Dehydration, Clearing and Impregnation

By

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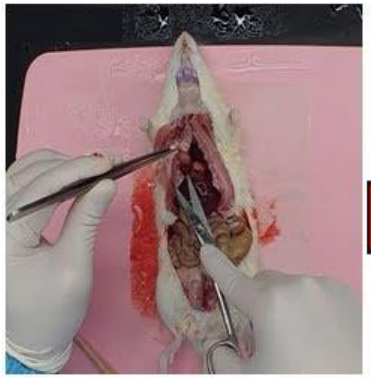
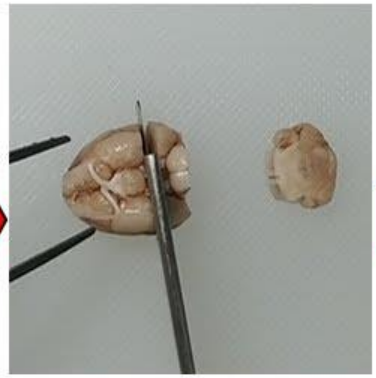
Steps of slide preparation



Necropsy

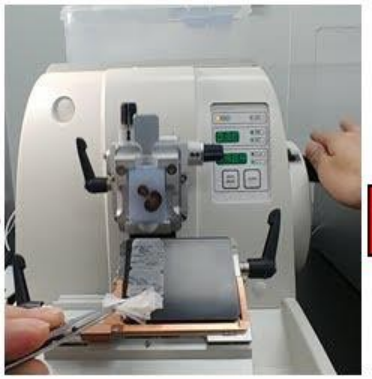
Fixation



Trimming

Tissue Processing

Embedding



Sectioning

Staining

Completion of slide preparation

Pay attention:-

A specimen brought to the laboratory is usually marked with an identifying number or name. Keep this identification with the specimen throughout processing. All identifying marks should be made with a soft lead pencil. Do not use ink or wax pencils

Tissue Processing Steps

Dehydration

- In this step water is removed from the tissue. Water is immiscible with wax, and therefore to infiltrate the tissue with wax, it is necessary to remove water.
- Gradual dehydration is necessary.
- Routine laboratory: 70, 90 and 100% alcohol for 2 h each (according to the type of tissue).
- Too much time in the dehydrating fluid: the tissue becomes hard and brittle.

Common dehydrating agents:

- Ethyl alcohol
- Methylated spirit
- Methanol
- Isopropyl alcohol

Clearing

This is needed to clear the dehydrating agent and to facilitate the transition of dehydration and impregnation stage. The clearing substance is usually miscible to both dehydrating agent (alcohol) and impregnating medium (paraffin wax).

Ideal Clearing Agent

- Low viscosity and high penetration rate.
- Low melting point.
- Miscible with both alcohol and molten wax.
- No tissue damage .

- Selection of appropriate clearing agent

Type of tissue, type of processor, processing condition (such as heat), safety factors and cost.

- Volume of clearing agent

40 times the volume of the specimen.

- Different clearing agents: Xylene, toluene, and chloroform.
- Total duration:

– Smaller biopsy: 1 hour.

– Larger tissue: Three changes in xylene, 60 min. each.

Impregnation of Embedding Medium

- Aims
- To provide support to the tissue.
- Principle
- Clearing agent is removed by the process of diffusion, and the tissue space is now infiltrated with the embedding media.

Ideal impregnating medium

- Miscible with clearing agent.
- Liquid in higher temperature and solid in room temperature.
- Homogenous and stable.
- Transparent.

Different embedding medium: Paraffin wax

Time list for Overnight Processing which differ according to the type of tissue:

- 70% ethanol: 1 h
- 90% ethanol: 1 h
- Absolute alcohol: 1 h
- Absolute alcohol: 1 h
- Absolute alcohol: (overnight)
- Xylene/toluene: 1 h
- Xylene/toluene: 1 h
- Xylene/toluene: 1 h
- Paraffin wax: 1 h … (oven 60 °C)
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Factors that affect Tissue Processing:-

- Size of the tissue

– The smaller the size, the better the processing.

