



How to do a skin smear examination for leprosy

ILEP Learning Guide Three

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.

Why take a skin smear?

- To confirm a diagnosis of skin smear-positive multibacillary leprosy in a suspect.
- To help diagnose multibacillary relapse in a patient who has previously been treated.
- To help with the classification of new patients.



Who can take a skin smear?

Anyone who has been trained to take a skin smear and who is authorised to do so.

Remember: Taking a skin smear is an invasive procedure. Wash your hands, wear gloves, and use sterilised equipment and a new blade for each patient. Do not take skin smears unnecessarily.

Principal authors: Dr Guido Groenen, Dr Paul Saunderson and Professor Baohong Ji on behalf of the ILEP Medico-Social Commission.

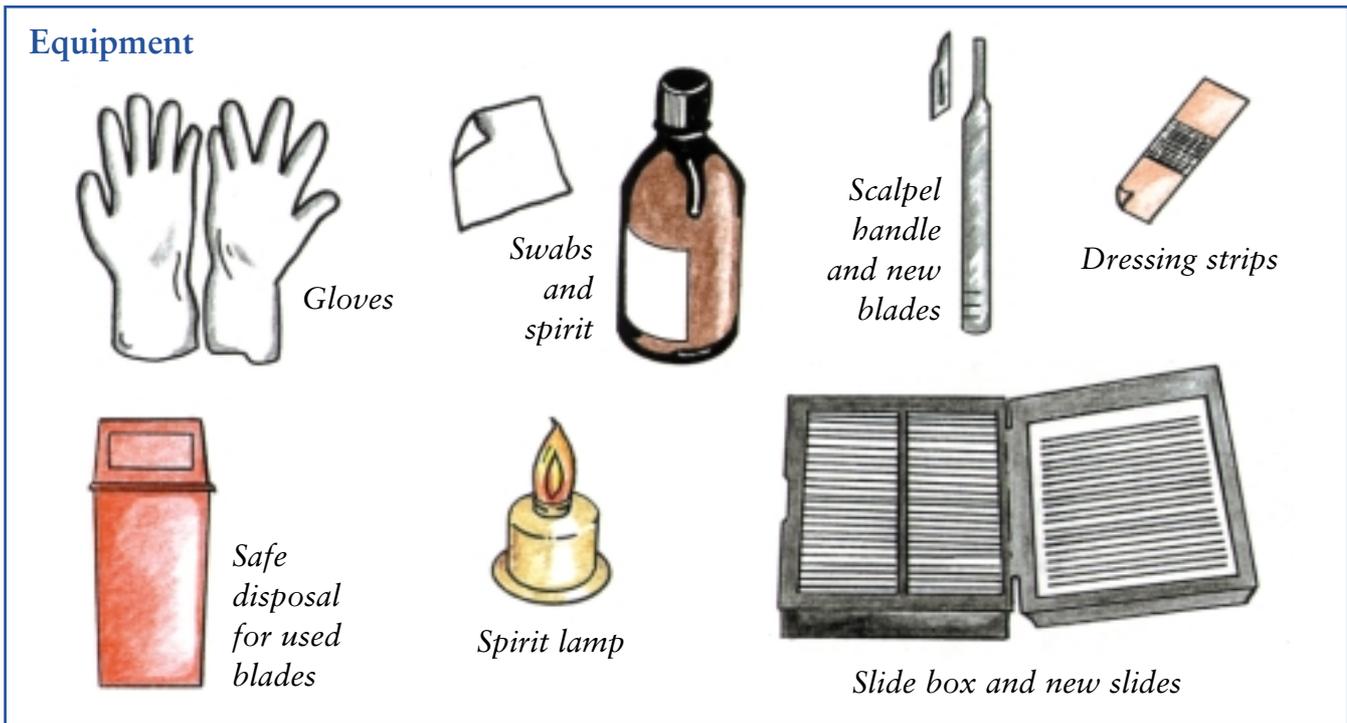
Illustrations: Brent Hawkins, from originals by Sandy Patience.

