PROTOCOL FOR the 2nd TB PREVALENCE SURVEY  
IN CAMBODIA (2010-2011)

INTRODUCTION

Tuberculosis control programs must be informed about the size of the tuberculosis problem, and, perhaps more importantly, the direction in which TB epidemiology is changing, i.e., are tuberculosis control efforts leading to a reduction of the tuberculosis problem. As a reduction in TB prevalence is one of the Millenium Development Goals (MDGs) and an indicator within the Global Stop TB Plan, TB prevalence surveys are an effective tool to monitor the impact of the program. The results of a series of high quality prevalence surveys may show the impact of national and international investments in TB control in Cambodia. The first national prevalence survey was carried out in 2002. After 8 years, the second survey is planned to measure both the current prevalence and any change in prevalence since the previous survey. The first survey suggested an impact of DOTS since 1994. The second survey is expected to show stronger evidence of a downward trend in TB prevalence in Cambodia due to DOTS expansion since 2001.

TB data in Cambodia are primarily based on case notification and WHO estimation efforts. As such, there are limited data with which to make assumptions about the true, current underlying TB epidemiology. While every effort is made by WHO expert groups to develop accurate estimates, there is a considerable range of uncertainty around these figures. There was a large discrepancy between WHO estimates and prevalence as measured by the 2002 prevalence survey. Therefore, the national TB program will conduct this second national TB prevalence survey in order to provide the program with updated and more accurate information on the current tuberculosis burden which can also serve as baseline information for future planning within the National Tuberculosis Control Program in the Kingdom of Cambodia. TB prevalence is also included in Milenium Developent Goals.

The study design is to be the same as that of the first survey for comparison purposes. However a few differences to the survey protocol have been made in light of the results of the first survey and current international recommendations for the conduct of tuberculosis prevalence surveys. A major difference is the age group of the survey population. In the first survey, the target population was those aged 10 years or older while in the second survey it will be those aged 15 years or older. This is because prevalence surveys are highly unlikely to detect TB cases among those under 15, making it more sensible to avoid increasing the study workload to include those younger. Because childhood tuberculosis is likely the result of tuberculosis within the household, no significant negative impact of this change is expected, assuming that contact examination is provided for children in households in which the survey detects a case of active tuberculosis.

A tuberculin survey was carried out as part of the first prevalence survey. While it may be possible to use tuberculin surveys to detect differences in prevalence of infection within subpopulations, tuberculin distribution curves are difficult to interpret and
extrapolate to estimate the true prevalence of infection and annual risk of infection. Due to this and the added burden of conducting a tuberculin survey which involves a significant number of subjects, no tuberculin survey will be carried out as part of the second prevalence survey.

1- OBJECTIVES

1.1 Primary objectives:
(1) To determine the prevalence of pulmonary TB among the population aged 15 years or older at a defined point in time (2010) in Cambodia as measured by:
   - smear-positive pulmonary TB
   - culture-positive pulmonary TB
   - bacteriologically-confirmed pulmonary TB
   - symptoms suggestive of TB
(2) To assess the trend in TB prevalence

1.2 Secondary objectives:
(1) To identify
   - Prevalence of TB suspects
   - radiological abnormalities suggestive of pulmonary TB
   - Health-seeking behaviour as defined by:
     ➢ Health-seeking behaviour of TB patients and individuals reporting chest symptoms
     ➢ Use of the private sector for TB care as reflected in the proportion of TB patients under treatment in the private sector
     ➢ Where the NTP is missing cases, by service area, demographics, etc.

2- STUDY DESIGN

2.1 Target areas:
The target area is the whole area of Cambodia. In the 1st survey, due to transportation problem and their relatively small size, four provinces (i.e. Mondul Kiri, Rattanak Kiri, Preah Vihea and Steung Steng) were excluded from the target areas of the survey. Because the transportation situation (i.e. accessibility by road) has been improved, these will now be included in the second survey. However, for purposes of comparison between the two surveys, these four provinces will be grouped into a stratum separate from other areas included in the first survey as mentioned below.

2.2 Stratification:
To maintain comparability with the first survey, the following stratification will be made. Note that strata 1 and 2 will be included for the comparision in prevalence between the first and second surveys.
- Stratum-1 (Urban areas): this stratum consists of areas categorized as urban in the 2008 census with the exception of four provinces (i.e. Mondul Kiri, Rattanak Kiri, Preah Vihea and Steung Steng)
• Stratum-2 (Rural areas): this stratum consists of areas categorized as rural in the 2008 census with the exception of four provinces (i.e. Mondul Kiri, Rattanak Kiri, Preah Vihea and Steung Steng)
• Stratum-3: this stratum consists of Mondul Kiri, Rattanak Kiri, Preah Vihea and Steung Steng which were excluded in the first survey.

