# Standard Operating Procedure for the National Prevalence Survey on Tuberculosis in Cambodia, 2010-2011

(Version: 20110315)

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1. Introduction

A National Tuberculosis Control Program (NTP) should know the size of the tuberculosis (TB) problem in the country and, even more importantly, its trend: whether TB control leads to a reduction of the TB problem. As TB prevalence is one of indicators of Millennium Development Goals (MDGs) and the Global Stop TB Plan, a TB prevalence survey is one of the most effective tools to monitor the impact of the program on the problems. The results from a series of quality prevalence surveys may show the impact of national and international investment in TB control in Cambodia. The first national prevalence survey was carried out in 2002 and eight years after which the second survey is planned to measure TB prevalence at time of the survey and difference from the first survey. The first survey suggested an impact of DOTS since 1994. The second survey is expected to show stronger evidence of “downward” trend in TB epidemiology in Cambodia owing to DOTS expansion since 2001.

The primary objectives of the coming national prevalence survey is to assess the prevalence of pulmonary TB among population aged 15 years or more at a defined point in time (2010) in Cambodia, including (1) sputum smear-positive pulmonary TB, (2) culture-positive pulmonary TB, (3) radiologically suggestive pulmonary TB, and (4) symptoms suggestive of TB. The secondary objective is to assess the trend of prevalence of pulmonary TB.

2. Overview of the procedure of the National Prevalence Survey, 2010/11

About 40,000 eligible individuals (i.e. 15 years old or older) from 62 representative clusters selected from districts of Cambodia are screened for TB by interviewing about TB related symptoms and chest X-ray examination. A person with any symptoms or chest X-ray abnormality suggestive of TB is further examined for sputum smear microscopy and culture tests according to the study protocol. The interview results and census data are entered into the computerised database and integrated with the test results of sputum and chest X-ray, and prevalence of smear-positive TB, culture-positive TB, and X-ray abnormality suggestive TB is calculated.

Three (3) survey teams are formed, the members of which are selected from the staff of CENAT. The team consists of the team leader, Census/interview unit, Informed consent (IC) unit, Radiology unit, and Laboratory unit (Table 1). Those three teams take a week of off-duty by rotation, thus at most two teams conduct the field operation. Because the field operation at one field site (cluster) is expected to take at least seven days, the team conducting a maximum of two survey sites (clusters) should leave the field to take rest for a week. The roles of each unit of the survey team are shown later subsections in detail.

Since at most two survey teams are on duty in the field during the survey period, it would take at least 32 weeks, probably almost 50 weeks, to finish the field operation.

Before the main field operation in a cluster, the representatives or a part of the survey team visit the cluster (pre-survey visits) twice: the first visit and the second visit.

### Table 1: The list of the survey staff

<table>
<thead>
<tr>
<th>Unit</th>
<th>The number of staff</th>
<th>Member</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survey team</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Team leader</td>
<td>1</td>
<td>CENAT senior physician</td>
</tr>
<tr>
<td>Informed consent unit</td>
<td>2</td>
<td>CENAT Administrative staff</td>
</tr>
<tr>
<td>Census / interview unit</td>
<td>3</td>
<td>CENAT health care staff</td>
</tr>
<tr>
<td>Department</td>
<td>Count</td>
<td>Position Description</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>Radiology unit</td>
<td>4</td>
<td>CENAT radiologist or respiratory physician 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Radiology technicians 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Radiology Assistant 1</td>
</tr>
<tr>
<td>Laboratory unit</td>
<td>2</td>
<td>CENAT laboratory technician and assistant</td>
</tr>
<tr>
<td>Driver</td>
<td>3</td>
<td>CENAT drivers</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>15</strong></td>
<td></td>
</tr>
<tr>
<td>Local survey collaborators</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local survey staff</td>
<td>3</td>
<td>Provincial (2:TB and lab) and OD (1) TB supervisors</td>
</tr>
<tr>
<td>Village leaders</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Regional volunteers for survey</td>
<td>8</td>
<td>Health volunteers of the village</td>
</tr>
<tr>
<td>Health centre staff</td>
<td>2</td>
<td>Person in charge of TB</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>15</strong></td>
<td></td>
</tr>
</tbody>
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3 Standard Operating Procedures (SOP) for the field operations
3.1 SOP for First Pre-survey visit

The purpose of the first pre-visit is as follows:

- To assess the feasibility of conducting the field operation at the selected village
- To meet with the village leader and to brief him/her about the NPS
- To estimate the number of eligible population (15 years old or older) in the selected village
- To select additional village(s) if the number of eligible population is less than 610
- To ask the local people for cooperation for the implementation of the survey
- To check the approximate adult population of the village if it is 640 or more
- To estimate the travel time from Phnom Penh to the selected village
- To list household group numbers for the survey
- To conduct mapping of the household groups in the selected village(s)
- To check the main industry and occupations in the village(s)
- To check the culture and customs of the village

3.1.1 Assessment of the feasibility of field operation

Two representatives of the Executive Committee meet with the Provincial and OD TB Supervisors who supervises the jurisdiction including the selected village. They hear the situation from the supervisors, including the access to the selected village by car and the seasonality.\(^1\) If the two representatives agree that field operation is not feasible to conduct at the selected village at the point, an alternative village is selected randomly from the same commune of the initially selected village according to the protocol. Then, the feasibility of conducting field operation at the alternative village is assessed with the Provincial and OD TB supervisors. After this, the mission proceeds to the health centre (HC) and the village.

At the village the mission assesses further, such as the availability of the venue of the field operation centre, including the accommodation for field operation team at the site, and supply of water. The field operation centre should be as close to the centre of the village as possible so that the villagers can access easily to the operation centre. The field team stays either at the operation centre throughout the operation or at a hotel nearby the operation centre. In the former case, the team needs to hire a house with sufficient space and rooms. In the latter case, the travel time between the hotel and the centre should be less than an hour.

3.1.2 Meeting with the Village leader

The mission meets with the village leader. At this point, the mission needs to provide just sufficient information, such as (1) the village was selected for NPS, (2) the census unit visits the household, (3) every household member who is 15 years old or older is taken chest X-ray to detect TB, (4) every TB suspect is taken further two specimens of sputum which are tested at the laboratory, and (5) every TB patient detected is on free TB treatment. Since the dates of visit by the field operation team have not been fixed, it is not possible for the mission to indicate when the field operation is conducted.

\(^1\) In NPS conducted in 2002, there were two villages that were initially selected and turned out to be inaccessible by car. Those villages eventually were replaced by alternatives.
3.1.3 Estimating the number of eligible population

Since every village has its own census data listed by the household group ID number, the mission estimates the number of eligible population for the survey (i.e. the number of the population with age 15 years old or older)

3.1.4 Additional village(s)

In case that the number of eligible population in the initially selected village is estimated to be less than 610, additional village(s) needs to be selected until the number becomes about 640. The method of the selection is based on the survey protocol: the additional village is randomly selected from the adjacent villages in the same commune as the initially selected village. After this, the mission proceeds clockwise around the initially selected village to select additional villages after the second until the number of eligible population becomes about 640 (610-670) or over.

3.1.5 Assessing the travel time to the village from the nearest health facility

Travel time from the nearest health facility (i.e. either health centre, OD or Provincial Referral Hospital) to the village is assessed and recorded.

3.1.6 Mapping of the selected village(s)

The selected village(s), including the household groups with its ID number, is mapped on a map.

3.1.7 Checking of the main industry and occupations of the village(s)

The main industry and occupations of the village(s) are checked. If most villagers are, for example, factory workers, the working hour of the field operation team needs to be adjusted for the villagers’ after work (e.g. 6-8 pm) to be able to achieve more than 90% participation rate. Likewise, if most villagers are farmers, the working hour may need to be shifted earlier (e.g. starting from 6 am).

3.1.8 Checking of the villagers’ customs regarding medical procedures

The norm or customs of the villagers are checked, particularly the villagers’ acceptance towards medical procedures (i.e. chest X-ray examination). Seasonality in the village is also assessed to foresee the feasibility of field operation in a certain season.
3.2 Second Pre-survey visit

The selected members of the field operation team which is actually conducting the survey in the selected village visits there as the second visit two to three weeks before the field operation. The mission consists of five persons: the team leader, Census Unit member (3), and the driver. The OD and Provincial TB supervisors and the Provincial TB Laboratory supervisor who oversee the village accompany the mission. Following items are brought to the village:

- Household register (Form01)(for 300 households)
- Leaflets about NPS (for 300 households)
- Brochure (notebook) about NPS (for 300 households)

The purposes of the second pre-visit are as follows.

- To meet with the village leader to ask to set up local collaborating team for the survey
- To count the number of eligible population (i.e. 15 years old or older) in the village(s)
- To decide in which part of the village the field operation is conducted (optional)
- To conduct on-the-job training for the volunteers to fill out household register (Form01)

3.2.1 Meeting with the village leader

The mission meets with the village leader and vice-leader and explains the procedures of the field operation of the NPS, including the dates on which field operation is conducted. When the field operation of the NPS is conducted in more than two villages, the mission meets with every village leader of the selected villages.

3.2.2 Organization of a local volunteer team

The village leader is asked to organize a local collaborating team for the survey consisting of at least 8 volunteers for the pre-survey census operation and the main field operation of the NPS. The candidates need to be informed in advance through OD TB Supervisor to come to the village leader’s office on arrival of the mission.

3.2.3 Verification of the number of eligible population in the village

The number of eligible population (i.e. 15 years old or older) in the selected village(s), including additional village(s) selected during the first visit, is counted using the village’s census data and verified if it is about 640 (610-670). If the number is not sufficient, an additional village is selected as has done in the first visit.

It should be reminded from the experience of the pilot surveys, the actual number of population in rural area, particularly that is near Phnom Penh, is a little smaller than is registered and thus it is recommended to enlist the eligible population as close to or more than 640 as possible. This is because some young people actually live in Phnom Penh and come back to the area in the weekends but they still are registered in the area. On the other hand, the actual resident population in urban area, particularly in Phnom Penh, tends to be larger than is registered for the same reason and thus enlisting more than 610 eligible population would be sufficient.
3.2.4 Deciding the part of village for survey

When the field operation is being done in a part of the selected village, either it is initially or additionally selected, the part (i.e. household groups) is being selected in one of the following manners.

As most villages have household groups with their ID numbers, the part of the village is selected by the IDs of household groups. First, a table with ID numbers of each household group and the total numbers of household members, including eligible population, is prepared. Second, the mission prepares two lotteries: one for going up or down and the other for the numbers from one through the total number of the household groups. Third, the village leader is asked to randomly pick one lottery for going up or down, and then the other for the ID number of household group from which the selection starts. For example, if the randomly selected number is five (5) after the leader has chosen going up, the household groups are selected from the number five of the household group going up to the number with which the total number of eligible population becomes sufficient. If the number reaches the top or the end of the household groups, then it starts again from the other side and the selection continues until the eligible population becomes sufficient.

If the village does not have household groups, the households are being selected for the survey by detailed mapping with an ID number allotted to each block, which desirably has an eligible population of nearly 200-300. And then, the starting block is randomly selected from the map of the whole village by lottery. From which, the same procedures as mentioned above are to be followed.

In either way, the method of the selection of the household groups needs to be recorded on the field operation report.

3.2.5 On-the-job-training on census taking for the volunteers

The mission provides on-the-job-training on how to fill out the household register (Form01) to the village leader and the volunteers, together with the OD TB supervisor. The Census Unit members of the mission and the volunteers are divided into subgroups and visit each household in the village where the main field operation is to be conducted. On arrival at the household, the census team explains the brief overview of the NPS, the census taking, the date of the main field operation, asks for the household member’s cooperation to the NPS, and provides with the brochure and leaflet.

The team instructs how to fill out the household registry as follows:

- The local authority should fill out the names of village, commune, district and province in the household registry (Form01).
- Every person, regardless of age, who has stayed at the house as of the date of arrival of the census team is recorded on the form (i.e. name, sex, date of birth, age, and occupation).
- The age of each household member should be recorded as accurate as possible.

The on-the-job-training would last only one day and the rest of the work should be left to the volunteers supervised by the OD TB supervisor. The whole work of drafting Form01 should be finished in two or three days. These household registries drafted by the volunteers before the field survey will be completed by the census team on the day 1 of the Survey.
3.2.6 Mapping of the selected village(s)

The selected village(s), including the household groups with their ID numbers, is mapped on a map. The tasks written from 3.1.5-7 are supposed to have been done during the first visit but it needs to be confirmed.

3.2.7 Checking of the main industry and occupations of the village(s)

The main industry and occupations of the village(s) are checked. If most villagers are, for example, factory workers, the working hour of the field operation team needs to be adjusted for the villagers’ after work (e.g. 6-8 pm) to be able to achieve more than 90% participation rate. Likewise, as most villagers are farmers, the working hour may need to be shifted earlier (e.g. starting from 6 am).

3.2.8 Checking of the villagers’ customs regarding medical procedures

The norm or customs of the villagers are checked, particularly the villagers’ acceptance towards medical procedures (i.e. chest X-ray examination). Seasonality in the village is also assessed to foresee the feasibility of field operation in a certain season.
3.3 Overview of the field operation

3.3.1 Day 0

The team leader, the member of the Census / interview unit, Informed consent (IC) unit, and the driver arrive in the village by 16:00 Sunday.

The team meets with the village leader and the volunteers. The Census unit receives the household registry which the village leader and the volunteers have drafted at the time of the team’s second visit.

The Census unit, together with the team leader and IC unit, then holds a brief meeting with the volunteers on the census taking to occur the next day.

After the meeting, the team does a couple of internal works: assigning dates of field operation to the household groups. The whole household groups are divided into six subgroups (am or pm of three days) depending on each size and assigned date and time to come to the field operation centre for interview and chest X-ray.

Table 2: The number of participants to be assigned to come to the field operation centre for interview and chest X-ray

<table>
<thead>
<tr>
<th></th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>120-130</td>
<td>120-130</td>
<td>120-130</td>
<td>For tracking</td>
</tr>
<tr>
<td>PM</td>
<td>80-90</td>
<td>80-90</td>
<td>80-90</td>
<td>non-responders</td>
</tr>
</tbody>
</table>

Then, the team prepares invitation letters to the participants for the interview and chest X-ray test conducted at the field operation centre. The letters are handed out to each family chief during each household visit for census taking. In the letter, the recommended date and time for each family to come to the field operation centre is indicated.

