
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:

Phosphotungstic acid	12.5gm
Picric acid	1.25 gm
95% alcohol	50.0ml

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

CAUTION: Flammable, Corrosive

2% Light Green Solution:

Light green, SF Y	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.

CAUTION: Possible carcinogen.

Working MacFarlane's:

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

Mix fresh, discard.

CAUTION: Flammable, Corrosive

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.
13. Differentiating solution, 20 quick dips.
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:

Luna L, Manual of Histologic Staining Methods of the AFIP, 3rdEd, 1968, pp 81-82, McGraw-Hill, NY
Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: _____ By: _____

Approved: _____ By: _____

PROCEDURE CARD

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Zenker's Fixative:

Purchased

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See Congo Red

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PROCEDURE CARD

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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

TECH: _____

DATE: _____

EXPIRATION: _____

1% Acid Fuchsin

TECH: _____

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

TECH: _____

DATE: _____

EXPIRATION: _____

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

TECH: _____

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.

TECH: _____

DATE: _____

EXPIRATION: _____

Celestine Blue

TECH: _____

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: _____

EXPIRATION: _____

Orange G-Picric Acid

TECH: _____

DATE: _____

Zenker's Fixative

TECH: _____

DATE: _____