262 Zoo-continue After Fixation Lecture 4

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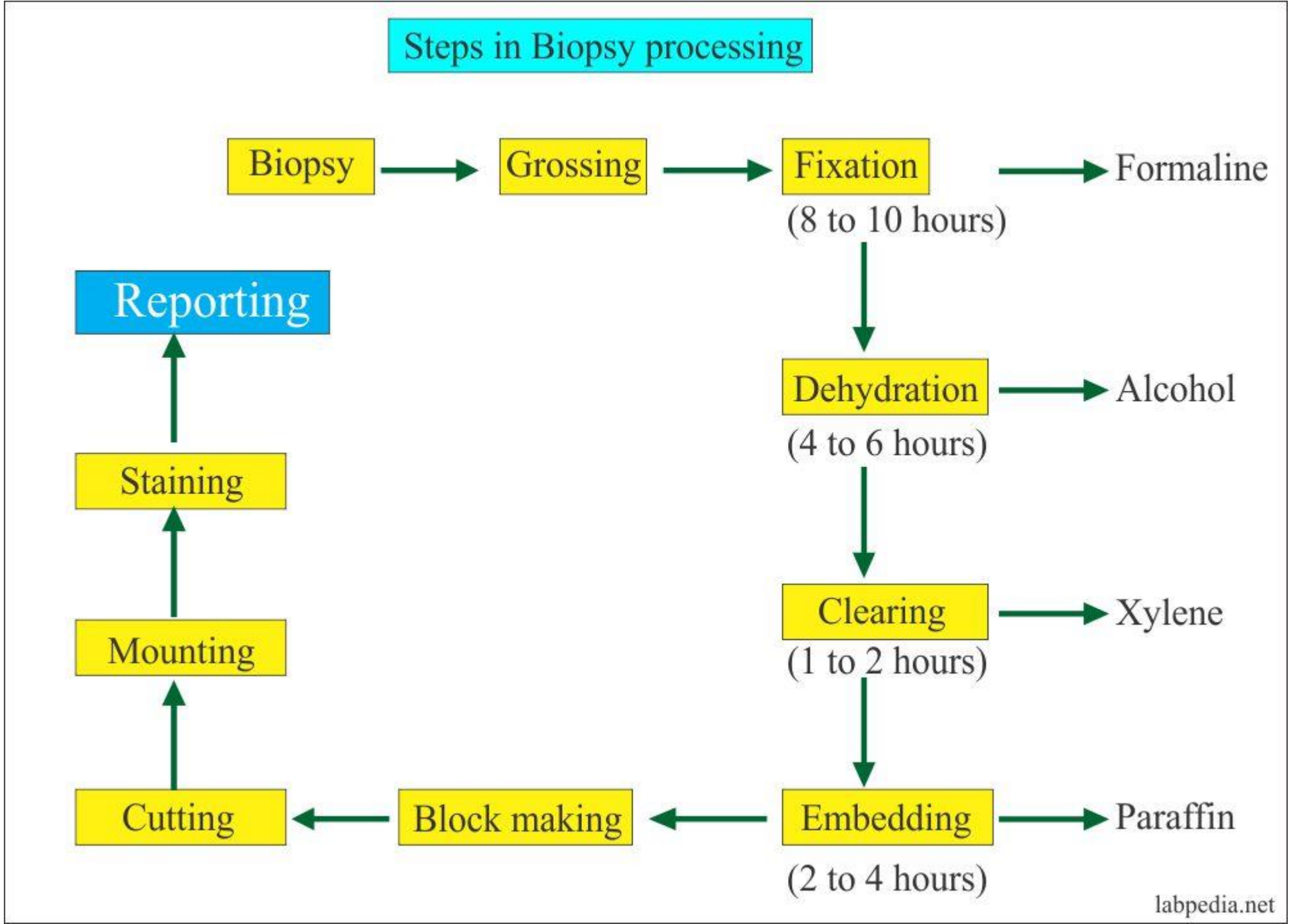
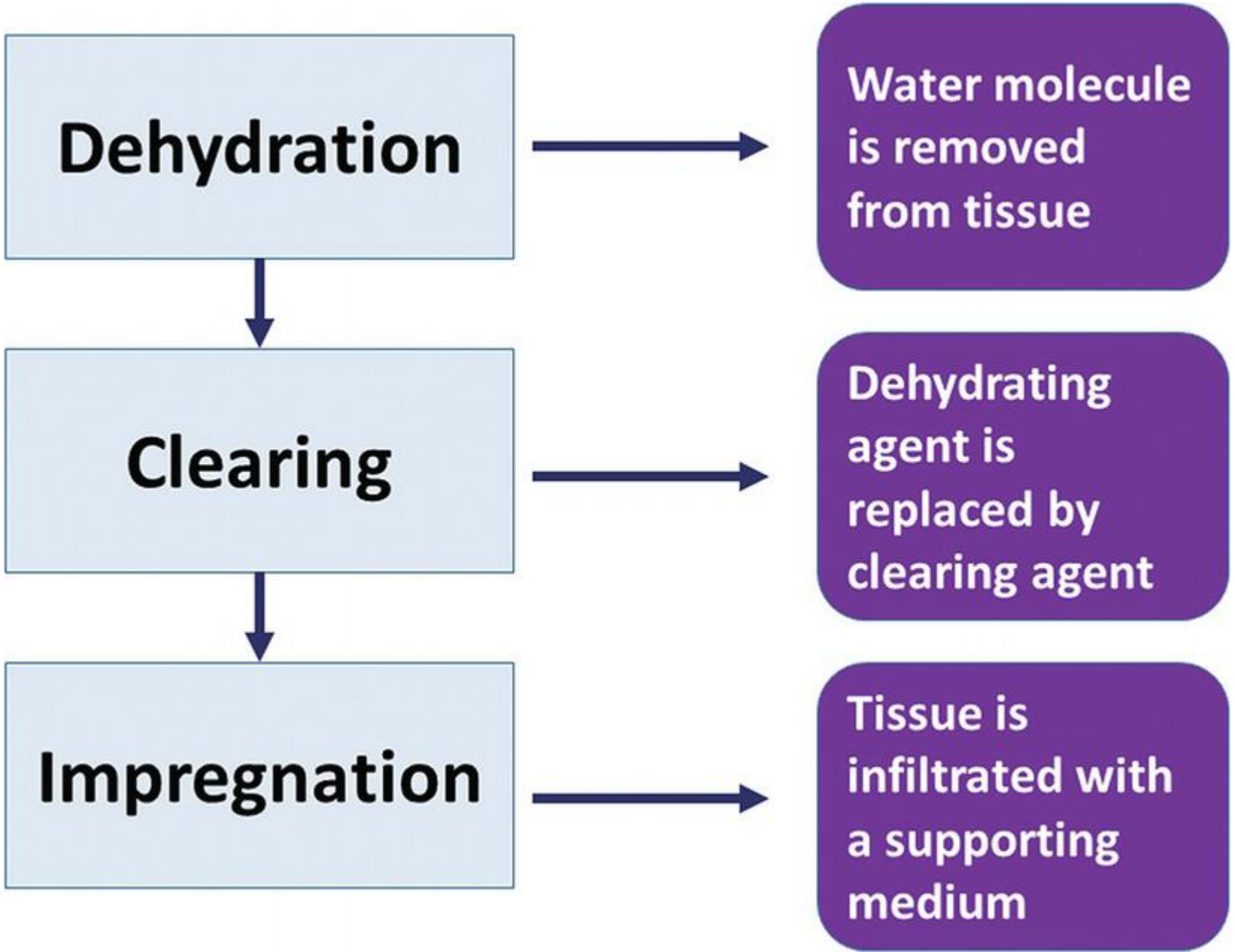
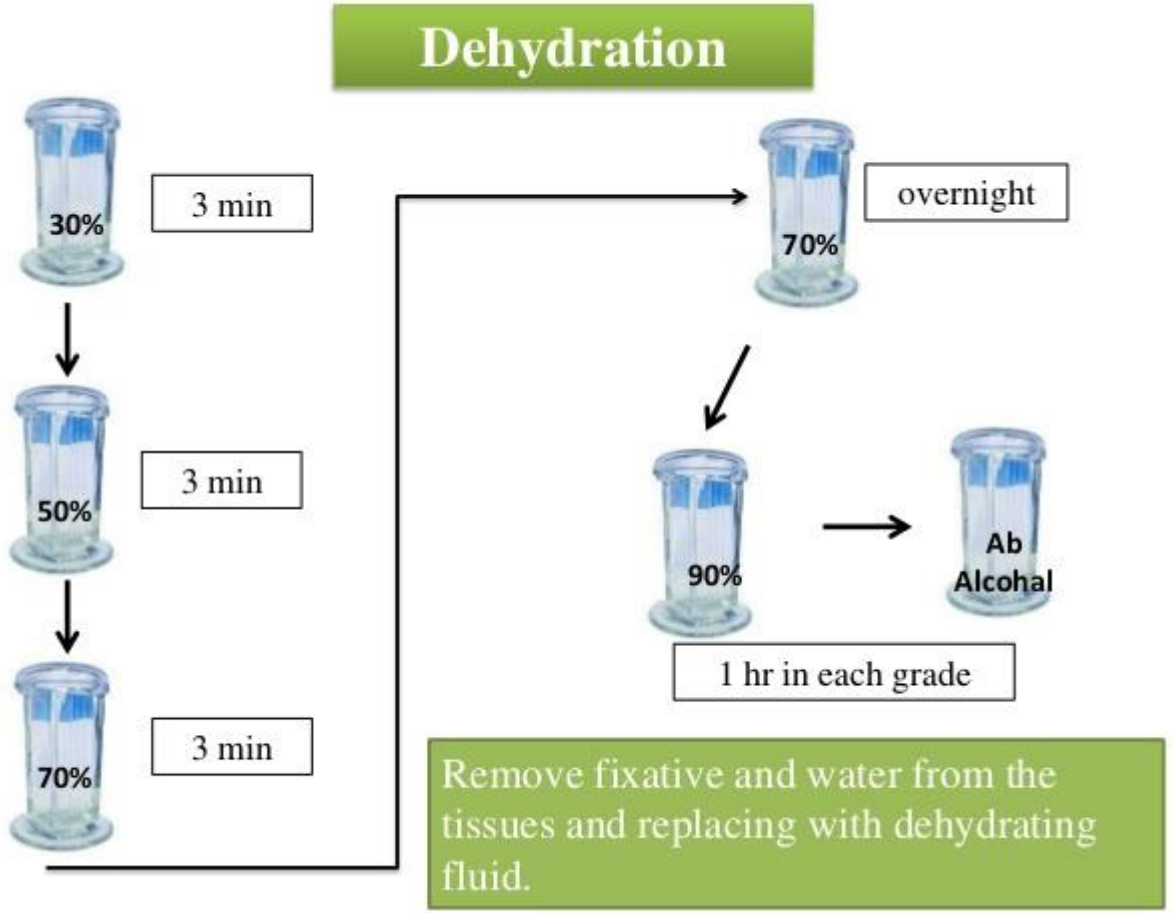