3.3.2 Day 1

3.3.2.1 Census taking

On the day 1, the Census unit, together with the team leader, IC unit, the volunteers, and TB supervisors, conducts census taking using household registry (Form01) drafted by local staff after the second visit of the team. The team, the volunteers, and the OD TB supervisor are divided into four subgroups: one for allotting and pasting a household number (Written on the Form02) to each house and three for census taking, each of which should have at least one member from central, either the team leader or a member of the Census unit. The Census team explains the tasks of census taking to the volunteers. This activity needs to be finished within one day.

After pasting Form02 on the door or a proper space with the household numbers, the team including TB supervisor gives the drafted household register (Form01) to the chief of household, which will be retrieved by the census team later coming.
The tasks of the subgroup of census taking are as follows:

- Each sub-census group visits all the households in the assigned area of the selected village(s).
- Every person, regardless of age, who has stayed at the house for two weeks or more before the arrival of the census team is verified (i.e. name, sex, date of birth, age, and occupation) one by one on the Form01 drafted by the volunteers beforehand. If any discrepancies are found, the team has to correct them accordingly.
- If there is any person who has stayed at the household for more than two weeks before the arrival of Census team and who has not been on the household registry yet, the person should be added at the end of the registry. If there is any person who is on the registry but has moved out of the household, has long been out for some reasons, or died, the person should be crossed out from the registry.
- If a person continuously stays at two locations, the person should be registered at the place he or she stays longer. If a person stays, for example, the dormitory of a school for weekdays, and spends weekends at home, the person should be registered at the location of the dormitory, not at the location of the home.
- The age of a person is recorded by YEAR. If the person is an infant, the age can be recorded by month but specified as XX month old. The age should be recorded as accurate as possible, particularly the person is nearly 15 years old. A big event occurred in 1995 or 1996 can be utilised to remind the person or his or her parents of the year of the person’s birth. If the age of the person is really not sure but seems between 14-16 years old, the person should be reminded of the birth day or year based on the information on such as the year animal or a specific memorial event. The animal-year-age table (Annex 7) would help in this regard.
- Each eligible person (i.e. ONLY 15 years old or older) is assigned a registration number, which has 7 digits (e.g. 15-103-08): the first two indicate the ID number of the cluster, the middle three digits the serial number of household in a cluster, the last two the serial number of family member in a household.
After the verification, the Census team members fill out the invitation letters (Form03), including the names of eligible members in the household, and gives it to each family chief. The Census team cordially asks the family chief that all eligible household members (i.e. those who are 15 years old or older) visit the field operation centre for interview and chest X-ray examination. The Census team needs to explain that the date and time are designated for the household members, however, they can actually visit whenever they like during the operation time (normally 7:00-12:00 and 13:30-17:00, day 2 through day 4). In the invitation letter, the date and time designated to the household member for the interview and chest X-ray is written previous day (i.e. day 0). Also, a brochure or a leaflet in which the details of the NPS are printed is provided to each household. Each household visit should be completed within five minutes so that the sub-groups of Census team can finish the task within one day.

3.3.2.2 Arrival of the X-ray and Laboratory units

The members of the X-ray and laboratory units arrive in the village by the sunset of the day 1. The X-ray unit members set up the X-ray machine, X-ray protection curtain, automatic processor, and a generator. The generator needs to be placed as far from the field operation centre as possible so as to prevent its noise from disturbing the operation. It normally takes 40 minutes to set up the X-ray facility. The detailed SOP for installation of the X-ray equipments is found in Section 3.6.

3.3.3 Days 2-4

On days 2-4, the team conducts screening for TB of the eligible population in the selected village(s). The field operation normally opens from 7:00-12:00 and 13:30-17:00. It should be noted that the working hour in the morning is longer than afternoon, thus morning sessions are more important than afternoon ones. The details should be decided by the team leader.

The whole household groups in the selected village(s) are divided into six groups on day 0 depending on each size and each subgroup of households are assigned date and time (either morning or afternoon and either day 2, 3, or 4) to come to the field operation centre for interview and chest X-ray.

Figure 2: Arrangement of the field operation centre

Flowchart of screening days (D2-D5)
3.3.3.1 Reception and informed consent

The participant is first lead to the reception by a volunteer (Figure 2). The receptionist is one of the IC unit members aided by a couple of local staff. If there is long queue in front of the reception, the participants are requested to wait at the waiting area, which needs to be prepared outside of the field operation centre with some seats and shading with tent.

The receptionist picks up the household registry (Form01) to which the participant belongs and verifies the participant’s invitation letter by referring to the household registry (Form01). Local staff provides the participant with an informed consent form (Form04) and an information sheet (Form14) for reading and acceptance of the participation in the survey. The local staff explains the participant the overview of the NPS and the procedures of interview, chest X-ray, and sputum collection (optional to symptomatic or abnormal shadow by chest X-ray), using the IC Form04 and the information sheet. The advantages and disadvantages of the survey participation are also fully explained to the participant here. After the explanation and clarifications, if any, the participant is requested to sign on the Form04. If the participant is 18 year old or younger, his/her guardian is requested to sign on the Form04, instead. The guardian is defined as the participant’s either one of parents or one of his/her close relatives who resides with the participant in case his/her parents live far away and thus either one of the parents are not able to attend at the field operation centre.

And then, the receptionist fills out the survey ID, name, age, sex and occupation of the participant in the individual survey card (Form05) with ID card (Form06) copied with carbon (Figure 3). The age of the participant is verified with the year of birth or the symbolic animal that represents the year of the birth, using year-animal-age table (Annex 7). He or she gives the individual survey card and ID card to local staff. The local staff brings these documents to interviewer and leads the participant there. It should be emphasised that the documents should never be given to the participant to avoid misplacing them somewhere or taking them to the participant’s home.

3.3.3.2 Interview

The interviewer conducts an interview using the individual survey card (Form05). The cluster number and the name of the cluster, commune, district, and province need to be printed in advance (Figure 3) on either the day 0 or at least the day before the participant is expected to come.

Figure 3. Filling in the names of village, etc. into the Survey sheet
The Survey ID number, the name, the sex, the age, and the occupation of the participant are verbally confirmed with the participant (The sections (1) through (5), see Figure 4 below).

**Figure 4. Filling in the Survey ID, the name, etc. into the survey sheet**

<table>
<thead>
<tr>
<th>Cluster No.</th>
<th>Survey ID No.</th>
<th>Name</th>
<th>Sex</th>
<th>Age</th>
<th>Occupation</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>(6) Symptoms and Duration (one month last)</th>
<th>(7) Health seeking behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>Yes</td>
</tr>
<tr>
<td>Sputum</td>
<td>Yes</td>
</tr>
<tr>
<td>Haemoptysis</td>
<td>Yes</td>
</tr>
<tr>
<td>Chest pain</td>
<td>Yes</td>
</tr>
<tr>
<td>Loss of B. W.</td>
<td>Yes</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Yes</td>
</tr>
<tr>
<td>Fever</td>
<td>Yes</td>
</tr>
<tr>
<td>Night sweats</td>
<td>Yes</td>
</tr>
<tr>
<td>Others</td>
<td>Yes</td>
</tr>
<tr>
<td>Interviewer comments for sputum collection</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(8) TB treatment history</th>
<th>(9) Radiology</th>
</tr>
</thead>
</table>

The actual interview starts with the symptoms suggestive of TB (the section (6), see Figure 4) which are selected from nine choices (1) cough, (2) sputum (production), (3) haemoptysis, (4) chest pain, (5) body weight loss, (6) fatigue, (7) fever, (8) night sweat, and (9) others. Multiple answers are allowed. If any “yes” in (1) cough, (2) sputum, and/or (3) haemoptysis is chosen, the duration of the symptom(s) needs to be indicated by number of days. Also, if “others” is chosen, the symptom(s) needs to be specified in text. After asking all symptoms mentioned here, the interviewer needs to decide whether the participant is eligible for sputum collection or not. The eligibility for this is defined as ‘cough lasting 14 days or longer’, or ‘haemoptysis’. If the interviewer wavers in the judgement, the team leader needs to be informed so that the leader can decide it.

Regarding the actions taken (the section (7), see Figure 4) to the symptom(s), just one is selected from three choices either 1) no action taken, 2) self medication of any kind, or 3) consultation to anybody. If “consultation” is selected, the facilities or person consulted with are also need to be chosen from a) Government Hospital, b) Health centre, c) Private clinic, d) Private hospital, e) Pharmacy, f) Traditional healer, g) Family member, h) Others. Multiple answers are allowed. If the participant either did not 1) take action, did 2) self medication of any kind or did not consult with a) Government hospital or b) Health centre, the interviewer further needs to clarify the main reason for this: i) not serious, j) economic reasons, k) far from house, l) prefer private, m) others.
Regarding TB treatment history, just one from either “Yes” or “No” needs to be selected by asking “Have you been treated for TB before or are you treated for TB now?” If the participant answers “Yes,” additional questions should be asked: “When? Are your receiving TB treatment at present or in the past?”. If the participant received TB treatment in the past, you should ask the latest year when the participant developed TB. If the participant is being treated at present, you should ask the months for which the participant has been on the current TB treatment. If the participant has both the current TB history and the past history(ies), you should confirm and record the both events independently. Also, the facility which provided the treatment needs to be selected from a) Government hospital, b) Health centre, c) Private hospital, d) Private clinic, e) Pharmacy, f) Traditional healer, or g) Others. Multiple answers are allowed. If he/she had more than one TB history, the most recent episode should be recorded.

After the interview, the interviewer gives the individual survey card (Form5) and the ID card (Form06) to a volunteer and the volunteer leads the participant to the radiology assistant. It should be emphasised that the individual survey card should never be given to the participant to avoid misplacing them somewhere or taking them the participant’s home. After taking the participant to chest X-ray, the volunteer gives the ID card (Form06) to the radiology assistant and the individual survey card (Form5) to the team leader.

3.3.3.3 Chest X-ray examination

The radiology assistant receives the ID card (Form06) from the census unit through a volunteer. After verifying the participant’s identity using the ID card, the assistant asks whether the participant has agreed to get chest X-ray examination. If so, he puts the participant’s X-ray ID number on the film cassette with adhesive cellophane tape (i.e. scotch tape). The radiology assistant also briefly explains the participant the procedure and its importance in the NPS. Then the participant is asked to take off any ornaments/metal objects, etc. from the body. If deemed necessary, the participant is asked to take off the shirt and change it to a T-shirt which does not produce artefact. Then the radiology assistant leads the participant to the X-ray room where the radiology technician is waiting. If the participant is female, the radiology technician needs to pay more attention so that she can change the cloth out of sight of other participants.
The technician makes the participant the position for chest P-A view, positions the X-ray tube at appropriate height and FFD, collimates the beam to area of interest and gives brief instructions on keeping being fully inhaled during the shot. If applicable, he shields the abdomen/pelvis with lead apron. He sets the X-ray exposure factors. Once ready, he takes the chest X-ray. He then informs the participant to relax and leads to the radiology assistant. He also gives the taken X-ray film cassette as well as the ID card to the radiology assistant. He asks the participant to change the cloth (if needed) and wait outside the chest X-ray area until the film is interpreted by the film reader.

Another radiology technician in charge of processing films receives the taken cassette from the radiology assistant. He or she enters in the darkroom, removes the exposed film from the cassette, inserts the film to the automatic film processor, and processes the film immediately and once developed, s/he verifies the ID number of the film against the ID card, and gives the film as well as the ID card to the chest X-ray reader.

The radiology assistant passes the ID card to the chest X-ray film reader once the chest X-ray has been taken for the participant, and the volunteer leads the participant to another waiting area set up near the chest X-ray film reader.

Figure 6. Filling out the Survey ID Card for X-ray examination

![Survey ID card](image)

The X-ray reader verifies the Survey ID number on the film and that on the ID card, and records the Survey ID number into the CXR Registry (Form 7, see Figure 7). Then the reader carefully interprets the film whether it has any abnormal shadow and records the findings of the film in the section of “Chest X-ray film reading” on the ID card (Form06, see Figure 6) as well as in the CXR registry (Form07). Primary result should be chosen from the three choices: “Normal,” “Abnormal,” and “Not taken.” If abnormal, it needs to be decided further whether the shadow is eligible for sputum collection: “Yes,” or not: “No.” For the purpose of screening, the reader needs to do over-reading but he or she does not need to give diagnosis such as active or healed TB or other disease. The eligibility for sputum collection in terms of chest X-ray is defined as all those with abnormal chest radiograph in the lung field or mediastinum other than a single small calcification nodule with a size less than 10 mm or pleural adhesion at cost-phrenic angle(s). Even if there are abnormal findings incompatible with TB such as broncho-pneumonia or bronchiectasis, sputum collection should be requested for the purpose of screening. Whenever the reader is undecided whether the shadow is eligible for sputum collection or not or whenever the participant is considered to need some urgent medical care, the reader must promptly consult with the team leader.
Figure 7. Registering the participant on the CXR registry

If the participant has an abnormal shadow eligible for sputum collection, the reader puts the CXR registry serial number on the film with a sticker for the later convenience, write down the CXR registry serial number on the ID card which is transcribed on the lab examination form (Form 09), and also needs to inform the team leader so that the leader can order sputum collection for the participant. If the reader suspects the participant having a disease which needs urgent action or treatment, the team leader needs to be informed as such and it should be recorded on a form for referral. These results are to be transcribed by the team leader to the individual survey card (Form05). The supplemental information on interpreting chest X-ray is found in Section 3.6.

3.3.3.4 Decision on sputum collection and QA for interview

The team leader receives the documents from the survey staff. The leader fills in the comment on sputum collection based on the symptoms recorded on the Individual Survey Card (Form 05) filled by an interviewer as well as comment of the X-ray reader on the ID card. The criterion of symptoms for sputum collection is either the participant has had cough for 14 days or more in the last month or haemoptysis. Also, he copies the comments of the film reader as well as his own on the ID card into the section (9) of the individual survey card.