2.3 Study population:
The study target population consists of all persons who are aged 15 years at time of survey or older who have resided at the selected survey sites for 2 weeks prior to the survey, except for those meeting the exclusion criteria mentioned below.

2.3.1 Inclusion criteria: Inclusion in TB screening will be made only with informed consent (see the section entitled ethical issues). If someone does not provide informed consent or does not appear for the interview/TB screening, they are categorized as non-participants but are included in the population of eligible individuals (study population). This is the denominator for assessing participation rate. Some individuals will be exempted from CXR examination (e.g. those who do not want to get chest X-ray test, those who have disability and can not take position for CXR, or those who are unable to show up for at the field operation centre to get chest X-ray for any reason). However, as long as they provide informed consent for participation, they are categorized as participants with missing information.

2.3.2 Exclusion criteria: Persons living at military and diplomatic compounds, hospitals and hotels will be excluded from the survey in sampling stage and/or during household census. Residents in dormitories (e.g school) and temporary settlements (e.g., accommodation facility for construction workers) will not be excluded a long as they have resided there for 2 weeks prior to the survey.

2.4 TB Screening methods:
Following current recommendation by the WHO Task Force on TB Impact Measurement, to detect prevalent tuberculosis cases, the following screening strategy is adopted:
• All eligible individuals will undergo an individual interview of TB symptoms and chest X-ray (CXR) examination. Exclusion criteria for the CXR is discussed in the methods section.
• TB suspects (those having TB symptoms and showing CXR abnormal shadow, as defined in the methods section) will submit two sputums, one on-the-spot and one the next morning. Sputum specimens are examined for smear and culture and, if culture is positive, an identification test is done.

2.5 Sampling methods:
Stratified multi-stage cluster sampling with population proportional to size (PPS) will be adopted.

2.5.1 Assumption for sample size for strata 1 and 2
Because DOTS expansion has been implemented into health centre levels and it has been observed in series of surveys in Korea and the Philippines, it is expected that prevalence smear-positive prevalence has been reduced predominantly. Because there is rather short period between the 1st and 2nd surveys and the first survey was designed for the point estimate of prevalence, the primary objective of this survey should provide point estimate with acceptable relative precision (i.e. 25% or less). Therefore sample size is determined to obtain the acceptable relative precision for a range of prevalence of smear-positive TB likely to be observed at time of 2nd survey. It is assumed that the range up to 42% reduction (corresponding 50% prevalence in 10 years after the 1st survey). To achieve relative precision of at least 25%, for this range sample size of 23932 is required under simple random sampling. With this sample size, 42% reduction from the prevalence in the 1st survey can be also detected with power of 80% and 95% confidence level. Although this level of reduction may not occur, it is thought this sample size is acceptable because, with this sample size, relative precision of point estimate achieve 25% or better (less than 25%) for prevalence of smear-positive TB.

The following assumptions are based on findings from the first survey and the population census of 2008:

- Participation rate >90%
- Final sampling unit and appropriate size of cluster: Considering operational issues, the village is to be the final sample unit. According to the 2008 census (total population: 13,395,682; total number of villages: 14 037; the proportion of those aged >= 15 yrs: 66.3%), the average population aged 15 years or more per village is 632. Taking into account the village population size and the capacity of the survey workers to process 150-180 participants/day (max 200), a cluster size of 600-650 is appropriate if each cluster's operation is to be completed within a week.

2.5.2 Design effect:
The summary of the first survey is shown in Appendix 1, on which the following discussion is based. The design effect (DEFF) for smear-positive tuberculosis was 1.15664 from svymean command of Stata 8.0. Thus intra-cluster correlation co-efficient (hereinafter ICC) is 0.000373 with an average cluster size of 420 and the equation: ICC = (DEFF – 1) / (cluster size –1). To decide DEFF in the second survey, it is necessary to assume whether or not, and the extent to which, ICC will be reduced or increased. The first survey indicated that the level of access to DOTS is inversely associated with the prevalence of smear-positive cases. During and after the survey, DOTS was expanded at the health centre level. It might therefore be sensible to assume that DOTS reduced the difference in smear-positive prevalence between surveys because prevalence was reduced more dramatically in areas of high prevalence at the time of the first survey. As a result, the ICC tends to be reduced. However because of sampling variability and the
slight difference in sampling between surveys, we consider the point estimate of ICC to be uncertain. It might be safe to assume an ICC in the second survey twice that of the first. If prevalence has been reduced more dramatically in areas with low prevalence at the time of the first survey, the ICC of the second survey will tend to be larger than that of the first. However, considering the inverse association between access to DOTS and prevalence found in the first survey, one may assume that the ICC may not have increased.

