GRAM BACTERIA - MODIFIED BROWN AND BRENN

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 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 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: 0.25% Basic Fuchsin:
Crystal violet 1.0 gm Basic fuchsin 0.25 gm
Distilled water 100.0 ml Distilled water 100.0 ml
Filter into a dropper bottle. Stable for 1 year. Filter into a dropper bottle. Solution is stable for 6 months.

CAUTION: Irritant, possible carcinogen CAUTION: Possible carcinogen.

Lugol's Iodine: Picric Acid-Acetone:
See Stock Solutions Picric acid, saturated 100.0 ml
CAUTION: Dermal sensitizer, irritant to eyes, skin and respiratory system. Acetone 900.0 ml
Discard after use.
CAUTION: Flammable, corrosive.

Acetone:
Purchased through Fisher.

CAUTION: Flammable.
MICROORGANISMS
GRAM STAIN

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:
Carsen F, Histotechnology: A Self-Instructional Text, 1990, pp 194-196, ASCP, Ill
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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RESULTS:
Gram-positive bacteria: blue
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Nuclei: red
Background: yellow

1% Crystal Violet:
Crystal violet 1.0 gm
Distilled water 100.0 ml
Filter into a dropper bottle. Stable for 1 year.

Lugol’s Iodine:
See Stock Solutions

CAUTION: Corrosive, irritant.

Acetone:
Purchased through Fisher

CAUTION: Flammable.
**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:__________________________

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**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:__________________________