Steps in Biopsy processing
Biopsy
Grossing
Fixation
Formaline
(8 to 10 hours)
Dehydration
Alcohol
(4 to 6 hours)
Clearing
Xylene
(1 to 2 hours)
Embedding
Paraffin
(2 to 4 hours)
Block making
Cutting
Mounting
Staining
Reporting
labpedia.net

Today lecture we will discuses



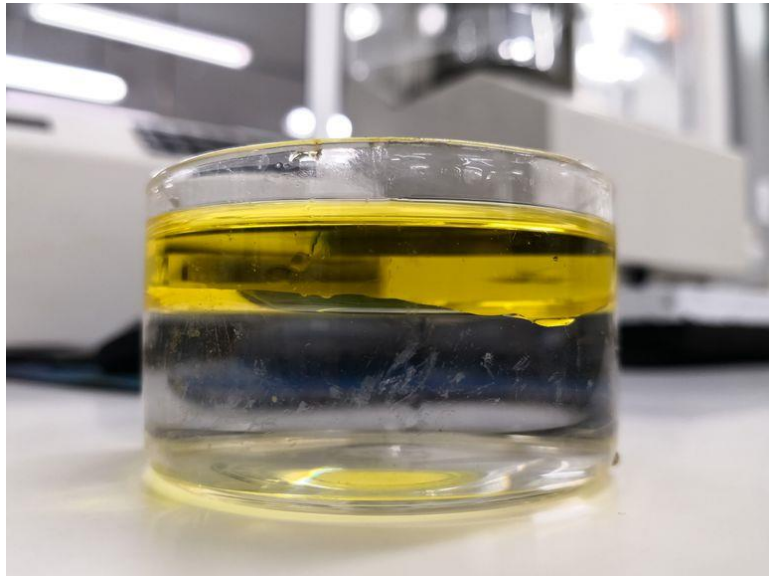
Dehydration (after Fixation)

- Tissues are placed in progressively increasing concentrations of a dehydrating agent (e.g., 70, 85, 95, and 100%) which is typically ethanol.
- Methanol, isopropanol, and acetone are alternative options.
- Two absolute alcohol (i.e., 100%) steps.



Dehydration (after Fixation)

- The dehydration step is critical, as water is immiscible with most embedding media.
- Dehydration will also remove some of the lipoidal material in the tissue. If the lipids are supposed to be visible, it will be necessary to use an appropriate fixative that will preserve the lipids prior to the dehydration step (e.g., osmium tetroxide).



Clearing

- The term “clearing” is related to the appearance of the tissue after it has been treated with a dehydrating agent.
- In this step, the dehydrating agent must be removed from the tissue and replaced with a solvent of wax.
- It is a wax solvent and must be miscible with both the dehydrating and embedding agents.

Clearing

- The clearing step can be more effective with the use of a vacuum system and should be carried out in a fume hood.
- Typical clearing agents include xylene, chloroform, and …….
- Some histological protocols have the potential option of processing the tissue without the use of a clearing agent (e.g., xylene) as a safe alternative to exposure to the hazardous effects of these chemicals.

Infiltration/impregnation

- The role of the infiltration agent is to remove the clearing agent from the tissue and to completely permeate the tissue with paraffin wax.
- Allow the tissue to harden and produce a wax block from which thin histological sections can be cut.
- **Complete infiltration is only possible after complete dehydration and complete clearing.**

Infiltration/impregnation

- Consistency of any solidified embedding medium???
- Paraffin wax is commonly used and heated to a temperature that is 2–3°C above its melting point.
- 20–25 times the volume of the tissue.
- Use graded mixtures of clearing agent and paraffin???
- Shrinkage and hardening will occur with more exposure.
- Numerous substances can be added to the molten paraffin to modify its consistency and melting point.

Tissue processing machine