The DEFF varies by cluster size under each value of ICC. As described in the next section, taking into account field operations and the average size of a village population, the appropriate cluster size is about 650 or less. If we adopt a cluster size of 640 and assume a participation rate of 90%, DEFF will be 1.4299, assuming the ICC of second survey is twice that of the first survey as shown in Annex 1.

2.5.3 Total sample size:
Based on the above requirements and assumptions, the following total sample size, cluster size and number clusters are adopted for strata 1 and 2:
- Total sample size of population aged 15 years or older: 38,400
- Number of clusters: 60
- Cluster size: 640
- 60 clusters are distributed to strata 1 and 2 proportional to their population sizes as shown in Table 1

As mentioned below, 2 clusters will be drawn from strata 3 (the four provinces excluded in the first survey). Therefore, the total sample size for all strata is 39,680 (62 clusters x 640 subjects per cluster).

<table>
<thead>
<tr>
<th>Stratum</th>
<th>Population aged ≥ 15yr: %</th>
<th>No. clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratum-1</td>
<td>1911597</td>
<td>13</td>
</tr>
<tr>
<td>Stratum-2</td>
<td>6642678</td>
<td>47</td>
</tr>
<tr>
<td>Sub-total (stratum-1&amp;2)</td>
<td>8554275</td>
<td>60</td>
</tr>
<tr>
<td>Stratum-3</td>
<td>322481</td>
<td>2</td>
</tr>
</tbody>
</table>

Because rounding the number of clusters has a small effect on representativeness between stratum 1 and stratum 2, while stratum 3 has reduced representativeness (about 10%), we will apply stratum-level weights (i.e. inverse values of selection probability for each stratum shown in Annex 1).

2.5.4 Procedure for sample unit selection within each stratum:
In Cambodia, there are 4 levels of administrative units: provinces, districts, communes and villages. Classification of urban and rural areas is made generally at the commune level. The sampling frame is census population with population
aged 15 years or over at districts, communes and villages. Sample units are selected by the multistage sampling method with probability proportionate to size (PPS) within each stratum as follows:

- **Primary Sampling Unit (PSU):** PSUs will be districts which were PSUs in the first survey. In the first survey, systematic sampling on the list of districts was used for sampling of PSUs with PPS. There is no district with more than one selected PSU because systematic sampling is made and sampling interval was more than population size of districts. Considering the benefit of PPS systematic sampling, it is applied to the 2nd survey. Five districts have a higher eligible population than the value of stratum population per the number of samples according to the sampling frame for this 2nd survey. Thus one or two PSUs are drawn from these districts if we use PPS systematic sampling. If two PSUs are selected from the same district, two SSUs will be selected without replacement.

- **Secondary Sampling Unit (SSU):** In the first survey, there was no SSU. However because more than half of the villages have an eligible population smaller than 640, and a proportion of selected villages will not have 610 (lower range of acceptable eligible population of 640) eligible, it is necessary to include other villages within these sampling units. For ease of operations, the villages to be included should be from the same commune. Therefore, considering the hierarchy of sampling units, it is better to introduce SSUs. Sampling of SSUs will be made with PPS (based on a size of commune population aged >= 15 years). One problem caused by using communes as SSUs is that some communes (less than 10 out 1400) have populations less than 950 (Thus, expected eligible population would be less than 610). Though exceptional; if such a small commune is selected in the first stage, randomly selected villages within bordering communes should be included in the same manner as mentioned below).

- **Third sampling stage:** One village within commune will be selected randomly. After selecting villages according to the size of the eligible population, the following procedures will be taken place:
  - If the selected village has significantly more than 640 individuals aged 15 years or older (e.g., larger than 800), the village will be divided into household groups by using existing household groups and paths, natural boundaries such as creaks. One of blocks will be selected randomly and then household groups will be selected in randomly selected direction (e.g. north) and clockwise direction until the required sample size is as close as possible to 640 (from 610 to 670).
  - If the selected village has significantly less than 640 individuals aged 15 years or older (e.g. 600), additional village(s) will be included within the same commune. Approximately 40-50% of selected villages are expected to
have less than 610 individuals aged 15 years or over. One of
the villages bordering on the originally selected village will be
randomly selected and the survey team will continue adding
village(s) in a clockwise manner, around the village originally
selected until the required number of participants is reached.

2.6 Information to be collected:
To estimate prevalence of tuberculosis and identify risk factors for prevalent tuberculosis,
the following demographic data and information on current health status/past history and
health-seeking behaviour of individual survey participants will be collected by interview:

- Age
- Sex
- Occupation
- Past history of tuberculosis diagnosis/treatment
- Current status of tuberculosis diagnosis/treatment
- Presence of symptoms (cough, sputum, haemoptysis, fever, loss of weight, night sweat) related to tuberculosis
- Health seeking behaviour (e.g. visit to hospital, health centre, private clinic, pharmacy, traditional healer) for those with symptoms

2.6.1 Chest x-ray examination results
All participants except those with criteria of exclusion from CXR receive the CXR
examination to identify eligible participants for sputum examination and to diagnose
bacteriologically negative tuberculosis.