Figure 8. Decision on sputum collection

If the leader finds “Yes” in the section 6.10 and/or in the section 9.3 of the individual survey card (Form05), he needs to tick “Yes” in the section 10.1 for sputum collection. Also, if the answer to the section 6.10 is “No” but the chest X-ray film suggests abnormal shadow suspected of TB, the leader verifies the symptoms (section (6)), the actions taken (section (7)), and TB treatment history (section (8)), again, using the identical questions printed on the backside of the individual survey card (see 3.3.3.2 Interview for more information). After the re-interview, the leader passes the ID card (Form06) to a volunteer. The volunteer leads the participant to the laboratory unit waiting outside.

The team leader also needs to re-interview about symptoms, action taken and TB treatment history for the quality assessment of the interview by systematically sampling at a given rate (5% or 10%) from the participants. If the leader finds any discrepancies between the first answer obtained by the interviewer and the answer which was given to the leader, more in-depth interview needs to be conducted for clarification and confirmation.
When the participant does not need sputum collection, the leader performs the final check of the documents (see 3.3.2.6 Final check before the participant leaves).

### 3.3.3.5 Sputum collection

First, the laboratory unit receives the ID card from the volunteer and write down the appropriate information on the TB suspect on a list of the TB suspects (Form08) with a duplicated-carbon copy (1st for being sent with sputum specimens, 2nd for the team leader and 3rd for being kept at laboratory site). The identical number of the lab examination form (Form09) are written on the designated row in Form08 (triplicated-carbon copy).

**Figure 9. TB Suspect list (Form08)**

<table>
<thead>
<tr>
<th>Kingdom of Cambodia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nation Religion King</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Operating site number</th>
<th>Cluster name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commune</td>
<td>District</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serial n°</th>
<th>Survey Code</th>
<th>Patient's name</th>
<th>Age M F</th>
<th>Date of specimen collection</th>
<th>Sticker of slip number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Second, the laboratory unit fills out the lab examination form (Form09) with a little more writing pressure because the form is tripli cate (i.e. the original is carbon-copied to other two sheets). On the Form09, the survey ID number, the name, age, and sex of the participant need to be filled out. Also, the label with a serial number (prepared in advance) of the form with the identical number printed in advance needs to be attached on the list of TB suspects (Form08) for reference. The laboratory unit attaches the label with the same serial number as the lab examination form on a sputum container and seals with scotch tape over the label, and writes down “D1” (i.e. day 1) on it with black permanent marker.

**Figure 10. The upper part of lab examination form (Form09)**

<table>
<thead>
<tr>
<th>To data management unit</th>
<th>Slip Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Laboratory Examination Forms</em> (Form 09)</td>
<td></td>
</tr>
<tr>
<td>at the site</td>
<td></td>
</tr>
<tr>
<td>1. Survey ID No.</td>
<td></td>
</tr>
<tr>
<td>2. Name (in full):</td>
<td></td>
</tr>
<tr>
<td>3. Sex: Male Female A.</td>
<td>Age: Year</td>
</tr>
<tr>
<td>5. Sputum collection data &amp; aspect</td>
<td></td>
</tr>
<tr>
<td>D1 Specimen: dd/mm/yy / / / Saliva Mucopurulent Bloody</td>
<td></td>
</tr>
<tr>
<td>D2 Specimen: dd/mm/yy / / / Saliva Mucopurulent Bloody</td>
<td></td>
</tr>
<tr>
<td>Date__________________</td>
<td>Sign by lab technician at site</td>
</tr>
</tbody>
</table>

And then, the laboratory unit gives the participant the instructions how to excrete good sputum into a sputum cup by using poster or leaflet, and asks to put sputum into the cup on the spot. The participant should produce sputum in open-air to avoid any possible TB infection to others. The laboratory unit staff fills out the date of collection on both Form08 and Form09, and checks the aspect of sputum (salivary, mucopurulent or bloody) on the Form09. The sputum cups with collected sputum should be tightly screw-capped and stored in an icebox with ice until transported to the culture centre. The ice box should contain sufficient amount of ice so that the temperature inside the ice box keeps less than 10 c (degrees centigrade). Also, it should be emphasized that the ice box should kept out of direct sunlight to prevent from heat.

The laboratory unit gives the TB suspect another sputum cup labelled the identical serial number and written down “D2” (i.e. day 2) on it with red permanent maker, and explains that the participant needs to take sputum next morning (D2) again and that he or she has to bring the cup to the field operation centre with ID card as soon as the sputum is taken. Then, the laboratory unit tells the participant to return to the team leader.

The lab examination form (Form09) has triplicate (i.e. the original is carbon-copied to other two sheets). The set of the form is to be sent to the designated culture centre together with the iceboxes including sputum cups. The collector needs to sign on the lab examination form (Form09).

After finishing the day laboratory staff receive individual survey sheet (Form 05) and fill the specimen collection date and her/his signature

### 3.3.3.6 Second collection of the sputum

Usually next morning, the TB suspect brings the sputum cup with second sputum
collected in the early morning at home to the laboratory unit of the field operation centre together with his/her ID card. When the laboratory unit receives the cup, the staff fills out the date of the second submission, the sputum aspect and his/her signature on the lab examination form (Form09). The date of sputum submission is written down on the list of TB suspects (Form08), as well. Finally, the laboratory unit tells the TB suspect to go and see the team leader together with ID card for the final checking and provision of the gifts.

**Backup system for culture examination**

In order to obtain backup for culture examination, an additional third specimen, called D3, will be collected throughout survey from the case who is strongly suspected for active TB on the CXR, for example, with cavitary or infiltration shadow. D3 specimens from the participant selected by X-ray expert must be kept in the freezer.

**During the survey;**

1) When the team leader or chest X-ray reader finds active TB case strongly suspected on CXR, he or she write down 'D3' (an additional specimen; call for the sake of convenience) on the ID card so that lab staff understands from whom the D3 specimen on the spot should be collected.

2) Lab staff writes down 'D3' on TB suspect list and the lab examination form (Form 09) of the subject in red ball-pen.

3) Give two conical tubes to the subject and ask the subject to bring two specimens in the morning of the next day.

4) Or when the subject brings D2 specimen on the next day, lab staff asks the subject to take D3 specimen on the spot. (The rate of the cases will be 3% of the participants at most which will be around 20 cases a cluster, 640x0.03=20).

5) If D3 specimen is difficult to collect in the field, field lab staff ticks cap of D2 (*or D1) the case in red marker. It means that this case has to collect additional specimen, but without D3, therefore culture staff needs to preserve D2 after inoculation instead of D3.

*Choose D1, if D2 specimen is not available.

**In the Laboratory (BTB, CENAT) ;**

6) The stocked specimen is basically one for case; treated D2 or untreated D3 specimen.

7) If culture staff finds D3 specimens, keep them in the deep freezer (freezer in Battambang) directly without inoculation process.

8) If culture staff finds a tick in red on the cap of D2 (that means it is the cases but no D3 specimen), the following treatment should be done for D2 specimen.

After the inoculation, add 5 times volume of the phosphate buffer (PB) solution than specimen in the sputum container already decontaminated by 4% NaOH. Then, mix it by vortex mixer. (Neutralization)

9) Keep the sputum containers with freezer plastic bag with zip lock into the ultra-deep freezer (a freezer at Battambang) until confirming contamination rate in next two weeks.
10) If complete contamination in all four tubes for a case is found out, defrost D2 or D3 specimen and inoculate.

11) According to capacity of deep freezer (freezer at Battambang), preserved specimen can be discarded after 2 months for CENAT, 1 month for Battambang.

3.3.3.7 Final check before the participant leaves

The team leader receives the ID card from the volunteer and performs the final check of the documents, particularly the individual survey card (Form05), if the necessary columns are accordingly filled out, before the participant leaves. If anything is missing, the leader asks the participant necessary inquiry and fills out the questionnaire or asks the participant to sign the IC Form04, etc. When everything is all right, the leader thanks the participant for his or her cooperation and provides with a gift, and the participant leaves the field operation centre for home.

If the participant needs to take sputum as TB suspect, the leader makes sure the participant comes back to the field operation centre next morning with the sputum cup with morning sputum taken. In such a case, a gift will be provided with the participant when he/she submits the second sputum to lab staff.

Apart from screening, the Census/interview group members conduct crosschecking of household registry with the individual survey sheets to verify actual attendance of the participants in the selected area every night after the screening activities are completed. The details of which are written in the section 3.5.

3.3.3.8 Specimen dispatch on Day 4

Sample will transport to designate the culture center twice a week. In the case of the field survey is planned from Monday to Saturday, the first shipment will be planed on Thursday (Day 4). Designate culture center is decided according to the location or capacity of culture centers. TB suspect list should be finalized and check samples and lab examination forms, if those are appropriate, fill the date of specimen dispatch, names and signature of laboratory staff and team leader.

The Samples (D1 collected in Day 2, 3 and D2 collected in Day 3, 4) will transfer. Laboratory field staff will send the follows;

1) Sputum Samples with enough ice in locked ice box
2) TB suspect list (Form 08) copy 1 white
3) Lab examination form (Form 09) all copies
* TB suspect list (Form 08) should be renewing (send all written with specimens and use new sheet) for D1 on Thursday.
* If D2 specimens have not received yet but all lab examination form (Form 09) should be send with specimens. Confirmation should be done by TB suspect list (copy 3)

3.3.4 Day 5

On day 5, the survey team conducts tracking-down of non-responders and prepares the field report. For example, a part of Census/interview unit and the volunteers visit households to ask the non-responders to come to the field operation centre for interview and chest X-ray examination. Also, a TB suspect who has not submitted the second sputum yet has to be tracked down to his or her house and to be asked on the spot to submit sputum. An elderly or a disabled person who cannot come to the field operation centre may be visited by a team member to take two sputum specimens on the spot to secure higher participation rate.

3.3.5 Day 6

On day 6, the sputum cups are transported to the designated culture centres with both finalised TB suspect lists (Form08) and laboratory examination forms (Form09) same as Day 4 (ref. 3.3.3.8. Specimens dispatch on Day 4). After completing all the field operations in the cluster, the team leader makes a summary report (Form10), which describes the number of eligible persons, non-respondents, attendees, etc. for every unit: interview, chest X-ray exam and sputum collection.

After the team shuts down the field operation centre and the team leader thanks the village leader, the volunteers, the OD and provincial supervisors, the team leaves the village and moves either back to Phnom Penh or to the next district where field operation is taking place.

3.3.6 Other issues

In operation in urban areas, the team has to consider conducting the field operations after office hours (17:00-20:00) depending on the participation rate. The team leader decides the details.
3.4 SOP for Team leader

The tasks of the team leader, a radiologist or respiratory physician, are as follows:

- To lead the second pre-survey visit, including assessing the feasibility of operation
- To supervise the survey team and field operation overall
- To coordinate the operation with the nearest OD TB supervisor, the village leader(s), and other local officials including the volunteers
- To read chest X-ray films, if necessary
- To make final check of the individual survey form (Form05)
- To fill out a report of field operation form (Form10)
- To coordinate post-field operation follow-up

See the related sections for more details (i.e. 3.2 for the second pre-survey visit, 3.3 for the field operation, 3.6 for chest X-ray reading).

Regarding the post-field operation follow-up, the team leader coordinates notification of active TB (i.e. smear-positive, culture-positive or CXR active result) from CENAT to the participant through respective OD TB Supervisor, who further arranges the TB treatment at the nearest health centre. When other diseases which need urgent treatment (e.g. pneumothorax) are detected during the field operation, the team leader and the OD TB Supervisor are in charge of coordination of the patient and the local health centre staff.
3.5 Census / interview unit

The unit consists of three health care staff of CENAT, with support from the village leader and volunteers of the village.

The overall tasks related to the census taking on the second pre-survey visit (3.2) and on the days 0-1 (3.3.1-3.3.2) are heavily written in the respective sections.

The overall tasks related to the interview are heavily written in the section 3.3.3 for field operation on days 2-4.

After operations on Day 2, 3, and 4, the Census/interview unit member has to conduct crosschecking of the household registry with the individual survey sheet. To do that, first all the individual survey sheets produced up to the moment of conducting crosschecking are sorted in order of the Survey ID number (i.e. thus in the order of household number). Second, cross-match of the individual survey sheets against the corresponding household registry is conducted for all the households in the area. Third, those who have not attended the screening are listed as “absentee list,” and on the Day 5, the Census/interview unit is to conduct tracing of absentees to increase the participation rate at the cluster. The SOP of tracking non-responders is written in the section 3.3.4, however, basically, a part of Census/interview unit and the volunteers visit households to ask the non-responders to come to the field operation centre for interview and chest X-ray examination. An elderly or a disabled person who cannot come to the field operation centre may be visited by a team member to take two sputum specimens on the spot to secure higher participation rate.
3.6 Radiology unit

The radiology unit consists of four persons: one radiologist or respiratory physician, two radiology technicians, and a radiology assistant. The roles of the unit are heavily written in the section 3.3.3 for days 4-6 of the field operation.

3.6.1 Equipment description and requirements

Equipment and accessories which will be part of the survey are described below:

3.6.1.1 X-ray machine:

For this survey each field unit is provided with one portable/mobile X-ray machine. Technical and other specifications are provided in Annex X. Emphasis has been on light weight for easy transportation as well as sturdiness for field work.

3.6.1.2 Film Processor

For developing and fixing X-ray films, each field unit is provided with one automatic film processor.

3.6.1.3 Protective devices

For protection of personnel and participants the following devices are used:

- Lead aprons with 0.25mm Pb thickness
- Lead screen for partition
- Radiation monitoring devices—the radiographers/X-ray technicians are encouraged to use their existing monitoring devices. In case of unavailability, good protection practices will be followed to ensure workers’ safety.

3.6.2 Installing and de-installing the X-ray equipments

3.6.2.1 Installation

The X-ray system (X-ray machine, film processor, chest stand, etc) is set up by the radiology technicians assisted by drivers and assistants under the supervision of the team leader.

- After arrival at the site, tally with the check list and ensure the safe arrival of all equipment. Assess any obvious signs of damage, and if any, report it to the team leader as soon as possible.

- Assess field conditions for optimum functioning in the X-ray area and also for adequate radiation protection. Plan for placing of the equipment and the generator.

- Prepare the area for setting up the equipment. Ensure adequate flat surface for documentation, and make arrangements for the participant’s changing areas. To ensure participant’s privacy, the changing area should be directly approachable from the shooting area.

