RETIC - GORDON AND SWEET'S METHOD - RETICULAR FIBERS

PURPOSE: A silver impregnation technique that demonstrates reticular fibers. Reticulum is a support function of the body and is abundant in liver, spleen, and kidney. In a normal liver the fibers are will defined strands, but necrotic and cirrhotic liver show discontinuous patterns. Reticulum also forms characteristic patterns in relationship to certain tumor cells.

PRINCIPLE: The tissue is oxidized, then sensitized with the iron alum, which is replaced with silver. The silver is reduced with formalin to its visible metallic state.

CONTROL: Normal liver.

FIXATIVE: 10% formalin

TECHNIQUE: Cut paraffin sections at 4m to 5m.

EQUIPMENT: Acid cleaned glassware, pipettes.

REAGENTS:

3% Sulfuric Acid:

Distilled water	100.0 ml
Sulfuric acid	3.0 ml

Mix well, label with initial and date. Solution is stable for 1 year.

CAUTION: Corrosive, avoid contact and inhalation.

Potassium Permanganate:

Potassium permanganate	0.5 gm
Distilled water	47.5 ml
3% Sulfuric acid	2.5 ml

Make fresh, discard after use.

CAUTION: Corrosive, avoid contact and inhalation.

1% Oxalic Acid:

Oxalic acid	1.0 gm
Distilled water	100.0 ml

Mix well, label with initial and date. Solution is stable for 1 year.

CAUTION: Corrosive, avoid contact and inhalation.

2% Iron Alum

Ferric ammonium sulfate	2.0 gm
Distilled water	100.0 ml

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

10% Silver Nitrate Stock

Silver nitrate	10.0 gm
Distilled water	100.0 ml

Acid clean all glassware and rinse well. Store in a brown bottle, keep in the refrigerator. Label with initial and date. Stable for 6 months.

CAUTION: Corrosive, possible carcinogen.

3% Sodium Hydroxide Stock:

Sodium hydroxide	1.5 gm
Distilled water	50.0 ml

Mix well, label with date and initials. Store in the refrigerator, stable for 3 months.

CAUTION: Corrosive, avoid contact and inhalation.

Ammoniacal Silver Working Solution:

10% silver nitrate	5.0 ml

Add ammonium hydroxide drop by drop until clear again.

3% sodium hydroxide	5.0 ml

Add ammonium hydroxide drop by drop until clear again.

Distilled water	40.0 ml

Using acid clean glassware, agitate solution continually while adding ammonium hydroxide. Make right before use, discard.

CAUTION: Corrosive, avoid contact and inhalation.

10% Formaldehyde:

Formaldehyde	5.0 ml
Distilled water	45.0 ml

Make fresh, discard after use.

CAUTION: Carcinogen.

0.5% Gold Chloride:

Gold chloride	1.0 gm
Distilled water	200.0 ml

Use acid clean glassware, label with date and initials. Store in the refrigerator. Stable for 1 year.

CAUTION: Avoid contact and inhalation.

5% Hypo

See Stock Solution

Nuclear-Fast Red (Kernechtrot)

Aluminum sulfate	25.0 gm
Distilled water	500.0 ml
Nuclear-fast red	0.5 gm

Dissolve the aluminum sulfate in distilled water, then the nuclear-fast red, using heat. Cool, filter, and add a few grains of thymol as a preservative. Label with date and initials. Stable for 1 year.

CAUTION: Irritant, avoid contact and inhalation.

SAFETY/PPE: Wear nitrile gloves, goggles and lab coat, avoid contact and inhalation.

Potassium permanganate: Skin and eye irritant, ingestion will lead to severe gastrointestinal distress. Strong oxidant.

Sulfuric acid: Strong irritant to skin, eyes and respiratory system. Inhalation can produce target organ effects on skin, respiratory, reproductive and fetal systems. Corrosive.

can cause severe burns of the eyes, skin or mucous membranes. Toxic by inhalation and ingestion. Target organ effects on kidneys and cardiovascular system, repeated exposure can cause dermatitis. Corrosive.

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

Ammoniacal silver solution can be explosive, discard down the drain followed by copious amounts of running water.

Ammonium hydroxide: severe eye irritant, irritating to respiratory system. Target organ effects on respiratory system. Corrosive. Work under hood.

Sodium hydroxide: severe skin and eye irritant. Corrosive.

Formaldehyde: known carcinogen.