2.6.2 Bacteriological information
For (a) those with either symptoms or CXR shadows which are eligible for sputum
collection and (b) the eligible subjects who accept participation but do not undergo
CXR (physically not possible, exempted because of pregnancy, declined), two
sputum specimens will be collected. Sputum smear status, culture status and
identification (M. tuberculosis or MOTT) are obtained by bacteriological examination
mentioned in the “Survey Procedures” section.

2.6.3 Information from patients detected by the survey versus patients detected from
routine NTP activities
To identify factors for not having been detected by routine NTP activities, detailed
information from these participants will be collected. This protocol will be prepared
separately and will be reviewed by an ethics committee.

3-ORGANIZATION
Two committees will be established, an Executive Committee and a Technical Committee.
3.1. Executive Committee (EC)
The Executive Committee is formed to take overall responsibilities of the survey and perform supervisory tasks. The director of the NTP is a chairperson. The committee consists of survey coordinators and other senior CENAT staff. The committee is technically supported by the advisers from core partner agencies such as WHO, JICA and RIT.

3.2. Technical Committee (TC)
The Technical Committee is responsible for the planning and execution of the work. Under the survey coordinators, it has five sub-committees: Statistical Analysis, Census, Chest X-ray, Bacteriological Examination, and Administration.

3.3 Bacteriological examination centres
Smear examination and culture examination will be carried out in two laboratories, the CENAT national reference laboratory and Battambang provincial laboratory. Identification test will be carried out in CENAT.

3.4 Survey Teams
At least three survey teams should be established to conduct field surveys in 62 areas within one year. Each team will have four units: the census/interview unit, x-ray unit, reception/informed consent unit and bacteriological examination unit. The team will be equipped with one portable x-ray unit and three vehicles. The total number of staff for each team is 15 persons. Considering backup, in addition to three regular teams, the same number of reserve members as one team should be established.

Staff of survey team (each team)

<table>
<thead>
<tr>
<th>Role/Designation</th>
<th>No.</th>
<th>Eligibility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Central Core Team</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Team Leader</td>
<td>1</td>
<td>Senior medical doctors of CENAT</td>
</tr>
<tr>
<td>Census • Interview unit</td>
<td>3</td>
<td>CENAT staff</td>
</tr>
<tr>
<td>CXR unit</td>
<td>4</td>
<td>Radiologist or Respiratory Disease Doctor x 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Radiological Technologist x 2?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Radiological Assistant x 1</td>
</tr>
<tr>
<td>Sputum collection unit</td>
<td>2</td>
<td>Laboratory technologist</td>
</tr>
<tr>
<td>Reception and Informed consent</td>
<td>2</td>
<td>CENAT staff</td>
</tr>
<tr>
<td>Drivers</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

| **Local Supporting Team** |       |                                                      |
| TB coordinator           | 3     | OD TB supervisor and Health Centre Staff            |
| Laboratory              | 1     |                                                      |
| Sputum collection        | 3     |                                                      |
Local volunteers | 6  
Village Health Volunteers |  
Security | 2  
Local police |  
Total | 15

Membership and role of each unit is as follows:

- **Team leader**: one medical doctor  
  His/her roles are:  
  - to supervise the field survey  
  - to visit and assess the village involved before the survey  
  - to randomly select the number of households  
  - to coordinate with the health center/health worker, volunteer health worker and local authorities for this purpose  
  - x-ray reading  
  - to collect all data from the forms, radiographs and sputum specimens, and send to the technical committee

- **The Census Unit**: 3 health staff from CENAT, local health worker or volunteer health worker or local authority. Role is to  
  - visit every household on the first day to:  
    - take census  
    - motivate for better cooperation  
  - interview and fill the questionnaire (individual survey forms) during TB screening

- **The X-Ray Unit**: 1 radiologist (CENAT), 2 x-ray technicians (CENAT), 1 assistant technician (hiring). This unit is responsible for taking chest x-rays of subjects aged 15 years or older, read x-ray film and record results in the form, noting the persons who need further sputum examination.

- **The Sputum Collection Unit**: 3 laboratory technicians (1-2 from CENAT and 1-2 from local), 1 driver. This unit is responsible for collection and packing of sputum specimen.

Local volunteers (some from the village level) can also participate in the survey.<community involvement>

4-TRAINING AND PILOT TESTING

All of the team members participating in the survey should be trained properly. Training for the survey teams consists of general issues of the survey (e.g. understanding the protocol) and contents specific to each unit. Contents specific to each field team unit will be based on SOPs. Trainings will be carried out in the second and third quarter of 2010.

