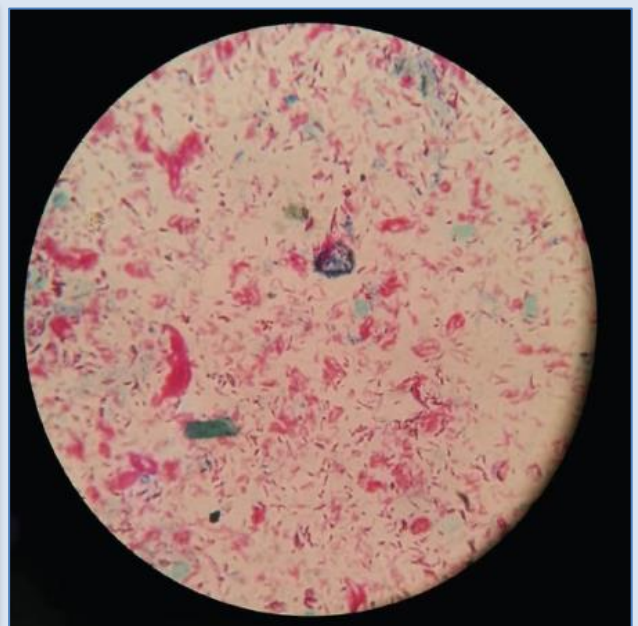




# Training Manual for Laboratory Technicians 2019



## National Leprosy Eradication Programme

Central Leprosy Division, New Delhi and Central Leprosy Teaching & Research Institute, Chengalpattu, TN

Directorate General of Health Services, Ministry of Health and Family Welfare

GOVERNMENT OF INDIA



**NATIONAL LEPROSY ERADICATION PROGRAMME  
(NLEP)**

**Training Manual for Laboratory Technicians**

**2019**



**Central Leprosy Division  
Directorate General of Health Services  
Ministry of Health and Family Welfare  
Government of India**



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दिनांक/Dated... 22/2/2019

### Foreword

It is my pleasure to introduce, the standardised "Training Module for Laboratory Technicians, 2019" developed with support of stakeholders of the National Leprosy Eradication Programme (NLEP), under the aegis of Central Leprosy Division, DGHS, MOHFW, GOI. This document is prepared to standardize the training procedure being followed by different govt. & non govt. institutes for capacity building of Laboratory Technicians to perform Slit Skin Smear (SSS) examination technique, which was not uniform till date.

The results of SSS examination i.e., morphological and bacteriological indices of lesions helps in diagnosis and better management of leprosy and also used as screening criteria for surveillance of drug resistance. Though it is a minimally invasive technique however chances of subjective variations and dilemma are there in view of the non-availability of a reference manual. Hence, to overcome these issues this module is prepared as a standard for internal quality assurance to check the subjective errors. The necessary formats for recording and reporting of SSS are also provided with this module that can be utilized by the laboratories performing this test. The module covers all aspects of SSS with clear demonstrations and colored images. It may be placed on the worktable for ready reference whenever needed.

With hopes and wishes that this module will get utilized by trainers and Laboratory Technicians, I cordially appreciate the hard work devoted by all technical experts in formulization of this module.

Anil Kumar

(Dr. Anil Kumar)



**Dr. VINEET K. CHADHA**  
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**Dated: 22 Feb 2019**

## ***PREFACE***

It gives me immense pleasure and proud to publish the “Training Module for Laboratory technicians, 2019”. Leprosy is a disabling disease, still prevailing in the communities. Leprosy is caused by *M. leprae*, a mycobacterium that multiplies very slowly. It is a disease with variable incubation period and there exist packets of high transmission. Though the prevalence has declined significantly after introduction of MDT, the notification rates have remained static for more than a decade. Therefore, the health care professionals should consider leprosy in their differential diagnosis and Slit Skin Smear examination is a useful tool in diagnosis.

Slit Skin Smear (SSS) is a simple, diagnostic test that can be utilized for early diagnosis of leprosy and necessary for implementing surveillance of drug resistance. This module has dealt in detail about all aspects of SSS like the required logistics, preparation of reagents, collection technique, microscopy and grading of results in terms of morphological and bacteriological indices.

In addition there is a color plate depicting the technique of collection of slit skin smear from a suspected patient. Ridley’s grade is provided in the form of a table as well as images for clear interpretation of grading. Most important, the manual includes sections on internal quality assurance, which will help to keep a check on the subjective errors. The recording and reporting formats for SSS have been provided, which need to be maintained by the laboratories performing this technique and for quality assurance.

I am confident that, the module designed for Lab. technicians will help in enhancing the skills and improve the performance regarding slit skin smear technique, thus contributing towards better-quality patient care.

I acknowledge the contribution of all the experts and technical persons involved in compiling this module, to deliver the intended objective.