- For radiation protection, house the X-ray machine in a manner that the primary X-ray beam is directed towards a thick concrete wall (if available) or towards an unoccupied area. Place the “radiation caution sign,” such that it is visible to participants. The other personnel than radiology staff and the participants are prohibited from three (3) meter perimeter from the radiation valve and the film to avoid unnecessary exposure to X-ray. It should also be avoided for anyone to continuously stay within five (five) meter perimeter from the same area.
• Unpack the X-ray machine, assemble a stand for the X-ray machine, place it at the identified place and install the X-ray machine to the stand. Connect the remote control cable to the X-ray machine. Connect the machine to input power and switch on the machine.

• Place the chest stand at appropriate focus film distance (FFD) from the X-ray machine. Measure accurately the FFD and also measure the distances between the focus and the right and left side of the chest stand. Place the lead screen between the X-ray machine and operator area.

• Unpack the automatic film processor and place it on a flat elevated surface or a long table. Assemble the racks for the developer and the fixer, and connect the tubes for them from the bottles to the racks. Pour the fluids of the developer, fixer, and clean water to the racks in the processor.

• Set up a darkroom back to back with the automatic film processor so that the exposed film can be removed from the cassette and inserted into the automatic developer.

• Developer and fixer are prepared according to the manufacturer’s manual with clean water brought from Phnom Penh or drinking water, if purchased locally. The developer and fixer is used for the first time after preparation, it may be recommended to add additives (i.e. starter), however, the amount of which should be limited to by half as indicated in the manufacturer’s manual.

• Before starting the generator, it needs to be checked according to the manufacturer’s manual. Switch on the generator after the driver/assistant has checked availability of fuel.

• The X-ray machine needs to go through “aging,” in which the voltage and milli-ampere are gradually increased from the minimum to the appropriate values.

• The processor also needs to go through aging, in which the temperature of the fluids have become appropriate at least five (5) “loss films” (i.e. used films) are processed and checked whether the films have been processed properly up to drying. This process should be repeated every morning before processing actually taken films, because during the night, the developer fluid gets oxidation and its capacity to develop films becomes weakened and this process said above ensures exchanging (or updating) part of the developing fluid so that the quality of developed and processed films more optimal than otherwise.

• Once the X-ray machine and the automatic film processor are ready, make a test exposure on the X-ray machine using a film and a human subject, if indicated. Process the film to ensure proper functioning of all equipment, including X-ray machine, processor, etc and verify whether expected quality of a chest X-ray film is obtained.

• The radiology technician needs to record the conditions of voltages and milli-amperes on the X-ray Registry. The various conditions of voltages and milli-amperes are also prepared as a table before going to the field and the condition is decided based on the table.

• At least two radiology technicians should wear the radiation exposure meters to measure the exposure during a field operation. One should reset the all data of the meter on the morning of Day 2 (the first day of screening) and should record the accumulated exposure at the end of the field operation of each cluster (i.e. Friday afternoon). Another radiation exposure meter is placed about three (3) meter away from the side of the radiation valve on the
morning of Day 2 and the result is recorded on the afternoon of Day 5 to monitor the exposure at a certain location.

3.6.2.2 De-installation

Switch off and uninstall the X-ray machine and carefully pack it in the respective case. Gather all accessories (chest stand, lead aprons, hangers, lead screen, table, chairs, radiation warning sign, cassettes, curtains, etc.) and pack them for transportation. Disconnect the generator and pack it for transportation. The automatic film processor is also turned off and all the fluids of developer and fixer are put into the bottles and tightly capped. Inside of the racks, tanks and tubes, and the rollers of the processor is tenderly brushed or wiped with a soft-sponge rinsing with clean water several times. After rinsing, the racks are wiped out and re-assembled for transportation.

3.6.3 Radiation safety

This subsection covers issues and practices related to radiation protection applicable for the purpose of this survey and field work. The overall objective is to ensure radiation safety for workers and participants in line with good radiation practices.

3.6.3.1 General practices

- X-rays will be performed only by trained individuals who have been sensitized about radiation safety
- All X-ray machines utilized for this survey will be pretested for radiation safety in line with international and Cambodian guidelines.
- Radiation protection devices (lead aprons, lead screens) will be used during X-ray procedures.
- As far as possible, X-ray procedures will be carried out in a closed concrete/brick walled area. If conducted in open air, it will be ensured that there is a minimum distance of 3 meters between persons (except radiographers and the participant undergoing X-ray procedure) and the X-ray tube (the recommended safe distance for radiography in hospital wards/ICU being 2 meters, refer to WHO publications in reference section).
- The team leader will supervise field work and supervisory visits will be made by the central X-ray team to ensure good radiation practices.

3.6.3.2 Participant/Public safety

- All participants are X-rayed only after obtaining informed consent.
- Survey consent form specifically includes consent for undergoing X-ray examination, and participants are informed about the insignificant risk carried by chest exposure (references can be found later in this document, in the References section).
- Female participants who are not sure of pregnancy/menstrual dates are X-rayed after shielding the abdomen/pelvis by using a lead apron/shield.
- The X-ray data sheet notes the factors (mAs and kV) used for each participant.
3.6.3.3 Workers’ safety

- Radiation warning sign is placed appropriately in the X-ray area.

- Pre-survey training of radiographers includes all aspects of radiation safety, and specifically concepts of ALARA (As Low As Reasonably Achievable) and TDS (Time, Distance and Shielding).

- Radiographers/X-ray technicians are encouraged to use their monitoring devices.

- Radiation protection devices (lead aprons, lead screens) are provided to all survey teams.

3.6.4 Interpretation of chest X-ray films

The primary purpose of using X-ray in this survey is to identify participants for bacteriological examination, and not to make a thorough radiological assessment for active case detection of TB and other lung diseases. For this end, TB implies pulmonary TB only and other forms of TB which may sometimes be reflected on CXR (like bone TB, spine TB, extra pulmonary lymph nodal or chest wall TB, etc.) are excluded by definition. Exceptions to this are isolated pleural effusion and pneumothorax (even without any demonstrable parenchymal involvement), which (for radiology purposes) will be considered part of pulmonary TB.

To keep in line with the primary purpose, following steps are adopted:

- Field level interpretation by medical doctor only classifies X-rays as (1) normal or (2) abnormal, and the participants as eligible for sputum collection (TB suspect) or not. These are the screening purpose only, so the reader does not need to give diagnosis such as active or healed TB, or other disease, but needs to pick up all TB suspect. Whenever the reader is undecided whether the shadow is eligible for sputum collection or not, or whenever the participant is considered to need some urgent medical care, he must promptly consult the team leader. If the participant has abnormal shadow eligible for sputum collection and is TB suspect, the reader needs to inform the team reader so that the leader orders sputum collection. If a disease which needs urgent action or treatment for the participant is suggested, the team leader needs to be informed as such.

- “Intentional Over-reading” is encouraged so that no suspected cases are left out for bacteriological examination.

- The final interpretation is made at the central level and this is described later in the SOP.

3.6.5 Quality assurance

This subsection deals with QA measures at the field setting. The QA for central reading is described in Section 4.3.

3.6.5.1 QA for X-ray procedure

- X-ray procedures are performed by trained individuals only.

- Field supervisory visits are carried out by the central X-ray team.
3.6.5.2 QA for interpretation:

- Images are interpreted in the field and later at the central level; retrospective analysis is carried out where applicable.

- The team leader re-examines all the films taken during the day at the night as a QA measure. In case of discordant interpretation, the participant is asked for sputum tests before the team leaves the site.
3.7 Laboratory unit

The laboratory unit consists of three persons: CENAT laboratory, CENAT car driver, and a health care staff at the nearest health centre.

3.7.1 Sputum collection

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

**Individuals from whom sputum specimens are to be collected**

Sputum specimens should be collected from TB suspect according to the protocol. A laboratory technician of the survey team must:

- fill out both the list of TB suspects (Form08) and the lab examination form (Form09) and stick a label with a slip number to the four correspondent areas (two for D1 cup, two for D2 cup);
- explain the reason why sputum examination is necessary;
- explain how to collect a sputum sample, with a pictorial poster or leaflet;
- confirm the sputum request on the ID card (Form06);
- attach the label with a slip number, and write down the “D1” or “D2” indicating the date of collection on the sputum containers;
- demonstrate how to open and close the screw cap;
- show how much sputum should be collected;
- tell TB suspect to show up next day with the 2nd sputum container and the ID card; and
- explain to the suspect the importance of sputum quality and proper amount in light of the need for accurate diagnosis.

**Number of samples and method of sputum collection**

The 1st specimen is on-spot sputum collected on a TB screening day, and the 2nd specimen is early morning sputum on the next day. The sputum sample should contain the volume of 3-5 ml, and be collected in a 50ml conical tube with a screw cap. The TB suspect should be instructed to do the utmost to collect a good quality and adequate amount of sputum.

**Place of sputum collection at the survey site (spot specimen)**

Sputum from subjects who come to the survey site should be collected at a place in the open air near the headquarters of the prevalence survey team, or outside the home of the subject during the visit.

**Reception of sputum specimen at the survey site**

Laboratory unit staff should wear the disposal gloves and laboratory white coat when they receive the sputum specimens. Sputum specimens that the subject brings from home should be checked for proper labelling and for any contamination outside the container. If contamination is
found, the container should be cleaned on the outside with a good-quality tissue paper soaked in 70% alcohol after the screw cap is tightly sealed, and labelled again if the label is not clear. Each sputum container should be placed in a vinyl bag with a leak-proof with rubber band. The sputum container with the cap tightly sealed, should then be placed in an icebox containing ice cubes. It should be emphasized that the sputum specimens from TB suspects must be stored in cool condition, thus the lid of the icebox should always be closed unless the cups need to be handled. Relating to this, the laboratory unit procures ice block from the nearest dealer everyday so that the content of the icebox should be kept cool. The sputum containers in the icebox should be away from the direct sunlight (UV light) by means of the black colour vinyl and rapping up with heat shield materials, for keeping the cool condition as well. The laboratory unit should arrange sputum stand in one-tier in an icebox, and the sputum cups in a sputum stand in order as indicated below. The laboratory staff places the sputum containers on D1 in a row, and then, the sputum containers on D2 in the adjacent row with a pair of two specimens from an identical TB suspect.

If the 2nd early morning sputum specimen has not been submitted, Health centre staff or volunteer has to visit home to collect the 2nd specimen as soon as possible.
Waste disposal

All possible contaminated materials and infectious waste should be collected in vinyl bags and burned or incinerated at the site.

Materials for sputum collection

The following materials are needed for sputum collection and handling:

- Two large iceboxes for specimen
- 50ml conical tubes with screw cap for sputum containers with stand;
- Leak-proof vinyl bags for sputum container;
- rubber bands;
- 70% alcohol in plastic bottle with screw cap;
- disposal gloves;
- cleansing tissue (tissue paper box);
- Styrofoam or small icebox for temporary storing sputum specimens;
- sputum container stands with base stand;
- ice cubes;
- vinyl bags for contaminated wastes;
- folding tables (1 set);
- folding chairs (3 sets);
- white desk cloth (2m);
- a tent;
- laboratory examination forms and leaflets;
- pens and permanent markers in red and blue colour;
- scotch tape with cutter;
- portable lighting devices;
- carry-on bags;
- thermo bottle;
- electronic kettle;
- plastic cups;
- clear holder;
- box files
- pure drinking water.
- Salt 1kg (500gm for one ice box)

3.7.2 Transport of sputum specimens

Before the transport of iceboxes, the laboratory unit has to drain water out of the icebox and replace old ice cubes with new ones to keep the specimens cool until arriving at the culture centre.

A car driver transports the icebox(es) containing sputum specimens, a carbon copy of lists of TB suspects (Form07) and laboratory examination forms (Form09) to the culture centre (CENAT or Battambang Provincial Hospital) on Thursday (D1 specimens on Tuesday, D2+D1 on Wednesday and D2 on Thursday) and Saturday (some D2+D1 specimens on Thursday, D2 on Friday and some remaining specimens) for one week of field operation. In case that additional days are required to complete the field operation at a cluster (e.g. cluster consist of more than 2 villages), the laboratory unit has to make sure that the inoculation of culture specimens takes place within 5 days after collection (It is recommended that inoculation of culture be done within
3 days of collection). In particular, before the long holiday or longer weekends consisting of three or more days off, the team leader, together with the laboratory unit staff, should carefully coordinate with the staff at the designated culture centre to prevent specimens from being belated inoculation and from ending up high-contamination rate and low-recovery rate.

3.7.3 Receiving of sputum specimens at culture centre

Once the specimens have arrived at the culture centre, they should immediately be stored in a refrigerator (4 degree centigrade). However, before that, the sputum cups should first be checked to see if any leakage has occurred and if the labelling is clear. If a specimen has leaked or contaminated others, disinfectant should be applied in a bio-safety cabinet (class II). After disinfection, the decontaminated specimens should be salvaged carefully. The outside of the salvaged sputum containers should be sterilized with cotton balls or tissue paper soaked in 70% alcohol, and then labelled clearly again. If contamination into other specimens is suspected, all the specimens affected should be discarded into an autoclave bag. The field survey team should be informed so that all the contaminated sputum specimens can be re-collected and replaced.

A laboratory technician at culture centre receives sputum specimens, 1st sheet of TB suspects list (Form08) and the triplicate laboratory examination forms (Form09 consists of LAB01, 02 and 03) from the survey teams twice a week to treat the specimens. Laboratory staff performs sputum smear examinations, culture examinations and identification test as described in the section 4.

Note: The number of sputum containers with TB suspect list (Form08) and the triplicate lab forms (Form 09) should be checked and consistency by the culture center staff when the icebox is arrived at the culture center. And the icebox condition also should be recorded the form.

