FIBRIN - FRASER-LENDRUM METHOD

PURPOSE: For the demonstration of fibrin.

CONTROL: Blood clot, specimen with injury.

FIXATIVE: Any well fixed tissue, Zenker fixed.

TECHNIQUE: Cut paraffin sections at 4 to 5 microns.

EQUIPMENT: Coplin jars, rinse all glassware in DI water, microwave.

REAGENTS:

Zenker’s Fixative:
See EVG Trichrome

Celestine Blue Solution:
Ferric ammon sulfate 2.5 gm
Distilled water 50.0 ml
Celestine blue 0.25 gm
Glycerin 7.0 ml

Dissolve the ferric ammonium sulfate in distilled water. Add the Celestine blue, boil for 3 minutes. Cool, filter and add the glycerin. Solution is stable for 6 months.

Caution: avoid contact and inhalation.

Orange G - Picric Acid Solution:
Picric acid 2.8 gm
80% alcohol 200.0 ml
Orange G 0.4 gm

Mix well. Label, stable for 1 year.

CAUTION: Flammable, Corrosive, Possible carcinogen

1% Acid Fuchsin:
Acid fuchsin 1.0 gm
Distilled water 100.0 ml
Acetic acid 1.0 ml

Mix well. Label with initials and date, solution is stable for 1 year.

Caution: avoid contact and inhalation

Differentiating Solution:
Orange G-Picric sol. 5.0 ml
80% alcohol 45.0 ml

Mix fresh, discard after use.

Caution: Flammable, Corrosive, Possible carcinogen

Mayer’s Hematoxylin:
See Congo Red
**Stock MacFarlane's Solution:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphotungstic acid</td>
<td>12.5 gm</td>
</tr>
<tr>
<td>Picric acid</td>
<td>1.25 gm</td>
</tr>
<tr>
<td>95% alcohol</td>
<td>50.0 ml</td>
</tr>
</tbody>
</table>

Mix well. Label with date and initial, solution is stable for 6 months.

**CAUTION:** Flammable, Corrosive

**Working MacFarlane's:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock MacFarlane's</td>
<td>10.0 ml</td>
</tr>
<tr>
<td>95% alcohol</td>
<td>30.0 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10.0 ml</td>
</tr>
</tbody>
</table>

Mix fresh, discard.

**CAUTION:** Flammable, Corrosive

**2% Light Green Solution:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light green, SF Y</td>
<td>2.0 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100.0 ml</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

Mix well. Label with initials and date, solution is stable for 1 year.

**CAUTION:** Possible carcinogen.

**SAFETY:** Wear nitrile gloves and lab coat, wear a particle mask or work under the hood. Keep all hot uncapped solutions under the hood. Avoid contact and inhalation.

Zenker’s contains; Mercuric chloride: Severe skin and eye irritant; target organ effects on reproductive, urogenital, respiratory, gastrointestinal and fetal systems via ingestion and inhalation routes.

Potassium dichromate: toxic by inhalation of dust and by ingestion; target organ effects on reproductive and fetal systems. Solid form is corrosive to eyes, skin and mucous membranes.

Do not discard Zenker’s fix down the drain. Dispose through Safety Services.

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

Picric acid: toxic, can become explosive if allowed to become dry.

Orange G and Light Green SF Yellowish, possible carcinogen.
PROCEDURE:
1. Deparaffinize and hydrate to distilled water.
2. *Zenker's, microwave Hi power, 45 seconds, let stand for 5 minutes.
3. Remove mercury pigments through Lugol's and hypo.
4. Wash in tap water, rinse in distilled.
5. Celestine blue, 5 minutes.
6. Wash in tap water.
7. Mayer's hematoxylin, 5 minutes.
8. Wash in tap water.
9. Orange G-picric acid solution, 5 minutes.
10. Wash in tap water.
11. Acid fuchsin, 5 minutes.
12. Wash in tap water.
14. Wash in tap water.
15. MacFarlane's Working solution, 5 minutes.
16. Wash in tap water.
17. Light Green, 1 minute.
18. Wash in tap water, dehydrate, clear, and coverslip.
   *Conventional method: 60°C oven for 1 hour.

