

**OLIVE VIEW-UCLA MEDICAL CENTER  
DEPARTMENT OF PATHOLOGY  
POLICY & PROCEDURE**

**NUMBER: 11876  
VERSION: 1**

**SUBJECT/TITLE:** SS 010 - AFB (FITE)

**POLICY:** The AFB (Fite) staining procedure shall be utilized when a diagnosis of Leprosy is required.

**PURPOSE:** For the detection of Mycobacterium Leprae.

**DEPARTMENTS:** PATHOLOGY & LABORATORY SERVICES

**DEFINITIONS:**

**PROCEDURE:** **FIXATION:**  
10% buffered neutral formalin.

**EQUIPMENT:**  
Coplin jars  
Filter paper  
Graduated cylinders  
Pipettes

**PROCEDURE:**

**I. Slide Preparation**

- Section paraffin blocks at 5 microns

**II. Quality control**

- Positive Control for Leprosy

**III. Solutions**

**A. Xylene-Peanut Oil Solution**

- Peanut Oil.....one (1) part
- Xylene.....two (2) parts

**B. Carbol Fuchsin – Ziehl Neelsen**

- Commercially prepared – Poly Scientific (S162-16)

**C. Decolorizing Solution (1% Acid Alcohol)**

- 99 ml of 70% Alcohol
- 1 ml of Hydrochloric Acid (conc.)

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- D. Fast Green – 0.01% Aqueous
  - Commercially prepared – Poly Scientific (s2252-8oz)

E. Xylene

#### **IV. Staining Procedure**

- A. Obtain and use a positive control.
- B. Deparaffinize through two changes of Xylene/Peanut Oil solution for 12 minutes each.
- C. Drain, wipe off excess oil and blot to opacity.
- D. Rinse in water until slide is clear.
- E. Stain in Ziehl-Neelsen Carbol Fuchsin solution for 30-60 minutes.
- F. Wash in tap water for 3 minutes
- G. Differentiate slides individually in 1% Acid Alcohol until the sections are a faint pink. (It is best to agitate the slides rather than allowing them to soak in the 1% Acid Alcohol.)
- H. Wash in running water for 3 minutes.
- I. Counter stain lightly with the Fast-Green solution.
- J. Rinse off excess Fast-Green solution in distilled water.
- K. Blot with a damp filter paper and allow to stand for a few minutes ensuring that the slides are thoroughly air-dried.
- L. Place the slides into Xylene for 5 minutes
- M. Place slides on the automated cover-slipper.

#### **V. Results**

|                    |   |             |
|--------------------|---|-------------|
| Acid Fast Bacilli  | - | Red         |
| Nocardia Filaments | - | Red         |
| Lepra Bacilli      | - | Red         |
| Back Ground        | - | Light Green |

#### **Nocardia Demonstration**

For the demonstration of Nocardia species, after deparaffinization to the Xylene/Peanut Oil solution, stain the slides for 10 minutes (timing is critical) in the Carbol Fuchsin solution. Decolorize in 1% **Aqueous Sulfuric Acid** for 5 to 10 minutes. Agitate slides frequently to remove background staining. Counterstain in **Methylene Blue**. Rinse in tap (to remove the excess Methylene Blue), blot dry and allow the tissue to air-dry. Coverslip utilizing a synthetic mounting medium.

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| References: 1. Manual of Histologic Methods, Lee Luna, Third Edition, pg. 217<br>2. Theory and Practice of Histotechnology, Dezna C. Sheehan, HT (ASCP), Second Edition, C.V. Mosby Co., 1980, pg. 237 |                  |
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