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 well 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.

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.

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.

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.

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.
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:
Crookham J, Dapson R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech
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.
17. Wash in running tap water.
18. Dehydrate, clear, and coverslip.

RESULTS:
Reticular fibers black
Nuclei red

3% Sulfuric Acid:
Solution is stable for 1 year.
CAUTION: Corrosive acid.

Potassium Permanganate:
Potassium permanganate 0.5 gm
Distilled water 47.5 ml
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.

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

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

Make fresh, discard after use.

### 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
- 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.
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.

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 stable 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:________________________

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 carcinogen.

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:_________________________