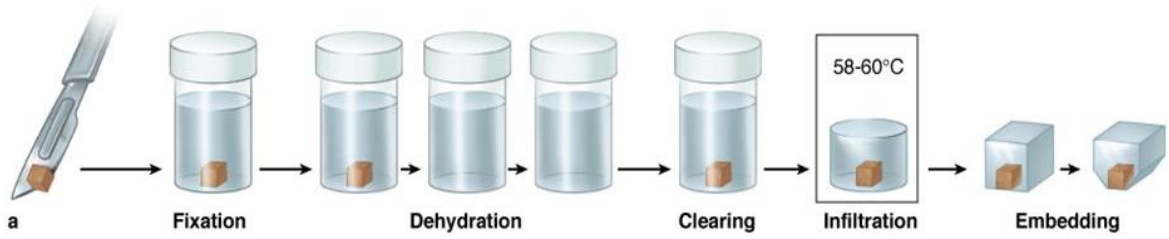
Asst. lecturer Ameer H. Gibreen
M.Sc. Pathology

# An Introduction to Specimen Processing

Microscopic analysis of cells and tissues requires the preparation of very thin, high-quality sections (slices) mounted on glass slides and appropriately stained to demonstrate normal and abnormal structures.

Tissue processing describes the steps required to take an animal or human tissue from fixation to the state where it is completely infiltrated with a suitable histological wax and can be embedded ready for section cutting on the microtome.




## Overview of the steps in tissue processing for paraffin sections

1. **Obtaining a fresh specimen**
    - Fresh tissue specimens will come from various sources.
    - It should be noted that they can very easily be damaged during removal from the patient or experimental animal.
    - It is important that they are handled carefully and appropriately fixed as soon as possible after dissection. Ideally, fixation should take place at the site of removal, perhaps in the operating theatre, or, if this is not possible, immediately the following transport to the laboratory.

**2. Fixation**

- The specimen is placed in a liquid fixing agent (fixative) sucha as formaldehyde solution (formalin).
- This will slowly penetrate the tissue causing chemical and physical changes that will harden and preserve the tissue and protect it against subsequent processing
- There are a limited number of reagents that can be used for fixation as they must possess particular properties that make them suitable for this purpose.

**3. Dehydration**

- Because melted paraffin wax is hydrophobic (immiscible with water), most of the water in a specimen must be removed before it can be infiltrated with wax.

- This process is commonly carried out by immersing specimens in a series of ethanol (alcohol) solutions of increasing concentration until pure, water-free alcohol is reached.
- Ethanol is miscible with water in all proportions so that the water in the specimen is progressively replaced by the alcohol.
- A series of increasing concentrations is used to avoid excessive distortion of the tissue.

A typical dehydration sequence for specimens not more than **4mm** thick would be:

| | |
|---|---|
| 70% ethanol | 15 min |
| 90% ethanol | 15 min |
| 100% ethanol | 15 min |
| 100% ethanol | 15 min |
| 100% ethanol | 30 min |
| 100% ethanol | 45 min |

❖ At this point, all but a tiny residue of tightly bound (molecular) water should have been removed from the specimen.

**4. Clearing**

- The tissue is now essentially water-free, we still cannot infiltrate it with wax because wax and ethanol are largely immiscible.
- We, therefore, have to use an intermediate solvent that is fully miscible with both ethanol and paraffin wax.
- This solvent will displace the ethanol in the tissue, then this, in turn, will be displaced by molten paraffin wax.
- This stage in the process is called “clearing” and the reagent used is called a “clearing agent”. The term “clearing” was chosen because of many (but not all).
- Another important role of the clearing agent is to remove a substantial amount of fat from the tissue, which otherwise displaces ethanol. Which presents a barrier to wax infiltration.
  A popular cleaning agent is a xylene, and multiple changes are required to completely

A typical clearing sequence for specimens not more than **4mm** thick would be:

| |
|---|
| 1. xylene 20 min |
| 2. xylene 20 min |
| 3. xylene 45 min |

## 5. Wax infiltration

- The tissue can now be infiltrated with a suitable histological wax. Although many different reagents have been evaluated and used for this purpose over many years,
- paraffin wax-based histological waxes are the most popular.
- A typical wax is liquid at 60°C and can be infiltrated into tissue at this temperature then allowed to cool to 20°C, where it solidifies to a consistency that allows sections to be consistently cut.

A typical infiltration sequence for specimens not more than 4mm thick would be:

| | |
|---|---|
| wax | 30 min |
| wax | 30 min |
| wax | 45 min |

## 6. Embedding or blocking out

- Now that the specimen is thoroughly infiltrated with wax, it must be formed into a "block" which can be clamped into a microtome for section cutting.
- This step is carried out using a "center" where a mold is filled with molten wax and the specimen placed into it. The specimen is very carefully orientated in the mold because its placement will determine the "plane of the section", an important consideration in both diagnostic and research histology.
- A cassette is placed on top of the mold, topped up with more wax, and the whole thing is placed on a cold plate to solidify.
- When this is completed, the block with its attached cassette can be removed from the mold and is ready for microtomy.
- It should be noted that, if tissue processing is properly carried out, the wax blocks containing the tissue specimens are very stable and represent an important source of archival material

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