

APPENDIX 1

Histology

1. Fixation

Intestine and liver for histopathological examination was placed in fixative (10% neutral buffer formalin saline) for at least 24 h prior to cassetting.

2. Cassettings

The allocated case number was entered on the cassette using a pencil. Tissue samples were trimmed to a suitable size taking care not to place too many tissue blocks in the cassettes as this can lead to ineffective dehydration and ultimately difficulty in sectioning. Small samples were wrapped in tissue paper before placing in the cassette. Cassetted samples were not allowed to dry out and were left in a bowl of water or fixative until loading into the processor.

3. Tissue processing

This procedure was carried out by placing the cassette into a basket, which was moved round automatically by a tissue processor at the appropriate time interval schedule

1. 50% Methylated spirit	1 h
2. 85% Methylated spirit	2 h
3. 100% Methylated spirit	2 h
4. 100% Methylated spirit	2 h
5. 100% Methylated spirit	2 h
6. 100% Ethanol	2 h
7. 100% Ethanol	2 h
8. Chloroform	2 h
9. Chloroform	2 h
10. Molten wax	1 h
11. Molten wax	2 h
12. Molten wax	2 h

Cassettes were removed from the processor and placed in molten wax until ready to block out. Tissues were trimmed and sections cut by microtomy.

4. Staining

Haematoxylin and Eosin

1. Xylere	5 min
2. Alcohol	2 min
3. Methylated spirit	1.5 min
4. Running tap water	2-5 min wash
5. Haematoxyline	5 min
6. Wash in tap water	3 min
7. Acid Alcohol	3 quick dips
8. Wash in tap water	3 min
9. Scott's tap water	30 sec
10. Wash well	
11. Eosin	5 min
12. Quick wash in tap water	10 sec
13. Methylated spirit	30 sec
14. Alcohol II	2 min
15. Alcohol I	1.5 min
16. Xylene	5 min

Sections were coverslipped after the last xylene in the staining series and mounting fluid allowed to dry before examination.