**Dr. Vineet K. Chadha**

## ACRONYMS

|      |  |
|------|--|
| NGO  | Non-governmental organization          |
| NLEP | National Leprosy Eradication Programme |
| SSS  | Slit Skin Smear                        |
| BI   | Bacteriological index                  |
| MI   | Morphological index                    |
| PB   | Paucibacillary                         |
| MB   | Multibacillary                         |
| AFB  | Acid fast bacilli                      |
| IQA  | Internal quality assurance             |

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## INTRODUCTION

**L**eprosy is a chronic mycobacterial disease caused by the bacterium named *Mycobacterium leprae*. It is a slow growing intracellular bacillus that infiltrates the skin, the peripheral nerves, the nasal mucosa and eyes. The incubation period of leprosy ranges from two to seven years and sometimes may be as long as twenty years. The leprosy bacillus was discovered by Norwegian scientist, Armaeur Hansen in 1873. Hence, it is also called as Hansen's disease. The bacillus measures about 3 to 5 micron in length and 0.2 to 0.5 micron in width and elongated forms can be seen sometimes. Infection probably occurs when leprosy bacilli are discharged through the nasal secretion. Infection occurs when the bacilli enters through nasal / oro-pharyngeal routes.

The main clinical features of leprosy are a variety of skin lesions and peripheral nerve trunk damage, which leads to anesthesia and paralysis causing deformities which are characteristic features of leprosy. Nerve damage occurs in untreated leprosy patients.

## **AIMS & OBJECTIVES OF TRAINING**

### **AIM OF THE SKIN SMEAR TRAINING:**

Training of laboratory technician in all aspects of skin/nasal smear and building up their confidence in performance and reporting Slit Skin Smear.

### **OBJECTIVES:**

- 1) To prepare skin smear in hospital and field conditions.
- 2) To stain smear with Ziehl-Neelson's stain.
- 3) To read and grade the smear according to Ridley's Scale and calculate Bacteriological Index and Morphological Index.
- 4) To provide requisite assistance to clinicians for diagnosis, classification and management of leprosy cases.
- 5) To assist in research in the diagnostic, clinical and operational aspects of leprosy.



# INDUCTION TRAINING AND REFRESHER TRAINING

## INDUCTION TRAINING

### Eligibility

Fresh in-service candidates working in the laboratories of Government / NGO / Autonomous institutions who had not earlier undergone a recognized skin smear training course.

### Number of Trainees per batch

The number of trainees per batch should not be more than ten. The number of trainees in the batch should depend on the available facilities and faculties in the Training Institutions.

### Duration of Training

The duration of induction training should be spread over 5 days.  
The topics should be in accordance with the training need assessment document as follows.

| Day | Sessions   | Duration |
|-----|--|----------|
| 1   | 1. Pre-test  | 1/2hour  |
|     | 2. To describe diagnosis, Pathogenesis, Clinical manifestations of leprosy | 2 hours  |
|     | 3. To describe National Leprosy Eradication Programme.                     | 2 hours  |
|     | 4. To describe the role of skin smear in NLEP.                             | 1 hour   |
| 2   | 1. To demonstrate slit skin smear technique                                | 4 hours  |
|     | 2. To calculate various indices in SSS                                     | 2 hours  |
| 3   | 1. To demonstrate slit skin smear technique                                | 4 hours  |
|     | 2. To describe bacteriological characteristics of <i>M. leprae</i>         | 1 hour   |
|     | 3. To describe management of laboratory wastes                             | 1 hour   |

|   |  |         |
|---|--|---------|
| 4 | 1. To demonstrate slit skin smear technique  | 4 hours |
|   | 2. To demonstrate preparation of various stains and reagents for slit skin smear                     | 1 hour  |
|   | 3. To discuss recent advances and its applications including molecular biology techniques in leprosy | 1 hour  |
| 5 | 1. To demonstrate slit skin smear technique in field conditions                                      | 4 hours |
|   | 2. To demonstrate various aspects in maintenance of quality of skin smears                           | 1 hour  |
|   | 3. Post test with discussion   | 1 hour  |

### Essential topics to be covered in theory classes

|                        |   |
|------------------------|---|
| 1) What is leprosy?    | 8) Microscopy                                 |
| 2) Causative agent     | 9) Bacteriology                               |
| 3) Incubation period   | 10) Sterilization                             |
| 4) Mode of spread      | 11) Taking smear (skin / nasal)               |
| 5) Signs and symptoms  | 12) Selection of sites                        |
| 6) Cardinal signs      | 13) Materials and equipments required         |
| 7) Indications for SSS | 14) Process of reading and grading            |
|                        | 15) Preparation of report including BI and MI |

### Field Visit

During field visit, the trainee has to be exposed for hands on training for collection, staining and reporting of skin smear in laboratory and field conditions.

## **REFRESHER TRAINING**

### **Eligibility**

In-service candidates working in the laboratories of Government / NGO / Autonomous institutions who had already undergone skin smear training in last 3-5 years. The training needs to be provided to Laboratory Technicians/Assistants as suggested by the higher officials (MO, DLO, SLO etc.)

