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Area	Laboratory-Histology
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Histology Special Stain - Brown and Brenn (B&B) - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for differentially demonstrating gram positive and gram-negative bacteria.

II. DEFINITIONS:

The cell walls of gram-positive bacteria, when compared with the walls of gram negative, are thicker, have more layers, have different lipoproteins and polysaccharides, and have a higher amount of an acidic substance. When both types of bacteria are stained, first with crystal violet then with iodine mordant, a dye lake is formed. Both types of bacteria are stained blue. When the acetone is applied, the thicker cells wall of the gram-positive bacteria, with its more impermeable lipoproteins, will resist decolorization longer than the gram-negative bacteria. Also, the high acidic substance in the cell walls of the gram-positive bacteria form a complex with the crystal violet and iodine, which helps in resisting decolorization. The basic fuchsin stains the gram-negative bacteria. Picric acetone stains the background.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue.

B. Processing
    1. Standard, overnight processing.

C. Section Thickness
    1. Routine specimens-5µ.

D. Slide Drying
    1. 60 minutes at 60°C.

E. Type of Slide
    1. Plain slides

IV. REAGENTS:

A. **Crystal Violet Solution**
Use vendor pre-made solution

B. **Gran Iodine Solution**
Use vendor pre-made solution

C. **0.25% Basic Fuchsin – Stock**

Basic fuchsin	**0.25 gm**
Distilled water	**100.00 mL**

Dissolve together. Store at room temperature; stable for months.

D. **Basic Fuchsin – Working**

0.25% Basic fuchsin, stock	**5.0 mL**
Distilled water	**45.0 mL**

Mix together; may be reused for one day only.

E. **Picric Acid – Acetone**

Saturated picric acid	**15.0 mL**
Acetone	**400.0 mL**

Dissolve together. Store at room temperature; stable for months.

V. EQUIPMENT:

A. Balance

B. Magnet stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Coplin jars

VII. QUALITY CONTROL:

Tissue with gram-positive and gram-negative bacteria.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Crystal Violet
    1. Harmful if swallowed.
    2. Causes serious eye damage.
    3. Suspected of causing cancer.

B. Gram Iodine
    1. May be fatal if swallowed.
    2. Irritant to eyes and skin.

C. Acetone
    1. Is an extremely flammable liquid and vapor. Vapor may cause flash fire.

D. Basic Fuchsin
    1. Is an irritant and a suspected carcinogen.

E. Picric Acid
    1. Is toxic, highly reactive (4) and an extreme fire hazard (4).
    2. Keep picric acid moist at all times.
    3. If dry around the top of the jar, wash off dry particles before opening.
    4. Store in an explosion proof cabinet.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Flood slides with crystal violet.	1 minute	Steps 2, 4 and 6 may be done by placing the slides flat on a staining rack. All other steps may be done in coplin jars.
3	Rinse slides in running tap water.	10 seconds	
4	Flood slides with Gram iodine.	1 minute	
5	Rinse slides in running tap water.	10 seconds	
6	Decolorize slides quickly with acetone.	1-2 seconds	This is best accomplished by pouring acetone on the slide over the sink and quickly rinsing with water. This should be done one slide at a time.
7	Rinse slides in distilled water.	10-30	

		seconds	
8	Counterstain with WORKING basic fuchsin.	1 minute	
9	Allow slides to drain.	20 seconds	
10	Dip slides in 1 change of picric acid - acetone to counterstain.	6-10 dips each	Picric acid-acetone may decolorize more of the background.
11	Dip slides in 2 changes of acetone to dehydrate.	6-10 dips each	
12	Dip slides in 2 changes of xylene to clear.	6-10 dips each	
13	Coverslip.		

X. LIMITATIONS:

A. The following may influence the validity of test results:
    1. Keep the time of decolorization to a minimum, as gram positive bacteria can be over-decolorized, and will appear as gram negative.
    2. Old or dead gram-positive bacteria may stain red instead of blue-black.
    3. Gram negative bacteria are difficult to see with this stain. However, due to the pale background, gram positive bacteria stand out very well.

XI. RESULTS:

A. Gram positive bacteria - **blue-black**
B. Gram negative bacteria - **red**
C. Background - **yellow**

XII. REFERENCES:

A. AFIP: Laboratory Methods in Histotechnology
B. Bancroft JD and Steven A: Theory and Practice of Histological Techniques, 3rd edition, New York, NY, Churchill-Livingstone, 1990.
C. Carson FL: Histotechnology: A Self-Instructional Text. Chicago, IL, ASCP Press, 1990.
D. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak