

Beaumont

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Histology
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Histology Special Stain - Gallyas - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of neurofibrillary tangles and thread-like nerve cell processes present in patients with Alzheimer's disease.

II. PRINCIPLE:

Sections are oxidized in a 5% solution of periodic acid and impregnated with alkaline iodide silver nitrate. Next, they are rinsed in a dilute solution of acetic acid before being placed in the developer.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation

1. Any well-fixed tissue.
2. 10% neutral buffered formalin preferred.
3. Avoid mercuric fixatives

B. Processing

1. Standard processing.

C. Section Thickness

1. Routine specimens-8-10µm.

D. Slide Drying

1. 60 minutes at 60°C.

E. Type of slide

1. Plain slides

IV. REAGENTS:

A. 5% Periodic Acid

Periodic Acid	50.0 gm
Distilled Water	1000.0 mL

Dissolve together. Store at room temperature. Stable for 1 year.

B. 1% Silver Nitrate

Silver Nitrate	5.0 gm
Distilled Water	500.0 mL

Dissolve together. Refrigerate. Stable for 6 months.

C. Alkaline Iodide

Sodium Hydroxide	20.0 gm
Potassium Iodide	50.0 gm
1% Silver Nitrate	18.0 mL

Add Silver Nitrate drop by drop while stirring. Dilute to 500 mL with distilled water.

D. Developer Stock A

Anhydrous Sodium Carbonate	5.0 gm
Distilled Water	100.0 mL

Dissolve together. Refrigerate. Stable for 1 year.

E. Developer Stock B

Ammonium Nitrate	0.19 gm
Silver Nitrate	0.20 gm
Tungstosilicic Acid	1.00 gm
Distilled water	100.00 mL

Dissolve together. Refrigerate. Stable for 1 year.

F. Developer Stock C

Ammonium Nitrate	0.19 gm
Silver Nitrate	0.20 gm
Tungstosilicic Acid	1.00 gm
Distilled Water	100.00 mL
37% Formaldehyde	0.65 mL (13 drops in 50 mL; 26 drops in 100 mL)

Dissolve together. Refrigerate. Good for 1 year.

*You can make double of Developer Stock B, measure out half and add formaldehyde to it to make developer Stock C.

G. Working Developer Solution (Prepare 10-15 minutes before use)

Developer Stock A	20.0 mL
Developer Stock B	8.0 mL
Developer Stock C	12.0 mL

1. VERY SLOWLY add solution "B" to solution "A" while stirring.
2. VERY SLOWLY add solution "C".
3. Stir constantly for 10-15 minutes before use.

H. 0.5% Acetic Acid

Acetic Acid 1.0 mL

Mix well. Store at room temperature. Stable for 6 months.

I. Stock Cresyl Echt Violet (this is the same solution used in the CEV staining procedure)

Cresyl echt violet (cresyl violet acetate) 0.50 g

Sodium acetate 0.18 g

Distilled water 500.00 mL

Acetic acid, concentrated 1.50 mL

1. Dissolve cresyl echt violet and sodium acetate in distilled water.
2. Slowly add acetic acid, drop by drop, to solution to reach a pH of 3.5.
 - a. If solution pH is below 3.5, add more sodium acetate.
 - b. If solution pH is above 3.5, add more acetic acid.
3. Filter.
4. Let stand overnight before using.
5. Store at room temperature.
6. Stable for months.
7. May be reused until weak.

V. EQUIPMENT:

- A. Balance
- B. 37°C oven

VI. SUPPLIES:

- A. Erlenmeyer flasks
- B. Graduated cylinders
- C. Acid clean coplin jars
- D. Non-metal forceps

VII. QUALITY CONTROL:

Use a section of brain medulla or peripheral nerve as a positive control.

VIII. SPECIAL SAFETY PRECAUTIONS:

- A. Periodic Acid
 1. Is a strong oxidizer.
 2. Store separately from all other chemicals.
- B. Sodium Hydroxide

1. May cause severe skin and eye burns.
 2. Harmful if inhaled.
- C. Potassium Iodide
1. Is an irritant.
- D. Silver Nitrate
1. Is harmful if inhaled.
- E. Sodium Carbonate
1. Is an irritant.
- F. Ammonium Nitrate
1. Is an oxidizer and an irritant.
- G. 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.
- H. Tungstosilicic Acid
1. May cause skin and eye burns.
 2. Irritating to respiratory tract.
- I. Acetic Acid
1. Is an acid.
 2. Add drop by drop to water.
 3. May cause skin and eye burns.
- J. Cresyl Echt Violet
1. Is an irritant

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Place in 5% periodic acid.	5 minutes	Begin making developer at this time, let it stir for at least 15 minutes before using.

3	Wash in distilled water.	5 minutes	
4	Place slides in alkaline iodide solution.	5 minutes	
5	Wash slides in several changes of 0.5% acetic acid.		
6	Place slides in developer at room temperature.	5 to 10 minutes (8 minutes appears to be optimal)	This solution should be constantly in motion using the shaker/rotating plate with the dial set to the half-way point.
7	Wash slides in 0.5% acetic acid.	5 minutes	
8	Wash in several changes of distilled water.		
9	Counterstain slides in stock cresyl echt violet	30 seconds	
10	Rinse in distilled water.		
11	Dehydrate through graded alcohols.		
12	Clear in two changes of xylene.		
13	Coverslip.		

X. LIMITATIONS:

Use acid-cleaned coplin jars and non-metal forceps or a dirty background may appear.

XI. RESULTS:

- A. Neurofibrillary tangles - **black**
- B. Senile plaques - **black**
- C. Neuropil threads - **black**
- D. Background - **purple**

XII. REFERENCES:

- A. Bancroft, John D.; Stevens, Alan; Theory and Practice of Histological Techniques, pp 355-356.

Approval Signatures

Step Description

Approver

Date

Medical Director	Kurt Bernacki: System Med Dir, Surgical Path	11/2/2022
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Policy and Forms Steering Committee (if needed)	Sharon Scalise: Supv, Laboratory	11/1/2022
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Applicability

Royal Oak

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