### **Number of Trainees per batch**

The number of trainees per batch should not be more than ten. The number of trainees in the batch should depend on the available patients, facilities and faculties in the Training Institutions.

### **Duration of Training**

The duration of induction training should be spread over 2 days and the topics should be in accordance with the training need assessment document as follows.

### **Topics to be covered for refresher training**

| <b>Theory<br/>(2 hours per day)</b>  | <b>Practical<br/>(4 hours per day)</b>   |
|--|--|
| In theory class, basics of leprosy focusing on laboratory aspects, signs and symptoms of leprosy should be dealt, cardinal signs and classification needs to be covered. | First day - collection & preparation of skin /nasal smear, staining and reading the smear.<br>Second day - grading the smear and interpreting the results. |

## CLASSIFICATION OF LEPROSY

**N**ow patients of leprosy are broadly classified into Paucibacillary (PB) and Multibacillary (MB) as per NLEP guidelines. The previous classification of Ridley & Joplings should be replaced with the above classification for all operation purposes under NLEP guidelines as follows.

### NLEP CLASSIFICATION:

| Sl. No. | Observations                     | PB       | MB                     |
|---------|----------------------------------|----------|------------------------|
| 1.      | Number of skin lesions           | 1-5      | More than 5            |
| 2.      | Peripheral Nerve Trunks involved | 1        | More than 1            |
| 3.      | Skin Smear                       | Negative | Positive (at any site) |

## SLIT SKIN SMEAR (SSS)

**A** skin smear is a diagnostic & prognostic test in which a sample of material is collected from a tiny cut in the skin and then stained for *M. leprae*.











Presence / demonstration of AFB in SSS is one of the cardinal signs of leprosy and the examination helps to diagnose / confirm difficult cases who are disseminating infection through nasal secretions and these patients can be diagnosed / put on treatment. Therefore, case load can be minimized in the long run.

### Applications of Slit Skin Smear

1. To confirm diagnosis of leprosy in a suspect where the case cannot be diagnosed clinically.
2. To determine the state of infectivity
3. To help in diagnosis of relapse of leprosy
4. For classification and prognosis
5. For certification of cured of leprosy

Performing skin-smear is an invasive procedure and requires all aseptic precautions. Washing hands, wearing gloves and using sterilized equipments and a new blade for each patient are mandatory requirements.

## REQUIREMENTS

|   |   |
|---|---|
| 1. Spirit Lamp<br>           | 2. Tincture Benzoin<br>                      |
| 3. Scalpel<br>               | 4. Sterile New surgical blade (No.15)<br>    |
| 5. Match box<br>             | 6. New glass slide (75mm X 25mm X 1.0cm)<br> |
| 7. Diamond Pencil<br>      | 8. Sterile Cotton Balls<br>                |
| 9. Sterile Swab sticks<br> | 10. Microscope<br>                         |

## PROCEDURE

|   |  |
|---|--|
| <p><b>A. Selection of sites for SSS</b></p> <ol style="list-style-type: none"> <li>1. Both ear lobes</li> <li>2. Both arms</li> <li>3. Both thighs</li> <li>4. One lesion (skin patch) - recently active</li> </ol> <p>In field conditions,</p> <ol style="list-style-type: none"> <li>1. One ear lobe</li> <li>2. The margin of lesion (skin patch)</li> </ol> | <p><b>B. Preparation of Slide</b></p> <ul style="list-style-type: none"> <li>➤ On a new slide, using a Diamond pencil write</li> </ul> <p>Top - Lab No.</p> <p>Bottom - Name of the patient and date</p> |
|---|--|

- Gently warm the slide. After warming, clean with Whatman's filter paper No.3. Do not use cotton.
- Insert a No.15 surgical sterile blade in scalpel using a Artery forceps
- Do not touch the blade.

### **C. Skin smear technique:**

- Thoroughly clean the selected site with Spirit or Ether to remove dirt and any saprophytes.
- Hold the skin pinched up and raise between the thumb and index finger of the left hand. This will squeeze out blood from the part and minimize bleeding when cut is made. Due to thickness of the skin, it is difficult to pinch up the skin, exert lateral compression with the two fingers stretching the bit of skin in between them.
- With the point of sterile scalpel make an incision of 5mm long and 2-3mm deep; scrape the bottom and the sides of the slit to obtain sufficient material for a smear.



- Transfer the material from the point of scalpel onto a clean new glass slide making a uniform and moderately thin smear of average 5-7mm diameter.
- Press the cut surface of the skin with a piece of cotton wool to stop bleeding and seal the part with tincture benzoin.
- Allow the smear to air dry, fix it by passing the slide twice or thrice over the flame, the heat should be bearable to the skin. Do not over heat, which results in charring or cracking of smear. Too little heat may not fix the smear efficiently and it may wash out.

