
BASEMENT MEMBRANES - JONE'S METHENAMINE SILVER

PURPOSE: For demonstrating the basement membrane of the glomerulus in the kidney. A routine stain on kidney biopsies.

PRINCIPLE: The periodic acid oxidizes the carbohydrate components of the basement membrane which produce aldehydes. The released aldehydes reduce the silver to a visible metallic silver.

CONTROL: Glomeruli from a normal kidney

FIXATIVE: Alcoholic Bouin's, 10% formalin

TECHNIQUE: Cut paraffin sections 1m-2m

EQUIPMENT: Acid cleaned glassware; coplin jars (no foil lined caps), graduated cylinders, Teflon forceps. Microwave, 60°C waterbath.

REAGENTS:

1% Periodic Acid:

Periodic acid	5.0 gm
Distilled water	500.0 ml

Mix well, label with initials and date. Solution is stable for 6 months.

5% Borax:

Sodium borate	5.0 gm
Distilled water	100.0 ml

Mix well, label with initials, date. Solution is stable for 6 months.

CAUTION: Avoid contact and inhalation.

Stock Methenamine Silver Solution:

3% Methenamine	400.0 ml
5% Silver nitrate	20.0 ml

Use acid clean glassware. Store in the refrigerator, solution is stable for 6 months.

Working Methenamine Solution:

Stock methenamine silver	50.0 ml
5% borax	6.0 ml

Prepare fresh, discard after use.

CAUTION: Corrosive, irritant

CAUTION: Corrosive, possible carcinogen.

CONNECTIVE TISSUE

JONE'S METHENAMINE SILVER

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0.2% Gold Chloride:

1% Gold Chloride 1.0 ml
Distilled water 50.0 ml

5% Hypo:

See Stock Solutions

Use acid clean glassware. Store in the refrigerator, solution is stable for 6 months.

Routine Hematoxylin and Eosin stains.

CAUTION: avoid contact and inhalation.

SAFETY: Wear gloves, goggles and lab coat. Keep hot uncapped solutions under the fume hood. Avoid contact and inhalation of dyes and chemicals.

Silver nitrate: severe skin and eye irritant. Oxidizer. Ingestion will produce violent gastrointestinal discomfort. Possible carcinogen: equivocal tumorigenic agent.

Sodium thiosulfate: Toxic on ingestion. Can irritate the stomach. Irritant to skin, eyes and respiratory tract.

Light Green SF Yellowish, possible carcinogen.

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. 1% Periodic acid, in 60°C waterbath, 15 minutes.
3. Distilled water, 3 changes.
4. *Working methenamine solution and one coplin jar of distilled water: microwave HIGH power for 60 seconds, dip slides in hot distilled water, agitate silver solution, add slides to silver solution.
5. Place coplin jar of silver solution, in 60°C waterbath, check every 2-5 minutes. Allow slides to remain in silver solution until sections become light brown, check under the microscope for black membranes.
6. Rinse in distilled water.
7. 0.2% Gold chloride, 20 dips, until gray.
8. Rinse in distilled water.
9. 5% Hypo, 1 minute.
10. Wash in water.
11. Counterstain in hematoxylin, 1-3 minutes.
12. Acid rinse, blue and wash in water.
13. Counterstain lightly in eosin.

14. Dehydrate, clear, and coverslip.

*Conventional Method: 4. Place in 60°C waterbath for 1 hour or until membranes have turned black.

RESULTS:

Basement membranes: black

Nuclei: blue

background: pink

NOTES: If tissue over stains, dip in a dilute (0.05 gm/50 ml water) potassium ferricyanide solution (solution should be a pale yellow color).

REFERENCES:

Sheehan D,Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp 186-187, Battelle Press, Ohio

AFIP MANUAL, pp 97

Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: _____ By: _____

Approved: _____ By: _____

PROCEDURE CARD

JONE'S METHENAMINE SILVER-BASEMENT MEMBRANES

CONTROL: Glomeruli from a normal kidney

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. 1% Periodic acid, in 60°C waterbath, 15 minutes.
3. Distilled water, 3 changes.
4. *Working methenamine solution (solution only no slides) and one coplin jar of distilled water: microwave HIGH power for 60 seconds, dip slides in hot distilled water, agitate staining solution, add slides.
5. Place coplin jar of silver solution, in 60°C waterbath, check every 2-5 minutes. Allow slides to remain in silver solution until sections become light brown, check under the microscope for black membranes.
6. Rinse in distilled water.
7. 0.2% Gold chloride, 20 dips, until gray.
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14. Dehydrate, clear, and coverslip.
*Conventional Method: 4. Place in 60°C waterbath for 1 hour.

RESULTS:

Basement membranes: black
Nuclei: blue

Cytoplasm: pink

1% Periodic Acid:

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5% Hypo:

See Stock Solutions

Working Methenamine Solution:

Stock methenamine silver	50.0 ml
5% borax	6.0 ml

Prepare fresh, discard after use.

CAUTION: Corrosive, irritant.

5% Borax:

Sodium borate	5.0 gm
Distilled water	100.0 ml

Mix well, label with initials, date. Solution is stable for 6 months.

0.2% Gold Chloride:

1% Gold Chloride	1.0 ml
Distilled water	50.0 ml

Use acid clean glassware. Store in the refrigerator, solution is stable for 6 months.

CAUTION: Avoid contact and inhalation.

STOCK 1% GOLD CHLORIDE:

Gold chloride 1.0 gm
Distilled water 100.0 ml

Mix well, label with initials and date. Store in the refrigerator, stable for 6 months.

CAUTION: Avoid contact and inhalation.

DATE: _____

TECH: _____

EXPIRATION: _____

O.2% GOLD CHLORIDE:

1% Gold Chloride 1.0 ml
Distilled water 50.0 ml

Use acid clean glassware. Store in the refrigerator, solution is stable for 6 months.

CAUTION: Avoid contact and inhalation.

DATE: _____

TECH: _____

EXPIRATION: _____

O.2% GOLD CHLORIDE

DATE: _____

TECH: _____

STOCK METHENAMINE SILVER:

3% Methenamine 400.0 ml
5% Silver nitrate 20.0 ml

Use acid clean glassware. Store in the refrigerator, solution is stable for 6 months.

CAUTION: Corrosive, avoid contact and inhalation.

DATE: _____

TECH: _____

EXPIRATION: _____

5% BORAX/SODIUM BORATE

Sodium borate 5.0 gm
Distilled water 100.0 ml

Mix well, label with initials and date. Solution is stable for 6 months.

DATE: _____

TECH: _____

EXPIRATION: _____

1% SODIUM METABISULFITE

DATE: _____

TECH: _____

1% PERIODIC ACID:

Periodic acid 5.0 gm

Distilled water 500.0 ml

Mix well, label with initials and date. Solution is stable for 6 months.

CAUTION: Avoid contact and inhalation.

DATE: _____

TECH: _____

EXPIRATION: _____

1% PERIODIC ACID

DATE: _____

TECH: _____