This course is for health workers involved in laboratory services in leprosy endemic areas.

This course is based on ILEP Learning Guide Three by Dr Guido Groenen, Dr Paul Saunderson & Prof. Baohang Ji. You can also download the full text of this Guide from the ILEP web page.
What is a skin Smear?

A skin smear is a test in which a sample of material is collected from a tiny cut in the skin and then stained for *M. leprae*, an acid-fast bacillus that causes leprosy. This test is done for:

- Confirming diagnosis of multi-bacillary leprosy in a suspect
- To help in diagnosis of relapse of leprosy
- To help with classification of new patients
- Taking skin-smear is an invasive procedure. Wash your hands, wear gloves and use sterilised equipment and a new blade for each patient.

Equipment & materials needed for taking a skin-smear - place all these materials on a clean table. You also need a slide marker.
**Explain to the patient**

Ask the patient to sit down. Explain what you want to do and why it is necessary. Answer any questions. Obtain the patient’s permission to proceed and enter the details on the laboratory examination request form.

**Select the sites**

The smear should be taken from two sites:

1. One ear lobe

2. One lesion: Select the most active looking lesion. 'Active' means lesions that are raised and reddish in colour. Smear should be taken from the most active part of the lesion, usually the edge.

Do not select a lesion on the face for taking smear.

If there is no suitable skin lesion, take the second smear from the other ear lobe, or from a site where active lesions were originally recorded or where a previous smear was positive.
How to take a Skin Smear (1)

* Wash your hands and put on gloves

* Take a new clean, unscratched microscope slide. Using a slide marker, write patient identification (ID) number at the bottom of the slide. This number must also be noted on examination request form.

* Clean the skin at the smear sites with a cotton wad drenched in alcohol. Let it dry.

* Light the spirit burner.

* Put a new blade on the scalpel handle. If you put the scalpel down, make sure the blade does not touch any thing.
Pinching the area for smear-taking (ear-lobe in this picture) before making incision

How to take a Skin Smear (2)

- Pinch the skin firmly between your thumb and forefinger; maintain pressure to press out the blood.

- Make an incision in the skin about 5 mm long and 2 mm deep. Keep on pinching to make sure the cut remains bloodless. If bleeding, wipe away the blood with a cotton wad.

- Turn the scalpel 90° and hold it at a right angle to the cut. Scrape inside the cut once or twice with side of the scalpel, to collect tissue fluid and pulp. There should be no blood in the specimen, as this may interfere with staining and reading of the slide.

- Stop pinching the skin and absorb any bleeding with a wad of cotton.
How to take a Skin Smear (3)

- * Spread the material scraped from the incision onto the slide, on the same side as the ID number. Spread it evenly with the flat of the scalpel, making a circle 8 mm in diameter.

  * Rub the scalpel with a cotton wool drenched with alcohol. Pass the blade through the flame of the spirit burner for 3-4 seconds. Let it cool without touching any thing.

  * Repeat the steps above for the second site for smear-taking. Spread the second smear next to, but not touching the first one.

  * Discard the scalpel blade safely.

  * Dress the wound and thank the patient.

How to take a Skin Smear (4)

- * Fix the smear by passing the slide, with the smears upwards, slowly through the flame of a spirit burner, 3 times. Do not over-heat. The slide should not be too hot to touch.

  * Put the slide in a slide box and send it to the laboratory with the skin smear request form.
How to stain a skin smear (1)

* Register the slide in the lab register

* Put the slide on the staining rack with smeared side upwards. Up to 10 slides can be stained together. Make sure the slides do not touch one another.

* Equipment needed for staining the skin smears with Ziehl-Neelsen method: a bottle each of 1% carbol fuchsin solution, 1% acid-alcohol and 0.2% methylene blue solution; spirit lamp, clock or watch, sink with running water, pipette, staining rods, slide rack, tissue paper and gloves.

How to stain a skin smear (2)

- A. Staining with Carbol Fuchsin

* Just before use filter the 1% carbol fuchsin solution through ordinary filter paper. Cover the whole slide with 1% carbol fuchsin solution.

* Heat the slide gently by holding a burning spirit lamp underneath it until the vapour begins to rise from carbol fuchsin. Repeat this three times during a period of 5 minutes. Make sure that the stain does not boil. If the stain dries, add more carbol fuchsin solution and heat again.

* Wash the slide gently under a running tap. Rinse until the run-off water is colourless, although the smears will remain dark red.
**How to stain a skin smear (3)**

**B. Decolourising**

* Cover the slide with 1% acid-alcohol for 10 seconds. An alternative method is to cover the slide with 5% sulphuric acid for 10 minutes.

* Rinse the slide gently with water.

**C. Counter-staining**

* Cover the slide with 0.2% methylene blue for 1 minute.

* Rinse the slide with water.

* Let the slide dry in the drying rack in an inclined position with smeared side downwards.

The slide is now ready to read.
**How to read a skin smear (1)**

* Using oil immersion objective of a microscope

  * Put the slide under the microscope with the smeared side upwards.
  * Focus the image using 10x objective of microscope.
  * Put a drop of immersion oil on the smear.
  * Switch to 100x objective till the oil immersion lens just touches the oil.
  * Open diaphragm completely and raise the condenser to its highest position.
  * Focus precisely with the fine adjustment screw of microscope.

**How to read a skin smear (2)**

* Using oil immersion objective of a microscope

With the help of oil immersion objective of the microscope, look for the presence of acid-fast bacilli. They appear as fine red rods against a blue background. They can be straight or curved. The red colour can be uniformly distributed (solid bacilli) or unevenly distributed (fragmented and granulated bacilli). Clumps of bacilli are called globi.

Solid bacilli suggest the presence of viable organisms and may be seen in new untreated cases and relapse cases.
How to read a skin smear

Calculating bacteriological Index (BI) of a skin smear

After examining the first field, move the slide to look at the next field. Examine approximately 100 fields per smear.

If acid-fast bacilli are seen in the skin smear, quantify them according to the scale shown on the left for the Bacteriological Index (BI). Calculate the BI for each smear separately.

Report the BI for both smears on the slide. For smear positive patients, either the average BI or the highest BI will be taken as the BI for that patient.

Write the result of both smears in the lab register.

How to dispose used slides

* Rinse the slide in Xylene. Do not wipe it.

* Store the slide in a slide box for future quality control.

* Slides that are not kept for quality control should be destroyed, or disinfected, boiled and washed for reuse in routine examinations (of stool or urine, for example). Slides should not be reused for other skin smears or for sputum examination.

<table>
<thead>
<tr>
<th>BI</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 bacilli in 100 fields</td>
</tr>
<tr>
<td>1+</td>
<td>1 - 10 bacilli in 100 fields</td>
</tr>
<tr>
<td>2+</td>
<td>1 - 10 bacilli in 10 fields</td>
</tr>
<tr>
<td>3+</td>
<td>1- 10 bacilli, on average in each field</td>
</tr>
<tr>
<td>4+</td>
<td>10 - 100 bacilli, on average in each field</td>
</tr>
<tr>
<td>5+</td>
<td>100 - 1000 bacilli, on average in each field</td>
</tr>
<tr>
<td>6+</td>
<td>&gt; 1000 bacilli, on average, in each field</td>
</tr>
</tbody>
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Calculating Bacteriological Index (BI)
Thank you for completing the online training course on how to do a skin smear examination.

You can download the full text of ILEP guide 3 on doing skin smear examination from the ILEP website by clicking here.

We appreciate receiving comments, criticisms and suggestions on this online course. If you want to send us your comments and suggestions by email, write to <sunil.Deepak@aifo.it>