
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 lipid capsule of the acid-fast organism takes up carbol-fuchsin and resists decolorization with a dilute acid rinse. The lipid 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.

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: Corrosive.

Methylene Blue**Stock Solution:**

Methylene blue	0.7 gm
Distilled water	50.0 ml

Methylene Blue**Working Solution:**

Methylene Blue Stock	5.0 ml
Distilled water	45.0 ml

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

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.

MICROOGANISMS

ZIEHL-NEELSEN-AFB

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RESULTS:

Acid-fast bacilli

bright red

Background

blue

REFERENCES:

Sheehan D,Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp235-237,Battelle Press, Ohio

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

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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: _____

METHYLENE BLUE

DATE: _____

TECH: _____

1% ACID ALCOHOL: FOR AFB

70% alcohol 990.0 ml
Hydrochloric acid 10.0 ml

Stable for 1 year.

CAUTION: Flammable, corrosive

TECH: _____

DATE: _____

ZIEHL-NEELEN

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: _____

CARBOL FUCHSIN

DATE: _____

TECH: _____