children might not be applicable to all age groups as the risk of infection has been observed to be age dependent. This has been demonstrated in some studies\textsuperscript{30,31}.

\textit{Interpretation of trends}

In a well functioning tuberculosis control programme with high levels of case-detection and cure of smear-positive cases, the risk of infection is expected to decline. The extent of annual decline in ARTI rates observed from repeat tuberculin surveys reflects on the programme efficiency. A declining trend also implies that more infected cohorts of individuals are being gradually replaced by the lesser infected ones. This would ultimately lead to a decline in incidence of TB cases.

An increasing trend in ARTI points towards poor performance of control programmes and/or epidemiological factors such as an increase in HIV seroprevalence in the community leading to a higher incidence of TB.

The observed estimates of ARTI and its trends should stimulate a review of the existing TB control policies as well as undertake appropriate actions for further strengthening of control activities.

7. \textbf{STRENGTHS AND LIMITATIONS}

The guidelines with respect to planning and organization, sampling, field procedures and analysis of tuberculin surveys presented have been formulated drawing from extensive experience while keeping the emphasis on practicability as well as scientific accuracy.

In areas with a high BCG immunization coverage, it has been suggested to conduct the surveys among children between 5-9 years, regardless of their BCG scar status. The influence of BCG-induced sensitivity on ARTI estimates is minimal in this age group when the estimation is done by \textit{mirror–image} technique\textsuperscript{6,10}. Inclusion of immunized children also augments the representativeness of the sample. At the same time the survey becomes cheaper as well as less time consuming.

The suggested sampling design is simple. It does not require knowledge of the population size of each cluster. Within individual clusters, sampling of children is not required making it easier for the field teams. Because of the self-weighing sampling design, analysis is also easier.

An unavoidable limitation of tuberculin surveys in children is that the results do not provide information on the risk of infection in other age groups. However, surveys in children make the study of trends in ARTI possible through repeat surveys.

Exclusion of certain areas from the sampling frame when unavoidable introduces a bias since these areas may be at different risks of infection. A similar influence is exerted by non-response, which should therefore be brought to a minimum by all efforts.
In some individuals, tuberculin sensitivity may wane with time. This may result in an underestimation of the prevalence of infection. However, its influence is expected to be minimal in children.

A possible limitation could be due to a proportion of the children without BCG scar actually having been immunized. This may happen due to either non-development of the scar after immunization or disappearance of the scar in later years. If the survey is designed to estimate ARTI exclusively among children without BCG scar, the small proportion of BCG-induced reactions that are larger than the anti-mode may lead to some overestimation of ARTI using the cut-off point method. However, its effect is neutralized when the estimation is done using mirror-image technique. This phenomenon is of no consequence when the survey has been designed to estimate ARTI among children of 5-9 years regardless of their BCG status.

Lastly, tuberculin sensitivity may be suppressed in a proportion of the children infected with HIV. However, the low prevalence of HIV among children in most countries of the WHO South-East Asia Region is unlikely to influence the ARTI estimates to any significant level.

8. PREPARATION OF SURVEY REPORT

A detailed report of the survey may be prepared for submission to the administrative and funding authorities as well as for dissemination of results. A broad outline should include the following sections: title, abstract, introduction, objectives, study population, methods, results and discussion.

The title of the report should be concise and at the same should reflect the objectives of the survey and the area where the survey was carried out.

The introduction to the survey report should include a brief review of the available information on the epidemiological situation of TB in the country including earlier estimates of the prevalence and risk of infection. Brief information on TB control activities forms part of the introduction. Broad aims of the study should be stated in this section.

The objectives for which the survey is planned should be stated clearly: baseline survey or a study of trends; to obtain a single estimate for an entire country or to independent estimates for different regions or strata.

The study population includes a description of the age group selected for the survey, whether it was a school or house-based survey and whether the survey was designed for estimating ARTI exclusively among children without BCG scar or regardless of BCG scar status. The rationale for choosing the study population should also be given.

The methods section of the report may be divided into three sub-sections: sampling, field procedures and statistical methods.

The sampling design and parameters considered for estimating the sample size should be sufficiently documented. The list of areas excluded from the sampling frame and reasons thereof should be documented.

Field procedures. The time period during which the field work is undertaken, the study area and organization of field work should be presented. Procedures of training the field workers and their evaluation should form part of the report. The dose and type of tuberculin used and tuberculin testing and reading techniques should be
adequately illustrated. Criteria adopted for excluding children from tuberculin testing, if any, should also be provided.

**Statistical methods.** Software used for data entry, methods of estimation and applied formulae and statistical tests are described in this section.

