

# Beaumont

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 Applicability Royal Oak

## Histology Special Stain - Rhodanine - Royal Oak

Document Type: Procedure

### I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of copper but may not be specific for copper. Copper is normally found in small amounts in the body. Abnormal deposits of copper can be found in Wilson disease, and in cirrhotic liver. In Wilson disease, copper accumulates in certain organs, such as the liver and brain, causing scarring and cirrhosis. In cirrhotic livers, the scarring may trap the copper, rather than the copper causing the scarring.

### II. PRINCIPLE:

The rhodanine may be specific for the protein to which the copper is bound. It may be demonstrating other metals. This stain is more sensitive than the rubeanic acid method but may not be as specific. Hematoxylin is used as a counterstain.

### III. SPECIMEN COLLECTION AND HANDLING:

#### A. Fixation

1. Any well-fixed tissue.
2. 10% neutral buffered formalin preferred.

#### B. Processing

1. Standard, overnight processing.

#### C. Section Thickness

1. Cut paraffin sections at 10μ.

- D. Slide Drying
  - 1. 30 minutes at 60°C.
- E. Type of Slide
  - 1. Plain slides

## IV. REAGENTS:

### A. Stock Rhodanine Solution

<b>Rhodanine (5(p-dimethylaminobenzylidene))</b>	<b>0.2 gm</b>
<b>Absolute ethanol</b>	<b>100.0 mL</b>

Stir together for several hours to make a saturated solution. Store at room temperature; stable for 4-6 months.

### B. Working Rhodanine Solution

<b>Stock rhodanine solution</b>	<b>3.0 mL</b>
<b>Distilled water</b>	<b>40.0 mL</b>

JUST BEFORE USE, mix together. Good for one day only.

### C. Hematoxylin

Use Mayer or Gill hematoxylin used in H&E set up.

### D. Dilute Ammonia Water

Use dilute ammonia water used in H&E set-up.

## V. EQUIPMENT:

- A. Balance
- B. Magnetic stirrer

## VI. SUPPLIES:

- A. Erlenmeyer flasks
- B. Graduated cylinders
- C. Forceps

## VII. SPECIAL SAFETY PRECAUTIONS:

- A. Rhodanine (5(p-dimethylaminobenzylidene))
  - 1. Should be kept away from sources of heat.
  - 2. Can violently decompose at elevated temperatures.
  - 3. May be harmful if swallowed.
- B. Hematoxylin
  - 1. Is incompatible with oxidizers and alkalies.

2. Store separately from them.

C. Ammonium Hydroxide

1. May cause severe skin and eye burns.
2. Vapors are irritating to eyes and respiratory tract.
3. Harmful if swallowed or inhaled.

## VIII. QUALITY CONTROL:

Section of tissue with copper.

## IX. LIMITATIONS:

- A. Sections should be 6-10 $\mu$  thick to locate minimal deposits
- B. Avoid fixatives with heavy metals (mercury, zinc), as possible false-negative staining have been reported.
- C. Fading of staining after coverslipping is common.

## X. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate sections through graded alcohol to distilled water.		
2	Place slides WORKING rhodanine solution in 60°C oven.	1 hour	Stain may also be done at 37°C; stain for 18 hours. It may also be heated in microwave oven on HIGH for 30 seconds, swirl than 30 seconds more. Allow to set on counter at room temperature for 5 minutes.
3	Rinse in distilled water, 3-5 changes.	5-10 seconds	
4	Stain lightly in alum hematoxylin.	10 seconds	
5	Wash in running water.	10 seconds	
6	Blue in dilute ammonia water.	2-3 seconds	
7	Wash in running water	5 minutes	
8	Dehydrate through graded alcohols, clear with xylene.	10 seconds	
9	Coverslip using a synthetic mounting media.		

## XI. RESULTS:

- A. Copper deposits - **orange/red**
- B. Heavy metals (mercury, silver, cadmium) - **orange/red**
- C. Bile - **yellow**
- D. Red blood cells - **yellow**
- E. Lipofuscin - **golden**
- F. Nuclei - **blue**

## XII. 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.
- 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

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## Applicability

Royal Oak

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