Preparation and Examination of Skin Smears

The skin smear is a valuable, cost-effective tool in the routine management of the patient with Hansen's disease. The smear is a means of estimating the number of acid-fast bacteria present, reported as the Bacterial Index (BI), and is important in determining the type and severity of disease as well as assessing the response to treatment.

General

- Initial skin smears are usually taken from 6 "routine sites" (both earlobes, elbows, and knees) as well as several typical lesions from the patient. Repeat smears are obtained from 3 to 4 of the most active sites previously tested to evaluate progress.
- The time interval between repeat smears is determined by the physician, but in general, annual smears are adequate for monitoring response to treatment and during the following-up period to detect any evidence of relapse.
- All microscopic slides on which skin smears are made should be pre-cleaned in 70% alcohol, acetone, or alcohol-acetone to remove amorphous debris. The slides are wiped dry with a clean hand towel. Blades that are used in smear taking are likewise cleaned.
- Slides should be air-dried and **NEVER** heat fixed.
- They may be sent in protective mailers to:

National Hansen's Disease Program
Attention: Clinical Laboratory – Skin Smears
1770 Physicians Park Drive
Baton Rouge, La. 70816
Phone: (225) 756-3735

Procedure for Obtaining Smears

1. Universal precautions should be observed in obtaining skin smears.
2. The skin is cleansed with 70% alcohol and air-dried or wiped dry with cotton. (Zephiran tends to make the skin too slippery and is not recommended.)
3. A fold of skin is made relatively avascular by pinching or mild clamping. If the skin cannot be grasped by pinching, it can be compressed. A surgeon's glove may aid in grasping.
4. Local anesthesia is generally unnecessary. (If there is not adequate decrease in sensation, obtain local anesthesia with 1% Xylocaine or Ethyl Chloride spray.) The compression of the skin by pinching aids in the anesthesia.
5. An incision 3-5 mm long and 2-3 mm deep is made with a alcohol cleansed, single-edged razor blade. A scalpel with a #15 Bard-Parker blade may also be used. Mild pressure to maintain relative avascularity is continuously applied to the area until an adequate smear has been obtained.

6. A small amount of blood does not interfere with the reading, but large amounts should be avoided and can usually be controlled by the amount of pressure of the pinch. If excessive bleeding occurs, it can be wiped away with a cotton swab.
7. After the incision is made, and before the blade is withdrawn, the inner surface of the wound is scraped with the blade held at a right angle to the incision. Upon scraping, tissue fluid and dermal tissue are obtained.
8. The material is transferred to the cleaned microscope slide. A moderately thick smear, with a visible uniform opacity is made. The smear is made in a circular manner on the slide, **no larger than a pencil eraser (5-7 mm)**, beginning peripherally and ending in the center, leaving a central "button" (2-4 mm) which can be easily focused upon with the microscope. Slides should be properly labeled as shown below in the sample diagram for 3 routine sites.

9. A Band-Aid is generally sufficient to protect the smear site.
10. A single technician takes all smears to insure more uniform and consistent results.
11. The smears are then sent to the National Hansen's Disease Program for reading.
12. A chart to diagram sites of the skin smears is linked from the top of this page.

Staining of Skin Smears

1. Dry the slide with smear at room temperature. **DO NOT HEAT FIX.**
2. Place slides on a staining rack and flood with 10% formalin for 15 minutes for fixation.
3. Gently rinse well with tap water. All formalin must be removed to prevent the formation of precipitates.
4. Flood slides with Ziehl-Neelsen carbol-fuchsin for twenty minutes. The carbol-fuchsin must be filtered before each use. Filtering can be accomplished by placing pre-cut filter paper strips on the slide prior to the addition of stain and left in place for the full twenty minutes.
5. After removing and discarding filter paper strips, gently rinse slides well with tap water to remove excess stain.
6. Decolorize with **2%** acid alcohol for 1 minute. This is best accomplished by placing slide into a two-slide plastic slide mailer filled with acid alcohol. Occasional up and down movement of the slide in the acid alcohol should remove all excess carbol fuchsin.
7. Gently rinse slides **thoroughly** with tap water.

8. Counterstain with alkaline methylene blue for 30 seconds to 1 minute.
9. Gently rinse well with tap water and air dry.

NOTE: Positive and negative control slides must be used each day for quality control purposes.

Z-N Carbol Fuchsin Stain:

Basic fuchsin ---------- 1.0 gm.
Phenol crystals (melted)----------5.0 mls.
95% ethanol ---------- 10.0 mls.
Water, to make ---------- 100.0 mls.

Dissolve stain in alcohol, and then add phenol/water mixture. Let stand overnight before use. Store in dark brown bottle. Stable for 1 year.

Acid alcohol:

Conc. HCl ---------- 2.0 mls.
95% ethanol ---------- 98.0 mls.

Alkaline Methylene Blue:

KOH (10%) ---------- 0.10 mls.
Methylene blue ---------- 0.35 gms.
95% ethanol ----------30.0 mls.
Water to make ----------100.0 mls.

Dissolve the stain in the alcohol, then add the KOH and water mixture and allow to sit overnight. Filter before use.

Microscopic Examination of Skin Smears

The stained smears are examined with a quality microscope using the oil immersion objective (x100) to determine the total number of bacilli. The same individual should read all smears for the purpose of consistency. The smear will have similar numbers of bacilli throughout. However, four separate quadrants of the smear are examined and averaged to establish the Bacterial Index.

Reporting the Bacterial Index

The results are reported on a 0 to 6+ semi-logarithmic scale using a descriptive phrase or numerical code. This is an indicator of the total bacillary load of the patient. It falls about 1 point per year during effective treatment as dead bacilli undergo lysis and are absorbed.

Very Numerous	(+6)	over 1000 bacilli per oil immersion field.
Numerous	(+5)	100 to 1000 bacilli per oil immersion field.
Moderate	(+4)	10 to 100 bacilli per oil immersion field.

Few	(+3)	1 to 10 bacilli per oil immersion field.
Very few	(+2)	1 to 10 bacilli per 10 fields.
Rare	(+1)	1 to 10 bacilli per 100 fields.
None found	(NF)	No AFB seen on entire site.

Revised by Clinical Lab 11/06/2008