3.7.4 Lab examination forms (triplicate sheets)

A lab examination form (Form09) is triplicate, i.e. the original sheet carbon-copied to the other two. Each pair of sputum cups (i.e. on-the-spot: D1 and the morning sputum: D2) goes with these forms to the culture centre.
4 SOP for in-house procedures

4.1 SOP for Laboratory

The appointed culture centre has to deal with sputum immediately after the arrival of the samples from the field operation centre. The culture centres directly perform smear microscopy, culture tests, and *M. tuberculosis* (MTB) identification tests (CENAT laboratory only). As each subject suspected of having TB is to submit 2 samples of sputum, 2 smear preparations are to be made from each TB suspect. Staining will be by auramine. Solid media is used for culture test, and seeding is made to 2 media from 1 sputum sample to increase the detection sensitivity. Once bacilli have grown on the slants, the samples will be sent to the CENAT laboratory to have further examinations. For the identification of TB, Capilia TB test will be carried out. At the culture centres, some overtime work is expected, especially on the days of twice a week sample arrival (planned on Thursday and Saturday), because accepting samples for smear microscopy and culture test is an additional task to the routine operation. In order to maintain a high recovery rate of TB strains from samples, it is necessary to treat samples as soon as possible to start cultivation, not to postpone it to the next day, even if some overtime work is required.

4.1.1 Safety of bacteriological examinations

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

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

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

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

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

4.1.2 Sputum reception and registration

A laboratory technician of culture centre receives sputum specimens with the triplicate examination forms (Form 09:LAB01, 02, 03) and TB suspects list (Form 08) from the survey teams twice a week to treat the specimens. The staff fills out the date of specimens’ reception and the consecutive lab culture number of the culture test registry (Form 13, LAB06) in the exam forms (LAB01). And then, the staff attaches a label with the slip number to the corresponding
raw of the culture test registry and transcribes the participant information on the culture registry. The record materials for use and the flow chart of them within CENAT for performing sputum smear examinations, culture examinations and identification test is as follows;

**Record materials**

- Examination forms (Form 09, triplicate sheets): consisting of 3 sheets
  - LAB01: for request from the operating site and the results of fluorescence microscopy (FM) sent to data management unit. (White colour sheet)
  - LAB02: for the results of FM, culture and identification sent to data management unit. (Green colour sheet)
  - LAB03: for the copy of LAB02 kept at the culture test section. (Pink colour sheet)
  - The attached sheet: 10 labels with an identical number (i.e. two for TB suspect lists, two for sputum cup, one for culture test registry, one for smear microscopy registry, three for backup)
- Request form for rechecking by Fluorescence microscopy (Form 11, duplicated sheets)
  - LAB04: for request for rechecking by FM in case of Smear-Negative and Culture-Positive (SNCP), and for the results submitted to culture test section. (White colour sheet)
  - LAB05: for the copy of LAB04 kept at the smear microscopy section. (Blue colour sheet)
- Culture registry (Form 13, LAB06): Logbook for recording of culture test results
- Smear registry (Form 12, LAB07): Logbook for recording of smear microscopy by both FM and ZNM results

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**Figure X: Flow Chart of laboratory forms**

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4.1.3 Smear preparation (See Annex-1 for details)

In the bio-safety cabinet (BSC) at the culture room after the registration, two direct smears per TB suspect are prepared from separate specimens collected on the first day and on the following early morning. The specimen is smeared with a size of approximately 2×3 cm with a sterilized bamboo stick. On the frosted area of the slide, the lab culture number and “D1” for 1st specimen or “D2” for 2nd specimen are written down with a pencil. After smearing, prepared unstained slides are contained in slide boxes and are sent to smear microscopy section with the triplicate examination forms (LAB01, 02, 03) for staining and reading.

4.1.4 Smear reading (See Annex-1 for details)

After receiving the slides with the triplicate examination forms (LAB01, 02, 03) from culture test section, information of the suspects is registered in the smear microscopy registry, and the slip number sticker is labelled on the same row as the information of the suspect on the registry.

Microscopy examinations are conducted with a binocular optical microscope (Primo Star iLED, Carl Zeiss Co.) using the fluorescence (Auramin-O) staining. The Auramin-O solution is prepared by each TB laboratory (CENAT, Battambang RH). The grading of fluorescent microscopy (FM) follows WHO/IUATLD recommendations. If a reader detects a positive slide, a second reader verifies it immediately. If a second reader is not available on site when positive slides are found, positive slides should be examined later by the senior staff of laboratory. Smear results are recorded in the smear registry (LAB07) and in the examination forms (LAB01, 02, 03). If a result is positive, the result should be written with a red colour ballpoint pen. The recorded LAB01 is submitted to the data management unit, while the others (i.e. LAB02 and 03) are sent to the culture test section. All smear slides should be kept in the slide boxes. If a positive smear is detected, LAB01 form should be sent to the data management unit as soon as possible so that a radiologist can check the chest X-ray film of the suspect and can inform the OD TB supervisor of the positive result.

4.1.5 Culture examination (See Annex-2 for details)

For primary cultures, the simple culture method (4% NaOH Ogawa method) with Kudoh medium is adopted for the comparison with the previous national survey. The Kudoh media are prepared twice a month. The number of necessary tubes depends on the rate of consumption of the tubes and the rate of sputum tests ordered by the survey team. Since two slants are placed for culture from each specimen, there are four culture tubes per subject. Inoculation on the medium is to be done at most within 5 days and strongly recommend to be done within 3 days after sputum collection to obtain appropriate recovery rate. The culture tubes are incubated for 9 weeks with weekly observations. The culture result is recorded in the culture test registry (LAB06), and the forms LAB02 and LAB03. If a result is positive, the result should be written with a red colour ballpoint pen. If a tube is contaminated, the result should be highlighted with a yellow highlighter. In case of smear-negative and culture-positive, additional request forms (LAB04 and LAB05; duplicated forms) need to be submitted to the smear microscopy section for the rechecking with fluorescent microscopy before the LAB02 is submitted to the data management unit.

Note: The person who performs the bacillary inoculation is divided by Day 1 and Day 2
specimens as the back-up system in order to avoiding of the un-recovery. Specimens should not be processed in batches of more than 10 samples because the simple methods are strictly time-dependent.

4.1.6 Identification test (See Annex-2 for details)

The identification of MTB is performed by a Capilia TB (Tauns Laboratories, INC.) and by the morphological characteristics of the suspected colonies in the culture tubes at CENAT. Typical colonies of \(M.\) \(tuberculosis\) are rough, crumbly, waxy, non-pigmented (cream coloured) and slow-growth. With doubtful cultures or when less experienced staff read cultures, the acid-fastness should be confirmed by Ziehl-Neelsen (ZN) staining. When \(M.\) \(tuberculosis\) is identified, the isolated colonies are suspended in a 2.0 ml cryovial containing 1.5 ml Middlebrook 7H9 medium for the further examination. On the cryovial, the lab culture number and the date of storage is recorded. And then, the vial is stored below -80c degree in the ultra-deep freezer. The laboratory staff records all results in the culture test registry (LAB06), LAB02 and 03. The LAB02 is submitted to the data management unit for data entry, and the LAB03 is kept at the culture test section. The Battambang culture centre does not perform identification test; thus the primary isolates in culture tubes are shipped to CENAT for the identification, basically twice a month, together with the copies of the corresponding page in the culture test registry (LAB06).

4.1.7 Re-checking of slides with Fluorescent Microscopy (FM)

When a specimen is turned out to be smear-negative but culture-positive, the slides derived from the specimen need to be rechecked with FM in order to avoid the false-negative smear result and to verify the original smear result. The request forms for this purpose are LAB04, 05: Request forms for rechecking of Fluorescence microscopy (duplicated sheets).

Since the original smear results have already been recorded in the culture test registry, culture test staff is the one to request rechecking to smear microscopy section by filling out the request forms when the staff finds a Smear-Negative and Culture-Positive (SNCP) result. The slide should be re-stained with Auramin-O and re-examined in the same way as the initial examination. After the rechecking, the result is returned to the culture test section by LAB04, while the copy (LAB05) is kept at the smear microscopy section. If the re-examined smear slide is turned out to be positive, the result needs to be reflected on the LAB02, 03 as well as both the culture and the smear registry with a red colour ballpoint pen.

4.1.8 Ziehl-Neelsen (ZN) examination to obtain results comparable with the 1st survey

It is recognised that fluorescent smear microscopic examination (FM) has the same or higher sensitivity compared to ZN microscopic examination (ZNM) and false positive may occur more often than ZNM. Therefore to keep comparability of the prevalence of smear positive cases between 1st and 2nd survey, ZNM will be performed to the sputum slides with positive results with FM and/or culture positive and randomly selected 10% of specimens with negative results with FM method. This cross-examination with ZNM should be performed only after the completion of re-checking with FM mentioned in the section 4.1.7 above. For this cross-examination procedure, FM results will not be informed of to the readers of ZNM to avoid bias due to knowing the FM results.
4.1.9 Storage of isolates and smear slides

All smear slides in the slides boxes and isolates in cryotubes will be kept at least until all the TB cases are determined. Isolates tubes in the cryobox will be kept in deep freezers (below -80 degree centigrade), then the date of storage is recorded in the culture registry. Disposal of smear slides and culture isolates will be made only by decision of the executive committee.

4.1.10 Transportation of isolates from Battanbang culture centre to CENAT

Twice a month, isolates (positive culture) will be transferred from Battanbang culture centre to CENAT National TB Reference Laboratory. Battanbang culture centre will sent the following items;

- Isolates tubes
- Dispatch list (Form 20)
- Laboratory examination form (Form 09) copy 1 white: smear results
- Laboratory examination form (Form 09) copy 2 yellow: culture results

When the isolates are sent, safe packaging and transportation should be consider. It should be done by using a double-box system. Steps of packaging are as follows;

1) Isolates tubes should be covered by “Parafilm”
2) (1) covered by absorbent (paper towel)
3) Put (2) into separate plastic zip lock bag
4) Put (3) upright into the smaller (inner) box and close the cover of the inner box tightly.
5) Put inner box upright into outer box and put some shock absorber between inner and outer boxes.
6) Close the cover tightly.
4.2 SOP for Data Management

The central data management unit (CDMU) is set up in a small room with a lock at the CENAT. The CDMU is headed by a focal point for the data management (the Data Manager) and has assistants with two computers.

The flow of the data is shown in Figure 13. The household register (Forms01), the individual survey form (Form05), and the TB suspect list (Form08) come to the CDMU directly from the field whereas CXR register and the laboratory request forms come through the Central Reading Panel with the final CXR reading and the Laboratory with smear and culture results, respectively. For the administrative purpose, the carbon-copies of TB suspect list are sent to the laboratory, however, as the data source, the original form sent from the field through the team leader is chosen.

An EpiInfo 3.5.1 (the Centers Disease Control and Prevention, Atlanta, Georgia, USA) is adopted as the database software, in which the data are entered by data enterer.

Figure 13. Flow of data among team and units

4.2.1 Receiving individual survey sheets and other forms

The CDMU staff receives the documents from the field team leader or his assistant on Saturdays. The data management staff counts the numbers of the three (3) separate documents: the household register (Form01), the individual survey sheets (Form05), and the TB suspect lists (Form08), and records the numbers on the data management logbook (Form15). Also, the staff who received the documents needs to sign on the logbook for the record.

The CDMU staff receives the results of laboratory examination (Form09) from Laboratory unit staff, once smear examination has been completed and for the second time the culture examination has been completed. The timing of the receiving results would be about a week after the completion of the cluster work for smear result and about two months after for culture result. Every time the staff receives the results from the laboratory, the forms need to be
checked the content and the number of sheets be counted, and the number need to be recorded on the logbook for the record. At the end of a line of the logbook, the staff needs to sign.

In addition, the CXR register (Form07) is submitted from the team leaders to the CDMU about a month after the completion of field operation. The CXR register contains the result of central reading on chest X-ray, as well. The staff of the CDMU needs to count the number of the sheets of the CXR register and records the number on the logbook for the record. As mentioned before, the staff needs to sign at the end of the line of the logbook.

4.2.2 Coordination of treatment of TB cases with research coordinator

The Data Manager or the designated assistant to the Manager informs the research coordinator once a smear-positive or a culture-positive TB case is reported from the laboratory. See more details of the coordination in section 4.4 Coordination of treatment of TB cases.

4.2.3 Back-up and filing of the documents sent from the field

The staff at the CDMU makes back-ups of some of the documents every time those are sent from the field. The household registry (Form01) and the individual survey (Form05) form, and CXR register (Form07) are scanned page by page into electric files (PDFs or portable document files) and the files are stored in a hard drive. The drive is kept in another room to secure the data in case of fire, storm, or flood. The suspect lists and the laboratory examination forms have carbon-copies and thus they are not necessary to have duplicates.

4.2.4 Data cleaning and data entry

Five separate electric databases are developed for data entry: the household register (Form01), the individual survey form (Form05), CXR register (Form07), the suspect list (Form08), and the lab examination form (Form09).

Data on the household register is single-entered on the database built on the computer. Specifically, the survey ID (two digits for cluster, three for household, and two for household members), the age (three digits), the sex (pull down), the occupation (the occupation is categorized into the four: a) Agriculture/Fishery/Forestry, b) Industry/Mining, c) Service sectors, d) Unemployed, before the data entry by staff), the attendance status (pull down), the reasons for non-eligibility and absence (each pull down) are picked up and entered into the data base. It should be emphasised that every household member should be entered regardless of age (i.e., including those less than 15 years old) or eligibility.

Data on the individual survey card is double-entered on the database by two separate data enterer. Specifically, the running card number (three digits), the survey ID (same as above), the sex, and the age are entered. The occupation needs to be coded based on the classification (a) Agriculture/Fishery/Forestry, b) Industry/Mining, c) Service sectors, d) Unemployed) before entry.

If such symptoms as cough, sputum, and haemoptysis are chosen, the duration (days) of the symptoms need to be entered in digits otherwise “No-s” are entered. The items from 6.4 through 6.8 are entered as yes/no. Symptoms other than those are listed need to be entered in text.

Regarding the health seeking behaviour, each item from 7.1 through 7.3g is entered as
yes/no. Also, items from 7.3i through 7.3l are entered with yes/no. If 7.3m is entered as yes, the reason needs further to be entered in text. If 7.3h is selected, the facility needs to be specified in text.

Regarding the TB treatment history, the item 8.1 is entered as yes/no. If the item 8.2 is entered as yes, the year needs to be specified in four digits or if the year is not specified, “forgot” is to be selected as yes. Also, the facility (a through f) is to be entered as yes. The similar process applies if the item 8.3 is entered as yes.