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Preparing to take a skin smear



Place all the material you need on a clean table.

You also need a slide marker and a laboratory request form.



Explain to the patient

Ask the patient to sit down and relax. 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 request form.

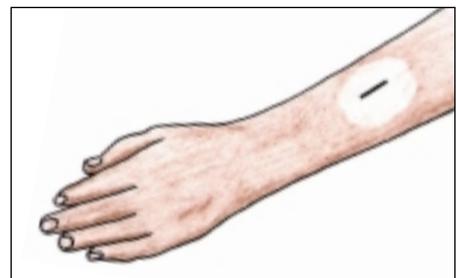
Select the sites

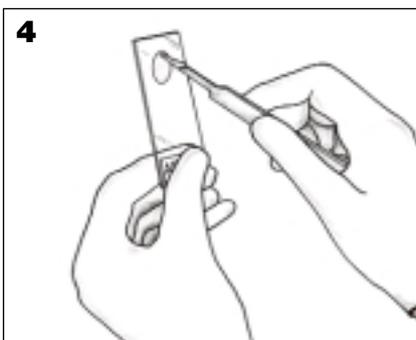
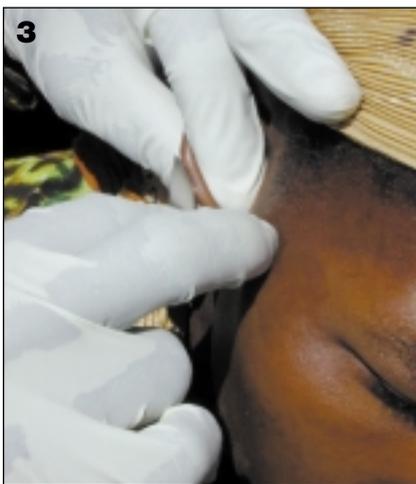
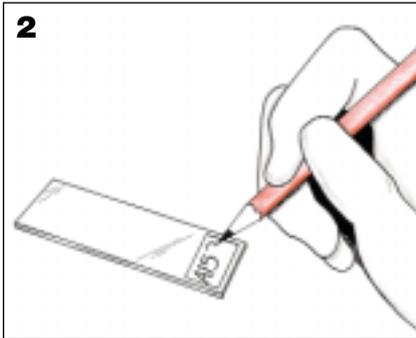
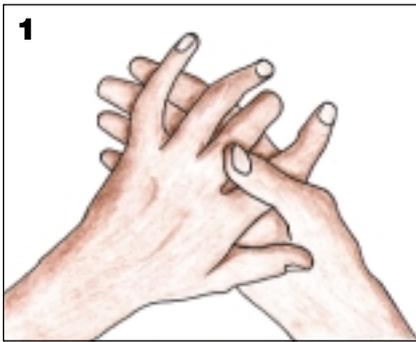
You should take a smear from two sites only:

1. One ear lobe.
2. One lesion. Select the most active-looking lesion, but not on the face. 'Active' means raised and reddish in colour. Take the smear in the most active area of the lesion (usually the edge).

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.

Many programmes traditionally took smears from four or even six sites, but two sites are now considered adequate in most cases.





How to take a skin smear

- Wash your hands (**1**) and put on gloves.
- Take a new, clean, unscratched microscope slide. Using a slide marker, write the patient identification (ID) number at the bottom of the slide (**2**). This number must be on the 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 anything.
- 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 (**3**). Keep on pinching to make sure the cut remains bloodless. If bleeding, wipe the blood away with cotton wad.
- Turn the scalpel 90° and hold it at a right angle to the cut. Scrape inside the cut once or twice with the 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.
- Stop pinching the skin and absorb any bleeding with a wad of cotton.
- 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 (**4**).
- Rub the scalpel with a cotton wad drenched in alcohol. Pass the blade through the flame of the spirit burner for 3 to 4 seconds. Let it cool without touching anything.
- Repeat the steps above for the second site. Spread this smear next to, but not touching, the first one.
- Discard the scalpel blade safely.
- Dress the wounds and thank the patient.
- Let the slide dry for 15 minutes at room temperature, but not in direct sunlight.
- Fix the smears by passing the slide, with the smears upwards, slowly through the flame of a spirit burner, 3 times (**5**). Do not overheat. The slide should not be too hot to touch.
- Put the slide in a slide box and send to the laboratory with the skin smear request form.