**Presentation of Results.** The response rate in terms of number of children test read satisfactorily out of those registered and the proportion of children with BCG scar is documented. The frequency distributions of reaction sizes are presented as histograms for different strata, separately for children without BCG scar, with BCG scar and for the combined group. The cut-off point and/or the mode used for estimating the prevalence are elaborated. The digit preference observed, if any, and the procedure adopted for overcoming the same should be described. The frequency distribution of reaction sizes among sputum smear-positive cases is also plotted. The estimates of the prevalence of infection and computed ARTI are presented as per the objectives. If repeat surveys were carried out, the estimated trend in the risk of infection, and whether statistically significant is provided.

The discussion section includes the interpretation of ARTI estimates and a comparison with any change from previous epidemiological status of TB. Information on BCG immunization policy, the performance of TB control programme and data on the HIV situation in the setting should form part of this section. Influence of possible limitations on survey results is discussed, such as exclusion of certain areas from the sampling frame, non-response, disappearance of BCG scar in a proportion of immunized children. This section should conclude with the implications of the survey results on the TB control activities.

The survey abstract gives in brief the objectives, study population, sampling, field procedures and main results with their implications. It should be prepared after writing the full report. It is placed at the beginning of the report document.

9. REFERENCES


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Annex 1

Sampling of districts by PPS method

In order to select districts, all districts are listed in an alphabetical order with their name in the first column and their population size in second column. A third column is added for cumulative population.

The sampling interval is then calculated by dividing the cumulative population of all districts by the desired number of districts to be selected. The first district is selected by randomly choosing a number between zero and the value of the sampling interval. The value of the sampling interval is then added systematically to this randomly selected number for the selection of remaining districts. With this method, a district may occasionally be selected more than once. In such case, a district may be divided into two parts, each of which may be considered as a separate district.

An example of PPS sampling is given in Table 1.

Table 1: example of PPS sampling

<table>
<thead>
<tr>
<th>District Name</th>
<th>District Population*</th>
<th>Cumulative population</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>245 000</td>
<td>245 000</td>
</tr>
<tr>
<td>B</td>
<td>64 000</td>
<td>309 000</td>
</tr>
<tr>
<td>C</td>
<td>640 000</td>
<td>949 000</td>
</tr>
<tr>
<td>D</td>
<td>125 000</td>
<td>1 074 000</td>
</tr>
<tr>
<td>E</td>
<td>72 500</td>
<td>1 146 500</td>
</tr>
<tr>
<td>F</td>
<td>234 500</td>
<td>1 381 000</td>
</tr>
<tr>
<td>G</td>
<td>386 000</td>
<td>1 767 000</td>
</tr>
<tr>
<td>H</td>
<td>45 000</td>
<td>1 812 000</td>
</tr>
<tr>
<td>I</td>
<td>190 000</td>
<td>2 002 000</td>
</tr>
<tr>
<td>J</td>
<td>74 500</td>
<td>2 076 500</td>
</tr>
<tr>
<td>K</td>
<td>100 000</td>
<td>3 076 500</td>
</tr>
<tr>
<td>L</td>
<td>70 500</td>
<td>3 147 000</td>
</tr>
</tbody>
</table>

* Since selection of districts is undertaken independently for the rural and urban strata, the district population should correspond to the rural/urban population as the case may be.

If three districts are to be selected, the sampling interval would be 3 147 000 ÷ 3 = 1 049 000. Therefore, a random number is selected between zero and 1 049 000, e.g. 242 200. Thus the first selected district is district A. For the next district, 1 049 000 is added to 242 200 which equals to 1 291 200, which falls in the cumulative population of district F. The sampling interval is added again to this number to select the third district that would be district I.
Annex 2

Work instructions for field teams

A. District level planning and organization

Prior to field work in a given district, the team leader should make an advance planning visit. During this visit, the team leader meets all relevant authorities and apprises them personally on the purpose of the survey, the time period during which the field work shall be carried out in the district and field procedures to be undertaken. Co-operation is sought for support in field work including accommodation of the field team, storage of tuberculin and transport. The list of schools/villages/urban blocks selected for the field work is provided to the district officials. The district health authorities and/or education department is urged to issue a circular to the respective health centres and heads of schools for extending cooperation to the field team.

The field team should carry with them all necessary requirements: study forms, tuberculin vials, tuberculin syringes, vaccine carriers, rulers, ink markers and the list of selected clusters. The entire list of primary schools/villages/urban blocks should also be available with the team leader since additional clusters may have to be selected in case of a shortfall in the number of tuberculin test read children. A complete list of material and equipments is provided in Annex 5

The team leader prepares in consultation with local health workers a tentative district work plan (Annex 3, Form 1) with the dates of planning, testing and reading for each selected cluster. The field work should be planned to proceed in the selected clusters in a contiguous manner. The planning, testing and reading activities proceed simultaneously in three different clusters each day.

District maps are provided to the field teams for easy location of the selected clusters. The field team should carry along with them a copy of the circular issued by the district authorities.

The health centres where children who require further medical assistance may be referred by the field team are decided in consultation with the district health authorities. District officials also ensure necessary supplies including drugs to these health centres.

