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Beaumont

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Area	Laboratory-Histology
Applicability	Royal Oak

Histology Special Stain - Fouchet - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

Bile and lipofuchsin appear as brown-yellow pigments. The purpose of this document is to provide a procedure for the demonstration of bilirubin (bile pigment) in tissue sections. Red blood cells, when destroyed, release their hemoglobin. This hemoglobin is broken down into globin (protein) and heme (iron). When the iron is removed from the heme portion, the residue is known as biliverdin. The biliverdin is transported to the liver where it is reduced to bilirubin, where it passes into the gallbladder, then into the duodenum. In the cases of biliary obstruction or extensive liver damage, bilirubin may build up in the liver.

II. PRINCIPLE:

Bile and lipofuchsin appear as brown-yellow pigments. This stain demonstrates bilirubin (bile pigment) in tissue sections. Red blood cells, when destroyed, release their hemoglobin. This hemoglobin is broken down into globin (protein) and heme (iron). When the iron is removed from the heme portion, the residue is known as biliverdin. The biliverdin is transported to the liver where it is reduced to bilirubin, where it passes into the gallbladder, then into the duodenum. In the cases of biliary obstruction or extensive liver damage, bilirubin may build up in the liver.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue, except Zenker.
    2. 10% neutral buffered formalin preferred.

B. Processing

1. Standard, overnight processing.

C. Section Thickness

1. Cut paraffin sections at 5µ.

D. Slide Drying

1. 30 minutes at 60°C.

E. Type of Slide

1. Plain slides.

IV. REAGENTS:

A. **10% Ferric Chloride**

Ferric chloride	**10.0 gm**
Distilled water	**100.0 mL**
OR	
29% Stock ferric chloride	**3.5 mL**
Distilled water	**6.5 mL**

Stir together with magnetic stirrer. Store at room temperature; stable for 1 month.

B. **Fouchet Reagent**

Trichloroacetic acid	**25.0 gm**
Distilled water	**100.0 mL**
10% ferric chloride	**10.0 mL**

JUST BEFORE USE, carefully add trichloroacetic acid to distilled water. Stir until dissolved. Add 10% ferric chloride. Filter before use.

C. **1% Acid Fuchsin**

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

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

D. **Saturated Aqueous Picric Acid**

Use vendor pre-made saturated aqueous picric acid.

E. **Van Gieson Counterstain**

1% acid fuchsin	**5.0 mL**
Saturated aqueous picric acid	**100.0 mL**

Mix together. Store at room temperature; stable for 4-6 months; may be reused until weak.

V. EQUIPMENT:

A. Balance

B. Magnet stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks
B. Graduated cylinders
C. Non-metalic forceps

VII. SPECIAL SAFETY PRECAUTIONS:

A. Trichloroacetic Acid
    1. Is an acid.
    2. Add slowly to water.
    3. May cause severe skin and eye burns.
    4. May be irritating to eyes and respiratory system.
B. Ferric Chloride
    1. Is a corrosive.
    2. May cause skin and eye burns.
    3. be irritating to respiratory tract.
C. Acid Fuchsin
    1. Is an irritant.
D. Picric Acid
    1. Is toxic, highly reactive (4 - NFPA) and an extreme fire hazard (4 - NFPA).

VIII. QUALITY CONTROL (QC):

Section of tissue with bile.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate sections through graded alcohol to distilled water.		
2	Place slides in freshly filtered Fouchet reagent.	5 minutes	Make Fouchet reagent just before use and filter.
3	Rinse in distilled water, 3-5 changes.	5-10 seconds	
4	Stain in Van Gieson counterstain.	1 minute	
5	Place slides directly into 95% alcohol, 2 changes each.	5-10 seconds	Placing the slides in water may decrease the amount of counterstain.
6	Dehydrate through graded alcohols, clear	10	

	with xylene.	seconds each	
7	Coverslip.		

X. RESULTS:

A. Bile or bilirubin - **shades of green**

B. Collagen - **red**

C. Cytoplasm, red blood cells - **yellow**

XI. REFERENCES:

A. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. New York, NY, Churchill Livingstone, 1990.

B. Carson FL: Histotechnology: A Self-Instructional Text, Chicago, IL, ASCP Press, 1990. (Source of procedure.)

C. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

D. Vacca L: laboratory Manual of Histochemistry. New York, NY, Raven Press, 1985.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak