ACID-FAST BACTERIA - ZIEHL-NEELSEN STAIN (AFB)

**PURPOSE:** Used in the demonstration of acid-fast bacteria belonging to the genus 'mycobacterium', which include the causative agent for tuberculosis.

**PRINCIPLE:** The lipoid capsule of the acid-fast organism takes up carbol-fuchsin and resists decolorization with a dilute acid rinse. The lipoid capsule of the mycobacteria is of such high molecular weight that it is waxy at room temperature and successful penetration by the aqueous-based staining solutions (such as Gram's) is prevented.

**CONTROL:** Any tissue containing acid-fast organisms. Use Millipore™ filtered water in the waterbath and staining procedure.

**FIXATIVE:** 10% formalin

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

**EQUIPMENT:** Rinse all glassware in DI water, coplin jars, microwave oven.

**REAGENTS:**

Ziehl-Neelsen Carbol-Fuchsin Solution:
- Basic fuchsin 2.5 gm
- Distilled water 250.0 ml
- 100% alcohol 25.0 ml
- Phenol crystals, melted 12.5 ml

Mix well, filter into brown bottle.
Label bottle with date and initials, solution is stable for 1 year.

1% Acid Alcohol:
- Hydrochloric acid 10.0 ml
- 70% Alcohol 990.0 ml

Mix well, label with date and initials, stable for 1 year.

**CAUTION:** Carcinogen, toxin.
### Methylene Blue Stock Solution:

- Methylene blue: 0.7 gm
- Distilled water: 50.0 ml

Mix well, filter into bottle. Label with date and initials, stable for 1 year.

### Methylene Blue Working Solution:

- Methylene Blue Stock: 5.0 ml
- Distilled water: 45.0 ml

Pour into coplin jar, stable for 2 months.

**CAUTION:** Avoid contact and inhalation.

### SAFETY/PPE:

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

Basic fuchsin: 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 and hydrate to distilled water.
2. *Carbol-fuchsin solution, microwave 80 power, 45 seconds, allow slides to stand in hot solution for 5 minutes. Filter solution once a week.
3. Wash in running tap water.
4. 1% Acid alcohol until light pink and color stops running.
5. Wash in running tap water for 5 minutes.
6. Rinse in distilled water.
7. Working methylene blue for 30 seconds.
8. Rinse in water.
9. Dehydrate, clear, and coverslip.

*Conventional Method: 60°C oven for 1 hour.
RESULTS:
Acid-fast bacilli: bright red
Background: blue

REFERENCES:
Carson F, Histotechnology: A Self-Instructional Text, 1990, pp189-191, ASCP, III
Crookham J, Dapson R, Hazardous Chemicals in the Histopathology Laboratory, 2nd Ed, 1991, Anatech

Prepared: ____________________  By: ____________________

Approved: ____________________  By: ____________________

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PROCEDURE CARD

ACID-FAST BACTERIA - ZIEHL-NEELSEN STAIN (AFB)

CONTROL: Any tissue containing acid-fast organisms. Use Millipore™ filtered water in the waterbath and staining procedure.

PROCEDURE:
1. Deparaffinize and hydrate to distilled water.
2. *Carbol-fuchsin solution, microwave 80 power, 45 seconds, allow slides to stand in hot solution for 5 minutes. Filter solution once a week.
3. Wash in running tap water.
4. 1% Acid alcohol until light pink and color stops running.
5. Wash in running tap water for 5 minutes.
6. Rinse in distilled water.
7. Working methylene blue for 30 seconds.
8. Rinse in water.
9. Dehydrate, clear, and coverslip.
   *Conventional Method: 60°C oven for 1 hour.

RESULTS:
Acid-fast bacilli bright red
Background blue

SAFETY/PPE: Open hot solution within the exhaust hood. Phenol will cause burns, wear nitrile gloves.

Basic fuchsin: carcinogen.

Ziehl-Neelsen
Carbol-Fuchsin Solution:
Basic fuchsin 2.5 gm
Distilled water 250.0 ml
100% alcohol 25.0 ml
Phenol crystals, melted 12.5 ml
Mix well, filter into brown bottle. Label bottle with date and initials, solution is stable for 1 year.

CAUTION: Carcinogen, toxin.

1% Acid Alcohol:
Hydrochloric acid 10.0 ml
70% Alcohol 990.0 ml
Mix well, label with date and initials, stable for 1 year.

CAUTION: Flammable, corrosive.

Methylene Blue
Stock Solution:
Methylene blue 0.7 gm
Distilled water 50.0 ml
Mix well, filter into bottle. Label with date and initials, stable for 1 year.

Working Solution:
Methylene Blue Stock 5.0 ml
Distilled water 45.0 ml
Pour into coplin jar, stable for 2 months.

CAUTION: Avoid contact and inhalation.
**METHYLENE BLUE**  
*Stock Solution:*
Methylene blue 0.7 gm  
Distilled water 50.0 ml  
Mix well, filter into bottle, add a crystal of thymol. Stable for 1 year.

*Working Solution:*
Methylene Blue Stock 5.0 ml  
Distilled water 45.0 ml  
Pour into coplin jar, stable for 2 months.

**CAUTION:** Avoid contact and inhalation.

**TECH:**  
**DATE:**  
**EXPIRATION:**  

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**ZIEHL-NEELSEN**  
**CARBOL FUCHSIN**
Basic fuchsin 2.5 gm  
Distilled water 250.0 ml  
100% alcohol 25.0 ml  
Phenol crystals, melted 12.5 ml  
Mix well, filter into brown bottle. Stable for 6 months.

**CAUTION:** Carcinogen, toxin, avoid contact and inhalation.

**DATE:**  
**TECH:**  
**EXPIRATION:**  

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**CARBOL FUCHSIN**

**DATE:**  
**TECH:**

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**1% ACID ALCOHOL: FOR AFB**
70% alcohol 990.0 ml  
Hydrochloric acid 10.0 ml  
Stable for 1 year.

**CAUTION:** Flammable, corrosive

**DATE:**  
**TECH:**