Staff

A field team consists of eight members who have been well trained and evaluated on all tasks to carry out the fieldwork:

1. team leader
2. planner
3. enumerator
4. tuberculin tester
5. tester secretary
6. tuberculin reader
7. reader secretary
8. (leave vacancy)
One health worker from the district may be deputed during the course of the field work to accompany the field team for liaison with local authorities and eliciting participation of the community.

B. Field work

The field work in selected clusters comprises of four major activities:

1. Planning of field activities
2. Registration of eligible children
3. Tuberculin testing
4. Reading of tuberculin reactions.

Each cluster is visited three times on different days: planning on the first visit followed by registration and testing on the second visit and reading on the third visit.

The field work for house-based surveys should be carried out in late afternoon or early evening hours when most children would be back from school. However, testing and reading should be performed under sufficient light.

B.1 Planning visit to clusters

The planner undertakes a planning visit to each selected cluster 2-3 days prior to the date of testing in order to solicit the support and consent of school authorities. The support of community leaders is solicited in case of a house-based survey. They are informed of the dates of testing and reading. A visit is also undertaken to the local health centre and the concerned health worker of the area is requested to assist the field team on the days of tuberculin testing and reading.

For guidance of the testing and reading teams, a cluster planning sheet (Annex 3, Form 2) is filled in. For house-based surveys, a rough sketch of the cluster showing different lanes and by-lanes with the approximate number of houses in each area is drawn on the reverse side of the form, in consultation with local people.

Information to parents (Annex 3, Form 3A) is sent through children in case of school based surveys. An Appeal form to residents (Annex 3, Form 3B) is freely distributed to the community leaders and residents in case of a house-based survey.

B.2 Registration of eligible children

In school-based surveys, the list of children enrolled in the first grade is obtained from the attendance register, and entered in school registration form (Annex 3, Form 4A).

In house-based surveys, the registration of eligible children is undertaken by house-to-house visits. The enumerator visits every household starting from one end of the first lane covering all the houses in the cluster in a continuous manner for registration of eligible children. A number is assigned to each household in the cluster, irrespective of the presence of eligible children. This number consists of the lane number written in Roman followed by a slash and the serial number of the household in that lane written in numerals (e.g. IV/12). The parents/guardians are explained the purpose of the survey and are informed that the test is given in the skin using a disposable syringe. They are explained that the test is safe though slight irritation may occur at the test site. They are also told that in case the child is found to be infected with TB and there are reasons for suspicion of TB disease, the child shall be further
investigated free of cost at the designated health center and provided treatment, if required. The relevant details including household number, number of eligible children along with their age are filled up in the household registration form (Annex 3, Form 4B). The parents/guardians are guided to bring the eligible children to the testing centre that has been temporarily set up within the cluster. If the child is not available at home, the time of his/her availability may be recorded in the remarks column.

### B.3 Procedures at the testing centre and tuberculin testing

The testing centre is set up under the shade at a convenient place in the cluster. An individual child card (Annex 3, Form 5) is opened for each eligible child available for testing. The items 1 to 11 are filled by the enumerator in case of a school-based survey and by the tester secretary in case of a house-based survey.

Each child is examined by the tester secretary on the upper third of both arms for presence/absence of a BCG scar – a pea-size (2 to 3 mm) hypopigmented shiny lesion raised above the skin. If a scar is present but does not have the characteristics of a BCG scar, it is recorded as *doubtful*. The result is entered at item 12 in Form-5.

The verbal consent of the parent/guardian is obtained before administering the test. In case of school-based surveys, written information has already been sent to the parents through children on the planning day and consent is assumed given in case no objection has been received by the day of testing.

Children with a history of skin rash in the recent past, suffering from high fever or receiving anti-TB treatment or children whose parents/guardians did not agree for the test are excluded.

**Tuberculin testing**

It is not required to sterilize the skin before injection. Children may be instructed to wash their forearms and allowed to dry before tuberculin testing.

The tuberculin tester administers 0.1 ml of tuberculin intra-dermally on the mid-anterior aspect of left forearm. In case of injury or scar on left forearm, the test may be given on the right arm. This should be recorded in the remarks column of Form 5.

The injection is given using a disposable 1-ml tuberculin syringe to which a needle of 26 gauge, 1-cm length and 20º bevel is attached. The needle point is inserted lengthwise with the bevel upward in superficial layer of the skin of the forearm while the skin is slightly stretched in the direction of the needle. The syringe is held by the barrel only and the plunger is not touched until the needle point has been satisfactorily inserted. A volume of 0.1 ml is slowly injected and the finger is removed from the end of the plunger before the needle is withdrawn.

The administration of each test is observed by the tester secretary. The testing status is recorded as follows:

- **S** (satisfactory): A satisfactory test is characterized by a flat pale wheal about 5 to 6 mm above the skin level with clearly visible pits and well-demarcated borders. The test should be strictly intra-dermal without any leakage of tuberculin;

- **U** (unsatisfactory): If there is leakage of tuberculin or the test has been given subcutaneously without formation of the wheal as described above.

