Silver Stain

(Additionally) Modified Yajima’s Methenamine Silver Stain

Use formalin or methacarn-fixed paraffin embedded 1-2 µm sections. If the tissue is human and fixed with methacarn, possibly immerse in Thiosemicarbazide. Use a water bath at a stable 60°C for the silver incubation step. Rinse all glassware with dH2O before use and use only acid-washed glassware for the silver stain step.

1) Turn on water baths and deparaffinize/rehydrate slides up to dH2O
2) Immerse in 1% Periodic acid for 15 minutes

Prepare Methenamine Silver Solution during incubation:
(our rectangular glass jars hold 45 mls solution and 14 slides each)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methenamine (3% sol’n)</td>
<td>34.2 ml</td>
</tr>
<tr>
<td>Stock Silver Nitrate (5% sol’n)</td>
<td>1.72 ml</td>
</tr>
<tr>
<td>Stock Sodium Borate (5% sol’n)</td>
<td>4.08 ml</td>
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<tr>
<td>DH2O</td>
<td>4.5 ml</td>
</tr>
<tr>
<td>2% gelatin solution</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Total</td>
<td>45 ml</td>
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</tbody>
</table>

** Mix well, and then add**

3) Wash slides with dH2O for 5 minutes

FOR ANIMAL TISSUE (do not use this Thiosemicarbazide step for formalin fixed human tissue. If it is exposed to Thiosemicarbazide, reduce silver time by 5-10 minutes)

* Immerse in 0.5% Thiosemicarbazide for 5 minutes
* Wash with distilled water for 5 minutes

4) Rinse all slides in the 60° dH2O container.

5) Incubate slides in the Silver Solution for approx. 20 minutes (check slides between 10 and 35 minutes, remove slides from solution when they become a dark orange/rust color)

6) Move all slides to room temperature dH2O
7) Immerse in 0.25% Gold Chloride Solution for 1 minute, then two dH2O washes
8) Place slides in 2% Nitric Acid Formalin for 1 minute, then two dH2O washes
9) Immerse in Fixer for 10 minutes, rinse in two dH2O washes
10) Stain with Hematoxylin for 2 minutes

- Rinse in three changes of tap water.
- Blue with five dips of Ammonia Water (or one minute in Tris)
- Rinse in two buckets of dH2O
- Rinse in one bucket of 70% ethanol
- Rinse in one bucket of 95% ethanol
11) Counterstain in 2% eosin for 10 minutes, dehydrate, clear and mount
Stock Solutions for Silver Stain:

1% Periodic Acid for tissue oxidation [WASTE: down drain]  \( \Leftarrow \text{Make fresh !} \)

Distilled water 200ml + Periodic Acid 2g

0.5% Thiosemicarbazide for tissue oxidation [WASTE: collect in bottle]

Distilled water 200mls + Thiosemicarbazide 1g

**Methenamine Silver Solution** [WASTE: Collect in bottle]

Stock methenamine 27.5ml + Stock silver 1.37ml + Stock sodium borate 3.3ml

3% Stock Methenamine Solution
Distilled water 500ml + Methenamine (hexamethylene tetramine) 15g

5% Stock Silver Nitrate (store in the dark at 4\(^\circ\)C)
Distilled water 50ml + Silver Nitrate 2.5g

5% Stock Sodium Borate (dissolves slowly, stir \(\sim\) 2 hrs; store room temp)
Distilled water 50ml + Sodium Borate (tetrahydrate) 2.5g
This is a saturated solution. It is OK to use after a long mixing, even if crystals are visible.

0.25% Gold Chloride Solution (store in the dark at 4\(^\circ\)C) [WASTE: Re-use until expiration, then check waste requirements (few months)]

Gold Chloride Solution 1g + Distilled water 400ml

2% nitric acid formalin [WASTE: Collect in bottle]

Nitric acid 10 ml + 37% formaldehyde 5ml + Distilled water 485ml

**Fixer, general purpose** (store at 4\(^\circ\)C and re-use) [WASTE: down drain]

Fixer 8.9g + Distilled water 500ml
Note: The water must be stirring \textit{before} the fixer is added, or it will be cloudy and not good to use.

**Ammonia Water**

DH2O 500 ml + Ammonium Hydroxide (29.3% as purchased) 1.28 ml

2% Eosin

Eosin 10 g + 95% ethanol 400 ml + dH2O 100 ml
Filter before use
TREATMENT OF GLASSWARE AFTER USE:
Any glassware that has been previously used in a silver run is cleaned in an acid wash first. This consists of a potassium dichromate-sulfuric acid mixture.

The following information is from the book THEORY AND PRACTICE OF HISTOTECHNOLOGY, SECOND EDITION by Sheehan

Potassium dichromate 200gm
distilled water 1 liter
concentrated sulfuric acid (cheapest grade) 750 mls

Use a heat-resistant glass container to prepare this solution! Dissolve the potassium dichromate in the water. While stirring this mixture with a glass rod, slowly pour in the sulfuric acid. Heat will be generated. When the solution has cooled, it may be stored in a glass-stoppered bottle. The solution is a dark red-brown and the mixture may be reused for soaking until it becomes dark green in color. Caution: Use this solution with extreme care - it is highly corrosive! We soak our glassware in this solution from a few minutes to overnight. Rinse well. The final rinse should be with distilled water.

DESTAINING Silver slides:
Use a dilute solution of Potassium Ferrocyanide

CHECKING SILVER STAINING UNDER THE MICROSCOPE:

Rinse the slide in hot distilled water (to reduce precipitate formation and excess background creation) then rinse the slides in room temperature water to view them. (so they don’t fog the lens). Rinse in hot water again before returning to the silver solution.