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Beaumont

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Histology Special Stain - Brown and Hopps (B&H) - 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. PRINCIPLE:

The cell walls of gram-positive bacteria, when compared with the wall 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 cell walls 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 acid-acetone removes more of the basic fuchsin from the background, and also counterstains 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. **Gram Iodine Solution**
Use vendor pre-made solution

C. **1% Basic Fuchsin**

Basic fuchsin	**1.0 gm**
Distilled water	**100.0 mL**

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

D. **Gallego Solution**

Formaldehyde (37%-40%)	**10.0 mL**
Acetic acid	**5.0 mL**
Distilled water	**500.0 mL**

Slowly add formaldehyde and acetic acid to distilled water. Mix together. Store at room temperature; stable for months

E. **Picric Acid – Acetone**

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

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

F. **Acetone – Xylene**

Acetone	**40.0 mL**
Xylene	**40.0 mL**

Mix together. Usually made in coplin jar and used that day.

V. EQUIPMENT:

A. Balance

B. Magnet stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Coplin jars

VII. 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.
    2. Vapor may cause flash fire.

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

E. Formaldehyde
    1. Is a poison.
    2. May be fatal or cause blindness if swallowed.
    3. Cannot be made non-poisonous.
    4. Possible cancer hazard.
    5. Irritating to eyes, skin, and respiratory tract.
    6. Can cause severe eye burns.

F. Acetic Acid
    1. Is an acid.
    2. Add drop by drop to solution.
    3. May cause skin/eye burns.

G. 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.

VIII. 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.	5-10 seconds	
4	Flood slides with Gram iodine.	1 minute	
5	Rinse slides in running tap water.	5-10 seconds	
6	Decolorize slides quickly with acetone until background is clear.	1-2 seconds	This is accomplished best by pouring the acetone onto the slide over the sink and quickly rinsing it off. This should be done one slide at a time.
7	Rinse slides in distilled water.	10-30 seconds	
8	Counterstain slides with 1% basic fuchsin.	5 minutes	
9	Rinse slides in tap water.	10 seconds	
10	Differentiate slides in Gallego solution, 2 changes.	1 minute each	Gallego solution will cause the red color to be removed from the background. This can be facilitated by jiggling the slides in the coplin jar, several times.
11	Rinse slides in running water.	10 seconds	
12	Place slides in acetone.	30 seconds	
13	Place slides in picric acid - acetone.	2-3 minutes	Picric acid – acetone may decolorize more of the background.
14	Place slides in acetone - xylene, 2 changes.	10 seconds	
15	Clear in two changes of xylene.		
16	Coverslip.		

IX. 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. Gram negative bacteria are difficult to see with this stain. However, due to the pale background, gram-positive bacteria stand out very well.
3. Old or dead gram-positive bacteria will stain red instead of blue-black.

X. RESULTS:

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

XI. REFERENCES:

A. Bancroft JD and Steven A: Theory and Practice of Histological Techniques, 3rd edition, New York, NY, Churchill-Livingstone, 1990.
B. Carson FL: Histotechnology: A Self-Instructional Text. Chicago, IL, ASCP Press, 1990.
C. 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