### **CHARACTERISTICS OF A GOOD SKIN SMEAR**

1. Should be approximately 5-7 mm in diameter
2. Should be uniform - should not be too thick in one area and too thin in other area. Rough estimate of acceptable thickness is that a print letter of hand on watch should be seen through, 80% of the area of the smear should be 80% transparent.
3. The point where the scalpel first touched the side will always be less transparent than the other areas.



## STAINING TECHNIQUE

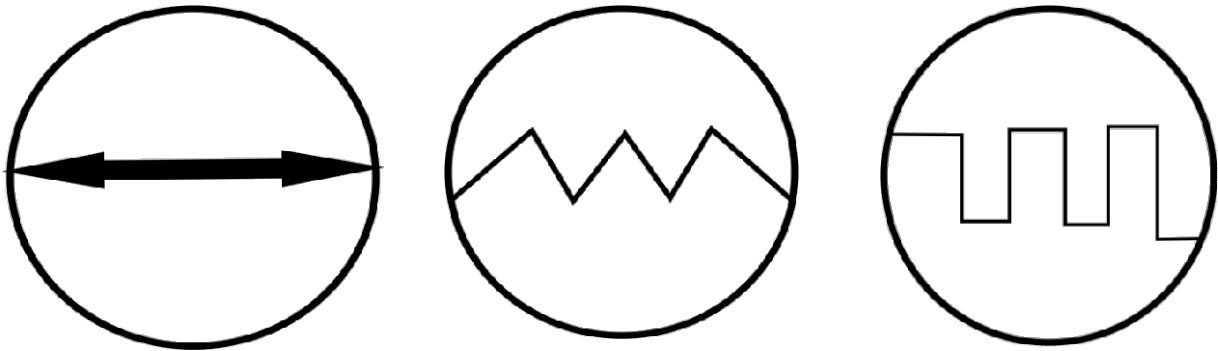
1. Place the slides on a pair of staining rods over a sink or a basin after making sure that the rods are horizontal. 6-8 slides may be stained together at one time.
2. Fold a filter paper into the shape of a funnel and filter carbol fuchsin on to the smears
3. Heat gently with the flame of a spirit lamp or candle, until steam begins to rise. **DO NOT HEAT TO BOILING.** Keep flooding the slides with carbol fuchsin and gently warm thrice in a period of 5 minutes. **DO NOT ALLOW THE STAIN TO DRY.** This causes the deposition of stain particles on the smear, making microscopic examination difficult.
4. Wash gently in running tap water. If a tap is available, attach rubber tubing to the tap and open the tap gently. Use a mug if no tap is available, but pour water gently.
5. Cover the slides with decolourising agent – 5% sulphuric acid. Let it stand for 10 minutes. (If using 1% acid-alcohol as decolorizing agent, let it stand for 5-10 seconds only).
6. Wash gently with running tap water
7. Cover the slides with methylene blue solution for 1 minute.
8. Wash gently with running tap water.
9. Wipe the under surface of the slides clean with cotton dipped in acid alcohol in order to remove any dried up stain deposits or soot.
10. Stand the slides on the blotting paper of the slide rack to drain off water drops.
11. Wait till the smears are dry.

The slides are now ready for examination.

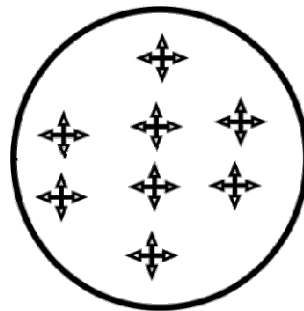
The lepra bacilli are seen as pink rods. The bacilli may be seen in single, small groups or closely packed bunches called globi. Scattered among the bacilli, irregular blue stained structure are seen. These are cells of various structures present in dermis. Smears are graded to report BI (Bacteriological Index) and MI (Morphological Index).

## EXAMINATION OF STAINED SKIN SMEAR SLIDE

1. Put the slide on microscope stage
2. Focus the smear under 10 X and observe uniformity/concentration of material.  
Select area which is not too thin or too thick.
3. Put oil on the smear and rotate objective to bring 100x in touch with oil.
4. Focus using fine adjustment
5. Start examining smear
6. Smear can be examined in different ways



Examine smear using any one style of the above. After about 80 fields, it is beneficial to select randomly i.e.



Always ensure that you have examined the center of the smear.

## **PRECAUTIONS**

- Avoid very fast movements of microscope stage.
- Avoid going to edge of the slide when using oil immersion objective.
- Clean the microscope stage and objectives before and after use.

## PREPARATION OF STAINING REAGENTS

### EQUIPMENTS REQUIRED

The equipment listed below is for a patient load of about 100 per week.