The items 13 to 15 and 20 of Form-5 are filled by the tester-secretary.
All children should be observed for at least 15 minutes to monitor for any of the rarely observed serious adverse reactions. In case of symptoms such as difficulty in breathing, cold clammy skin or fall in pulse rate, the child should be rushed to the designated medical facility.

At the end of the day, all forms and cards are checked again for correctness and completeness of all entries before leaving the cluster.

**B.3 Reading of tuberculin reactions**

Reading of reactions is done about 72 hours after administering the test. It may be undertaken anytime between 48 to 96 hours in case of exigencies.

The reader secretary collects all child cards from the testing team. In house-based surveys, reading should be done by visiting each child in the home and under sufficient light. The reader-secretary verifies the identity of tested child. The BCG scar status of the child should not be revealed to the reader while reading the reaction.

The reader locates the testing spot by slowly palpating the edges of induration. The induration is easily recognizable when firm and well circumscribed. In case of soft ill-defined swelling, the margins are required to be identified very carefully. The maximum transverse (perpendicular to long axis of the forearm) diameter of induration is measured in millimeters using a transparent ruler. For marking the transverse edges of induration, a ballpoint is drawn from a point 5-10 mm away from the margin of induration towards the induration until a resistance is felt to the movement. The procedure is repeated on the opposite side. If there is no induration, “0” is recorded. Care should be taken not to measure the erythema.

The reader also examines for the presence of complications such as bullae, vesicles, necrosis or lymphangitis. The reader dictates all his observations to the reader secretary who notes them under items 16-19 in Form 5. For those tested children whose reactions have not been read, the reasons should be recorded under item 20.

All cards and forms at the end of each day’s work are examined for correctness and completeness.

On some festival days, school attendance may be low on the day of testing. In such case, another day of testing may be planned. Similarly, if the proportion of tested children available for reading is low, home visits may be undertaken to supplement the coverage.

**Referral to the designated health centre**

For children having reactions ≥10 mm, parents are enquired about the current health status of the child especially for presence of chest symptoms, loss/failure to gain weight, history of anti-TB treatment and history of contact with a known TB case. Children with any of these are referred to the designated health centre with a child referral form (Annex 3, Form 6) for further investigations and treatment, if required.

**B.4 Field Report**

A cluster summary form (Annex 3, Form 7) is filled in on completion of the field work in each cluster. These are consolidated into a district summary form (Annex 3, Form 8).
In case the number of children registered is below the district target, the required number of additional clusters are selected by SRS.

On completing the work in a given cluster, all forms are duplicated as a safeguard against loss in transit. The team leader shall crosscheck all duplicated entries. The originals are dispatched to the central office periodically.

C. Tuberculin vials and maintenance of the cold chain

The required numbers of tuberculin vials are transported to the district in vaccine carriers containing ice packs preferably by air where possible. At the district level, vials are stored in a refrigerator. The team Leader should check the thermal regulator and thermometer as well as the reliability of electric current before placing the vials in the refrigerator. Care should be taken to maintain the temperature of the refrigerator between 2-8 °C. Tuberculin must not be allowed to freeze. The vials are carried to the clusters on the days of testing in vaccine carriers. Care should be taken not to expose the vials to sunlight or heat. The testing centre should be set up at cool shaded places. A vial once opened must be used on the same day or the next day. The vials have to be used within the expiry date.

D. Maintaining standard of field work

An important aspect of the survey is to maintain a high standard in carrying out all tasks as per the protocol and work instructions without any changes or deviations. The team leader should ensure satisfactory and smooth conduct of the field work. The principal investigator and co-investigators should closely supervise the fieldwork.
Annex 3

Forms

ARTI Survey

FORM 1 – DISTRICT WORK PLAN

District code: ______________________
District name: ______________________
Stratum: ______________________

<table>
<thead>
<tr>
<th>Cluster Serial Number</th>
<th>Cluster Name*</th>
<th>Date of Planning</th>
<th>Date of Testing</th>
<th>Date of Reading</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

* school/village /urban block
**ARTI Survey**

**FORM 2 – CLUSTER PLANNING SHEET**

Date of planning visit: _____________________

<table>
<thead>
<tr>
<th>District code: ___________________________</th>
<th>Date of testing: ______________________</th>
</tr>
</thead>
<tbody>
<tr>
<td>District name: __________________________</td>
<td>Date of reading: ______________________</td>
</tr>
<tr>
<td>Cluster serial number: __________________</td>
<td>Special information, if any: ____________</td>
</tr>
<tr>
<td>Name and address of cluster*: ____________</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratum (rural/urban): __________________</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Name and designation of persons met:</td>
<td>Road description to reach the cluster:</td>
</tr>
<tr>
<td>1. ______________________________________________________________________</td>
<td></td>
</tr>
<tr>
<td>2. ______________________________________________________________________</td>
<td></td>
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<tr>
<td>3. ______________________________________________________________________</td>
<td></td>
</tr>
<tr>
<td>4. ______________________________________________________________________</td>
<td></td>
</tr>
</tbody>
</table>