After training a pilot test will be carried out 1-2 months prior to the implementation of the survey. The pilot test will be carried out in two sites (urban and rural settings) which are
not selected for the survey in order to identify weaknesses in the protocol and SOPs and to revise them by going through each step of the survey procedures in the field.

Training for x-ray readers and technicians and other survey participants should be conducted in the second quarter of 2010. Training for x-ray technicians consists of how to use portable x-ray machines, fixing and developing films. The length of training needed is 5 days. Training for x-ray reading (6 trainees) is estimated to take five days. Training for six laboratory technicians and six census staff from CENAT is estimated to take three days.

5-SURVEY PROCEDURES

5.1 Procedures before the field survey:
- The Executive Committee (EC) selects 62 clusters in the manner as mentioned in the design section
- 5 or 6 months prior to the commencement of survey operation, the team leaders, with provincial TB supervisors, visit the selected sites and investigate feasibility in terms of security and accessibility
- The EC replaces areas if there are serious problems such as road conditions, poor security, etc.
- The EC finalizes enumeration areas for field survey
- The EC communicates with concerned provincial health directors and local authorities to cooperate in the survey
- Forms (household registry, personal survey cards, area map, poster, and leaflet) are sent to the local administrative office of selected areas through Provincial Health Department 14 days prior to the first day of the survey. Household lists are filled in at the local authorities office, which is transferred to the Census Unit on taking census during the second pre-visit.
- Prior to the survey start, a team leader and Census Unit visit the commune (second pre-visit) to explain the study rationale and procedure and to identify which villages will be involved. Also, the Census Unit provides with on-the-job training to local officials and volunteers how to take census and fill out the Household lists for a day and the rest of the work are to be completed by the local officials and volunteers.
- 1 or 2 weeks before receiving the survey team, the health centre workers and local authorities in each selected area should conduct a communications campaign by pasting posters, distributing leaflet, public announcements, etc. to each household

5.2 Field survey procedures:
The amount of time needed is expected to be one week per cluster if the population aged 15 or more in the selected village is more than 610. Some sites requiring involvement of two or more villages may require a few additional days. The decision will be made at the time of the first field visit. Another consideration is operating hours for urban areas. Fieldwork is usually more difficult in urban areas as the population tends to be more mobile and busy and thus less likely to collaborate with a survey. This may be taken into
account by allowing sufficient time for follow-up of non-attendants and considering adjusting hours (e.g., include work in the evening).

5.2.1 Census taking:
- On the first day of a field operation in the village, the census group will receive the household registry from local field workers or commune health workers.
- The census team visits every household to confirm the list of persons staying there with age and sex and the eligible subjects on the name list of the household registry. If eligible persons are not included, they will be added to the list. In this situation, local field workers should motivate the eligible subject for better cooperation to attend survey. To average the workload of each examination day, one examination day is assigned to each household, but they are informed that they will be accepted on any examination days if they are unable to attend on the assigned day.
- Every household is given a serial number on the list and paste the number label by census group on the door or the gate of household (Annex-5.Form-1).
- Census unit member and field workers interview one head of household or the most appropriate person about household level information (e.g., size of house) and record it in the form.
- Registration number will be given to each subject regardless of their availability on the survey day; XX-####-OO: cluster number-household number-individual number.
- Although those aged 15 or more are eligible for the survey, children aged less than 15 years are recorded in household registry.

5.2.2 Registration and informed consent:
When eligible subjects attend the examination site, the receptionist asks them to provide informed consent. The results of informed consent are recorded on the household registry (attended and accept, attended but refused).

5.2.3 Interview on the survey examination spot
After informed consent, the interview will be conducted according to the individual survey form (Form-2). If the participant’s symptoms meet the criteria of symptoms eligible for sputum examination, interviewers mark the corresponding section of the individual survey form and inform the participant that he or she needs to submit sputum after CXR examination. All interviewed subjects except those exempted (who refuse or can not receive, e.g. due to disability) will be referred for x-ray examination (see the next section).

5.2.4 Chest x-ray examination:
- A chest x-ray examination will be carried out using size of 350mm x 350mm.
- X-ray technician takes x-ray of subjects aged 15 years or older.
- All eligible inhabitants will undergo x-ray examination if they do not
decline it; follow-up of non-attendants will be undertaken to reduce the number of non-attendance as minimum as possible.

- X-ray assistant technician fixes and develops chest x-ray films immediately after taking the CXR film.
- The field x-ray reader and second leader (team leader or another medical doctor) will interpret the chest films immediately after CXR film is developed. If only one reader is available, a second reader will check films to prevent false negative judgement, at latest, at night.
- The results will be recorded on personal survey card and x-ray examination registry (Form-3).
- Films will be categorized into those with shadows eligible for sputum collection and those without. Chest radiograph shadows eligible for sputum collection is defined as follows: (1) any abnormal shadow in lung field and mediastinum <describe details of abnormal shadow here or in SOP>, (2) pleural effusion except pleural thickness
- Shadows will be also categorized for on-site diagnosis: normal, active tuberculosis, suspected tuberculosis, healed tuberculosis, other lung disease (record most possible diagnosis), heart disease, others (record most possible diagnosis).
- All the radiograph films taken should be sent for central reading (CENAT) after the end of each field operation.

