2-10. SCHIFF STAINS

a. **Aldehyde Blockade with Chlorous Acid**
b. **Feulgen Reaction for Nucleic Acids**
c. **Periodic Acid Schiff**
d. **Schiff Reagent**

Staining Procedures For Plastic Embedded Tissue
Verified at the Applications Laboratory of the Biomedical Division, Sorvall Microtomes

a. Aldehyde Blockade with Chlorous Acid (Ref 7)

SOLUTIONS:

Solution A: Sodium Chlorite Solution
2.19g Sodium chlorite
78.0ml Distilled water
Solution B: 5 N Acetic Acid
28.57ml Glacial acetic acid
100.0ml Distilled water
Blockade Working Solution
All Solution A
20.0ml Solution B

BLOCKING PROCEDURE:

1. Treat slides from overnight to 48 hours.
2. Rinse well in tap water.
3. Proceed to Schiff stains.

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b. Feulgen Reaction for Nucleic Acids (Refs 5,3)

SOLUTIONS:

Aldehyde Blockade with Chlorous Acid
See formula in procedure 2-10, a.

1 N HCl
83.5ml Hydrochloric acid
916.5ml Distilled water

Schiff Reagent
Use commercial solution or see formula in procedure 2-10, d.

1% Fast Green
1.0g Fast green
100.0ml Distilled water

STAINING PROCEDURE:

1. Perform aldehyde blockade in chlorous acid.
2. Wash in running tap water for 10 minutes.
3. Rinse in distilled water for 2 minutes.
4. Treat in HCl at 60ºC for 10 minutes.
5. Rinse in distilled water for 5 minutes.
6. Stain in Schiff reagent for 15-20 minutes.
7. Wash in running tap water for 20 minutes.
8. Rinse in distilled water for 2 minutes.
9. Counterstain in fast green at room temperature for ten seconds for yellow stain; or at 60ºC for 10 seconds for green stain.

Rinse in distilled water to clear plastic for 5 seconds.
Blow dry.
Mount

RESULTS:

Nuclear chromatin and some inclusion bodies -- red; other structures -- yellow or green depending on the method of counterstaining.

c. Periodic Acid Schiff (Refs 2, 7)

SOLUTIONS:

Aldehyde Blockade with Chlorous Acid
See formula in procedure 2-10, a.
1% Periodic Acid
1.0g Periodic acid
100.0ml Distilled water
Schiff Reagent
Use commercial solution or see formula in procedure 2-10, d
Sulfurous Acid
5.0ml Hydrochloric acid, 1 N
5.0ml Sodium metabisulfite, 10%
100.0ml Distilled water
Make fresh.
0.3% Methyl Green
0.15g Methyl green
50.0ml Ethyl alcohol, 95%
0.5% Acetic Acid
0.5ml Glacial acetic acid
100.0ml Distilled water
Harris' Hematoxylin
See formula in procedure 2-7, b

STAINING PROCEDURE:

1. Perform aldehyde blockade in chlorous acid.
2. Wash in running tap water for 10 minutes.
3. Rinse in distilled water for 2 minutes.
4. Treat in periodic acid for 10 minutes.
5. Wash in distilled water for 5 minutes.
6. Stain in Schiff reagent for 15-20 minutes.
7. Bleach in sulfurous acid for 2 minutes.
8. Repeat.
9. Rinse in distilled water for 5 minutes.

Counterstain in methyl green for 3-7 minutes.
Destain in acetic acid.
Rinse in distilled water.

Blow dry.
Mount.

OR:

Counterstain in Harris' hematoxylin for 10 minutes.
Follow routine procedure to distilled water (see procedure 2-7, b, omitting steps 8 and 9).
Blow dry.
Mount.

RESULTS:

Polysaccharides -- red; other tissue components stained according to the counterstain.

d. Schiff Reagent

900.0ml Distilled water

Boil in 2 liter flask. Slowly add:
5.0g Basic fuchsin

Swirl for 1 minute. Vacuum filter through two layers of filter paper in a buchner funnel into a 1 liter flask. Cool to 50ºC. Slowly, while swirling, add:
100.0ml Hydrochloric acid, 1.0 N
10.0g Potassium metabisulfite, $K_2S_2O_5$

Swirl 2 minutes. Cloudy red solution will become clear blood red. Stopper and store in refrigerator for 24 hours. Then remove from refrigerator, warm to room temperature, and add:
3.75g Activated charcoal

Shake vigorously for 1 minute. Vacuum filter immediately. The reagent should be clear and colorless. If not, repeat the charcoal step. Store Schiff reagent in the refrigerator. May be used in 48 hours; remains stable for months.

Warning: Some of the chemicals used for the staining procedures given in this section may be hazardous if misused. For this reason, read and observe all warnings and cautions provided by the manufacturer for each chemical before proceeding with a staining procedure.

Note: In order to prevent sections from loosening from the slides during staining, all sections should be heat-fixed (60ºC to 100ºC) to the slides for a minimum of 2-5 minutes prior to staining, preferably at the time the sections are mounted on the slides.