* school/village/urban block

Signature:

Name: _________________________________

Note: in house-based survey, a sketch of the cluster with lanes and by-lanes with the approximate number of houses in each should be drawn on the reverse side.
The Department of Health is carrying out a tuberculin survey in ______________________ (name of the school). Children attending the first grade will be examined by Mantoux test, which is given in the skin using a disposable syringe. This is a safe test though a slight irritation may occur at the test site. Any child suspected of suffering from tuberculosis shall be further investigated free of cost at the designated health centres and treated, if required. The parents are requested to co-operate and ensure that the child attends the school on the dates given below.

The survey results will help to further improve the delivery of anti-TB services in the country.

Date of testing: ______________________
Date of examining the test result: ______________________

(Signature)
FORM 3B – APPEAL TO RESIDENTS

The Department of Health is carrying out a tuberculin survey in ______________________________ (name of the village/urban block). Children of _____ (age group involved) years old will be examined by Mantoux test, which is given in the skin using a disposable syringe. This is a safe test though a slight irritation may occur at the test site. Any child suspected of suffering from tuberculosis shall be further investigated free of cost at the designated health centre and treated, if required. The residents are requested to co-operate and ensure that the children are available at home on the time and dates given below.

The survey results will help to further improve the delivery of anti-TB services in the country.

Time and date of testing: ______________________________

Time and date of examining the test result: ______________________________

(Signature)
**FORM 4A – SCHOOL REGISTRATION FORM**

Date: ______________________

Cluster number: ___________  Name of the school: _____________________________

District name: _________________________  Stratum (rural/urban): ________________

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Grade</th>
<th>Name of the child</th>
<th>Age*</th>
<th>Sex</th>
<th>Father’s name</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

* Age in completed years

Name of enumerator: _____________________

Signature:
FORM 4B – HOUSEHOLD REGISTRATION FORM

Date: ______________________

Cluster number: _______________ Name of village/urban block: ______________________

District name: _________________ Stratum (rural/urban): ___________________

Date of birth eligibility for registration: ______________________

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Lane number/ household number</th>
<th>Name of the child</th>
<th>Age*</th>
<th>Sex</th>
<th>Father’s name</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

* Age in completed years

Name of enumerator: _____________________

Signature: ___________________________
FORM 5 – CHILD CARD

Instructions on how to fill in the child card

Item 1: district code as allotted
Item 2: cluster is coded in numerals as allotted in the list of selected clusters
Item 3: name and address of cluster
Item 4: write “R” if the cluster lies in a rural stratum and “U” in case the cluster is located in an urban stratum
Item 5: the lane number is written in Roman followed by a slash and the serial number of the household written in numeral (e.g. VI/09)
Item 6: the child number is written in four digits, starting from 0001 for the first child tested in each district and continues sequentially throughout the district
Item 7-8: self explanatory
Item 9: date of birth is obtained from the school register/parent/guardian and in case of doubt substantiated by appropriate document, if available
Item 10: age of the child is written in completed years. Care must be taken to record the age as accurate as possible
Item 11: write “M” for boy (male) and “F” for girl (female)
Item 12: write “S” for scar present, “N” for scar absent (no scar) and “D” for doubtful scar
Item 13: date on which the tuberculin test is given
Item 14: enter the tester code (each tester is assigned a code)
Item 15: write “S” for satisfactory testing and “U” for unsatisfactory testing
Item 16: enter the reader code (each reader is assigned a code)
Item 17: date on which the reaction is read
Item 18: reaction size in mm
Item 19: mention unpleasant manifestations, if any. (“V” for vesicle, “B” for bulla, “N” for necrosis, “L” for lymphangiitis and “A” for no unpleasant reaction
Item 20: any remark that the team wants to indicate, e.g. test given on right forearm, child registered but not tested (reason thereof), etc.
**ARTI Survey**

**FORM 5 – CHILD CARD**

<p>| | | | | |</p>
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<tr>
<td>___________________</td>
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<td>___________________</td>
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<tr>
<td>2. Cluster code:</td>
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<td>5. Lane number/household number:</td>
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<tr>
<td>7. Name of the child:</td>
<td></td>
<td>8. Name of Father or guardian:</td>
<td>9. Date of birth:</td>
<td></td>
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<tr>
<td>___________________</td>
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<td>____________________</td>
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<td>Day Month Year</td>
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<tr>
<td>15. Testing status:</td>
<td>16. Reader code</td>
<td>17. Date of reading:</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Day Month Year</td>
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<tr>
<td>18. Reaction (size in mm)</td>
<td>19. Unpleasant reactions</td>
<td>20. Remarks:</td>
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(Signature of tester) (Signature of reader)
FORM 6 – CHILD REFERRAL FORM

To:
the Medical Officer in-charge
________________________________
________________________________
(write the name and address of the health centre)

During the conduct of the tuberculin survey for estimation of the annual risk of tuberculosis infection (ARTI), the below-mentioned child is found to be infected with *Mycobacterium tuberculosis*. The child is being referred to you for further investigations and necessary action, as you may deem fit.

