Slide Preparation

All techniques are needed to collect, prepare, and mount samples on slide to preserve it using microscopic. These techniques included: Anesthetization, Fixation, Embedding and cutting, mounting onto slides, Staining, Washing, mounting and preservation

Anesthetization

Animal experiments are necessary for a better understanding of diseases and for developing new therapeutic strategies. Animal experiment include Mouse, Rat, Rabbit, Hamster,....ect. The mouse is currently the most popular laboratory animal in biomedical research. Experimental procedures on animals often require anesthesia and/or analgesia to obtain adequate immobilization and to reduce stress or pain to collect samples in two ways:

1. Under anesthetization many samples can collect without kill the animals such as: blood, biopsy from different organs, and interpretonium fluid.

2. Under anesthetization can kill animal, then collect the blood or any other body fluids, tissues, and organs

Anesthesia types and dosage choice depend on several factors including the animals' size, weight, and metabolic rate. There are many types of Anesthesia:

1- Freezing: used for the new born mice and rat by put the baby in 4°C for 1min to the Mouse and 2min to the Rat.

2- CO_2 gas: used only for adult animals by respirator for 20-30 min. It rarely use because it can kill the animals.

3- Ether drop: used for the young and adult animals by put some drops in the can, transport the animal in this can, and cover it for few second. The animal sleep fast but also weak up fast.

4- Opioide injection: mix from many drug type (Pethidin, Buprenophin, and Piritamid). These drugs mix and inject ip or iv. The animal didn't weak up fast but stile has pain.

5- Ketamin injection: the best type used for all ages except newborn of mice. It leads to deep sleep, relaxation, normal breath, and no muscles contract.

Fixation

It is a process by which the tissue, internal and external structures of the cells, and microorganisms are preserved and fixed in position.

This process inactivates enzymes that might disrupt the tissue and cell morphology and toughens cell structures so that they do not change during staining and observation. There are three types of fixation processes depending on the initial specimen:

1. **Heat fixation**: Heat-fixed bacterial smears by gently flame heating an air-dried film of bacteria and archaea. It preserves overall morphology but not internal structures. It cannot be used in the capsular stain method as heat fixation will shrink or destroy the capsule (glycocalyx) and cannot be seen in stains.
2. **Air drying**: usually with subsequent chemical post-fixation, is applicable for cell smear, cytopins and cryosection.
3. **Snap freezing**: usually in liquid nitrogen is routinely employed for tissue probed for subsequent cryosectioning.
4. **Immersion**: The sample of tissue is immersed in fixative solution of volume at a minimum of 20 times greater than the volume of the tissue to be fixed. This is a common technique for cellular applications.
5. **Perfusion**: Fixation via blood flow. The fixative is injected into the heart with the injection volume matching cardiac output. The fixative spreads through the entire body, and the tissue doesn't die until it is fixed. This has the advantage of preserving perfect morphology, but the disadvantages that the subject dies and the cost is high (because of the volume of fixative needed for larger organisms)

Chemical Fixation

In both immersion and perfusion fixation processes, chemical fixatives are used to preserve structures in a state (both chemically and structurally) as close to living tissue as possible. It fixatives penetrate cells and react with cellular components, usually proteins and lipids, to render them inactive, insoluble and immobile. There are many types of Chemical fixation such as: alcohols, acetone, and formaldehyde.

Embedding and cutting

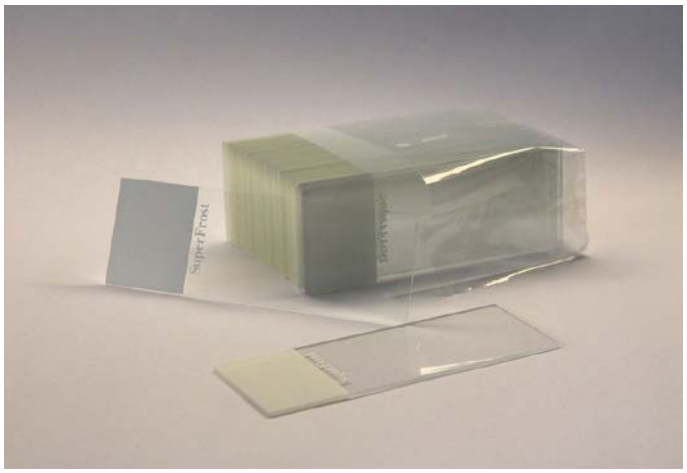
The fixation of the tissues has to be followed by dehydration, clearing, embedding, and cutting.

