PRACTICAL 1

FROZEN SECTION
HAEMATOXYLIN & EOSIN STAIN, POLYCHROME METHYLENE BLUE

A. Objective

- To section fresh tissues using a cryostat.
- To stain the frozen sections with haematoxylin and eosin stain.

B. Requirement

- Cryostat
- Fresh tissues
- Harris's haematoxylin, HCL, ammonia, alcohol, eosin, xylene, DPX
- Dissecting board, scalpel & blades, self adhesive label, glass markers
- Beakers 100 ml & 250 ml, measuring cylinder 1000 ml & 10 ml
- Disposable gloves, mask, goggles
- Paper towel, old newspapers
- Slides, cover slips, staining dish, staining rack

C. Practical exercise

- Section the fresh tissues using the cryostat as demonstrated by the MLT or lecturer in charge.
- Stain the sectioned tissues according to the H&E procedures below :

Step	Solution	Time
1	Rinse slide in running tap water	Few dips
2	Immerse slide in Haematoxylin 560 MX	1 minute
3	Wash in running tap water	Few dips
4	Differentiate in Define MX-aq	30 seconds
5	80% Alcohol	10 dips
6	Blue Buffer for bluing (section turn into blue)	10 dips
7	Eosin 515 LT	10 dips
8	Absolute Alcohol	10 dips
9	Absolute Alcohol	10 dips
10	Absolute Alcohol	10 dips
11	Xylene	10 dips
12	Xylene	10 dips
13	Xylene	10 dips

- The method of polychrome methylene blue staining is recommended for rapid diagnosis with frozen sections.

1. Stain the section with polychrome methylene blue for 30 to 60 seconds.
2. Rinse with water.
3. Dehydrate, clear and mount.

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PRACTICAL 2

PARAFFIN SECTION
MASSON'S TRICHROME STAIN

A. Objective

- To stain tissues with the Masson's trichrome stain.

B. Requirement

- staining dish, staining rack
- absolute alcohol, xylene
- beakers 100 & 250 ml, measuring cylinder 1000 & 10 ml
- slide, coverslip, DPX, self-adhesive label, glass marker
- diposable glove, mask, goggle
- paper towel, old newspapers

C. Practical exercise

Reagent

1. Weigert's iron haematoxylin stain

   solution A :

haematoxylin	1 g
absolute alcohol	100 ml

   solution B :

29% ferric chloride	4 ml
distilled water	95 ml
hydrochloric acid	1 ml

- Mix equal parts of solution A and solution B before use. Put solution A first in the beaker and then solution B.

2. 1% Acid alcohol

hydrochloric acid	2 ml
70 % alcohol	198 ml

3. Biebrich scarlet – acid fuchsin solution

Biebrich scarlet aqueous 1%	180 ml
Acid fuchsin aqueous 1%	20 ml
Glacial acetic acid	2 ml

4. Phosphomolybdic – phosphotungstic acid solution

Phosphomolybdic acid	5g
Phosphotungstic acid	5g
Distilled water	200ml

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5. 2.5% aniline blue in 2% acetic acid solution

Aniline blue	5 g
Acetic acid	4ml
Distilled water	196ml

6. Acetic acid solution 1%

Glacial acetic acid	2 ml
Distilled water	198ml

Staining procedure

1. Deparaffinise
   [(200 ml) xylene - xylene for 5 min each]
2. Take section to water
   [(200 ml) alcohol 100% - 90% - 70% - water for 30 sec each]
3. Stain nuclei with Weigert's iron haematoxylin for 10 - 40 min
4. Differentiate to a pure nuclear stain with acid alcohol 1% for 30 sec
5. Wash in running tap water until blue
6. Rinse in distilled water (do not use tap water)
7. Stain in biebrich scarlet – acid fuchsin for 15 min
8. Rinse in distilled water
9. Place in phosphomolybdic acid - phosphotugstic acid solution for 10 - 15 min
10. Drain and stain in 2.5% aniline blue in 2% acetic acid solution for 5 to 10 min
11. Rinse in distilled water
12. Differentiate in acetic acid solution 1% for 3 - 5 min , discard the acid solution.
13. Dehydrate [(200 ml) alcohol 70% - 95% - 100% - 100% for 30 sec each]
14. Clear [(200 ml) xylene - xylene for 30 sec each]
15. Mount with DPX

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PRACTICAL 3

PARAFFIN SECTION
WEIGERT-VAN GIESON STAIN

A. Objective

- To stain tissues with the Weigert-Van Gieson stain.

B. Requirement

- staining dish, staining rack
- absolute alcohol, xylene
- beakers 100 & 250 ml, measuring cylinder 1000 & 10 ml
- slide, coverslip, DPX, self-adhesive label, glass marker
- disposable glove, mask, goggle, electronic balance
- paper towel, old newspapers

C. Practical exercise

Reagent

1. Weigert's iron haematoxylin stain

   solution A :

haematoxylin	1 g
absolute alcohol	100 ml

   solution B :

29% ferric chloride (29 g in 100 ml water)	4 ml
distilled water	95 ml
hydrochloric acid	1 ml

- Mix equal parts of solution A and solution B before use. Put solution A first in the beaker and then solution B.

2. Van Gieson's solution

1% acid fuchsin aqueous solution (1 g in 100 ml water)	20 ml
picric acid (saturated solution)	200 ml

3. 1% Acid alcohol

hydrochloric acid	2 ml
70% alcohol	198 ml

4. 95% alcohol 200 ml
   add picric acid

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Staining procedure

1. Deparaffinise
   [(200 ml) xylene - xylene for 5 min each]
2. Take section to water
   [(200 ml) alcohol 100% - 90% - 70% - water for 30 sec each]
3. Stain nuclei with Weigert's iron haematoxylin for 20 - 40 min
4. Wash in running tap water for 30 sec
5. Differentiate in 1% acid alcohol for 30 sec
6. Blue in tap water for 30 sec and examine
7. Counterstain in Van Gieson's solution for 3 - 5 min
8. Rinse rapidly in distilled water do not use tap water
9. Differentiate in 95% alcohol saturated with picric acid for 30 sec
10. Dehydrate [(200 ml) alcohol 70% - 95% - 100% - 100% for 30 sec each]
11. Clear [(200 ml) xylene - xylene for 30 sec each]
12. Mount with DPX

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