Standard Laboratory Operating Procedures in the Disease Prevalence Survey

A Misra*

Abstract

The study of epidemiological situation of a communicable disease like Tuberculosis (TB) is of paramount importance for formulating the disease control strategies. It provides an insight into the impact of the control programmes on the disease situation in the community. Bacteriology is one of the fundamental aspects of National Tuberculosis Programmes (NTPs) and a key component of the DOTS strategy. Serious deficiencies in the laboratory operations may result in the quality of outcome, leading to both under-diagnosis or over-diagnosis of the disease. Under-diagnosis of the disease in patients leads to life threatening illness and increased rates of infection transmission whereas over-diagnosis exposes the individuals to unnecessary treatments, expenses and stigma associated with Tuberculosis.

Keywords: Prevalence, Symptomatic, Smear microscopy, Sputum culture, Fluorescent Microscopy.

Background

National Tuberculosis Institute, Bangalore proposed the Disease Prevalence Survey (DPS) among adults of ≥14 years of age residing in rural areas of Nelamangala Taluk of Bangalore rural district in the year 2006 with the objective to estimate the point prevalence of Pulmonary Tuberculosis (PTB). An earlier survey in the taluk in 1975, using Mobile Mass Miniature Radiography (MMR) and symptom-screening followed by sputum examination had revealed the prevalence of culture positive PTB at 4.8/1000 population in the age group of ≥14 years. Nelamangala taluk is a pre-dominantly rural taluk with total population being 2,74,445 of which 2,49,239 is rural and 25,206 is urban.

The Disease Prevalence survey is being carried out in all the villages in randomly selected panchayats. The field work is being carried out by three field teams (Team-I for Planning, Team-II for Enumeration, Symptomatic screening and X-Ray and Team-III comprises the Laboratory personnel for Sputum collection) supported by a head quarter co-ordinating team. The field work is regularly supervised by the Field In-charge, Team leaders, Senior Research Fellow (SRF) and Senior Epidemiologist.

Prior to the start of the survey, Deputy Commissioner of the District, Tehsildar, District Health Officer, District Tuberculosis Officer, Taluk Medical Officer/MOTC, CEO Zila Panchayat and Executive Officer Taluka Panchayat Office were contacted personally by SRF and briefed about the purpose of survey in order to ensure their fullest cooperation and support for carrying out the survey. They were requested to instruct all their subordinate officials at village level to extend the necessary assistance to the field teams. Village planning was undertaken 3-4 days prior to the proposed dates for the survey in the village. The planner records all the required

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information in Village Planning Report form including hamlets of the village, road description, population as per 2001 census and present population according to the panchayat, number of houses, distance from camp/HQ and draw a rough sketch of the village including hamlets on the back side of the form. The village is divided into different sectors like A, B, C, D, etc. and these sectors are allotted to each enumerator by the field in-charge. A house to house enumeration of all individuals residing in the study areas for ≥6 months as on the date of registration is carried out in a clockwise direction and the numbering of houses is done from 01-99. The enumeration is undertaken for all permanent residents. An informed written consent pertaining to examination from all the individuals is obtained on reverse of RF/3 (Individual Card). All eligible individuals (≥15yrs) are registered by the enumerator and screened for cardinal symptoms suggestive of pulmonary TB before being X-rayed.

A chest symptomatic is defined as an individual with one or more of the following:

- Persistent cough for ≥ two weeks
- Fever for ≥ one month
- Chest pain for ≥ one month
- Haemoptysis within the last 6 months.
- History of previous anti-TB treatment

All X-ray films shall be read independently by two trained readers and classified as under:

N - Normal
TI - Technically inadequate
A - Lung pathology other than tuberculosis
B - Tuberculosis inactive
C - Tuberculosis active

In case of disagreement between the two readers, the X-Ray will be referred to an umpire reader. All individuals who are either chest symptomatic or have abnormal shadow on x-ray as read by either of two independent readers shall be subjected to sputum examination.

Objective

To describe the Standard Laboratory Operating Procedures in the Disease Prevalence Survey.

Sputum Collection and Transportation

Two sputum specimens (one spot and one overnight) are collected from each eligible individual and sent to the laboratory. The samples are collected in pre-numbered sterilized sputum cups and are transported to laboratory as soon as possible after collection. If delay is anticipated a suitable preservative (1% Cetyl - Pyridinium Chloride-CPC and 2% Sodium Chloride) in equal amount is recommended. After collecting the sputum sample, the sputum supervisor cross checks the specimen numbers of each bottle with the individual cards which contains information of the sputum eligible individual. They reach the laboratory on the same day of collection and cold chain is maintained till the sample is processed.