How to stain a skin smear

Stain the smears using the hot Ziehl-Neelsen method. Stain with 1% carbol fuchsin, which colours everything red. Wash out the stain with 1% acid-alcohol, which removes the stain from everything except *M. leprae*. Counter-stain the slide with 0.2% methylene blue. The leprosy bacilli will be seen as red rods on a blue background.

Equipment

A bottle each of:

1% carbol fuchsin solution

1% acid-alcohol

0.2% methylene blue solution

Spirit lamp

Clock or watch

Sink with running water

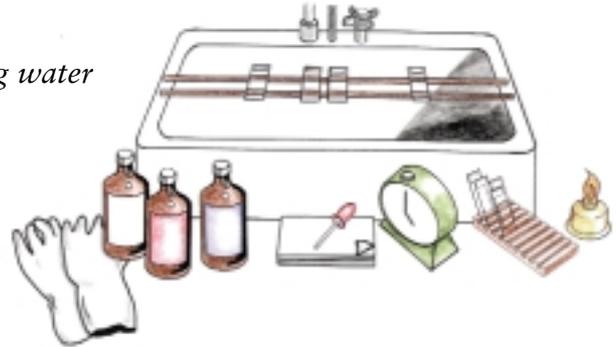
Pipette

Staining rods

Slide rack

Tissue paper

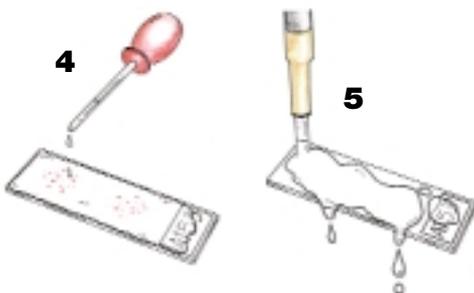
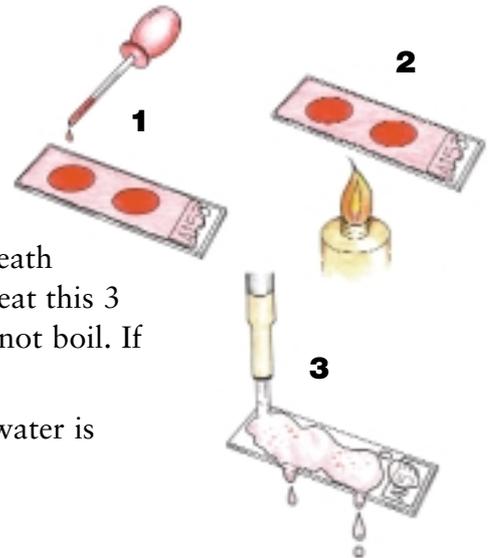
Gloves



- Register the slide in the lab register.
- Put the slide on the staining rack with the smeared side upwards. Up to 10 slides can be stained together. Make sure that the slides do not touch one another.

Staining

- Just before use, filter the 1% carbol fuchsin solution through ordinary filter paper.
- Cover the whole slide with 1% carbol fuchsin solution (1).
- Heat the slide gently by holding a burning spirit lamp underneath it until vapour begins to rise from the carbol fuchsin (2). Repeat this 3 times during a period of 5 minutes. Make sure the stain does not boil. If the stain dries, add some more reagent and heat again.
- Wash gently under a running tap (3). Rinse until the run-off water is colourless, although the smears will remain dark red.

