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**GRAM BACTERIA - MODIFIED BROWN AND BRENN**

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**PURPOSE:** For demonstrating gram-negative and gram-positive in tissue.

**PRINCIPLE:** Both bacteria's, positive and negative, cell wall is composed of peptidoglycan,(the gram-positive has a thicker wall) and both will take up the crystal violet. The gram-negative has a a layer of lipopolysaccharide external to the peptidoglycan wall, which is disrupted in the acetone rinse, allowing the crystal violet to be differentiated out. This allows the the gram-negative bacteria to take up the basic fuchsin stain.

**CONTROL:** An infected appendix, or any tissue containing both negative and positive gram rods.

**FIXATIVE:** 10% formalin

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

**EQUIPMENT:** A staining rack, coplin jars, dropper bottles, bibulous paper. Rinse all glassware in DI water.

**REAGENTS:**

**1% Crystal Violet:**

Crystal violet	1.0 gm
Distilled water	100.0 ml

Filter into a dropper bottle. Stable for 1 year.

**0.25% Basic Fuchsin:**

Basic fuchsin	0.25 gm
Distilled water	100.0 ml

Filter into a dropper bottle. Solution is stable for 6 months.

**CAUTION:** Irritant, possible carcinogen **CAUTION:** Possible carcinogen.

**Lugol's Iodine:**

See Stock Solutions

**CAUTION:** Dermal sensitizer, irritant to eyes, skin and respiratory system.

**Picric Acid-Acetone:**

Picric acid, saturated	100.0 ml
Acetone	900.0 ml

Discard after use.

**CAUTION:** Flammable, corrosive.

**Acetone:**

Purchased through Fisher.

**CAUTION:** Flammable.

**SAFETY/PPE:** Wear gloves, goggles and lab coat. Avoid contact and inhalation of dyes and chemicals.

Crystal violet/Gentian violet: carcinogen, skin irritant.

Iodine: Dermal sensitizer, irritant to eyes, skin and respiratory system. Corrosive.

Basic fuchsin: carcinogen, work in a well ventilated area.

Picric acid: can become explosive if allowed to dry out. Toxic through skin exposure.

Acetone: moderately toxic on ingestion. May cause dizziness, headaches and irritation to respiratory system on inhalation. Defatting and dermatitis on skin contact.

**PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. Place slides on staining rack, drop crystal violet stain onto tissue section, stain for 1 minute.
3. Wash in tap water.
4. Lugol's iodine, 1 minute.
5. Wash in tap water.
6. Blot sections dry, breath on section then quickly pour acetone over section until no color runs off.
7. Wash in tap water.
8. Place slides on staining rack, drop Basic fuchsin on tissue sections, stain 3 minutes.
9. Wash in tap water, blot gently but not completely dry.
10. Dip quickly into acetone, 2 dips.
11. Dip directly into picric acid-acetone mixture until a 'salmon' color.
12. Dip quickly into two changes of acetone.
13. Air dry, dip into xylene, and coverslip.

**RESULTS:**

Gram-positive bacteria	blue
Gram-negative bacteria	red
Nuclei	red
Background	yellow

**REFERENCES:**

Luna L, Manual of Histologic Staining Methods of the AFIP, 3rd Ed, pp 222, McGraw-Hill, NY

Carsen F, Histotechnology: A Self-Instructional Text, 1990, pp194-196, ASCP, III

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

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Downloaded from WebPath: Internet Pathology Laboratory  
<http://www-medlib.med.utah.edu/WebPath/webpath.html>



**Saturated Picric Acid:**

Picric acid 13.0 gm  
Distilled water 1000.0 ml

Mix well, some precipitate should remain at the bottom of the bottle.

CAUTION: Toxic, do not allow to dry out.

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

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

EXPIRATION: NONE

**Picric Acid-Acetone:**

Picric acid, saturated 100.0 ml  
Acetone 900.0 ml

Discard after use. Stable for 1 year.

CAUTION: Flammable, corrosive.

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

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

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

**0.25% Basic Fuchsin:**

Basic fuchsin 0.25 gm  
Distilled water 100.0 ml

Filter into a dropper bottle. Solution is stable for 6 months.

CAUTION: Carcinogen.

**0.25% BASIC FUCHSIN**

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

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

**Picric Acid-Acetone:**

Picric acid, saturated 100.0 ml  
Acetone 900.0 ml

Discard after use. Stable for 1 year.

CAUTION: Flammable, corrosive.

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

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

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

**1% Crystal Violet/Gentian:**

Crystal violet 1.0 gm  
Distilled water 100.0 ml

Filter into a dropper bottle. Stable for 1 year.

CAUTION: Irritant, possible carcinogen.

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

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

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

**1% CRYSTAL VIOLET**

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

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