Regarding the radiology, the status of chest X-ray is entered from the pull-down menu. If the “d. others” is selected in item 9.1, the status is specified in text.

Data on the CXR register is double-entered on the database. Specifically, the running radiology number (three digits), the survey ID (two-three-two digits format, the same as the individual survey card), the status read by the field reader (normal or abnormal from the pull down menu), the decision on sputum collection (yes/no), and the results of central reading (pull-down menu) are entered. If “other pulmonary” or “others” is selected, the name(s) of other pulmonary disease or other disease needs to be entered in text.

Data on the TB suspect list is single-entered on the database, in which the survey ID is entered as two-three-two digits format as mentioned above.

Data on the laboratory examination form is double-entered on the database. Specifically, the running laboratory specimen number (i.e., slip number, four digits) and the survey ID (two-three-two digits format) are entered.

The initial results of smear microscopy are selected from the pull-down menu. If “b. Scanty” is selected, then the number of bacilli needs to be specified in two digits from 01 to 29. If “f. NA (i.e., not available)” is selected, the reason for unavailability needs to be specified in text. Since normally two specimens are collected from a suspect, smear result needs to be entered for the two specimens separately (i.e., D1 and D2).

Likewise, the results of culture examinations are selected from the pull-down menu. If “b. Scanty” or “h. NA” is selected, either the number of colonies or the reason for unavailability of the result is entered as in digits or in text, respectively. Since normally two tubes are inoculated from a specimen, culture result needs to be entered for the two tubes separately (i.e., Kh1 and Kh2 for each specimen, resulting in four tubes in total).

In case of a positive result of culture examination, the identity test result (Capilia test) needs to be entered additionally.

Also, in case that culture result is positive but the initial smear result has been negative (smear-negative but culture-positive or SNCP), the slides of the specimen need to be rechecked and the result needs to be entered.

In principle, the data on the individual survey forms should be entered within a week after receiving forms from the team leader.

At about the end of the whole survey period, all the slides with a positive result and 10% slides with a negative are re-stained with a Ziel-Neelsen method. The results need to be entered into another database.
After matching the two databases by Survey ID as a key for matching, inconsistent values will be detected by comparing values between the databases. Original forms will be reviewed when inconsistent values are detected.

4.2.5 Backup and security of data

Two computers will be used only for the two survey databases and locked with password known to individuals designated by the technical committee. The computers are kept in the CDMU room with lock. Every week backup will be made in an external hard-drive and the drive is kept in a separate room for precaution.

4.2.6 Confidentiality of the personal information

The individual survey card, the data on CXR and laboratory, and other data should be treated with good care and are kept in the CDMU room with lock. Every time when the staff leaves the room, the door must be locked. The key of the lock should be kept by a designated data management personnel.
4.3 SOP for Central reading of chest X-ray films

All the chest X-ray films taken in the field are sent for central reading to the CENAT after each field operation.

As QA measures at the central level, all films with shadow categorized as eligible for sputum collection and 10% of films with shadow categorized as non-eligible for sputum collection are checked by the CXR central team consisting of at least three X-ray readers.

The second level reading is carried out for all films taken in the field 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 reader and following the QA reading at CENAT mentioned above. The CXR results are categorized into (1) normal, (2) active TB (Active TB is strongly suspected), (3) TB suspect (possible TB lesion or stable lesion such as tuberculoma), (4) healed TB, (5) suggestive of other respiratory disease with differentiating diagnosis, (6) suggestive of other heart disease with differentiating diagnosis, and (7) others with differentiating diagnosis.
4.4 Coordination of treatment of possible TB cases

The focal point of the central data management unit (i.e. Data Manager) informs the research coordinator once a smear-positive or a culture-positive TB case is reported from the laboratory. The research coordinator then coordinates the treatment of TB cases with the relevant OD TB supervisor and health centre staff so that the TB patient can get TB treatment accordingly. To facilitate the process, the Data Manager assigns his assistants to take turns to visit the laboratory and review the laboratory register to see if a new smear-positive or a new culture-positive case is recorded.
References

- Assessing tuberculosis prevalence through population-based surveys, WHO, 2007
- TB bacteriology examination to stop TB, Fujiki A, 2001
- TB CAP Laboratory toolbox—Strengthening TB laboratory services, TBCAP, 2009
- National TB Prevalence survey, 2002 Cambodia, Ministry of Health, Cambodia, 2005
Annex 1: Forms  
List Forms for second National Prevalence Survey in Cambodia

<table>
<thead>
<tr>
<th>Name</th>
<th>Form N°</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household registry</td>
<td>Form01</td>
<td>Triplicate carbon-copy</td>
</tr>
<tr>
<td>Household number</td>
<td>Form02</td>
<td></td>
</tr>
<tr>
<td>Invitation card</td>
<td>Form03</td>
<td>Backside with survey information</td>
</tr>
<tr>
<td>Informed consent form</td>
<td>Form04</td>
<td></td>
</tr>
<tr>
<td>Individual survey sheet</td>
<td>Form05</td>
<td>Interviewing sheet</td>
</tr>
<tr>
<td>ID Card</td>
<td>Form06</td>
<td>Mini Carbon-copy interviewing sheet</td>
</tr>
<tr>
<td>X-ray registry</td>
<td>Form07</td>
<td></td>
</tr>
<tr>
<td>TB suspects list</td>
<td>Form08</td>
<td>Triplicate (specimen transportation, Lab-unit, Team leader)</td>
</tr>
<tr>
<td>Lab-examination Form</td>
<td>Form09</td>
<td>Triplicate</td>
</tr>
<tr>
<td>Summary report of each surveyed cluster</td>
<td>Form10</td>
<td>After finishing each cluster operation</td>
</tr>
<tr>
<td>Lab-rechecking registry</td>
<td>Form11</td>
<td>Duplicate</td>
</tr>
<tr>
<td>Smear registry</td>
<td>Form12</td>
<td></td>
</tr>
<tr>
<td>Culture registry</td>
<td>Form13</td>
<td></td>
</tr>
<tr>
<td>List of cluster TB patient registered for TB treatment at OD</td>
<td>Form14</td>
<td></td>
</tr>
<tr>
<td>Central Data Management Unit Logbook</td>
<td>Form15</td>
<td></td>
</tr>
<tr>
<td>Information Sheet</td>
<td>Form16</td>
<td></td>
</tr>
<tr>
<td>Smear positive</td>
<td>Form17</td>
<td></td>
</tr>
<tr>
<td>Smear negative culture positive</td>
<td>Form18</td>
<td></td>
</tr>
<tr>
<td>Smear negative culture negative CXR positive</td>
<td>Form19</td>
<td></td>
</tr>
<tr>
<td>Dispatch sheet of positive culture</td>
<td>Form20</td>
<td></td>
</tr>
</tbody>
</table>
HOUSEHOLD REGISTRY
(FORM 1)

Name of sample area: …………………Commune………………District………………Province………………

Cluster No: [ ] [ ] Number of household: [ ] [ ] [ ] [ ]

Filled by: ……………………………………………………………………………………………

<table>
<thead>
<tr>
<th>Serial No</th>
<th>Registration No *</th>
<th>Name</th>
<th>Sex (M/F)</th>
<th>Date of birth</th>
<th>Age</th>
<th>Occupation</th>
<th>Participated</th>
<th>Remark</th>
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</thead>
<tbody>
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<td></td>
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<td></td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>4</td>
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</tr>
<tr>
<td>5</td>
<td>0100105</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All forms must be filled with a pen.

*: Every subject eligible for the survey must be given his/her own number which has 7 digits: 00 000 00
   The first two digits indicate the number of the survey area (sample unit) which is 1 to 64
   The middle three digits indicate the serial number of households in a survey area.
   The last two digits indicate the serial number of family member in a household.

**: No survey ID number means no eligibility and the reason should be explained in the remarks. If adult,
   delete the name by line. Children under 15 is ‘no code and no deletion’

***: Participated: when the eligible person attends the survey, please tick. R: refuse and A: absence.

****: Categorize?: occupation and remarks (the reason for R or A)
# 001

Please participate TB survey on ........../........../201 .........., at ..........

Notice: You can come on the different mentioned date, if you are on-duty.

Participation in TB survey means to promote yours and your family health!

# 002

Please participate TB survey on ........../........../201 .........., at ..........

Notice: You can come on the different mentioned date, if you are on-duty.

Participation in TB survey means to promote yours and your family health!
Invitation card (Form03)

Participation for TB Survey means to promote your family health and community

Invitation card (Form03)

Household number __ __ __
Name of participant __________________________
______________________________
______________________________
______________________________

Please participate TB survey on ........day........month.........year........., at ...............  
Notice: Please carry this card to show our survey team while arriving at the operating center

Invitation card (Form03 back side)

Knowledge on TB Survey
1 - TB is a deadly disease because those who are not get diagnosis and treat on time. This survey is to assess the burden pulmonary TB disease in Cambodia. It also provides the invaluable information to National TB Program as well as to develop appropriate strategic plan for TB control.

2 - Your participation for is very important, not only giving knowledge about your health condition but also for other people as well, especially for improvement of future health status.

3 - Eligible person will be interviewed by health officers on respiratory and chest x-ray in order to find out the lesion sign. If you are suspected of TB, you will be requested sputum for smear microscopy.

4 - In order to swift for time consuming, you can wear t-shirt for chest x-ray.

5 - After the examination, you will get the result on-spot about your respiratory health. If you are found of TB, you will be provided free of charge for TB treatment at the nearby HC. You also be given the good explanation while having other respiratory diseases.

Thanks in advance!
Informed consent form (Form04)

(Information part)
This informed consent form is for the household members who are invited to participate in TB prevalence survey in the selected clusters of Cambodia.

The aim of this survey is to assess the disease burden of active pulmonary TB. The community from the selected clusters will be screened for TB by interviewing about the TB symptoms and Chest X-ray examination. If a participant is suspected of having TB, sputum is taken for TB examinations and the results will be given back later. The information that we collect from this survey will be kept confidential. The respondents are entitled to the medical benefits and treatment for TB if necessary.

The findings of the survey will provide valuable information on the programme impact and contribute to developing appropriate plans and strategies for the National TB Programme.

(Declaration part 1)
I have read the above explanation and the information leaflet, or they has been explained to me by health staff. I have had the opportunity to ask question about it and all the questions that I have asked were answered to my satisfaction. I have been informed that the risks by the survey are minimal. I know that I will be able to receive treatment at health centre or referral hospital if I have TB. I have agreed to participate in this survey with understanding that I have right to reject any interview/screening and withdraw from the participation without affecting my further medical care.

Name of participant ........................................
Signature or thumb print ...................................
Date ......../....../......

(Declaration part 2)  If a participant is unable to read:
I have witnessed that the participant was fully explained about the accurate consent form and that the individual had the opportunity to ask any questions. I hereby confirm that the individual has been given informed consent to participate in the survey.

The witness must sign (if possible, this person should be selected by the participant out of the research team). The participant should leave his/her thumb print as well.

Name of witness .................................
Signature of witness ..........................
Date ......../....../......
<table>
<thead>
<tr>
<th>(1) Survey ID Nº</th>
<th>(2) Name</th>
<th>(3) Sex</th>
<th>(4) Age</th>
<th>(5) Occupation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sign by receptionist: ____________________________

(6) Symptoms (last one month) and Duration

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoptysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of B.W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night sweats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.10 Interviewer comments for sputum collection

Yes ☐ No ☐

Signature: ____________________________

(7) Health seeking behavior

7.1 No attention ☐

7.2 Self-medication ☐

7.3 Consultation If not either 7.1, 7.2, 7.3a

a. Government hospital ☐

b. Health centre ☐ i. Not severe ☐

c. Private clinic ☐ j. No money ☐

d. Private hospital ☐ k. Far distance ☐

e. Pharmacy ☐ l. Times waiting ☐

f. Traditional healer ☐

m. Others (specify) ☐

g. Family member ☐

h. Other facility (specify)

………………………………………………………………

(8) TB treatment history

8.1- Yes ☐ No ☐

If yes (duration)

8.2 Past ☐ 8.3 Present ☐

Year……… Month……

forget ☐ forget ☐

a. Government hospital ☐

b. Health centre ☐

c. Private clinic ☐

d. Private hospital ☐

e. Pharmacy ☐

f. Traditional healer ☐

g. Others

………………………………………………………………

(9) Radiology

9.1 Chest X-ray taking 9.2 Result

a. X-ray taken a. Normal

b. Refuse b. Abnormal

c. Unable for x-ray 9.3. Necessity to collect sputum
d. Others Yes ☐ No ☐

Reader

signature: ____________________________

(10) Sputum collection:

10.1 Comments by Team leader for sputum collection:

Yes ☐ No ☐

Specimen-1 ☐ ………….………………/…………….

Specimen-2 ☐ ………….………………/…………….

Signature of Lab-technician: ____________________________
Participation for TB Survey means to promote your family health and community

Survey ID card (Form06)

<table>
<thead>
<tr>
<th>Chest X-ray film reading:</th>
<th>Normal □</th>
<th>Abnormal □</th>
<th>Not taken □</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments by field reader for sputum collection:</td>
<td>Yes □</td>
<td>No □</td>
<td>Signature: ........................................</td>
</tr>
<tr>
<td>Comments by team leader for sputum collection:</td>
<td>Yes □</td>
<td>No □</td>
<td>Signature: ........................................</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(1) Survey ID Nº</th>
<th>(2) Name</th>
<th>(3) Sex</th>
<th>(4) Age</th>
<th>(5) Occupation</th>
</tr>
</thead>
<tbody>
<tr>
<td>__ __ __ __ __ __</td>
<td>______</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
</tbody>
</table>

Notice: Please carry this card when you go to the operating site
<table>
<thead>
<tr>
<th>Nº</th>
<th>Survey Code</th>
<th>Name</th>
<th>Sex</th>
<th>Address</th>
<th>Field reading-specimen collection</th>
<th>Central reading</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
<td>Request sputum</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Use ✓ in the box for every reading by field or central level
<table>
<thead>
<tr>
<th>Nº</th>
<th>Survey Code</th>
<th>Patient's name</th>
<th>Age</th>
<th>Date of specimen collection</th>
<th>Others</th>
</tr>
</thead>
</table>
|    |             |                | M   | F                           | Specimen-1 .../....../.........
| 1  |             |                |     |                             | Specimen-2 .../....../.........
| 2  |             |                |     |                             | Specimen-1 .../....../.........
| 3  |             |                |     |                             | Specimen-2 .../....../.........
| 4  |             |                |     |                             | Specimen-1 .../....../.........
| 5  |             |                |     |                             | Specimen-2 .../....../.........
| 6  |             |                |     |                             | Specimen-1 .../....../.........
| 7  |             |                |     |                             | Specimen-2 .../....../.........
| 8  |             |                |     |                             | Specimen-1 .../....../.........
| 9  |             |                |     |                             | Specimen-2 .../....../.........
| 10 |             |                |     |                             | Specimen-1 .../....../.........
| 11 |             |                |     |                             | Specimen-2 .../....../.........
| 12 |             |                |     |                             | Specimen-1 .../....../.........
| 13 |             |                |     |                             | Specimen-2 .../....../.........
| 14 |             |                |     |                             | Specimen-1 .../....../.........
| 15 |             |                |     |                             | Specimen-2 .../....../.........
| 16 |             |                |     |                             | Specimen-1 .../....../.........
| 17 |             |                |     |                             | Specimen-2 .../....../.........
| 18 |             |                |     |                             | Specimen-1 .../....../.........
<table>
<thead>
<tr>
<th></th>
<th>Specimen-1</th>
<th></th>
<th>Specimen-2</th>
</tr>
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<tbody>
<tr>
<td>19</td>
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<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Date:............./............./.............