Processing of direct sputum samples

Two slides are prepared for smear microscopy before processing the sputum samples. Homogenization and Decontamination of sputum samples is done by using 4% NaOH (Modified Petroff's Method) as per RNTCP guidelines\(^1\). In this procedure sputum (x) is transferred to a McCartney bottle and double the amount (2x) of 4% NaOH is added. This is incubated in the shaker for 20 minutes. Approx. 20ml of sterile distilled water (SDW) is added to the suspension. Centrifuged it at 3000g for 15mins. The supernatant is decanted and the deposit is inoculated onto 2 slopes of Lowenstein-Jensen (LJ) medium and the cultures are incubated at 37°C until growth is observed. Slopes which are
grossly contaminated are discarded. Cultures are read every week till sufficient growth appears for identification tests. 3mm loopful of growth is required to proceed for identification tests. A smear positive specimen is incubated for 12 weeks before declaring it as negative whereas a smear negative specimen is incubated and read for 8 weeks before declaring it as negative.

**Bacteriological examination**

The smears are examined and graded by fluorescent microscopy (FM). Before processing the sample direct smears are made from each sample and stained using Auramine O (0.1%) and Potassium permanganate (0.5%) for fluorescence microscopy, grading is done as per the RNTCP guidelines as Negative, Scanty, 1+, 2+ or 3+ at a magnification of 200X. If there is uncertainty about the presence of bacilli because of lower magnification, a higher (400X) Magnification can be used for conformation of the suspected bacilli. FM has replaced the Ziehl-Neelsen (ZN) staining as it takes less time to read the slide (slide load per day is approx, 30-40 slides) and it has been found better in sensitivity to detect low positive specimens.

### Grading for FM

<table>
<thead>
<tr>
<th>AFB* COUNT</th>
<th>RESULT AFB COUNT (200X) magnification, 1 length(\text{<strong>}=30) fields=300 HPF</strong>(\text{**}))AFB</th>
<th>RESULT AFB COUNT (400X) magnification, 1 length=40 fields=200 HPF)AFB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Zero AFB/1 Length</td>
<td>Zero AFB/1 Length</td>
</tr>
<tr>
<td>Scanty</td>
<td>1-29 AFB/1 Length</td>
<td>1-19 AFB/1 Length</td>
</tr>
<tr>
<td>1+</td>
<td>30-299 AFB/1 Length</td>
<td>20-199 AFB/1 Length</td>
</tr>
<tr>
<td>2+</td>
<td>10-100 AFB/1 field</td>
<td>5-50 AFB/1 field</td>
</tr>
<tr>
<td>3+</td>
<td>&gt;100 AFB/1 field</td>
<td>&gt;50 AFB/1 field</td>
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-Acid fast bacilli. **1 length corresponds to 20mm long sputum smear on the slide. ***High power field.

Cultures are read weekly till sufficient growth appears for identification tests. 3mm loopful of growth is required to proceed for Identification tests. If the growth is insufficient a subculture should be done on a fresh LJ medium touching all the colonies. Culture reports should be qualitative (positive or negative) as well as quantitative.

The following scheme is recommended:

### Reading for Primary Culture

<table>
<thead>
<tr>
<th>Report</th>
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<tbody>
<tr>
<td>No growth</td>
</tr>
<tr>
<td>1-100 colonies</td>
</tr>
<tr>
<td>&gt;100 discrete colonies</td>
</tr>
<tr>
<td>Confluent growth</td>
</tr>
<tr>
<td>Contaminated</td>
</tr>
<tr>
<td>NTM colonies</td>
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</table>
*Mycobacterium tuberculosis* is identified on the basis of the following characteristics:

- Growth rate is slow,
- No growth on LJ medium containing p-nitro benzoic acid (PNB),
- Growth temperature 35° - 37° C only,
- No pigmentation and Niacin Positive.

The results of bacteriological examination (smear microscopy and culture) are entered in referral form (RF/3) by the Lab Technician and finally handed over to the Statistical Assistant. Cross-checking at TRC, Chennai ensures quality control. In case both the sputum samples get contaminated, Lab Coordinator prepares a new duplicate sputum eligible card and handovers the same to the sputum field team for recollection of sputum specimen.

**Reporting of smear results**

Smear results are reported at the end of each week (Preferably Monday forenoon) but for smear positive cases, reports are sent immediately. Culture results are reported at the end of 8 weeks for smear negative cases and at the end of 12 weeks for smear positive cases.

All the individuals found positive after bacteriological examination are referred to the nearest RNTCP centers for further follow up and treatment as per the guidelines.

**Analysis**

The results of Smear microscopy and culture are analysed for Bacillary and Abacillary cases and disease prevalence is estimated using statistical methods by the statistician.

**Estimation of disease prevalence**

a) **Bacillary cases:** Prevalence of bacillary cases of PTB shall be estimated while defining a case found positive on sputum smear microscopy and/or culture. These cases shall be further classified as:

- Smear and culture positive
- Smear negative culture positive
- Smear positive and culture negative

b) **Abacillary cases:** Prevalence of abacillary PTB shall be estimated considering those individuals whose X-rays are read as C by two of the three readers and found negative on sputum smear microscopy or culture.

**References**