1. Child's name: ____________________________________________
2. Father's / guardian's name: ____________________________________________
3. Age: _____________________
4. Address: ____________________________________________
5. Tuberculin reaction size: _____________________ mm
6. Unpleasant reaction: ____________________________________________
7. BCG status _____________________
8. History of contact with a TB case: ___________________________________________
9. Symptoms present: ____________________________________________

(Signature of team leader / test reader)
**FORM 7 – CLUSTER SUMMARY FORM**

1. District code: ____________________  
2. District name: ______________________________

3. Cluster serial number: ___________  
4. Cluster name: ____________________________

5. Number of children registered\(^f\): _____________________

6. Number of children tested: __________ (satisfactorily); __________ (unsatisfactorily)

7. Out of children tested satisfactorily, number of children read: _________

8. Number of children referred to health centre for further investigation: _________

(Signature of the team leader)

---

\(^f\) This number should correspond to the number enrolled in the school register/registered in the household registration form
**FORM 8 – DISTRICT SUMMARY FORM**

1. District code: _____________________  2. District name: _______________________________

3. Details of clusters surveyed:

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name</th>
<th>Total registered</th>
<th>Total tested</th>
<th>Total referred to health centre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Satisfactory</td>
<td>Un satisfactory</td>
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</tbody>
</table>

* out of satisfactorily tested

4. Details of additional clusters surveyed, in case of shortfall:

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name</th>
<th>Total registered</th>
<th>Total tested</th>
<th>Total referred to health centre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Satisfactory</td>
<td>Un satisfactory</td>
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</tbody>
</table>

* out of satisfactorily tested

Name team leader: ___________________________

Signature:
<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Name</th>
<th>Address</th>
<th>TB Reg. No.</th>
<th>Age</th>
<th>Sex</th>
<th>Date of testing</th>
<th>Date of reading</th>
<th>Reaction size (mm)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>
Annex 4

Protocol for training field teams

Adequate training of field teams is one the most important components of a tuberculin survey. Otherwise, the entire effort may end up in wrong results and go for waste. Especially the large-scale inter-reader as well as intra-reader variation in measuring the reaction size may create problems in the interpretation of the survey results.

All the team members should be trained together by personnel with vast experience in tuberculin surveys including tuberculin testing and reading.

A tuberculin survey field team should consist of eight members: seven field workers and a team leader who coordinates all activities.

The optimum period of training a newly recruited team is about 25 working days. When already experienced tuberculin testers and readers are chosen for the field work, this period may be reduced to 12 working days to familiarize them with the field procedures and study forms and to standardize testing and reading techniques.

For a newly recruited team, the training may be imparted in four phases.

**Phase I**

During the first 2-3 days, all participants are acquainted with the purpose of the survey, the study population and different components of the field work such as planning, registration, tuberculin testing and reading. The trainers demonstrate the procedures for identification of a BCG scar, administration of the tuberculin test, reading of the reaction size including measurement of maximum transverse diameter of induration and recording of data in the appropriate study forms.

**Phase II**

During the next 2-3 days, all participants are given practice in identification of BCG scar, communication skills to mobilize community participation and in tuberculin testing and reading. This gives an opportunity to the trainers to identify the potential testers and readers. Four testers and four readers should be identified from the group based on their aptitude for respective tasks. They should be imparted further training in those tasks.

**Phase III**

Over the next 12-14 days, further practice is given in testing and reading

Each tester performs about 200 tests in this period under the close supervision of trainers. On-the-spot corrective actions are taken in case of errors in testing technique.

Each reader is made to read about 400 tuberculin reactions in this phase under the close supervision of expert readers. Proper technique of palpating the
margins of induration and use of ruler is emphasized to the trainees. On-the-spot corrective action is taken in case of errors in reading and measurements of reaction sizes. Training is also imparted for recognition of unpleasant reactions such as bullae, vesicles, necrosis and lymphangitis.

In case of already experienced tuberculin testers and readers, Phase II and III may be skipped.

**Phase IV**

This phase is to assess the trainees and should be completed in 6-8 days.

Each tester performs about 150 tests in this period under observation of a trainer. Each test is recorded as satisfactory or unsatisfactory.

The testers with less than 5% unsatisfactory test results qualify as testers for the study. The two testers with the lowest number of unsatisfactory tests should be chosen as testers for the survey.

Each reader reads about 300 reactions independently. To assess intra-reader consistency, each reader reads every reaction twice with a sufficient time gap between the two readings to avoid recall bias. The trainer also reads each reaction once independently.