- (a) Glassware
- (b) Other equipment

#### (a) GLASSWARE

| Item                                      | Capacity                | Number |
|---|-------------------------|--------|
| Conical flasks                            | 1 L                     | 4      |
| Stock solution bottles with screw caps    | 1 L                     | 4      |
| Dropper bottles                           | 150-200 ml              | 4      |
| Funnels                                   | Large/Medium            | 2      |
| Pipettes (for acid)                       | 10ml                    | 2      |
| Stock solution bottle for distilled water | 3-5 L                   | 1      |
| Beaker                                    | 50ml                    | 2      |
| <b>Measuring cylinders</b>                |                         |        |
| for acid, phenol and rectified spirit     | 50ml                    | 2      |
| for preparing stock solutions             | 500ml                   | 4      |
| <b>Glass rods</b>                         |                         |        |
| for staining                              | to fit the mouth of the | 2      |
| for stirring                              | sink                    | 4      |

#### (b) OTHER EQUIPMENT:

| Item           | Capacity               | Number |
|----------------|------------------------|--------|
| Common Balance | upto 1 gm. sensitivity | 1      |
| 1 Hot plate    | -                      | 1      |

|                            |                       |                     |
|----------------------------|-----------------------|---------------------|
| <b>Filter paper</b>        | (available in sheets) | <b>2 or 3 boxes</b> |
| <b>Spirit lamp/candles</b> | -                     | <b>As needed</b>    |
| <b>Timer</b>               | -                     | <b>1</b>            |
| <b>Box of Matches</b>      | -                     | <b>As needed</b>    |

## STAINS

The reagents needed for the preparation of stains are:

- Basic Fuchsin
- Phenol
- 95% Ethyl alcohol
- Distilled water
- Concentrated sulphuric acid
- Concentrated hydrochloric acid
- Rectified spirit
- Methylene blue

### WATER FOR PREPARATION OF STAINS

Use distilled water

If not available, use boiled, cooled and filtered water: If filter paper is not available, a double fold of cloth freshly boiled, separately, may be used as a filter.

**DO NOT USE TAP WATER**

**Three solutions have to be prepared for staining by the acid fast method**

1. **The Primary stain:** *1% carbol fuchsin*
2. **The decolourising agent:** *1% acid alcohol solution*
3. **The counter-stain:** *1% Methylene blue*

### **1. PRIMARY STAIN (1% CARBOL FUCHSIN: 1 LITRE)**

#### **Materials Needed**

- a. Basic fuchsin

Weigh 10gms of Basic fuchsin in the balance

- b. Melted Phenol

Place a few crystals of phenol in a small beaker. Place the beaker in a container with water and heat it on a Hot plate until the phenol melts.

Measure out 50ml.

c. 95% Ethyl alcohol

Measure 100ml. of 95% alcohol

d. Distilled water

Measure 850 ml of distilled water.

e. Glass Rod, Conical flask, Funnel & Filter Paper

### **Procedure:**

1. Place basic fuchsin in a large conical flask
2. Add alcohol
3. Shake or stir with a glass rod to mix well
4. Add distilled water and shake or stir until all basic fuchsin is dissolved.
5. Add the remaining water and Mix well
6. Add phenol and stir to mix
7. Filter using a funnel with a filter paper placed in it. Place the funnel in a conical flask.
8. Collect the filtrate
9. Store in a screw-capped labeled bottle

### **2. DECOLOURISING AGENT: (1% ACID ALCOHOL SOLUTION)**

Concentrated Hydrochloric Acid - 3ml

70% alcohol - 97ml

### **3. COUNTER-STAIN: (1% METHYLENE BLUE)**

a. Methylene blue - 10 gms.

b. Distilled water - 1000ml.

**Procedure:**

1. Add methylene blue to distilled water and stir until dissolved.
2. Filter using a funnel with a filter paper placed in it. Place the funnel in a conical flask.
3. Store in a screw-capped labeled bottle
4. Pour into labeled dropper bottles and use as needed

All stock solutions may be stored for approximately one month. Wash and dry stock solution bottles once a month.



## RIDLEY'S GRADING OF SKIN SMEAR

| <b>Bacteriological<br/>Index</b> | <b>Criteria</b>  |
|----------------------------------|--|
| <b>1+</b>                        | 1-10 bacilli on an average in 100 oil immersion fields                                 |
| <b>2+</b>                        | 1-10 bacilli on an average in 10 oil immersion fields                                  |
| <b>3+</b>                        | 1-10 bacilli on an average in each oil immersion field                                 |
| <b>4+</b>                        | 10-100 bacilli on an average in each oil immersion field                               |
| <b>5+</b>                        | 100-1000 bacilli on an average in each oil immersion field                             |
| <b>6+</b>                        | > 1000 bacilli on an average in each oil immersion field / innumerable bacilli / globi |

## **BACTERIOLOGICAL INDEX (B.I.)**

The density of bacilli in smears is known as Bacteriological Index and includes both living and dead bacilli. It speaks of the presence of bacilli in the patients of MB variety.

Positivity depends upon the degree of bacterial load. Negativity attains gradually at the rate of 1+ per year, after adequate and effective MDT.

Presence of positive BI, after completion of course of treatment does not mean patient is active and therefore does not require more treatment.