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

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Potassium permanganate solution, 5 minutes.
3. Wash in water.
4. 5% oxalic acid until clear.
5. Wash in distilled water.
6. Iron alum solution, 10 minutes.
7. Wash in running tap water, rinse in distilled, 3 changes.
8. Silver solution, 7 dips, shake excess solution off slides.
9. Distilled water, 2 changes, 3 quick dips each.

10. 10% formaldehyde solution until gray black, 30 seconds.
11. Wash in distilled water.
12. 0.5% Gold chloride, 1 minute.
13. Rinse in distilled water.
14. 5% hypo, 1 minute.
15. Wash in tap water.
16. Nuclear-fast red solution, 5 minutes.
17. Wash in running tap water.
18. Dehydrate, clear, and coverslip.

RESULTS:

Reticular fibers	black
Nuclei	red

NOTES:

1. Use acid clean glassware, or rinse 5x with distilled water.
2. When making working silver solution, if over 30 drops of ammonium hydroxide are used to turn the solution, then the ammonium hydroxide is too old. Start over with fresh ammonium hydroxide.
3. When adding the 3% sodium hydroxide solution to the silver solution it should turn black, if not make fresh sodium hydroxide.
4. Because of the alkalinity of the solution, it may cause some tissues to fall off the slides, celloidinize (see Stock Solutions).
5. Change distilled water after every slide , step '9'.

REFERENCE:

Bancroft J,Stevens A, Theory and Practice of Histological Techniques, 2nd Ed, 1982, pp142-143, Churchill Livingstone, NY

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

Prepared:_____By: _____

Approved:_____By:_____

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Solution is stabel for 1 year.
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3% Sulfuric acid	2.5 ml

Make fresh, discard after use.

1% Oxalic Acid:
Solution is stable for 1 year.

2% Iron Alum
Solution is stable for 6 months.

10% Silver Nitrate Stock
Store in the refrigerator. Label with initial and date. Stable for 6 months.

CAUTION: Corrosive.

PROCEDURE CARD

3% Sodium Hydroxide Stock:
Store in the refrigerator, stable for 3 months.

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Working Ammoniacal Silver Solution:

10% silver nitrate	5.0 ml

Add ammonium hydroxide drop by drop until clear again

3% sodium hydroxide	5.0 ml

Add ammonium hydroxide drop by drop until clear again

Distilled water	40.0 ml

Using acid clean glassware, agitate solution continually while adding ammonium hydroxide. Make right before use, discard.

10% Formaldehyde:

Formaldehyde	5.0 ml
Distilled water	45.0 ml

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0.5% Gold Chloride:

Store in the refrigerator. Stable for 1 year.

5% Hypo:

See Stock Solutions

Nuclear-Fast Red (Kernechtrot)

Aluminum sulfate	25.0 gm
Distilled water	500.0 ml
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Dissolve the aluminum sulfate in distilled water, then the nuclear-fast red, using heat. Cool, filter, and add a few grains of thymol as a preservative. Label with date and initials. Stable for 1 year.

CAUTION: Irritant, avoid contact and inhalation.

SODIUM HYDROXIDE STOCK:

Sodium hydroxide	1.5 gm
Distilled water	50.0 ml

Mix well, label with date and initals. Store in the refrigerator, stable for 3 months.

CAUTION: Corrosive.

DATE:_____

TECH:_____

EXPIRATION:_____

3% SULFURIC ACID:

Distilled water	100.0 ml
Sulfuric acid	3.0 ml

Mix well, label with initial and date. Solution is stabel for 1 year.

CAUTION: Corrosive acid.

DATE:_____

TECH:_____

EXPIRATION:_____

0.2% GOLD CHLORIDE

DATE:_____

TECH:_____

NUCLEAR-FAST RED

DATE:_____

TECH:_____

2% IRON ALUM

Ferric ammonium sulfate	2.0 gm
Distilled water	100.0 ml

Mix well, label with date and initial. Solution is stable for 6 months. Discard when iron alum precipitates.

DATE:_____

TECH:_____

EXPIRATION:_____

2% IRON ALUM

DATE:_____

TECH:_____

10% SILVER NITRATE

Silver nitrate	40.0 gm
Distilled water	400.0 ml

Mix well. Pour into an acid cleaned brown bottle. Refrigerate, stable 1 yr.

CAUTION: Corrosive, irritant, possible cacinogen.

DATE:_____

TECH:_____

EXPIRATION:_____

1% OXALIC ACID

Oxalic acid	4.0 gm
Distilled water	400.0 ml

Solution is stable for 1 year.

CAUTION: Avoid contact and inhalation.

TECH:_____

DATE_____

EXPIRATION:_____

1% OXALIC ACID

DATE:_____

TECH:_____