FITE'S ACID FAST STAIN - LEPROSY

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.

PROTOCOL:
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.
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:
Crookham J, Dapson R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: __________________________ By: __________________________

Approved: __________________________ By: __________________________
PROCEDURE CARD

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

**CONTROL:** Leprosy positive tissue.

**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-fuchsins, 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.
9. Wash in tap water.
10. Blot and air dry.
11. Dip in xylene and coverslip.

**RESULTS:**
- Acid-fast bacilli: red
- Background: blue

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

Basic fuchsin: carcinogen

**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.

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

**Ziehl-Neelsen Carbol-Fuchsin:**
- See Ziehl-Neelsen AFB

**1% Acid Alcohol:**
- See Ziehl-Neelsen AFB

**Working Methylene Blue:**
- See Ziehl-Neelsen AFB
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:_____________________