Bacteriological Index (BI) – It is the total number of bacilli at all sites divided by number of sites.

There are two methods commonly employed for this purpose:

- I. Dharmendra's method
- II. Ridley's method

### **BI Depends Upon:**

1. Depth of the scrape
2. Amount of tissue fluid removed
3. Size and thickness of the smear etc.

## **MORPHOLOGICAL INDEX (M.I.)**

Lepra bacilli when stained by Ziehl-Neelson's method take pink colour (stain). But most of the bacilli are stained irregularly and only a few are uniformly stained and the uniformly, intensely stained bacilli are viable and capable of multiplying and that all the other forms are dead or dying and are incapable of multiplying. The percentages of solid bacilli are called M.I.

### **METHOD**

In a smear stained by Ziehl-Neelson's method for AFB 100 individually recognizable bacilli should be identified as showing uniform or irregularly stained. Normally no difficulty will be experienced in identifying whether a bacillus is uniform or irregularly stained and clumps / globi are not included during MI. Four criteria given below should be strictly fulfilled to label a bacillus as solid.

1. The whole length of the bacillus is stained uniformly and densely
2. The sides of the bacillus are parallel
3. The ends of the bacillus may be rounded, straight or pointed
4. The bacillus is at least four times as long as its width.

The changes in the morphology of human leprosy bacilli serve as useful indicator of progress during treatment. For example under the influence of a potent anti-leprosy drug the bacilli lose their ability to stain intensely and uniformly with carbol fuchsin and show such features as irregular staining, beading and breaking up into fragments etc. Organisms which reveal such characteristics in their morphology are considered to be non-viable at least in their ability to propagate in another living host.

Further, such morphological alterations occur in much shorter time than the reduction in number of organisms so that these changes are apparent much before the BI begins to fall. Therefore, we have the MI as a useful measure, which enables us to assess the suitability of any therapeutic regimens against leprosy in shorter period.

Presence of MI indicates whether disease is active or not and there is a possibility of bacterial resistance.

### **M.I Depends Upon:**

The availability of sufficient free stained bacilli.

# NASAL SMEAR

## METHOD

A part of the lining of the nose is gently scraped and the fluid obtained is smeared on a microscopic slide. This is stained by Ziehl-Neelson method and examined under the microscope. The nasal smear is painful, therefore it has to be collected with care.

## TECHNIQUE

1. Seat the patient on a low stool
2. Insert a sterile cotton swab stick (dipped in normal saline) gently into a nostril
3. Scrape over the inferior turbinate bone
4. Smear the material on Microscopic glass slide (Oval shape)
5. Allow the slide to dry at room temperature
6. Fix the slide by gentle heat method
7. Stain the slide by Ziehl-Neelson cold method
8. Allow it dry at room temperature
9. Examine under 100x in a microscope

## REPORT

Positive / Negative

Grading (if positive)

## INTERNAL QUALITY ASSURANCE OF SLIT SKIN SMEAR

The quality control of SSS is important to maintain the validity and reliability of results, from the effective management point of view. The skin smear is usually done by the laboratory technician after a brief training of 2-5 days. The empowerment of laboratory technician in the periphery, need to be ensured about maintaining quality of skin smear as critical decisions are dependent on the results of SSS. IQA of SSS also serves a good purpose as a training tool for laboratory technician to introspect in the view of exchange of skills of slit skin smear.

Therefore the internal quality assurance of slit skin smear is required to be implemented at all the laboratories involved in slit skin smear in at least two tiers.

| <b>LEVEL</b>                | <b>LEVEL 1</b>   | <b>LEVEL 2</b>   | <b>LEVEL 3</b>   |
|-----------------------------|--|--|--|
| Facility                    | PHC/Peripheral lab   | DLO  | Medical College  |
| <b>Who will do?</b>         | <b>Lab. Technician</b>   | <b>Assessor</b>  | <b>Referee</b>   |
| What they will do?          | Lab. Tech. collect, stain and report the slit skin smear and maintain all the slides | All positive slides and 10% of negative slides to give a reasonable quantity. (Random Blinded Cross-Check) | Senior person trained and involved in performing and reporting SSS and having a good repute (usually a teaching faculty in microbiology/ pathology from Medical College) |
| <b>Quantitative IQA SSS</b> | <b>All +ve &amp; 10% of –ve slides</b>   | <b>Concordance / Discordance</b>   | <b>Discordant slides to be examined by referee</b>   |
| Qualitative IQA             | --   | Quality of smear   | Quality of smear   |
| <b>Duration</b>             | <b>Compilation of monthly slides</b>   | <b>Once a month</b>  | <b>As and when required. In discordant slides.</b>   |

## ANNEXURE

### NATIONAL LEPROSY ERADICATION PROGRAMME

#### Laboratory Requisition Form for Slit Skin Smear

Date: .....