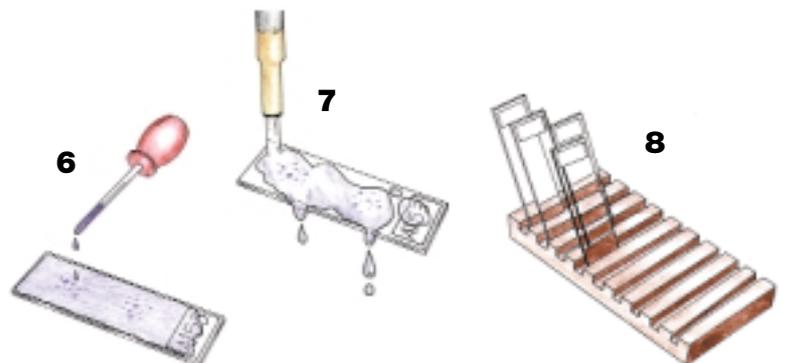


Decolourising

- Cover with 1% acid-alcohol for 10 seconds (4). An alternative method is to cover with 5% sulphuric acid for 10 minutes.
- Rinse gently with water (5).

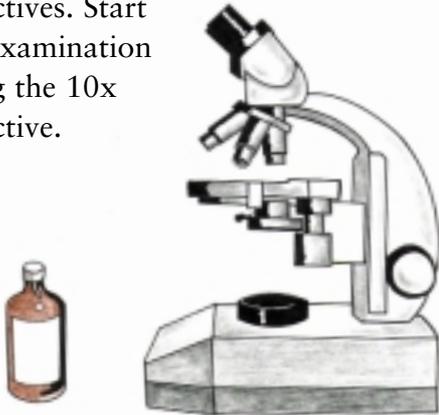
Counter-staining

- Cover with 0.2% methylene blue for 1 minute (6).
- Rinse with water (7), and let the slide dry in the drying rack in an inclined position, with the smeared side downwards (8).
- The slide is now ready to be read.

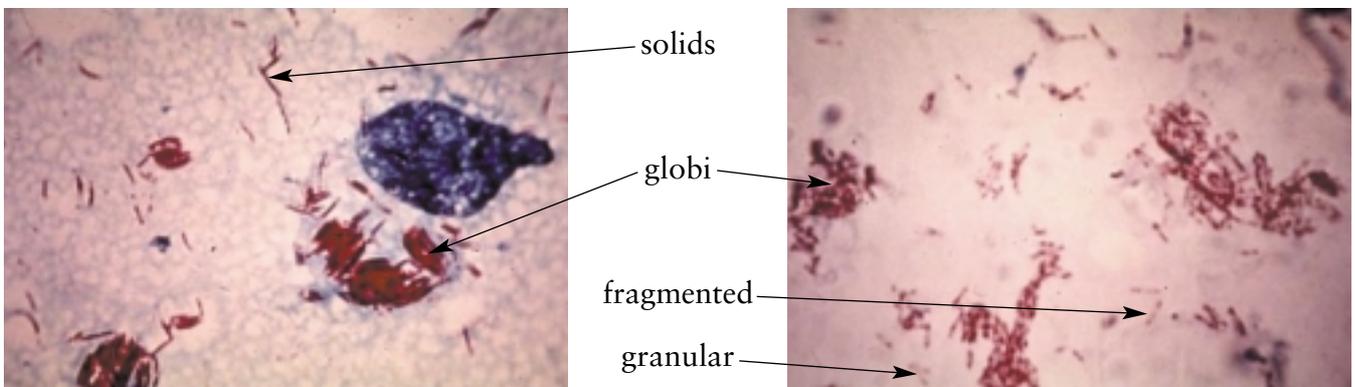


How to read a skin smear

You need a microscope with a 10x eye piece and 10x and 100x objectives. Start the examination using the 10x objective.



