

PRACTICAL -3

SELECTION OF THE TISSUE BLOCK

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Before the specimens come to the laboratory:

1. Immediate fixation & the suitable fixative.
2. Enough amount of fixative.
3. Suitable container.
4. Filling the request form with necessary data to accompany the specimen.
5. Labeling the container with necessary information.

When receiving the specimen check for:

- If the specimen is for histology.
- If it is the correct specimen.
- If the specimen is in the suitable fixative.
- Make sure that all the necessary information is complete both the request form and the container.
- Registration (giving number).
- The selection of the suitable block.
- Adequate fixation.
- Decalcification if necessary.
- Neutralization if decalcified.
- Processing: dehydration, clearing, wax impregnation.
- Embedding (orientation of tissue blocks in paraffin wax) to make a paraffin wax blocks.

◆ Decalcification:

Decalcification mean:

- The removal of calcium salts. Even the tissue contain calcium salts naturally or due to some disease.

Calcium salts should be removed (Why?)

- a) it is difficult to end up with a satisfactory section.
- b) if calcium salts not removed. Knives will be destroyed.

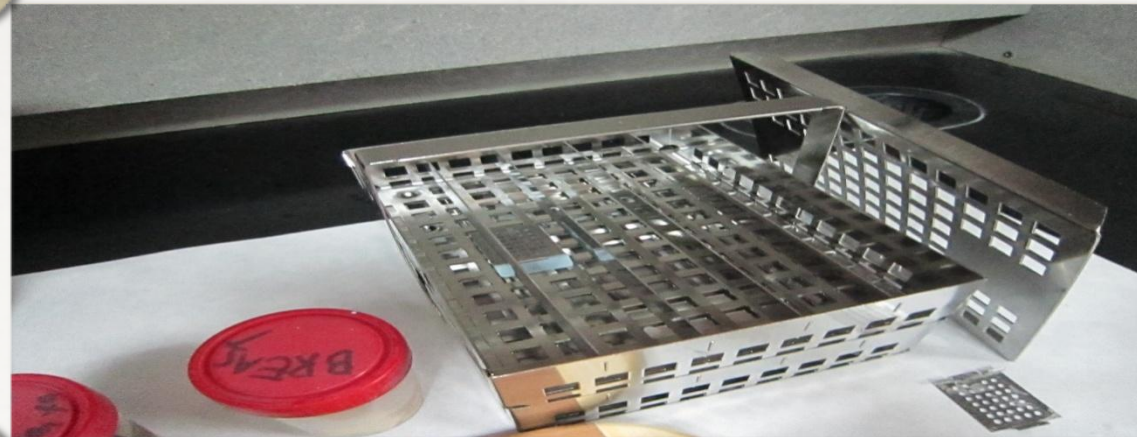
The criteria of a good decalcifying agent:

1. Complete removal of calcium salts.
2. Absence of damage to tissue, cells fibers or other constituents.
3. Non-impairing of subsequent staining.
4. Reasonable speed of decalcification.

The process of decalcification:

1. The selection of the tissue blocks.
2. Adequate or complete fixation of the tissue blocks.
3. Decalcification using the decalcifying solution.
4. Testing the end point of decalcification to get sure that it is complete (removal of calcium salts).
5. Neutralization of the acid using 5% lithium or sodium sulphate for 12-18 hours or by washing in running water.
6. Washing in running tap water to remove the alkaline solutions used for neutralization to avoid interfering with staining.





➤ Principle of tissue processing

- The aim of tissue processing is to embed the tissue in a solid medium firm enough to support the tissue and enable thin sections to be cut.
- The most satisfactory embedding material for routine histology is paraffin wax.

The stages involved are:

- 1. DEHYDRATION.**
- 2. CLEARING.**
- 3. IMPREGNATION OR INFILTRATION.**

1. DEHYDRATION

To remove fixative and water from the tissue and replacing them with dehydration fluid.

The first stage of processing is the removal of aqueous fluids from the tissues by various compounds, many of which are alcohols of various types. There are numerous dehydration strengths.

Using ethanol for instance, tissue is immersed first in **70% ethanol, 95% to 100% ethanol.**

2. CLEARING

Replacing the dehydration fluid with a fluid that totally miscible with both the dehydration fluid with the embedding medium.

- Most clearing agents are flammable liquids.
- Prolonged exposure to most clearing agents causes the tissue to become brittle and therefore more difficult to section.

THE CRITERIA FOR CHOOSING A SUITABLE CLEARING AGENT ARE:

- 1.Speedy removal of dehydration agent.
- 2.Ease or removal by molten paraffin wax.
- 3.Minimal tissue damage.
- 4.Flammability.
- 5.Toxicity and cost

THE CLEARING AGENTS SUITABLE FOR ROUTIN USE:

- **XYLENE** generally suitable for most routine histology schedules of less than 24 hr and when blocks is less than 5 mm in thickness.
- **TOLUENE** has similar properties to xylene, although it is less damaging with prolonged immersion of tissue.
- **CHLOROFORM** is slower in action than xylene but causes less brittleness. Thicker tissue blocks can be processed, even those up to 1cm in thickness.

3. IMPREGNATION OR INFILTRATION

Replacing the clearing agent with the embedding medium. The medium of choice is Paraffin wax .It is of different melting points about 40-70°C.

Soft wax has a lower melting point, whereas hard wax higher melting point.

□ **Some substances are added to paraffin wax these include:**

Bees, Wax, Cerecin, Rubber, Plastic.

THE ADDITION OF THE SUBSTANCES:

- Increase the hardness of the wax in order to cut thinner sections.
- To aid the production of serial sections.
- Alter the crystalline structure of paraffin wax.
- Increase the hardness to give the necessary support when cutting harder tissues.

Factors influence the rate of processing:

1. **AGITATION**: the rate of agitation
2. **HEAT**: increase the rate of penetration and hence interchange
3. **VISCOCITY**.
4. **VACUMM**: can be applied to speed up wax impregnation.