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**FITE'S ACID FAST STAIN - LEPROSY**

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**PURPOSE:** To demonstrate mycobacterium leprae (leprosy), which are acid fast organisms.

**PRINCIPLE:** This technique combines peanut oil with the deparaffinizing solvent (xylene), minimizing the exposure of the bacteria's cell wall to organic solvents, thus protecting the precarious acid-fastness of the organism.

**CONTROL:** Leprosy positive tissue.

**FIXATIVE:** 10% formalin

**TECHNIQUE:** Cut paraffin sections 4-5 microns.

**EQUIPMENT:** Rinse all glassware in DI water, coplin jars, bibulous blotting paper.

**REAGENTS:**

**Xylene/Peanut Oil Solution:**

Xylene	50.0 ml
Peanut Oil	50.0 ml

Mix well. Label with date and initials, solution is stable for 1 year.

**CAUTION:** Flammable, irritant.

**Ziehl-Neelsen Carbol-Fuchsin:**

See Ziehl-Neelsen AFB

**1% Acid Alcohol:**

See Ziehl-Neelsen AFB

**Working Methylene Blue:**

See Ziehl-Neelsen AFB

**SAFETY/PPE:** Wear nitrile gloves, goggles and lab coat. Keep hot, uncapped, solutions in the exhaust hood. Avoid contact and inhalation.

Basic fuchsin: possible carcinogen.

Phenol: toxic by ingestion, inhalation and skin absorption. Readily absorbed through skin, causing increased heart rate, convulsions and death. Will burn eyes and skin, analgesic action may cause loss of pain. Target organ effects in digestive, nervous and urinary systems.

Hydrochloric acid: strong irritant to skin, eyes and respiratory system.

Target organ effects via inhalation on skin, respiratory, reproductive and fetal systems.

Methylene blue: produced deleterious effects on fertility in rats.

**PROCEDURE:**

1. Deparaffinize in xylene/peanut oil mixture, 2 changes, 10 minutes each.
2. Drain slides, blot off excess oil.
3. Rinse in distilled water until slide clears.
4. Carbol-fuchsin, 30 minutes, room temperature.
5. Wash in tap water.
6. Acid alcohol until pale pink, dip until stain stops running.
7. Wash in tap water.
8. Counterstain in Working Methylene blue, 30 seconds.
9. Wash in tap water.
10. Blot and air dry.
11. Dip in xylene and coverslip.

**RESULTS:**

Acid-fast bacilli           red  
Background                 blue

**NOTE:** Mineral oil may be substituted for peanut oil.

**REFERENCES:**

Sheehan D, Hrapchak B, Theory and practice of Histechnology, 2nd Ed, 1980, pp237, Battelle Press, Ohio  
Bancroft J, Stevens A, Theory and Practice of Histological Techniques, 2nd Ed, pp 285, Churchill-Livingstone, NY  
Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: \_\_\_\_\_ By: \_\_\_\_\_

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PROCEDURE CARD  
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**SAFETY:** Keep hot solutions under fume hood.

Basic fuchsin: carcinogen

**Xylene/Peanut Oil Solution:**

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50.0 ml  
P        e        a        n        u        t  
Oil 50.0 ml

Mix well. Label with date and initials,  
solution is stable for 1 year.

**CAUTION: Flammable.**

**NOTE:** Mineral oil may be substituted for  
peanut oil.

**Ziehl-Neelsen Carbol-Fuchsin:**

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**1% Acid Alcohol:**

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**Working Methylene Blue:**

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**Xylene/Peanut Oil Solution:**

Xylene 50.0 ml

Peanut Oil 50.0 ml

Mix, solution is stable for 1 year.

**CAUTION:** Flammable, irritant.

TECH: \_\_\_\_\_

DATE: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**XYLENE/PEANUT OIL**

TECH: \_\_\_\_\_

DATE: \_\_\_\_\_