The reaction sizes recorded by each of the trainee-readers and reference reader during Phase IV are recorded in a tabular form. A format for the table is given below. The analysis of the data in respect of each trainee is undertaken as follows:

(i) A scatter plot is drawn separately for each trainee where X-axis represents reading of the trainer (called reference reader) and the Y-axis the first reading of the trainee reader. This gives a visual impression of the agreements and differences between the readings recorded by the trainer and the trainee. An example is shown in Figure 6. In a perfect correlation, all readings by both lie on the line. The measurements by the trainee located on the left side of the line are under-read by the trainee and vice versa. The distance from the line represents the difference between the trainee and trainer reading. The pattern of under or over-reading may be different for small and large reactions.

(ii) The percent agreement at ± 2 mm is estimated between the first reading of the trainee-reader and the trainer.

(iii) The first and second readings by each reader are compared similarly for intra-observer consistency by generating a scatter-plot and calculating the percent agreement.

(iv) The frequency distribution curves are plotted for the trainee reader to find out any digit preference. Some readers have propensity to measure reactions in even numbers and some others may have preference for digits ending with 0 or 5.

The trainees with less than 90% percent agreement with the trainer and similar levels of intra-observer consistency should not do reading during the survey; they may be assigned other tasks in the field work. The two best performing trainees

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ballpoint pen markings by each reader should be erased with a suitable ink remover before the next reader reads the reaction
on the above counts and with the minimum levels of digit bias should qualify as readers. Of those two readers, one should be the *regular reader* during the survey. Preferably this reader should read all tests given by his team. Only in case of exigency, the other reader should be asked to read the reactions. This is to minimize the influence of inter-reader variation.

Table 2: Assessment of readers

<table>
<thead>
<tr>
<th>Child No.</th>
<th>Tuberculin reaction size in mm as measured by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trainee reader A</td>
</tr>
<tr>
<td></td>
<td>I</td>
</tr>
</tbody>
</table>

I  First reading  
II Second reading
Fig. 6: Scatter-plot of tuberculin reaction between standard reader (trainer) and trainee reader.
Annex 5

Resource requirements

The resources required for conducting a tuberculin survey in terms of staff, material and equipments, time and budget heads are described below.

A. Human resources

1. Principal investigator (preferably epidemiologist) – 1
2. Co-investigators – 2
3. Statistician – 1
4. Data entry operator – 1
5. Office assistant-cum-accountant – 1
6. Field teams – one for each stratum: composition given below.
7. Field support staff
8. Expert assistance

The principal or chief investigator is responsible for management of the entire survey including preparation of the project proposal, recruitment of field teams, training, planning, organizing, implementation of the field work, data analysis and report writing. The co-investigators assist the principal investigator in the above tasks and supervise the fieldwork periodically.

A statistician should be involved in all stages of the survey, i.e. sample size estimation, sampling, designing of study forms and analysis. A data entry operator is provided at the central office.

The office assistant has to maintain office files and accounts, provide secretarial assistance to the principal investigator and receive the study forms dispatched by the field teams.

Field team

One field team is required for each of the strata. If the entire country is divided into two strata, than two teams are recruited. If an independent survey is planned in each area, than separate teams are recruited for each area and stratum. The field team members are assigned with the allocated responsibilities:

1. team leader – 1
2. planner – 1
3. enumerator – 1
4. tester – 1
5. co-tester – 1
6. reader – 1
7. co-reader –1
8. for leave vacancy – 1

The team leader has to undertake the advance planning visit to each district to review arrangements for the storage of tuberculin, transport and accommodation of the field team and to solicit further support from the district authorities. The team leader has to prepare a tentative time schedule and coordinate all activities during the field work, check the study forms at the end of each working day for completeness and correctness. Other responsibilities include liaising with the district authorities, maintaining accounts in the field and dispatching study forms to the central office. The team leader should preferably be of the level of a health supervisor with experience in field surveys.

The planner is for field planning in individual clusters. The planner visits each cluster 2-3 days in advance to locate the cluster, elicit participation of the community and local health authorities and inform about the dates of testing and reading. During the visit, the planner draws a road route and a rough sketch (in case of house-based survey) of the cluster showing lanes and by-lanes.

The job of enumerator is to register the children and guide them to the testing centre in the cluster.

The tester and the co-tester are responsible for the identification of the BCG scar, administration of tuberculin test and recording the testing status.

It is desirable that a single reader reads all reactions during the entire course of the field work. The other designated reader (co-reader) shall normally function as co-reader and record the reaction sizes and unpleasant manifestations, if any, as dictated by the reader. The co-reader may be asked to read the reactions in case of exigencies.

The other member acts as a replacement during leave vacancy for planning, enumeration or as co-tester/co-reader.

**Field support staff**

The field support staff consist of a local health worker, two drivers and peripheral health workers.