Have seen

Laboratory

Survey team leader

Signature-name

Signature-name
Laboratory examination form (Form 09)

**at the site**

1. Survey ID No.: 
2. Name (in full): 
3. Sex: [ ] Male [ ] Female
4. Age: __________ Year
5. Sputum collection data & aspect
   - **D1 Specimen:** dd/mm/yy ______/_____/_____
   - **D2 Specimen:** dd/mm/yy ______/_____/_____
   - Saliva [ ] Mucopurulent [ ] Bloody [ ]

   Date: __________ Sign by lab technician at site: __________

**at the laboratory (CENAT or Battambang RH)**

6. Specimens reception date at Lab: dd/mm/yy ______/_____/_____
7. Lab culture number: 

8. Result:

<table>
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<th>FLUO MICROSCOPY</th>
<th>CULTURE</th>
<th>ID TEST</th>
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<tr>
<td><strong>Result</strong></td>
<td><strong>Result</strong></td>
<td><strong>Result</strong></td>
</tr>
<tr>
<td><strong>Reacting</strong></td>
<td><strong>Reacting</strong></td>
<td><strong>Reacting</strong></td>
</tr>
<tr>
<td><strong>Fluorochrome</strong></td>
<td><strong>Fluorochrome</strong></td>
<td><strong>Fluorochrome</strong></td>
</tr>
<tr>
<td><strong>Stain</strong></td>
<td><strong>Stain</strong></td>
<td><strong>Stain</strong></td>
</tr>
<tr>
<td><strong>Result</strong></td>
<td><strong>Result</strong></td>
<td><strong>Result</strong></td>
</tr>
<tr>
<td><strong>Reacting</strong></td>
<td><strong>Reacting</strong></td>
<td><strong>Reacting</strong></td>
</tr>
<tr>
<td><strong>Fluorochrome</strong></td>
<td><strong>Fluorochrome</strong></td>
<td><strong>Fluorochrome</strong></td>
</tr>
<tr>
<td><strong>Stain</strong></td>
<td><strong>Stain</strong></td>
<td><strong>Stain</strong></td>
</tr>
</tbody>
</table>

**D1**

- a. [ ] Neg
- b. [ ] Scanty
- c. [ ] 1+
- d. [ ] 2+
- e. [ ] 3+
- f. [ ] NA

**D2**

- a. [ ] Neg
- b. [ ] Scanty
- c. [ ] 1+
- d. [ ] 2+
- e. [ ] 3+
- f. [ ] NA

Date: __________ Sign by lab-technician: __________
To data management unit

Slip Number

LAB 02

Laboratory Examination Forms (Form 09)

at the site

1. Survey ID No.

2. Name (in full):

3. Sex: ☐ Male ☐ Female

4. Age: ____________ Year

5. Sputum collection data & aspect

D1 Specimen: dd/mm/yy /_____ /_____ Saliva ☐ Mucopurulent ☐ Bloody ☐

D2 Specimen: dd/mm/yy /_____ /_____ Saliva ☐ Mucopurulent ☐ Bloody ☐

Date: _______________ Sign by lab technician at site: ____________________________

at the laboratory (CENAT or Battambang RH)

6. Specimens reception date at Lab: dd/mm/yy /_____ /_____ 

7. Lab culture number: ____________________________

8. Result:

<table>
<thead>
<tr>
<th>FLUO MICROSCOPY</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>Rechecking (if SNCP)</td>
</tr>
<tr>
<td>D1</td>
<td>a. [] Neg</td>
</tr>
<tr>
<td></td>
<td>b. [] Scanty ( )</td>
</tr>
<tr>
<td></td>
<td>c. [] 1+</td>
</tr>
<tr>
<td></td>
<td>d. [] 2+</td>
</tr>
<tr>
<td></td>
<td>e. [] 3+ f. [] JNA ( )</td>
</tr>
<tr>
<td></td>
<td>f. [] JNA ( )</td>
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<tr>
<td>D2</td>
<td>a. [] Neg</td>
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<td>b. [] Scanty ( )</td>
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<td>c. [] 1+</td>
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<td></td>
<td>d. [] 2+</td>
</tr>
<tr>
<td></td>
<td>e. [] 3+</td>
</tr>
<tr>
<td></td>
<td>f. [] JNA ( )</td>
</tr>
</tbody>
</table>

Date: _______________ Sign by lab-technician: ____________________________
Laboratory Examination Forms (Form 09)

at the site

1. Survey ID No.: [Blank]
2. Name (in full): [Blank]
3. Sex: [ ] Male [ ] Female
4. Age: [Blank] Year
5. Sputum collection data & aspect
   **D1 Specimen:** dd/mm/yy [Blank]/[Blank]/[Blank] Saliva [ ] Mucopurulent [ ] Bloody [ ]
   **D2 Specimen:** dd/mm/yy [Blank]/[Blank]/[Blank] Saliva [ ] Mucopurulent [ ] Bloody [ ]
   Date: [Blank] Sign by lab technician at site: [Blank]

at the laboratory (CENAT or Battambang RH)

6. Specimens reception date at Lab: dd/mm/yy [Blank]/[Blank]/[Blank]
7. Lab culture number: [Blank]
8. Result:

<table>
<thead>
<tr>
<th>FLUO MICROSCOPY</th>
<th>CULTURE</th>
<th>ID TEST Capilia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FLUO MICROSCOPY</strong></td>
<td><strong>CULTURE</strong></td>
<td><strong>ID TEST Capilia</strong></td>
</tr>
<tr>
<td><strong>Result</strong></td>
<td><strong>Rechecking (if SNCP)</strong></td>
<td><strong>Kudoh tube</strong></td>
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<tr>
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<tr>
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<td>b. [ ] Neg</td>
<td>a. [ ] Neg</td>
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<tr>
<td>b. [ ] Scanty</td>
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<td>b. [ ] Scanty</td>
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<tr>
<td>c. [ ] 1+</td>
<td>d. [ ] 2+</td>
<td>c. [ ] 1+</td>
</tr>
<tr>
<td>e. [ ] 3+</td>
<td>f. [ ] NA</td>
<td>e. [ ] 3+</td>
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<td>b. [ ] Neg</td>
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<td>f. [ ] NA</td>
<td>e. [ ] 3+</td>
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<td>( )</td>
<td>f. [ ] NA</td>
</tr>
</tbody>
</table>

Date: [Blank] Sign by lab-technician: [Blank]
Summary report of each survey cluster

Cluster ID [ ] [ ] [ ]

1. Census taking
   - Eligible person : persons
   - Person age less than 15 years old : persons
   - Total population of the cluster : persons
   - Number of eligible household : households

2. Registration
   - Consented person : persons
   - Refused person : persons
   - Absentee : persons

3. Interview
   - On-site interviewed person : persons
   - Outreach interviewed person : persons
   - Sputum request by interview : persons

4. Chest X-ray
   - X-ray taken person : persons
   - Non x-ray taken person : persons (Refused: persons)
   - Result of x-ray reading
     - Normal : cases
     - Abnormal : cases
       - Sputum collection : cases
       - Not required sputum : cases

5. Sputum collection
   - Request for sputum collection : cases
   - Collected sputum specimen
     - 1st Specimen : cases
     - 2nd Specimen : cases

6. Shipment of sputum specimen
   - 1st time, Date........./........./......... : cases : containers
   - 2nd time, Date........./........./......... : cases : containers

7. Nº of TB patients per cluster which registered for TB treatment at OD
   - 2009 : persons
   - 2010 : persons

Date.............../........./..................
Survey team leader (signature and name)
Request Form (Form11)
(Rechecking of Smear Slide)

1. Lab culture number: ____________________________

2. Specimen number requested:
   D1 □  D2 □  or Both □

3. Result:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td>D1</td>
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</tr>
<tr>
<td>D2</td>
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</tbody>
</table>

Date: __________ Sign by smear microscopy staff: ______________
Request Form (Form 11)
(Rechecking of Smear Slide)

1. Lab culture number: ________________________________

2. Specimen number requested:

   □ D1  □ D2  □ or Both  □

3. Result:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
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Date: ____________ Sign by smear microscopy staff: ______________________
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<th>Specimen number</th>
<th>1st reader</th>
<th>2nd reader</th>
<th>ZN microscopy</th>
<th>Microscopy</th>
<th>Remarks</th>
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Laboratory Smear Register (Form 12)
Laboratory culture register (Form 13)
List of cluster TB patient registered for TB treatment at OD
(Form 14)

Cluster Nº……………… Cluster name…………………… Commune…………………… District…………………… Province……………………

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<tr>
<th>Serial Nº</th>
<th>Name of patient</th>
<th>Age</th>
<th>Sex</th>
<th>Disease type</th>
<th>Date of treatment</th>
<th>Treatment result</th>
<th>Others</th>
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</table>

Date……………../…………../……………..

Have seen
OD TB Supervisor ＿Signature-name＿
Survey team leader ＿Signature-name＿
<table>
<thead>
<tr>
<th>Serial number</th>
<th>Date (dd-mm-yy)</th>
<th>Cluster name</th>
<th># of household registers (Form01)</th>
<th># of individual survey sheets (Form05)</th>
<th># of TB suspect list sheets (Form08)</th>
<th># of result of smear microscopy forms (Form09)</th>
<th># of result of culture forms (Form09)</th>
<th># of CXR registers (Form07)</th>
<th>Signature of receiver at CDMU</th>
<th>Remarks</th>
</tr>
</thead>
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</table>
Information sheet (Form16)

This is to be used for explaining overview of the National Prevalence Survey.

Thank you for coming today for the second National Tuberculosis (TB) Prevalence Survey (the Survey). From now on, we would like to explain to you what will be done during the Survey, because you are one of the household members who are invited to participate in the Survey in the selected clusters of Cambodia. Please listen to me carefully and understand well about the procedures. After the explanation, if you agree, you will be asked for your signature or thumb printing on the consent sheet below. If you do not agree to the participation in the survey, you can have the right to decline.

- The aim of the Survey is to assess the disease burden of active pulmonary TB in the country.
- All the residents with age 15 years old or older in the randomly selected community all over the country are screened for TB by interviewing about symptoms suggestive of TB and chest X-ray test.
  - If a participant is suspected of having TB, two sputum specimens (on-the-spot and morning) are taken for laboratory test.
  - The results will be given back to the participant later.
- The information collected from the participant for the Survey will be kept confidential.
- The participants are entitled to the medical benefits and treatment for TB, if deemed necessary.
- The findings of the Survey will provide the National TB Programme with valuable information on how effectively control TB in the country.
Smear positive (Form 17)

<table>
<thead>
<tr>
<th>No</th>
<th>Survey ID (Lab No)</th>
<th>Name (Villages: if 2 or more)</th>
<th>Age</th>
<th>D1</th>
<th>D2</th>
<th>Central reading (CXR No)</th>
<th>Sputum by symptom (YN)</th>
<th>History (Past or Current)</th>
<th>Remarks</th>
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</table>
Smear negative culture positive (Form 18)

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<th>Age</th>
<th>M</th>
<th>F</th>
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<th>D2</th>
<th>Central reading (CXR No)</th>
<th>Sputum by symptom (Y/0)</th>
<th>History (Past or Current)</th>
<th>Remarks</th>
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Total No of sample send to CENAT: __________

Date of dispatch: _______ / _______ / _______  
* PLEASE KEEP COPY IN YOUR LABORATORY

Shipper: _________________________________

Signature: _______________________________

Date of received: _______ / _______ / _______

Consignee: _______________________________

Signature: _______________________________
Annex 2: Smear Microscopy

Smear preparation

In the safety cabinet (class II), wipe the outside of the sputum container with a cotton ball soaked in 70% alcohol without erasing the label if necessary. After confirming the identification number of the sputum specimen, next, carefully open the container to pick and transfer a mucopurulent specimen with a sterile bamboo stick onto a clean and correctly marked slide glass. Make an appropriate size (3 cm in length and 2 cm in width) and thickness of smear and cover the container tightly with the screw cap. After completing the smear preparation, place the stick into a discard container and use a new stick for each specimen.

Smear fixation

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

Fluorescent microscopy (FM)

After smear fixation, the slide with laboratory exam forms (LAB01, 02, 03) should be forwarded to smear microscopy section.

Staining

Place fixed smear slides on staining rods in a sink and apply fluorochrome (0.1% Auramine O (See Annex 3) over the slides by flooding the entire surface. Staining should take place for 20 minutes. Rinse stained slides with clean water and drain it before decolorizing.

Decolorizing

Decolorize stained slides with 0.5% acid-alcohol (See Annex 3) is necessary and may be repeated if required. And then rinse with clean water. Drain water before proceeding to the next step.

Counterstaining

Counterstain the stained slides with 0.5% potassium permanganate (See Annex 3). Rinse the slides with clean water and then drain.

