

APPENDIX A: PREPARATION OF CHEMICALS

a) PREPARATION OF FIXATIVES:

Bouin's Solution:

| | |
|-------------------------|--------|
| Picric acid (Saturated) | -75ml |
| Formaldehyde (37-40%) | - 25ml |
| Glycical acetic acid | - 5ml |

10 Percent Nutreal Buffered Formaldehyde Soutlion (pH7.0)

| | |
|--|--------|
| Concentrated Formaldehyde Solution (40%) | -100ml |
| Distilled water | -900ml |
| Acid sodium phosphate, monohydrate | -4g |
| Anhydrous disodium phosphate | -6.5gm |

b) PREPARATION OF SERIES OF MULTI-CONCENTRATION ALCOHOL SOLUTION:

70% Alcohol Solution:

| | |
|----------------------|-------|
| 95% alcohol solution | -70ml |
| Distilled water | -25ml |

80% Alcohol Solution:

| | |
|----------------------|-------|
| 95% alcohol solution | -80ml |
| Distilled water | -15ml |

d) PREPARATION OF CHEMICALS FOR HARRIS HEMATOXYLIN AND EOSIN STAINING:

PREPARATION OF CHEMICALS

1% Acid Alcohol Solution (for differentiation):

| | |
|-------------------------|--------|
| Hydrochloric acid ----- | 1 ml |
| 70% ethanol ----- | 100 ml |

Mix well.

0.2% Ammonia Water Solution (Bluing):

| | |
|---|---------|
| Ammonium hydroxide (concentrated) ----- | 2 ml |
| Distilled water ----- | 1000 ml |

Mix well.

Lithium Carbonate Solution (Saturated):

| | |
|-------------------------|--------|
| Lithium carbonate ----- | 1.54 g |
| Distilled water ----- | 100 ml |

Mix well.

Eosin-Phloxine B Solution:

Eosin Stock Solution:

Eosin Y ----- 1 g
Distilled water ----- 100 ml
Mix to dissolve.

Phloxine Stock Solution:

Phloxine B ----- 1 g
Distilled water ----- 100 ml
Mix to dissolve.

Eosin-Phloxine B Working Solution:

Eosin stock solution ----- 100 ml
Phloxine stock solution ----- 10 ml
Ethanol (95%) ----- 780 ml
Glacial acetic acid ----- 4 ml
Mix well.

Alternate for Eosin-Phloxine B Solution

Eosin Y Solution:

Eosin Y Stock Solution (1%):

Eosin Y ----- 10 g
Distilled water ----- 200 ml
95% Ethanol ----- 800 ml
Mix to dissolve and store at room temperature.

Eosin Y Working Solution (0.25%):

Eosin Y stock solution ----- 250 ml
80% Ethanol ----- 750 ml
Glacial acetic acid (concentrated) ----- 5 ml
Mix well and store at room temperature.

Hematoxylin Solution (Harris):

Potassium or ammonium (alum) ----- 100 g
Distilled water ----- 1000 ml

Heat to dissolve. Add 50 ml of 10% alcoholic hematoxylin solution and heat to boil for 1 minute. Remove from heat and slowly add 2.5 g of mercuric oxide (red). Heat to the solution and until it becomes dark purple color. Cool the solution in cold water bath and add 20 ml of glacial acetic acid (concentrated). Filter before use.

e) PERPARATION OF CHEMICAL FOR MASSON'S TRICHROME STAINING:

PREPARATION OF NOT SUPPLIED CHEMICALS:

- a. Working Phosphotungstic/Phosphomolybdic Acid Solution: Mixing 1 volume of Phosphotungstic Acid Solution and 1 volume Phosphomolybdic Acid Solution with 2 volumes of distilled water. (Discard after one use).
- b. Weigert's Iron Hematoxylin:
Mixed equal parts of stock solution A and B. This working solution is stable for 3 months.

Solution A:

| | |
|-------------|--------|
| Hematoxylin | - 1g |
| 95% alcohol | -100ml |

Solution B:

| | |
|--------------------------|-------|
| Ferric chloride in water | -1.2g |
| Distilled water | -95ml |
| 1% Acetic acid | -1ml |

- c. Bouin's solution:
Picric acid (Saturated) -75ml
Formaldehyde (37-40%) - 25ml
Glycial acetic acid - 5ml
- d. 1% Acetic Acid Soutlion:
Acetic acid, glacial -1ml
Distilled water -99ml