Name of the Lab:

Address of Lab.:

Referring centre:

Referring Dr.:

Patient's Name:

Age: M/F

Hospital Ref. No:..... OPD/IPD

Provisional diagnosis:

Routine sites

| S. No | Sites          | Please tick the sites smeared |
|-------|----------------|-------------------------------|
| 1     | Right ear lobe |                               |
| 2     | Left ear lobe  |                               |
| 3     | Right arm      |                               |
| 4     | Left arm       |                               |
| 5     | Right thigh    |                               |
| 6     | Left thigh     |                               |

Other sites (e.g. nasal mucosal scrapping, forehead scrapping, skin patch etc.)

| S. No | Please specify the sites |
|-------|--------------------------|
| 1     |                          |
| 2     |                          |
| 3     |                          |
| 4     |                          |

Signature of Medical Officer

# NATIONAL LEPROSY ERADICATION PROGRAMME

## Report of Slit Skin Smear Examination

Name of the Lab:

Address of Lab:

Referring centre:

Referring Dr.:

Patient's Name:

Age: ..... M/F

Hospital Ref. No: ..... OPD/IPD

Provisional diagnosis:

Laboratory No.: .....

Date: .....

| S. No         | Sites          | BI* | MI (%)* |
|---------------|----------------|-----|---------|
| 1             | Right ear lobe |     |         |
| 2             | Left ear lobe  |     |         |
| 3             | Right arm      |     |         |
| 4             | Left arm       |     |         |
| 5             | Right thigh    |     |         |
| 6             | Left thigh     |     |         |
| 7             |                |     |         |
| 8             |                |     |         |
| 9             |                |     |         |
| 10            |                |     |         |
| Average BI/MI |                |     |         |

BI\*-Bacteriological index, MI\* (Morphological index)

### Ridley's grading of Skin smear

Neg No bacteria seen in 100 oil immersion field, when BI is 0.00, then %MI is not applicable

BI = 1+ 1-10 bacilli on an average in 100 oil immersion fields

BI = 2+ 1-10 bacilli on an average in 10 oil immersion fields

BI = 3+ 1-10 bacilli on an average in each oil immersion field

BI = 4+ 10-100 bacilli on an average in each oil immersion field

BI = 5+ 100-1000 bacilli on an average in each oil immersion field

BI = 6+ > 1000 bacilli on an average in each oil immersion field / innumerable bacilli/ globi

MI = % score of morphologically intact (solid) bacilli

Signature of Reporting Officer

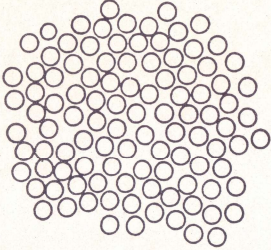
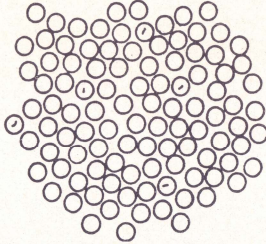
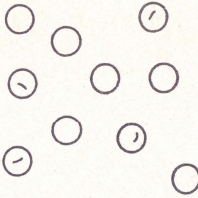
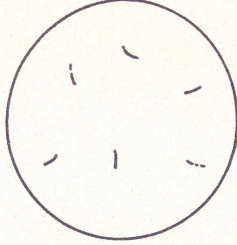
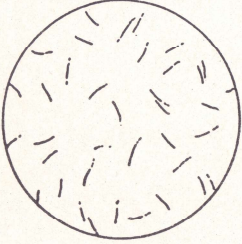
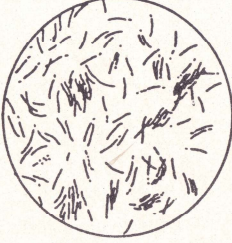

Name:

Designation:

# NATIONAL LEPROSY ERADICATION PROGRAMME

## Ridley's grading of slit skin smear

**LEPROSY SMEARS: BACTERIOLOGICAL INDEX - 'BI' (Ziehl-Neelsen stain)**

|  |   |  |  |
|--|---|--|--|
| <p><b>BI = 0</b><br/>No bacilli in 100 oil immersion fields</p>  <p>Examine 100 oil immersion fields</p>            | <p><b>BI = 1</b><br/>1-10 bacilli, on average, in 100 oil immersion fields</p>  <p>Examine 100 oil immersion fields</p> | <p><b>BI = 2</b><br/>1-10 bacilli, on average, in 10 oil immersion fields</p>  <p>Examine 100 oil immersion fields</p>    | <p><b>BI = 3</b><br/>1-10 bacilli in an average oil immersion field</p>  <p>Examine 25 oil immersion fields</p>   |
| <p><b>BI = 4</b><br/>10-100 bacilli in an average oil immersion field</p>  <p>Examine 25 oil immersion fields</p> | <p><b>BI = 5</b><br/>100-1000 bacilli in an average oil immersion field</p>  <p>Examine 25 oil immersion fields</p>   | <p><b>BI = 6</b><br/>1000 or more bacilli in an average oil immersion field</p>  <p>Examine 25 oil immersion fields</p> | <p>Original reference to the Bacterial (or Bacteriological) Index: Ridley DS, Bacterial Indices. In Cochrane, RG and Davey, TF (Editors), 'Leprosy in Theory and Practice' Bristol; John Wright and Sons Ltd, 1964, pages 620-22.</p> <p>Details of taking, fixing, staining, and reading smears, including the BI, are given in 'Technical Guide for Smear Examination for Leprosy by Direct Microscopy' by Leiker, DL, and McDougall, AC. Published by Leprosy Documentation Service (INFOLEP), Wibautstraat 135, 1097 DN, Amsterdam, Netherlands.</p> |