If the slides cannot be examined immediately after staining, keep them in the dark, closed slide box, to prevent rapid fading.

Microscopy

Before reading stained smears, check the microscope to see if there is any physical functional or mechanical defect in the optical outfit or objective/ocular lens. See to it that every outfit in the light path is functioning. After they are completely dry, read the stained slides under a fluorescence microscope and record and report the results quantitatively. It is to read fluorochrome stained smears within 24 hours after staining, always keeping the slides away from light (especially ultraviolet light).

Examine one length of a slide, using 400 x magnifications, to cover 40 fields in one length, equivalent to 200 fields at 1000x magnification (high-power fields or HPF).
Recording and Reporting
Record the microscopy results on the laboratory exam form (LAB01) and smear registry quantitatively by standardized grading. Report the results to the local TB centre through the data management team for the immediate registration of the cases found for treatment. After completing microscopy, keep all slides in slide boxes and send the laboratory request form to the culture section.

Ziehl-Neelsen staining and microscopy for Rechecking

Staining
Apply carbol-fuchsin (Ready-made: RAL kit®) over the slides by flooding the entire surface. Warm over the flame (with an alcohol soaked cotton plug at the end of iron bar or wire) until vapor appears. Staining should take place for 5 minutes. Pour off stain and rinse gently with clean water. Drain water carefully using a forceps.

Decolorizing
Flood the stained and washed slides with 3% acid-alcohol for 3 minutes, then rinse with water and drain. Repeat the decolorizing step if too much red remains, and then rinse with water. Drain water before proceeding to the next step.

Counterstaining
Flood the decolorized slide with 0.3% methylene blue and stain for about 1 minute. Drain the stain and rinse with clean water.

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

Microscopy
Before reading stained smears, check the microscope to see if there is any physical functional or mechanical defect in the optical outfit or objective/ocular lens. See to it that every outfit in the light path is functioning. After placing a stained slide on the stage, focus a clean microscopic field under the low-power objective lens with a coarse knob and adjust the light intensity to the most comfortable level using the lamp intensity and diaphragm. After finding the clearest field with the fine-focus knob, apply a drop of immersion oil over the stained smear and focus the field. Fit the right eyepiece to the right eye by turning the fine-focus knob and then the left eyepiece using the diopter adjustment. Examine at least 300 microscopic fields by moving from one end of the smear, if the smear is negative to 1+. An appropriate examination of 100 fields, even by a skilled microscopist, takes at least five minutes. To prevent possible carry-over, wipe off immersion oil from the lens with a lens tissue only after examining a positive smear.
Recording
Record the microscopy results on the register book quantitatively by standardized grading. Report the results to the data management section. After completing microscopy, keep all slides in slide boxes with a layer of lens tissues to absorb immersion oil dripping from the slides.

### Reporting of smear microscopy results

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<th>Report</th>
<th>FLM</th>
<th>Z-N</th>
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<tbody>
<tr>
<td></td>
<td>400x magnification</td>
<td>1000x magnification</td>
</tr>
<tr>
<td>Negative</td>
<td>Zero AFB / 40fields</td>
<td>Zero AFB / 300 fields</td>
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<tr>
<td>Scanty (actual No#)</td>
<td>1–19 AFB / 40fields</td>
<td>1–29 AFB / 300 fields</td>
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<tr>
<td>1+</td>
<td>20-199 AFB / 40fields</td>
<td>10-99 AFB / 100 fields</td>
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<tr>
<td>2+</td>
<td>5-50 AFB / 1 field on average</td>
<td>1-10 AFB / fields at least 50 fields</td>
</tr>
<tr>
<td>3+</td>
<td>&gt;50 AFB / 1 field on average</td>
<td>&gt;10 AFB / fields at least 20 fields</td>
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</table>
Annex 3: Culture and Identification test

Simple culture method (NaOH Ogawa method)

**Materials**
- 4% NaOH (sodium hydroxide)
- Kudoh - 2% modified Ogawa medium (see formula in Annex 12)
- Sputum specimens in the container
- Disinfectant with autoclave bag in a jar
- Cotton balls soaked in 70% alcohol
- 5% Phenol
- Safety cabinet (class II)
- Vortex mixer
- Bunsen burner
- Incubators
- Refrigerators
- Deep freezer
- Slanting beds
- Culture tube racks
- Sputum container racks
- Sterile plastic pipettes
- Autoclave
- Gloves
- N95 mask
- Gown

**Preparations**
- 4% NaOH: Dissolve 40 grams of NaOH in 1000 ml of DW and sterilize by autoclaving.
- Label all media.

**Procedure**
- In the safety cabinet (class II), clean the outside of the sputum container with cotton balls soaked in 70% alcohol if necessary, after confirming the identification (ID) number and name of the TB suspect.
- Open the screw cap carefully and add an approximately equal volume of 4% NaOH to the sputum sample, but 2 volumes to thick and viscous sputum. After tightly recapping the bottle, vortex the NaOH containing sputum for 15 - 30 seconds until the sample is completely homogenized. Leave the sample for 15 minutes to decontaminate at room temperature. If there are large numbers of specimen, handle in batches so that the NaOH treatment time must not be longer than 20 minutes.
- Inoculate the NaOH-treated sputum specimen on to two slopes of modified Ogawa medium with 0.1 ml or 4 drops (measure the volume as 0.1 ml) of solution. Confirm the labelling of the culture media before inoculation. Spread the inoculum over the entire surface of the medium in a horizontal position in the slanting bed. Leave the tubes slightly open during incubation for 2-3 days at 37°C in order to evaporate excess water.
- After checking for any evaporation of excess liquid, tightly cap the inoculated media and incubate for up to 9 weeks before discarding as a negative (no growth) culture. Thereafter, if space is needed in the incubator, tubes could be placed upright in the rack.
Examine all cultures one week after incubation in order to detect rapidly growing mycobacteria. Record any growth quantitatively with a description of colony morphology. Thereafter, examine weekly of incubation until 9 weeks before discarding the media as no-growth (negative).

- Record and report the results quantitatively according to the grading recommended by WHO and the International Union against TB and Lung Disease.
- After completing the reading of the cultures, autoclave all culture tubes and discard unless further testing or subculture is planned.

### Reporting of culture results

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<th>Report</th>
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<tr>
<td>No growth</td>
<td>Neg</td>
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<tr>
<td>1 – 19 colonies</td>
<td>Report actual figures</td>
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<tr>
<td>20 – 100 colonies</td>
<td>1+</td>
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<tr>
<td>100 – 200 colonies</td>
<td>2+</td>
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<tr>
<td>200 – 500 colonies (difficult to count, but discrete colonies)</td>
<td>3+</td>
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<tr>
<td>&gt;500 colonies (confluent growth)</td>
<td>4+</td>
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<tr>
<td>Contaminated (complete contamination)</td>
<td>Contaminated or “C”</td>
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</tbody>
</table>

**Identification test**

MTB must be differentiated from other mycobacterial isolates. All mycobacterial isolates should be subcultured for storage and further tests. The isolates will be identified as MTB through “Capilia TB®” (Tauns Laboratories, INC.).

**Capilia TB®** (Tauns Laboratories, INC.)

#### SAMPLE HANDLING PROCEDURES

From Sub-culture on solid L-J medium (3-4 weeks)
- Carefully examine the culture slants to make sure the mycobacterium growing and appearance of the visible colonies.
- Add 0.2 ml of the sterile distilled water into a test tube (sterile plastic tube).
- Take one full loop of the colonies using the inoculating loop.
- Dip the loop full of the colonies into the tube containing the sterile distilled water.
- Vortex the tube with the lid tightly capped, mix it well to be used as test sample.

#### TESTING PROCEDURES

Allow the Capilia TB Test Device Kit and prepared test samples to equilibrate to room temperature (15-30 degree centigrade) prior to testing.
- Remove test device from the sealed pouch just prior to the testing and lay flat on work bench.
- Carefully apply 100μl culture sample into sample well.
- Read results at 15 minutes and disregard after 60 minutes.

#### RESULTS INTERPRETATION

**POSITIVE:** One red line appears in the control region (C), and one red line in the test region...
The shade of colour may vary, but it should be considered positive whenever there is even a faint line. This indicates that the sample contains detectable amount of MPB64 protein.

NEGATIVE: One red line appears in the control region (C), and no line in the test region (T). This indicates that there is no detectable MPB64 protein in the sample.

INVALID: No red line appears in the control region (C). The test is invalid even if there is a line on test region (T). Insufficient sample volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the test procedure and repeat the test using a new test plate. If the problem persists, discontinue using the test kit immediately and contact the local distributor.

【PRECAUTIONS】
For In Vitro Diagnostic Use.
Do not use after the expiration date.
Ensure foil pouch containing test device is not damaged before opening for use.
Perform the test at room temperature 15 to 30 degree centigrade.
Wear gloves when handling the samples, avoid touching the reagent membrane and sample window.
Use universal precautions when testing. The sample handling and testing must be preceded in bio-safety cabinet.
Annex 4: Laboratory Reagents and Media

Smear microscopy
Staining reagents

All staining reagents are stored in screw-capped bottles (preferably 500ml) at room temperature. Each bottle should be carefully labelled with the name of the reagent as well as the preparation and expiry dates.

Fluorochrome staining

Fluorochrome staining reagents should be stored in tightly stoppered amber bottles for 3 months. But, the working solutions should be used within 1 month.

Fluorochrome stain: 0.1% auramine O in 3 % phenol

- Wear the gloves
- Dissolve 1.0 g of auramine O in 100 ml of 95% ethanol (technical grade) (A).
  Note: Direct contact with auramine O powder or solution must be avoided because it has been shown to be a carcinogen.
- Dissolve 30 g of phenol crystals in the flask containing 870 ml of distilled water (B).
- Mix well 100 ml of auramine O solution (A) with 900 ml of phenol solution (B) and store in a screw-capped amber bottle away from heat and light.
- Label the bottle "0.1% auramine", add the date and sign with initials.

Decolorizing agent: 0.5% acid-alcohol

- Carefully and slowly add 5 ml of concentrated hydrochloric acid in the flask containing 1000 ml of 70% ethanol (technical grade).
- Label the bottle "0.5% acid–alcohol", add the date and sign with initials. The date the bottle is first opened must be written on the label. This solution may be kept indefinitely.

Counterstains: 0.5% potassium permanganate

- 0.5% potassium permanganate: Dissolve 5 grams of potassium permanganate (KMnO4) in 1000 ml of distilled water.
- Label the bottle "0.5% potassium permanganate", add the date and sign with initials. The date the bottle is first opened must be written on the label. Solution should be used within 6 months.

Ziehl-Neelsen (Z-N) staining

Ready-made kit (RAL kit ®) is used as Ziehl-Neelsen staining solution.

Quality control of freshly prepared stains

The quality of newly prepared staining reagents must be checked with known unstained AFB positive (1+) and negative smears before use.

Culture examination

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Reagents for the decontamination

**Materials**
- Sodium hydroxide (NaOH), 4%
- Distilled water
Dissolve NaOH in the distilled water. Aliquot in 4ml amounts. Sterilize by autoclaving at 121°C degree for 15 minutes.

Media preparation for culture and subculture

**Materials**
- Blender
- Dispenser
- Stirrer, magnetic
- Refrigerators with freezer
- Inspissator with steel slanting beds
- Oven
- Autoclave
- Indicator tape for sterilization
- Various glass or plastic ware items (beakers, flasks, funnels, measuring cylinders, serological pipettes)
- Forceps and gauzes
- Petri dishes
- Culture tubes with screw caps
- Gloves
- Timer
- Plastic bags for storage (e.g. Ziploc)
- Various chemicals for media preparation (see formula)
- Fresh eggs (not more than one week old) from hens not fed with antibiotic-containing feed

### Formulas for Culture media

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<tr>
<td>Malachite green (2%)</td>
<td>4ml</td>
<td>20ml</td>
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</tbody>
</table>
Mineral salt solution: Dissolve mineral salts in distilled water by heating the mixture, and then add glycerol. Autoclave at 121°C for 30 minutes to sterilize. Cool to room temperature.

Malachite green (MG) solution: Add 10 grams (1%) or 20 grams (2%) of malachite green to 1000 ml of sterile DW and dissolve in the incubator for 1–2 hours. Always use freshly prepared MG solution.

Homogenized whole eggs: Wash fresh hens’ eggs with a plain alkaline soap by scrubbing thoroughly with a brush in warm water. Rinse eggs thoroughly under running water and wipe them with gauze soaked in 70% ethanol. Crack the eggs one by one into a sterile petri dish and check freshness before combining in a sterile blender for homogenization.

Preparation of medium: Mix mineral salt solution, fresh malachite green solution, and fresh egg homogenate in the ratio shown in the table above, and dispense 6ml of medium to each culture tube. Place media tubes without babbles in the slanting beds and inspissate for 60 minutes at 90c degree.

Sterility tests of newly prepared media should be done by incubating in an incubator at 37 c degree for 2–3 days. Put media with no contamination into plastic bags (e.g. Ziploc) and store in a refrigerator. Media can be stored for several weeks, but should be used only if not dried and not contaminated.

Middlebrook 7H9 for storage of strains

Materials
Middlebrook 7H9 broth
Glycerol
Distilled water
Erlenmeyer flask
Autoclave

<table>
<thead>
<tr>
<th>Formula for Middlebrook 7H9 medium</th>
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</thead>
<tbody>
<tr>
<td>Middlebrook 7H9 broth</td>
</tr>
<tr>
<td>Distilled water</td>
</tr>
<tr>
<td>Glycerol</td>
</tr>
</tbody>
</table>

Suspend 4.7g of the powder in 900ml of distilled water (containing 10ml-15ml of Glycerol). Autoclave at 121 degree centigrade for 10min
Annex 5: Field level interpretation

- Assess the image quality using the parameters mentioned in the training (positioning, side marker placement, inclusion of complete lung fields, motion blurring, overall brightness and contrast, demographic data)

- Perform interpretation, so as to classify the X-ray as normal, active TB (Active TB is strongly suspected), TB suspect (possible TB lesion or stable lesion such as tuberculoma), healed TB, suggestive of other respiratory disease, suggestive of other heart disease, and other.

- Document QA and interpretation on the X-ray data sheet and on the survey questionnaire

- Hand over the X-ray data sheet to the radiographer for filing