RESULTS:
Fibrin, keratin, some cytoplasmic granules:  red
Erythrocytes:  orange
Collagen:  green

REFERENCES:
Crookham J, Dapson R, Hazardous Chemicals in the Histopathology Laboratory, 2nd Ed, 1991, Anatech

Prepared: ___________________________ By: ___________________

Approved: ___________________________ By: ___________________
PROCEDURE CARD
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RESULTS:
Fibrin, keratin, Erythrocytes: orange  Collagen: green
some cytoplasmic granules: red

Zenker’s Fixative:
Purchased

Celestine Blue Solution:
Ferric ammonium sulfate  2.5 gm
Distilled water  50.0 ml
Celestine blue  0.25 gm
Glycerin  7.0 ml
Dissolve the ferric ammonium sulfate in distilled water. Add the Celestine blue, boil for 3 minutes. Cool, filter and add the glycerin. Solution is stable for 6 months.

Orange G - Picric Acid Solution:
Picric acid  2.8 gm
80% alcohol  200.0 ml
Orange G  0.4 gm
Mix well. Label with initials and date, solution is stable for 1 year.

CAUTION: Flammable, Corrosive

1% Acid Fuchsin
Acid fuchsin  1.0 gm
Distilled water  100.0 ml
Acetic acid  1.0 ml
Mix well. Label with initials and date, solution is stable for 1 year.

Mayer's Hematoxylin:
See Congo Red
Stock MacFarlane's Solution:
- Phosphotungstic acid: 12.5 gm
- Picric acid: 1.25 gm
- 95% alcohol: 50.0 ml

Mix well. Label with date and initial, solution is stable for 6 months.

CAUTION: Flammable, Corrosive

Working MacFarlane's:
- Stock MacFarlane's: 10.0 ml
- 95% alcohol: 30.0 ml
- Distilled water: 10.0 ml

Mix fresh, discard.

Differentiating Solution:
- Orange G-Picric acid solution: 5.0 ml
- 80% alcohol: 45.0 ml

Mix fresh, discarded after use.

2% Light Green Solution:
- Light green, SF yellowish: 2.0 gm
- Distilled water: 100.0 ml
- Acetic acid: 1.0 ml

Mix well. Label with initials and date, solution is stable for 1 year.
1% Acid Fuchsin:
Acid fuchsin 1.0 gm
Distilled water 100.0 ml
Acetic acid 1.0 ml
Dissolve. Stable for 1 year.
Caution: Avoid contact and inhalation

Stock MacFarlane's Solution:
Phosphotungstic acid 25.0 gm
Picric acid 2.5 gm
95% alcohol 100.0 ml
Mix well, label and date. Stable for 6 months.
CAUTION: Flammable, Corrosive

Technician: __________________________
Date: __________________________
Expiration: __________________________

1% Acid Fuchsin
Technician: __________________________
Date: __________________________

2% Light Green Solution:
Light green, SF yellowish 8.0 gm
Distilled water 400.0 ml
Acetic acid 4.0 ml
Stable for 1 year.
Caution: Possible carcinogen

Technician: __________________________
Date: __________________________
Expiration: __________________________

Celestine Blue Solution:
Ferric ammonium sulfate 5.0 gm
Distilled water 100.0 ml
Celestine blue 0.5 gm
Glycerin 14.0 ml
Dissolve the ferric ammonium sulfate in distilled water. Add the Celestine blue, boil for 3 minutes. Cool, filter and add the glycerin. Solution is stable for 6 months.
Caution: Avoid contact and inhalation.

Technician: __________________________
Date: __________________________
Expiration: __________________________

Celestine Blue
Technician: __________________________
Date: __________________________
**Orange G - Picric Acid Solution:**

Picric acid 2.8 gm
80% alcohol 200.0 ml
Orange G 0.4 gm

Dissolve picric acid in alcohol, add Orange G. Stable for 6 months.

**Caution:** Flammable, Corrosive, Possible carcinogen

TECH:____________

DATE:____________

**Zenker's Fixative**

TECH:____________

DATE:____________

**Orange G-Picric Acid**

TECH:____________

DATE:____________