***SOP for 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 mL.

95% ethanol --------------------------- 10.0 mL.

Water, to make ---------------------- 100.0 mL.

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 mL.

95% ethanol -------------------------- 98.0 mL.

Alkaline Methylene Blue:

KOH (10%) -------------------------- 0.10 mL

Methylene blue ---------------------- 0.35 gm.

95% ethanol ---------------------------30.0 mL.

Water to make -----------------------100.0 mL.

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.

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| 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. |