5.2.5 Sputum collection, storage and shipment:

- Sputum collection: Two sputum specimens will be collected from TB suspect (persons with either symptom or CXR shadow eligible for sputum collection). A first specimen is on-spot specimen and will be collected on TB screening day and a second specimen will be early morning specimen. It will be collected at home and submitted to survey team at survey examination site.
- Team leader or medical doctor will explain sputum examination to TB suspects based on the results of symptom screening and chest x-ray examination. Team leader and/or medical doctor in the team also explain the results of CXR when they find participants who need medical attention.
- Storage of specimens at survey sites and during shipment to culture centres: Submitted specimens will be kept in ice box after collection until reaching culture centres.
- Recording: Record the number of specimen and necessary information in the sputum smear examination forms (Form-5).
- Tracing persons who are eligible for sputum collection but haven’t submitted early morning sputum specimen: Health centre staff or volunteer visit their home to collect second specimen as soon as possible.
- Shipment of specimens to the designated culture centre: The sputum specimens and sputum smear examination forms are shipped to culture centre (CENAT or Battambang) on Wednesday and Saturday for one week of field operation. If additional days are required to complete the survey (e.g. cluster consists of more than two villages), shipment should be made to make it
possible to culture specimens within five days after collection (it is recommended culture be done within three days whenever possible).

- Sputum is collected from those who decline CXR if they have any symptom.
- Sputum is collected from those who are handicapped or sick with TB suggesting symptom and can not come to the examination site.

5.2.6 Re-interview of TB suspects:
For each of TB suspects eligible for sputum examination, who are detected by symptom screening and/or chest x-ray examination, interview of the same questions about symptoms as the first interview will be made again by team leader or another interviewer, who has not interviewed him/her at TB screening, so that more accurate information on TB suspects will be collected.

TYPICAL SCHEDULE OF SURVEY BY EACH SURVEY TEAM

1st DAY: Arrival and setting up with local collaborators
2nd DAY: Census
3rd DAY: Examination-1
4th DAY: Examination-2 & sputum shipment-1 to culture the centre
5th DAY: Examination-3
6th DAY: Examination-4 mainly for non-attendance
7th DAY: Sputum collection from TB suspects and sputum shipment-2 to culture the centre Move to another sites

It is estimated to take a week to complete the field operation of one cluster. In special cases such as involving two or three villages in one cluster, seven weeks will not be enough to complete one cluster so extension of duration (e.g., an additional three days) may be needed. For clusters in urban areas, the field operation needs to be extended until early evening to make it possible for participants who are paid workers to participate in the survey.

5.3 Central level procedure following field survey:

5.3.1 Bacteriological examination:
The laboratory technician of the laboratory test committee receives sputum from the survey team. For both of two sputum specimens (spot and morning), laboratory staff conducts sputum smear examination, culture examination and identification test. Laboratory staff record the results in the laboratory registers. Detailed standardized procedures are described in SOPs of bacteriological examinations.

- Smear examination:
First examination will be made by fluorescence microscopic examination (FLM). FLM is adopted to reduce workload and turn-around time and to avoid false negatives. If a reader find a positive slide, a second reader will confirm it immediately. If a second reader is not available on site when
positive slides are found, positive slides will be examined later by senior laboratory staff.
- Culture examination and storage:
   Inoculation on the media is to be done at latest within seven days of collection though it is strongly recommend that it be done within five days in order to obtain appropriate recovery rate.
- Shipment of isolates from Battambang to CENAT:
   Primary isolates will be shipped to CENAT for further examination (procedures for storage after recovery and shipment will be provided in SOPs)
- Identification test
  - Identification:
    Identification (M. tuberculosis or Non-tuberculous mycobacteria) will be made by Niacin Test and Capilia at CENAT.
  - Re-checking of slides by FLM:
    Smear slides which have been judged as negative but for which culture is positive will be re-examined by FLM.
  - Ziehl-Neelsen (ZN) examination to obtain results comparable with the first survey:
    It is recognised that FLM has the same or higher sensitivity compared to ZNM microscopic examination and that false positives may occur more often than with ZNM. Therefore, in order to maintain the comparability of smear positive prevalence between first and second surveys, ZNM will be performed on sputum slides with positive results by FLM and/or those that are culture positive and also on another randomly selected 10% of specimens with negative results by the FLM method. This cross-examination by ZNM should be made only after completion of re-checking by FLM mentioned above. For this cross-examination procedure, FLM results will not be provided to the readers of ZNM to avoid bias.
- Storage of isolates and smear slides:
  All smear slides and isolates will be kept at least until determination of the presence of tuberculosis cases (see next section) is made. Isolates will be kept in deep freezers. Disposal of smear slides and culture isolates will be made only by decision of the executive committee.

