

Silver Stain

Alpers lab 11/15/01

(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)

	<u>Amount</u>
Methenamine (3% sol'n)	34.2 ml
Stock Silver Nitrate (5% sol'n)	1.72 ml
** Mix well, and then add**	
Stock Sodium Borate (5% sol'n)	4.08 ml
DH ₂ O	4.5 ml
<u>2% gelatin solution</u>	<u>0.5 ml</u>
Total:	45 ml

- 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] ←**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 °C)

Distilled water **50ml** + Silver Nitrate **2.5g**

5% Stock Sodium Borate (dissolves slowly, stir ~ 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°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°C and re-use) [WASTE: down drain]

Fixer **8.9g** + Distilled water **500ml**

Note: The water must be stirring *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.