One health worker from the district may be deputed during the course of the field work to accompany the field team and to liaise with local authorities and the community.

Two drivers are required, one for the planning and reading team and one for the registration and testing team.

The help of the peripheral health worker (multipurpose health worker) should be sought for the days of testing and reading in the clusters under his/her jurisdiction.
Technical assistance

An expert with vast experience in conducting and analyzing tuberculin surveys may be required for peer-review of the protocol as well as for sampling of clusters, supervision of the pilot study and for facilitating data analysis and report writing.

A reference reader for standardization of trainee readers is also required.

B. Materials and equipments

During training

The materials needed during training are given in Table 3.

Table 3: Materials needed during training

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready-to-use 2 ml vials of PPD RT 23</td>
<td>160</td>
</tr>
<tr>
<td>Disposable tuberculin syringes</td>
<td>1800</td>
</tr>
<tr>
<td>Planning sheets</td>
<td>10</td>
</tr>
<tr>
<td>School/household registration forms</td>
<td>80</td>
</tr>
<tr>
<td>Individual child cards</td>
<td>1800</td>
</tr>
<tr>
<td>Cluster summary forms</td>
<td>10</td>
</tr>
<tr>
<td>Child referral forms</td>
<td>20</td>
</tr>
<tr>
<td>Files</td>
<td>11</td>
</tr>
<tr>
<td>Transparent plastic rulers (10 cm long, calibrated in mm)</td>
<td>8</td>
</tr>
<tr>
<td>Writing boards</td>
<td>6</td>
</tr>
<tr>
<td>Permanent ink markers *</td>
<td>10</td>
</tr>
</tbody>
</table>

* only for house-based training

During field work

Table 4 shows the materials required for every 1000 children included in the survey.

Table 4: materials needed during field work (for 1000 children)

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready-to-use 2 ml vials of PPD RT 23</td>
<td>120</td>
</tr>
<tr>
<td>Disposable tuberculin syringes</td>
<td>1100</td>
</tr>
<tr>
<td>Planning sheets (10 clusters)</td>
<td>11</td>
</tr>
<tr>
<td>School/household registration forms</td>
<td>50</td>
</tr>
<tr>
<td>Individual child cards</td>
<td>2200</td>
</tr>
<tr>
<td>Cluster summary forms (10 clusters)</td>
<td>22</td>
</tr>
<tr>
<td>Child referral forms</td>
<td>22</td>
</tr>
<tr>
<td>Files (10 clusters)</td>
<td>11</td>
</tr>
<tr>
<td>Transparent plastic rulers (10 cm long, calibrated in mm) (per team)</td>
<td>4</td>
</tr>
<tr>
<td>Writing boards</td>
<td>6</td>
</tr>
<tr>
<td>Permanent ink markers *</td>
<td>As required</td>
</tr>
</tbody>
</table>
Other (pen, pencils, erasers, writing papers, stapler, punch hole machine, envelopes, sealing tape, etc.) | As required

* only for house-based surveys

**At the central office**

The following materials and equipments are required at the central office:

- Computer-cum-printer
- Data storage devices (one external hard disk, ten rewritable CDs, 20 floppy disks)
- Printing paper
- Other stationeries

**C. Budget**

The budget requirement will depend upon the expected duration of the survey, the sample size, transportation and local costs. The required budget may be estimated with the budget lines as indicated in Table 5.

<table>
<thead>
<tr>
<th>Budget heading</th>
<th>Item</th>
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</thead>
<tbody>
<tr>
<td>Travel</td>
<td>Travel for training: 8 nos.</td>
</tr>
<tr>
<td></td>
<td>Planning visits by team leader: 1 to each district</td>
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<tr>
<td></td>
<td>Supervision visits by the principal investigator/co-investigators: 1-2 per district</td>
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<tr>
<td></td>
<td>Inter-districts travel by field teams (depending on number of districts)</td>
</tr>
<tr>
<td>Salaries, honorarium, per diems</td>
<td>Salaries of contractual staff recruited for survey, if any</td>
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<tr>
<td></td>
<td>Honorarium for regular staff, as applicable</td>
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<tr>
<td></td>
<td>Per diem for field visits of principal investigator, co-investigator and field team members</td>
</tr>
<tr>
<td>Transport, accommodation</td>
<td>For field work during the training</td>
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<tr>
<td></td>
<td>For field work during the main study</td>
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<tr>
<td></td>
<td>Camp shifting</td>
</tr>
<tr>
<td></td>
<td>Accommodation for the field teams</td>
</tr>
<tr>
<td></td>
<td>Courier charges for dispatching study forms to the central office</td>
</tr>
<tr>
<td>Printing</td>
<td>Printing of study forms, survey report</td>
</tr>
<tr>
<td>Supplies</td>
<td>Tuberculin vials</td>
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<tr>
<td></td>
<td>Tuberculin syringes</td>
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<td></td>
<td>Stationary items</td>
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</tbody>
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