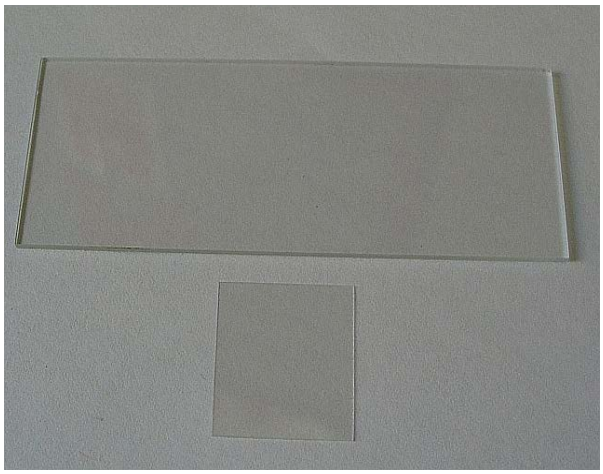
1. Dehydration is the first step in the processing of fixed tissue included the removal of water to allow complete infiltration of tissues with clearing agent. Unless all traces of water are removed, infiltration is incomplete, the tissues appear opaque and bacterial decay ultimately sets in. If the water carried out too rapidly, dehydration causes distortion and shrinkage, especially of delicate tissues, by setting up violent diffusion currents. It should therefore be done gradually and sufficient time allowed for the complete extraction of water. Dehydration is commonly effected by immersion tissues in series diluting solutions of alcohol bingeing with 75% and ending with absolute alcohol 100% alcohol.
2. Clearing is the removal of alcohol to be infiltrated with the paraffin wax by immersion the tissue in clearing agents such as: are1, 2- dimethylbenzene (xylene) for small soft tissues; clove oil and cedar wood oil for thick tissues. Toluene is another good clearing agent but it is a bit costly.
3. Embedding is the removal of clearing agent to make tissue blocks by paraffin wax which stored without any chemical or morphological tissue changes and easily cutting. Embedding begin by immersion tissues in hot paraffin and then transport the tissues to the template full of hot paraffin, finally leave to cool and isolated the paraffin tissue block from the template.

4. Cutting of the tissue to thin spaces which could be mounted onto slid. It include two types:
    - Cutting of paraffin-embedded tissues by microtome at 3-10µm.
    - Cutting of frozen tissue blocks by cryostat (a microtome mounted in freezing cabinet) at 5-10µm, after quickly frozen of tissue samples with or without freeze-embedding medium, tissue teak or miles laboratories, and stored at -80°C.

Mounting section onto slides

A **microscope slide** is a thin flat piece of glass, typically 75 by 25 mm (3 by 1 inches) and about 1 mm thick, used to hold objects for examination under a microscope. Typically the object is placed or secured (mounted) on the slide, and then both are inserted together in the microscope for viewing. This arrangement allows several slide-mounted objects to be quickly inserted and removed from the microscope, labeled, transported, and stored. Microscope slides are often used together with a cover slip or cover glass, a smaller and thinner sheet of glass that is placed over the specimen. Slides are held in place on the microscope's stage by slide clips.

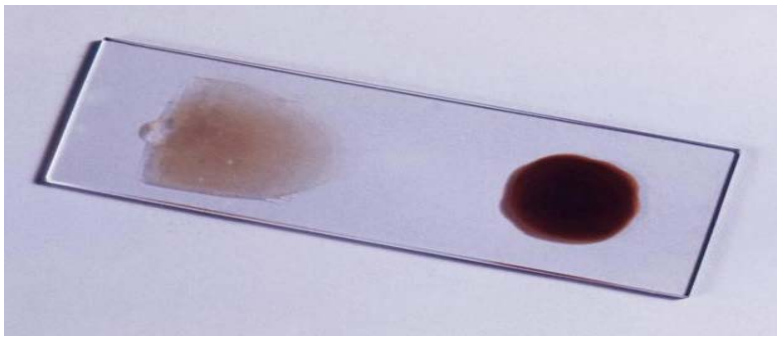




Microscope slides are usually made of optical quality glass, such as soda lime glass or borosilicate glass, but specialty plastics are also used. Fused quartz slides are often used when ultraviolet transparency is important, e.g. in fluorescence microscopy. To

help adherence to the glass and decrease the chances of sections dissociating from slides, paraffin tissue section should mounted on tissue-adhesive slides such as Super Frost Plus which especial important for paraffin tissue sections under-going heat-induced antigen retrieval. It can also used silanized slides by washing the normal slides with amino-propyl-tri-ethoxy-silane (APES)

- Paraffin sections mounted onto slides using deionized water in the water bath in order to avoid sectioning artifacts. The sections must be dried to increase adhesion of tissue sections to the surface of the glass slide. For drying of paraffin sections, it was put on hotplate or oven at 56°C for 1h, and then can put it in the oven at 37°C overnight or longer up to 3 days, this is even better. Mounted paraffin tissue sections must be deparaffinized and rehydrated before use, to ensure that the staining have full access to the tissue
- Blood or any other body fluid mounted onto slid directly by put a drop on the slid, make a smear, and leave it to dry at room temperature. This sometimes need to fixe by 75% alcohol. This method is also commonly used, for example, to view microscopic organisms that grow in pond water or other liquid media, especially when studying their movement and behavior.



- Solid samples mounted onto slid by put water drop on it, make a smear. This sometime need to dry and fix by heat like bacteria, others like stool sample did not need to dry or fix it. This method is commonly used, for example, to view microscopic organisms that grow in pond water or other liquid media, especially when studying their movement and behavior