Winter School on

'RECENT ADVANCES IN DIAGNOSIS AND MANAGEMENT OF DISEASES IN MARICULTURE'

7th to 27th November, 2002

Course Manual

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HISTOPATHOLOGICAL TECHNIQUES

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Histology, the study of the microanatomy of specific tissues, has been successfully employed as a diagnostic tool within medical and veterinary science since the first cellular investigations were carried out in the mid -19th century (Virchow 1858). Due to the rapid rate of autolysis of fish tissues compared to that of homeotherms, they must be handled rapidly to prevent degenerative changes within the specimen, making ultimate diagnosis either unreliable or impossible.

For satisfactory histological preparations only freshly killed or moribund fish should be considered.

Fixation.

Proper fixation is fundamental to satisfactory histological preparations. The primary objective of fixation is to preserve the morphology of the tissue in a condition as near as possible to that existing during life. The most widely used fixative is formaldehyde.

Phosphate-Buffered formalin

40% formaldehyde	- 100 ml
Tap/distilled water	- 900ml
NaH2 PO4H2O	- 4g
Na2HPO4	- 6 g

Bouin's fluid

Saturated aqueous picric acid	- 75ml
Formalin	- 25ML
Acetic acid	- 5 ml

Shrimp tissues can be preserved in Davidson's fixative.

Davidson's fixative.

95% of ethyl alcohol	- 330ml
100% formalin (formaldehyde 37-39%)	- 220ml
Glacial acetic acid	- 115ml
Distilled water or tape water	- 335ml

Decalcification

The primary objective of decalcification is to remove calcium ions from bony components of the specimen without damaging other components, so that sectioning is facilitated. The use of formic acid (8%) in distilled water is found to be effective in decalcification. The solution should be renewed frequently (every 8-12 hr) and when decalcification is complete the tissue should be transferred to 70% alcohol. Trichloro acetic acid as a 5% aqueous solution can also be used for decalcification.

Processing

Processing of the fixed tissue involved dehydration through ascending grades of alcohols, clearing in a wax miscible agent such as xylene or chloroform and finally impregnation with wax.

Schedule

1. Fix tissue
2. Wash in water
3. 70% alcohol – 4-8hrs
4. 90% alcohol - 4hr
5. Absolute alcohol - 1-2hr
6. Absolute alcohol II - 3hrs
7. Absolute alcohol III - 3hrs
8. Chloroform I - 1hr
9. Chloroform II - 1hr
10. Wax I - 2hr
11. Wax II - 2hr
12. Wax III - 2hr

Place in embedding molds to form blocks.

Sectioning

Sections are cut on a microtome. Generally blocks of fish tissue are cut at 5µm. Sections are floated from the microtome on a water bath usually held at 48°C and mounted on a clean slide coated with a thin layer of section adhesive such as glycerin albumin. Keep on the Dryer bench for heat fixing.

Staining

1. Xylene I - 5min
2. Xylene II - 3 min
3. Absolute alcohol - 3min
4. 90% alcohol - 3 min
5. 70% alcohol - 3 min
6. 50% alcohol - 3min
7. Distilled water - 1-2 min
8. Haematoxylin stain - 5-8 min
9. Tap water - 0.5 to 1 min
10. Acid alcohol - few dips
11. Tap water - 0.5-1min
12. Scott's tap water - 1-2 min
13. Eosin stain - 1-3 min
14. Absolute alcohol - 2 min
15. Absolute alcohol - 2 min
16. Xylene I - 5 min
17. Xylene II - 3 min
18. Mount in DPX mountant

Scots tap water - 3.5g of $NaHCO_3$ and 20g of $MgSO_4$ dissolve in 1000 ml distilled water.

Eosin stain - 1000ml of 70% alcohol + eosin 1g +glacial acetic acid 5ml

Acid alcohol - 1% HCl in alcohol