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**BILE - HALL'S BILIRUBIN STAIN**

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**PURPOSE:** Bilirubin is the principal bile pigment, and is a normal product of red cell degradation. Excessive amounts of bile pigment in the liver may be found in cases of hepatic or extrahepatic biliary obstruction.

**PRINCIPLE:** The oxidizing action of Fouchet's reagent converts the bile pigment to green biliverdin (if it has been transported to the liver and reduced, it is referred to as bilirubin.). Colors range from olive green to emerald green, depending on the concentration of bile pigment present.

**CONTROL:** Tissue with a known positive control, an obstructed bile duct. Coverslip slide with Permount, the fast drying media leeches out the staining reaction.

**FIXATIVE:** 10% formalin

**TECHNIQUE:** Cut paraffin sections 4m.

**EQUIPMENT:** Balance, weigh boats, acid cleaned glassware.

**REAGENTS:**

**10% Ferric Chloride:**

Ferric chloride	0.5 gm
Distilled water	5.0 ml

Make FRESH.

**CAUTION:** Avoid contact and inhalation.

**Fouchet's Reagent:**

Trichloroacetic acid	12.5 gm
Distilled water	50.0 ml

Mix well, then add:

10% ferric chloride	5.0 ml
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**Van Gieson's Solution:**

1% Acid fuchsin	1.0 ml
Picric acid, saturated	45.0 ml

Make FRESH discard after use.

**CAUTION:** Corrosive, avoid contact and inhalation. Can cause burns.

Mix well, stable for 2 WEEKS.

**CAUTION:** Avoid contact and inhalation.

**SAFETY:** Wear gloves, goggles and lab coat. Avoid contact and inhalation.

Ferric chloride; causes GI distress when ingested. Solution reacts with metal.

Trichloroacetic acid; Corrosive to skin, can cause burns. Can cause damage to the respiratory system on inhalation.

Picric acid; Toxic by skin exposure. Can become explosive when dry.

**PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. Fouchet's reagent, 5 minutes.
3. Rinse in tap water, followed by distilled water.
4. Van Gieson's solution, 3-5 minutes.
5. Rinse in distilled.
6. Dehydrate, clear, and mount in PERMOUNT, or the coverslipper.

**RESULTS:**

Bile pigment: green  
Muscle and cell cytoplasm: yellow  
Collagen: red

**REFERENCES:**

Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp 219, Battelle Press, Ohio  
Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: \_\_\_\_\_ By: \_\_\_\_\_

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## PROCEDURE CARD

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#### **RESULTS:**

Bile pigment: green  
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Collagen: red

#### **Fouchet's Reagent:**

Trichloroacetic acid 12.5 gm  
Distilled water 50.0 ml

Mix well, then add:

10% ferric chloride 5.0 ml

Make FRESH, discard after use.

**CAUTION: Corrosive, avoid contact and inhalation. Can cause burns.**

#### **10% Ferric Chloride:**

Ferric chloride 0.5 gm  
Distilled water 5.0 ml

Make FRESH.

**CAUTION: Avoid contact and inhalation.**

#### **Van Gieson's Solution:**

1% Acid fuchsin 1.0 ml  
Picric acid, saturated 45.0 ml

Mix well, stable for 2 WEEKS.

**CAUTION: Avoid contact and inhalation.**