5.3.2 Central reading of the radiographs and determination of tuberculosis cases:
The 2nd reading is made for all films at CENAT after the field operation. The x-ray examination committee consisting of at least three x-ray readers reads all films except for those judged as normal by the field team and following the second reading at CENAT. The CXR results will be categorized into normal, active tuberculosis, suspected tuberculosis, healed tuberculosis, other lung diseases, heat diseases or other. The central diagnostic committee will establish the final consensus regarding the x-ray findings and determination of tuberculosis cases based on both x-ray results and bacteriological examination. < Description of case definitn here >

5.3.3 Data management:
- Technical sub-committee of Statistical Analysis at CENAT is responsible for data management with technical support from JICA, WHO and RIT.

- Data entry and data cleaning:
  During the field operation, all individual survey forms should be checked every evening to avoid missing information and to obtain the necessary information before leaving the survey site. All forms will be brought back to CENAT. An electronic database will be maintained for survey forms, the CXR register, laboratory register, and a non-participation list will be developed (details will be described in SOPs). All variables will be entered using double entry with the exception of variables collected in more than one source of information. Survey identification number, age and sex of eligible individuals listed in the survey household registry but who did not participate in the survey will also be entered in the manner of double entry. After matching the databases by survey ID, inconsistent values will be detected by comparing values between databases and between double entered data. Original forms will be reviewed when inconsistent values are detected for validation of data.

- Backup and security of data
  Original forms will be kept in a locked room accessible only to persons designated by the executive committee. Two computers will be used only for survey databases and locked by password known only to individual(s) designated by the executive committee. They will also be kept in the locked room. Each time the database is modified (entry and/or correction), it will be backed-up via external storage.

5.3.4 Statistical analysis:
Statistical analysis will consist of the estimation of prevalence, situation analysis of health seeking behaviour of TB suspects and risk factors for tuberculosis. These will include:
- Prevalence of radiological confirmed pulmonary TB among persons aged 15 years and above
- Prevalence of bacteriologically confirmed pulmonary TB among persons aged 15 years and above
- Prevalence of sputum smear-positive pulmonary TB among persons aged 15 years and above
- Prevalence of TB symptomatic individuals
- Health seeking behavior of TB symptomatic individuals
- Coverage of health services for TB symptomatic individuals
- Association between tuberculosis prevalence and possible risk factors

When estimating prevalence, appropriate weights should be assigned to obtain representative figures. Weights are proportional to the inverse of selection probability. For stratum level weighting, as shown in Annex-1, the nature of PPS, samples are self-weighted (i.e. no explicit weighting is required) when sizes
of all clusters are identical. Because actual cluster size may vary, weighting is expected to be required even if all selected villages have more than 640 eligible population. Association of possible risk factor (e.g. age, sex, type of area) will be made by using logistic regression model in which survey design is incorporated (e.g. svy command in Stata (StataCorp, Texas)).

As primary analysis, prevalence will be estimated based on the number of TB cases detected among participants. To correct for missing data and non-participation, the influence of missing data and non-participation on the results will be assessed using weighted analysis and multiple imputation. In addition, the post-stratification is made adjusting for demographic difference between survey population and population census of 2008 to explicate survey results to current population.

For comparison with survey of 2002, because the sampling of 2nd survey is made independently of 1st survey, primary analysis is made by logistic regression incorporating survey design (e.g. svy command in Stata) by handling with two surveys as different strata. To take into account demographic change between survey, age and sex are included as covariates in the logistic regression.

Two surveys are dealt with as different strata

5.3.5 Follow-up of TB cases identified in the survey:
Information on smear and culture results will be informed to team leaders and other medical doctors of the survey immediately once positive specimen is detected. They will inform district TB supervisors of the results with advice on diagnosis and treatment based on the bacteriological results, symptom and chest x-ray finding. For participants with chest X-ray suggesting TB, they also inform for treatment or further examination. To confirm TB cases identified in the survey receive proper care, central team member visit the facility responsible for them.

6-QUALITY CONTROL

6.1 Bacteriological examination
- Smear examination: After ZN examination, specimens with positive results by either FL or ZN, those that are culture positive, and 10% of ZN-negative specimens will be blindly re-examined by ZN methods.
- Culture examination: the contamination rate and recovery rates will be assessed by smear positivity. The recovery rate for smear-positive cases should be 90% or more. If the contamination rate is too high (over 5%) or too low (close to 0%), the decontamination process will be checked. If the contamination rate is over 5% and the recovery rate for smear-positive specimens is lower than 85%, suspension of the survey until correcting this will be considered.