- Put the slide under the microscope with the smears upwards and the ID number to the left.
- Focus the image using the 10x objective.
- Put a drop of immersion oil on the smear.
- Switch to the 100x objective. This will touch the immersion oil (if necessary, move the coarse adjustment screw to make sure that the oil immersion lens just touches the oil).
- Open the diaphragm completely and raise the condenser to its highest position.
- Focus precisely with the fine adjustment screw.



Look for the presence of acid-fast bacilli. They appear as fine red rods against a blue background. They can be straight or curved, and the red colour can be uniformly distributed (solid bacilli) or unevenly distributed (fragmented and granulated bacilli). Clumps of bacilli are called globi. Solid bacilli may suggest the presence of viable organisms and may be seen in new, untreated cases or in relapse cases.

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

- If acid-fast bacilli are seen, quantify them according to the following scale for the Bacteriological Index (BI). Calculate the BI for each smear separately:

Bacteriological Index (BI)	
0	0 bacilli in 100 fields
1+	1 – 10 bacilli in 100 fields
2+	1 – 10 bacilli in 10 fields
3+	1 – 10 bacilli, on average, in each field
4+	0 – 100 bacilli, on average, in each field
5+	100 – 1000 bacilli, on average, in each field
6+	> 1000 bacilli, on average, in each field

- Write the result of both smears in the lab register.
- 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 re-use in routine examinations (of stool or urine, for example). Slides should not be re-used for other skin smears or for sputum examinations.
- Give the result to the health worker who requested the skin smear.

Note: 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.

How to prepare the staining reagents

Material needed to prepare the reagents

Basic fuchsin powder

95% ethyl alcohol*

Phenol crystals

Hydrochloric acid (HCl)

Methylene blue powder

Clean water

Graduated 1000 ml cylinder

Two 10 ml syringes

Three brown 1 litre flasks

Balance

**If unavailable, methyl alcohol or denaturated alcohol may be used instead.*

Preparation

These instructions are for 1 litre of the reagent. If you need to prepare less than 1 litre, adapt the quantities of the ingredients accordingly. Prepare new reagents once a month.

You can keep the reagents in large brown bottles. To do the actual staining, a smaller quantity of the reagent is poured into a staining bottle, such as a drop bottle of 250 ml.

1% carbol fuchsin solution

Step 1. Make 100 ml of saturated fuchsin solution:

- Add 10 g basic fuchsin powder to 100 ml 95% ethyl alcohol.
- Mix well by stirring.

Step 2. Make the working solution of 1% carbol fuchsin:

- Put 50 g of phenol crystals in a flask.
- Put this flask in a basin with warm water until the crystals become liquid.
- Pour the liquid (50 ml) into a graduated 1000 ml cylinder.
- Add water to 900 ml.
- Add 100 ml of saturated fuchsin solution to 1000 ml and mix well.

Keep in a dark bottle. Filter before use or pour onto the slide through a small funnel with filter paper.

1% acid-alcohol

- Fill a 1 litre cylinder with 1000 ml of 95% ethyl alcohol.
- Remove 10 ml of the alcohol with a 10 ml syringe.
- Pour a small quantity of hydrochloric acid (HCl) in a small bowl. Attention! This is a caustic and toxic substance. Do not touch it! Do not inhale!
- Take 10 ml of the HCl with a **dry** 10 ml syringe.
- Very slowly add the HCl to the alcohol in the cylinder. **Never add alcohol to the acid.**
- Return the leftover acid in the bowl to the bottle of acid.
- Pour the acid-alcohol from the cylinder into a large dark flask.

An alternative to 1% acid-alcohol is 5% sulphuric acid (H₂SO₄) solution:

Take 950 ml distilled water. Slowly add 50 ml concentrated sulphuric acid; **never** add water to the acid. Store in a dark bottle.

0.2% methylene blue solution

- Add 2 g methylene blue powder to 1 litre water. Mix well.

Keep in a dark bottle. Filter regularly.