



Washing

It is remove the excess of un combined fixative.

✚ All tissues after fixation in fluids containing potassium dichromate, mercuric chloride should be washed before next procedure.

Example:

Zenker's fixative

The tissue must be washed with running tap water (14-24) hrs because precipitate protein does not remove by tap water.

Bouin's fixative

Ttissue not must be washed with tap water, but treated with 70 % directly for (3-8) hrs because formation of sore water.

Dehydration

It is removing excess water from tissue.

Dehydrating agent:

Is substance freely miscible with water and capable of replacing it.

e.g Ethanol .

✚ The concentration of alcohol depend on:

- 1) The fixation.
- 2) Size of the tissue.
- 3) Type of the tissue.



Notes:

- Delicate tissue like brain, spinal cord and embryos need to be dehydrated slowly and starting first with 50 % alcohol, but most tissue may be put in to 70 % alcohol.
- Put tissue specimens in high concentration 100 % of alcohol cause shrinkage , due to the rapid removal of the water.
- Treatment in graded alcohol will depend on size and type of tissue. Thus diluted alcohol will not harm tissue, indeed tissue may store in 70 % alcohol after fixation.

Clearing

It is process of replace the alcohol in the dehydrated tissue with a solvent of the wax in which the tissue, later embedded before it can be cut, therefore it is important that using clearing agent must be freely miscible with alcohol.

✚ The time for complete clearing depend on:

- 1) Type of the clearing agent.
- 2) Size of tissue.
- 3) Thickness of tissue.

Clearing agent:

A. Xylene

- 1) Highly inflammable
- 2) Rapid in action.
- 3) Prolong treatment for solid tissue.
- 4) Brittle cause for soft brain and spleen.
- 5) No toxic.
- 6) May cause dermatitis.
- 7) Cause excessive shrinkage of delicate tissue

**B. Benzene:**

Similar to xylene but it does not cause soft tissue brittle and it is toxic in low concentration .

C. Toluene:

Some like xylene and benzene but less toxic cause little hardening and destruction for tissues.

D. Chloroform:

- 1) Not inflammable.
- 2) Slow in action .
- 3) Toxic vapour.
- 4) Little hardening effect.

E. Cedar wood oil:

- 1) Toxic.
- 2) Very slow in action.
- 3) Suitable for large pieces as brain and lymph gland.

✚ If the tissue cleared quickly the clearing **agent don't reach the center.**