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Tissue Processing Protocol for Formalin Fixed-Paraffin Embedded Specimens

Materials
(All materials are available at Histology, 11G1, Tupper Bldg. at no extra charge)

- 10% Acetate Buffered Formalin
  - 0.2 L 37% Formaldehyde
  - 1.8 L Distilled H_2O
  - 46.1g Na Acetate-$3H_2O$
- Embedding cassettes
- Foam pads (for very small specimens)
- Cassette containers

Procedure

- Label cassettes with experiment # in pencil only (do not use pens or markers). Try to minimize information on these cassettes by using a simple system (ie: SB-1 or similar).
- Dissect tissues and place in cassettes.
- Immerse in containers filled with fixative and leave for 24-48 hours.
- Replace formalin with 2 washes of 70% ethanol, take cassettes to Histology (11G1, Tupper Bldg.) for processing. If necessary cassettes can be brought to Histology in a different concentration of ethanol, or in fixative. For safety of handling please indicate what solution the specimens are in.
- Fill work requisition sheet indicating embedding orientation, fixative, experiement #, and other relevant information. This sheet will be returned to you upon completion of the work for your records.
- Store paraffin blocks at room temperature.

Processing in the Histology Lab

This process includes dehydration, clearing and paraffin wax infiltration using the Autotechnicon tissue processor.

Fixed specimens are *dehydrated* as follows:

70% ethanol 1 ½ hr.
95% ethanol 1 ½ hr.
95% ethanol 1 ½ hr.
100% ethanol 1 ½ hr.
100% ethanol 1 ½ hr.
100% ethanol 1 ½ hr.

Clearing

50:50 (100% ethanol: xylene)	1hr.
xylene	1hr.
xylene	1hr.

Infiltration (embedding) media

Paraffin wax (Tissue Prep, Fisher Sci., melting point 56-57°C)	1hr.
Paraffin wax	1hr.

Casting or blocking

Specimens are embedded in paraffin using embedding rings and orienting tissue to area of interest. This step is most critical in embedding. Blocks are placed at 4°C for 15 min. to solidify.

Microtome cutting

5 μm sections are cut using a Reichert-Jung rotary microtome. Cut sections are placed in a 45°C water bath and put on silinated slides. Slides are allowed to dry in a 37°C oven overnight before staining takes place. Hematoxylin and Eosin stains are usually done at the Histology Lab.