A Colin McDougall, Department of Dermatology, Slade Hospital and David Webster, Department of Medical Illustration, John Radcliffe Hospital, Oxford, UK, July 1985 (REVISED)



## NATIONAL LEPROSY ERADICATION PROGRAMME

### Lab register for slit skin smears for leprosy

Year .....

Month.....

| S. No. | Date | Name | Age/<br>Sex | Lab. no. | Smear result |        |        |        |          |          |        |        |        |        | BI | MI (%) | Signature of<br>technician |
|--------|------|------|-------------|----------|--------------|--------|--------|--------|----------|----------|--------|--------|--------|--------|----|--------|----------------------------|
|        |      |      |             |          | R. ear       | L. ear | R. arm | L. arm | R. thigh | L. thigh | Site 7 | Site 8 | Site 9 | Site10 |    |        |                            |
| 1.     |      |      |             |          |              |        |        |        |          |          |        |        |        |        |    |        |                            |
| 2.     |      |      |             |          |              |        |        |        |          |          |        |        |        |        |    |        |                            |
| 3.     |      |      |             |          |              |        |        |        |          |          |        |        |        |        |    |        |                            |
| 4.     |      |      |             |          |              |        |        |        |          |          |        |        |        |        |    |        |                            |
| 5.     |      |      |             |          |              |        |        |        |          |          |        |        |        |        |    |        |                            |
| 6.     |      |      |             |          |              |        |        |        |          |          |        |        |        |        |    |        |                            |
| 7.     |      |      |             |          |              |        |        |        |          |          |        |        |        |        |    |        |                            |
| 8.     |      |      |             |          |              |        |        |        |          |          |        |        |        |        |    |        |                            |
| 9.     |      |      |             |          |              |        |        |        |          |          |        |        |        |        |    |        |                            |
| 10.    |      |      |             |          |              |        |        |        |          |          |        |        |        |        |    |        |                            |

N-negative

BI-bacteriological index

MI-morphological index

## NATIONAL LEPROSY ERADICATION PROGRAMME

### Internal quality assurance of slit skin smears for leprosy

| S. No. | Date | Slide no. | No. of fields examined | Smear result |    | Specimen Adequacy & quality | Smear quality (Good/Average/ Poor) |                |                 |                  | Overall smear quality (1-5) | Agreement statistics & Remark |                        |
|--------|------|-----------|------------------------|--------------|----|-----------------------------|------------------------------------|----------------|-----------------|------------------|-----------------------------|-------------------------------|------------------------|
|        |      |           |                        | Positive     |    |                             | Negative                           | Evenness (GAP) | Thickness (GAP) | Smear size (GAP) |                             |                               | Staining quality (GAP) |
|        |      |           |                        | BI           | MI |                             |                                    |                |                 |                  |                             |                               |                        |
| 1.     |      |           |                        |              |    |                             |                                    |                |                 |                  |                             |                               |                        |
| 2.     |      |           |                        |              |    |                             |                                    |                |                 |                  |                             |                               |                        |
| 3.     |      |           |                        |              |    |                             |                                    |                |                 |                  |                             |                               |                        |
| 4.     |      |           |                        |              |    |                             |                                    |                |                 |                  |                             |                               |                        |
| 5.     |      |           |                        |              |    |                             |                                    |                |                 |                  |                             |                               |                        |
| 6.     |      |           |                        |              |    |                             |                                    |                |                 |                  |                             |                               |                        |
| 7.     |      |           |                        |              |    |                             |                                    |                |                 |                  |                             |                               |                        |
| 8.     |      |           |                        |              |    |                             |                                    |                |                 |                  |                             |                               |                        |
| 9.     |      |           |                        |              |    |                             |                                    |                |                 |                  |                             |                               |                        |
| 10.    |      |           |                        |              |    |                             |                                    |                |                 |                  |                             |                               |                        |

GAP= Good/ Average/Poor



**Central Leprosy Division,  
New Delhi  
Directorate General of Health Services  
Ministry of Health and Family Welfare  
Government of India**

**Central Leprosy Teaching & Research Institute,  
Chengalpattu, Tamil Nadu  
Directorate General of Health Services  
Ministry of Health and Family Welfare  
Government of India**