6.2 CXR reading for detecting the eligible for sputum collection
All films with shadow categorized as eligible for sputum collection and 10% of films
with shadow categorized as non-eligible for sputum collection will be checked by the CXR central team.

7-ETHICAL CONSIDERATIONS

The survey will be designed and carried out following the internationally established methods for TB screening and diagnosis. Considering the relatively low prevalence among children, it has been decided to exclude the population aged under 15 years. The subjects will be properly informed of the purposes and methods of the survey, and their rights to reject will be guaranteed. Participation in the survey will be made only after obtaining informed consent. The objective and procedures of survey, risk/benefits will be informed by the explantion material and explanation by survey team. For minors (persons aged under 18 years old), informed consent will be obtained from his/her parent (or guardian) and assent will be obtained from him/herself. However, if both parents and guardian are not available with them, considering minimal risk of procedures by survey and benefit of TB screening in high burden country, they will participate after his/her consent. This type of situation may be faced, for example, when some young persons migrate from rural to urban to seek the job.

Bacteriologically confirmed subjects and those with CXR suggestive of tuberculosis will be informed of the result through a local health official, and they will be provided treatment or further examination free of charge under the DOTS programme. Since 2005, DOTS is available in primary health care centres in village level across the country. For bacteriologically-confirmed caseae, health officials (district TB supervisor) should be informed as soon as possible within 7 days after the results become available. Those with other medical conditions will be referred for medical services. While harm due to exposure to radiation in taking one CXR film is regarded minimal, appropriate protection procedures will be adopted to reduce unnecessary exposure including covering abdomen of women participants by lead-material. While CXR examination is to non-abdominal and non-pelvic regions, it is regarded as not significantly damaging to a fetus; however, regardless of known pregnancy, participant have right to reject CXR and other survey procedures after participating in the survey. This issue is included in the informed consent. Because TB is curable disease and it can affect patient’s famry and others, TB treatment will be provided if necessary for participants as mentioned in the “Survey procedure” section. This is also included in the informed consent. Approval of the protocol will be obtained from the Cambodian Ministry of Health, the WHO Task Force for TB Impact Measurement and the institutional review board of the Research Institute of Tuberculosis, Japan.

8-TIME SCHEDULE (Annex-4)

1st quarter 2010
- Complete draft of proposal for prevalence survey and submit to MOH and WHO Task Force
- Submit necessary items and personnel expenses
- Develop draft SOPs for survey implementation
Nominate team leaders and technical team members
Establish Executive Committee and Technical Committee members

2nd and 3rd quarter 2010
- Sample sites
- Visit selected sites to assess feasibility and coordination with local authorities
- Conduct workshop
- Conduct training
- Conduct field test and pilot study
- Modify protocol and SOPs based on pilot study

October 2010 – July 2011
- Field operations

November 2011
- Assess preliminary results of survey

9-BUDGET FOR NECESSARY ITEMS AND PERSONNEL EXPENSES

Draft of budget is Annex-5.
The budget for the survey consists of the following items and personnel expenses:
- Budget for equipment
- Budget for training
- Budget for pilot study
- Budget for field survey, central X-ray reading and laboratory work
- Budget for data entry and analysis.

10-TECHNICAL ASSISTANCE

The following technical assistance will be provided.

- JICA and RIT/JATA: With the agreement between the Government of Japan and the Royal Government of Cambodia, JICA launched a 3 year project to provide a comprehensive technical assistance package, including dispatches of technical experts in different areas. Under the contract between JICA and RIT/JATA, JICA will work as the leading technical assistance agency to assist the NTP to design, prepare and implement the survey in collaboration with various country partners such as TBCAP and the US Centers for Disease Control (CDC) Cambodia office. In collaboration with the WHO, it will assist the NTP to analyse and disseminate the survey results promptly.

- RIT/JATA will provide technical support both as an implementing agency under the JICA project and as a primary WHO task force member for the survey in Cambodia.

- WHO Stop TB Task Force on TB Impact Measurement (the Task Force): With
the country and regional offices, WHO Stop TB, TB Monitoring & Evaluation (STB/TME) and the Task Force will facilitate international technical support. Team members will provide information on the international guidelines for prevalence survey and will lead the certification and analytical processes of the survey.

- US-CDC: The US-CDC regional office in Bangkok will participate in the protocol review, mid-term review of survey operations and analytical processes as an external reviewer in collaboration with the Task Force and US CDC headquarters.

REFERENCES


2. Pregnant or Potentially Pregnant Patients: in ACR PRACTICE GUIDELINE. 2008

3. Protection of Pregnant Patients during Diagnostic Medical Exposures to ionizing Radiation. HPA. The Royal College of Radiologists. 2009