- Agitation

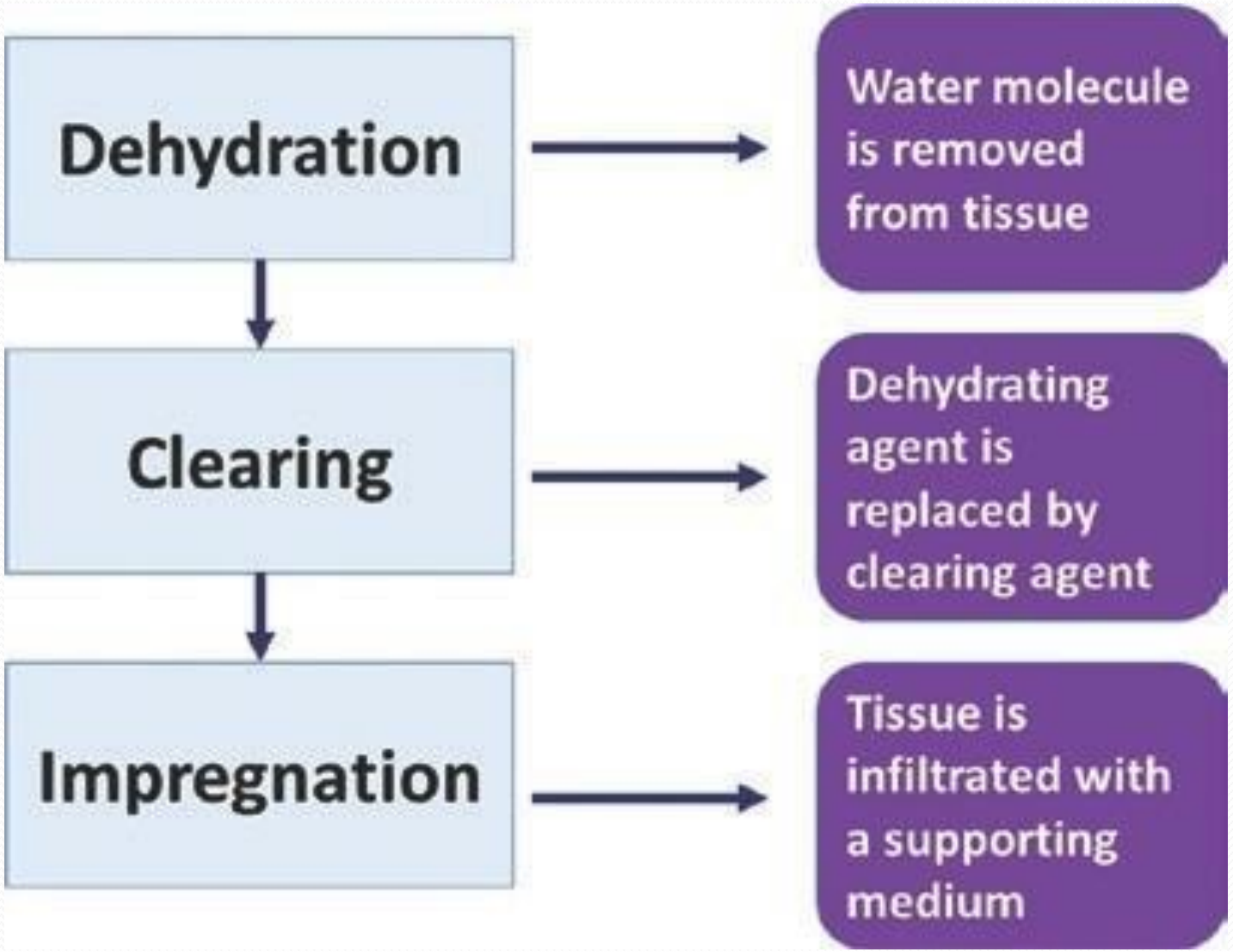
– Agitation facilitates the contact of tissue with fresh solution.

- Heat

– Increases the better penetration of fluid.

- Viscosity

– The higher the viscosity of the medium, lower the penetration


Dehydration
Water molecule is removed from tissue
Clearing
Dehydrating agent is replaced by clearing agent
Impregnation
Tissue is infiltrated with a supporting medium