MANUAL TISSUE PROCESSING IN HISTPATHOLOGY

1. *Introduction*

Processing of tissue is an important step in Histopathology. The preparatory treatment of tissue manually before section cutting, entailing specimen impregnation with an embedding medium to provide support and a suitable consistency for microtomy includes fixation, dehydration, clearing, impregnation and embedding (casting and blocking). This preparatory treatment is known as "Manual Tissue-Processing".

2. *Procedure or Stages of Tissue Processing (Before Micrototomy)*

a. Gross Examination, Dissection and Selection of Tissue

- Gross Examination - the gross specimen is weighed, measured, and may be photographed.
- Dissection - 3 mm sections of tissue are cut from the specimen and placed into cassettes, then returned to formalin.
- Selection of tissue - pieces of tissue for histological examination are selected from the gross specimen. A brief description of the nature of the tissue and site of the origin should be recorded.

b. Labeling of Specimen

- The specimen is labeled and precisely identified by a penciled not to dissolve during processing. They are added into tissue cassettes. Small fragments of tissue or friable tissue are wrapped in a thin paper.

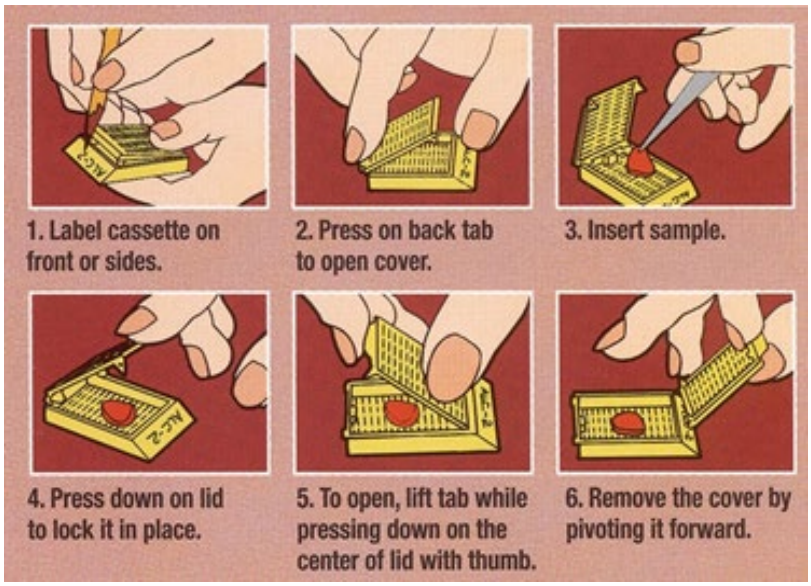


Figure 1 Procedures in mounting of oral specimen

3) Fixation

- After specimen collection, the specimen should be fixed in 10% formal saline for suitable time according to the size and type of the tissue to preserve the tissue nearest to its living state.

c. Decalcification *(If necessary)*

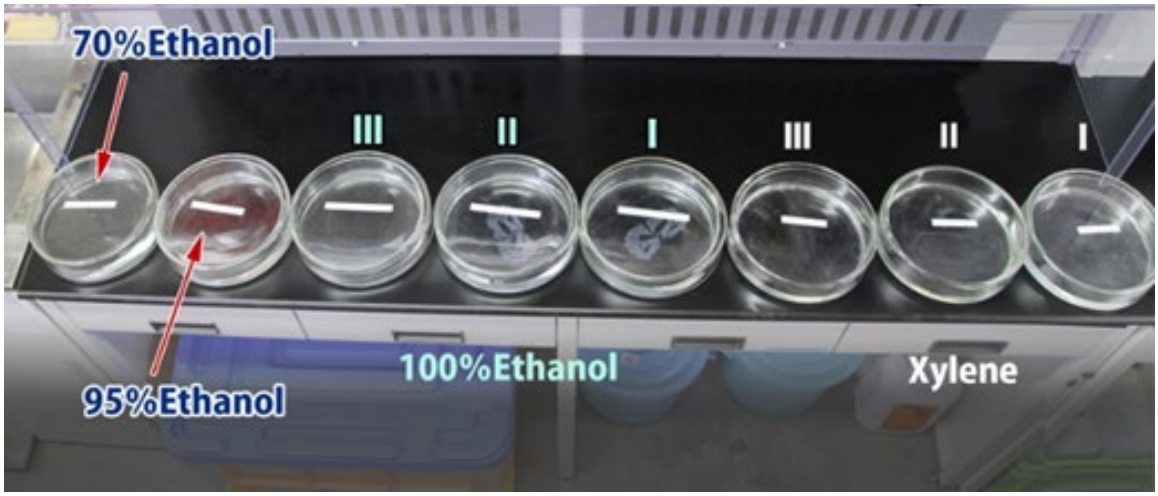
- 5-10 % HNO3 solution is routinely used as decalcification reagent for bony or calcified tissue biopsy. After complete decalcification, the tissues should be neutralized by sodium sulfate for 12 hours before dehydration.

d. Dehydration and Clearing

- Necessary to remove water. A commonly used range of dehydrating solutions is 70% ethyl alcohol, 95% ethyl alcohol and 100% ethanol I, II and III. Normal biopsy specimens 1 - 4 hours in each grade 70%, 95% & then 3 changes of 1 - 4 hours in absolute ethanol.

- The time required for dehydration will vary from a few minutes to many hours depend on the type of tissue.

- Needed to clear the dehydrating agent. Common clearing agents are xylene, benzene, toluene, chloroform. The period for clearing of tissue depends upon thickness and density of tissue and the reagents employed.



e. Impregnation & Embedding

- Needed to provide adequate rigidity of the tissue. The tissue cassette is removed from the clearing fluid and gently bottled with filter paper and then both are transferred to a container containing molten paraffin wax and placed in the oven. The amount of wax should be 25 - 50 times the volume of tissue. The tissue must be submitted to 3 changes in wax. The temperature of the wax bath should be 2 - 3°C above the melting point of wax.
- Open the tissue cassette, check the tissue pieces.
- Select the appropriate size of the mould for the tissue.
- Fill the mould with paraffin wax.
- Chill the mould on the cold plate, orienting the tissue and firming it into the wax with warmed forceps.
- Insert the identifying label or place the labeled embedding ring or cassette base onto the mould and complete fill with the wax.
- Set the combine mould and cassette on to the cold plate
- Remove the block from the mould and clean the block from excess paraffin

Reference

- https://www.google.com/url?sa=i&url=https%3A%2F%2Fparamedicsworld.com%2Fhistological-techniques%2Fimpregnation-tissue-embedding%2Fmedical-paramedical-studynotes&psig=AOvVaw0dMWE1KdkzlzubuwKl1-kS&ust=1637732104742000&source=images&cd=vfe&ved=0CAkQjhxqFwoTCJDP7NXhrfQCFQAAAAAdAAAAABAD
- University of Medical Technology, Department of Medical Technology, Histopathology Practical Manual Handbook