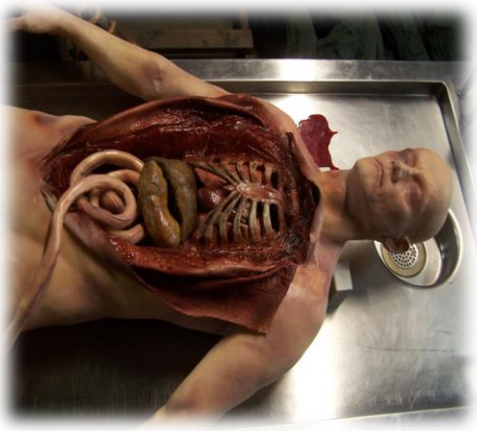
Obtaining the sample to be examined from living person or animals can be done by

A. **Surgical resection specimens:** are obtained by the therapeutic surgical removal of an entire diseased area or organ (and occasionally multiple organs) after anesthetization.



All specimens removed during surgery must be submitted to surgical pathology for **gross and/or microscopic examination**. This includes major surgery performed within the hospital and minor surgeries performed in outpatient clinics or hospital units.

Gross examination is the process by which pathology specimens are inspected with the naked eye to obtain diagnostic information.

Gross examination is a description of what tissue taken during a biopsy looks like without using a microscope.

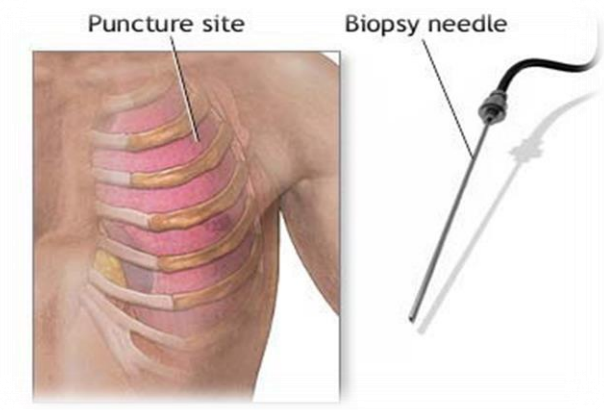
The gross description may include the

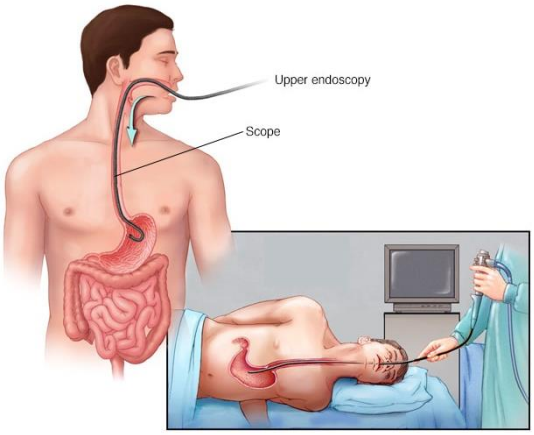
1. **Size** of the tissue sample
2. **Shape** of the tissue sample
3. **Color** of the tissue sample
4. **Weight** of the tissue sample.

Pathological analysis of surgical resection specimens is critically important in confirming:

1. Previous diagnosis
2. Staging the extent of malignant disease
3. Establishing whether or not the entire diseased area was removed
4. Identifying the presence of unsuspected concurrent diseases
5. Providing information for postoperative treatment, such as chemotherapy in the case of cancer.

B. Biopsy: is a medical procedure that involves taking a small sample of body tissue so it can be examined under a microscope.





Biopsy is a medical test commonly performed by a surgeon, interventional radiologist, or an interventional cardiologist.

There are many different types of biopsy procedures. The most common types include:

1. **Incisional biopsy**, in which only a small sample of tissue is removed
2. **Excisional biopsy,** in which an entire lump or suspicious area is removed
3. **Needle biopsy,** in which a sample of tissue or fluid is removed with a needle

Fixation

In the fields of **histology**, **pathology**, and **cell biology**, fixation is a chemical process by which biological tissues are **preserved** from decay, either through autolysis or putrefaction. Fixation terminates any ongoing biochemical reactions and may also increases the mechanical strength or stability of the treated tissues. Examining cells, fragments of tissues or organs, after their vital processes have been stopped by fixation. This allows the examination of the structure and main characteristics of cells.

Fixation is achieved by exposing the tissue to chemical compounds called fixatives.

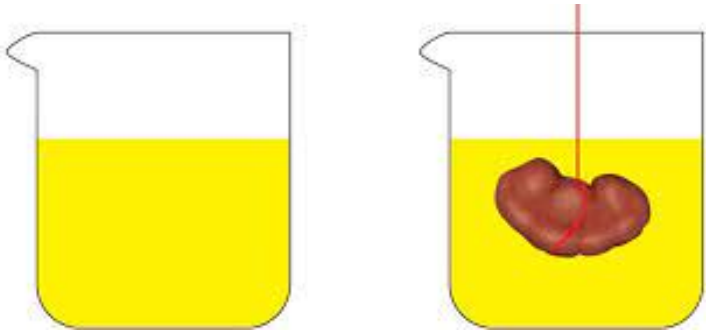
Objective: The broad objective of tissue fixation is to preserve cells and tissue components and to allow for the preparation of thin, stained sections.

What will happen with removed tissues without doing preservation?

1. Once the tissue is removed from the body it will go through a process of self-destruction. This process is known as **autolysis**.
2. If tissue is left without any preservation, then a microbial attack will occur, the process is known as **putrefaction**.

Amis and effects of fixation:

1. Fixation stops autolysis. (Autolysis means destruction of the cell components by their enzymes).
   - The tissue size should be small to avoid from autolysis and it should be around 5*5*5 mm
2. Fixation prevents bacterial decomposition. (As fixatives are toxic for microorganisms)
   - The tissue pieces should be submerged within fixative.



3. Fixation reduces the cell shrinkages and cell modifications.
4. Fixation makes the cells suitable for sectioning (make the tissue hard) and staining (make it possible to have clear stain).
   - Poorly fixed specimens are almost always more difficult to section than those that are well fixed.
5. Fixation increases the visibility of cell components.

Properties of perfect fixative

1. High ability to preservation.
2. High ability to penetration.
3. Easy available.
4. Low cost.
5. Not toxic to worker.
6. Without effect on tissues.

Factors affecting fixation:

1. PH and buffering
2. Penetration rate of fixative
3. Volume of fixative
4. Temperature
5. Concentration
6. Time
7. Specimen dimensions

Examples of fixatives

1. **Formaldehyde solution (formalin solution 10%)**
   - Formaldehyde (40%) - 10 ml
   - Distilled water - 90 ml

2. **Formal Saline**

- Formaldehyde (40%) - 100 ml
- Sodium Chloride - 9 gm
- Distilled Water - 900 ml

3. **10% Buffered Formalin**

- Formaldehyde (40%) - 10 ml
- Sodium dihydrogen phosphate - 0.4 gm
- Disodium hydrogen phosphate (anhydrous) - 0.65 gm
- Distilled water - 90 ml

4. **Bouin's solution**

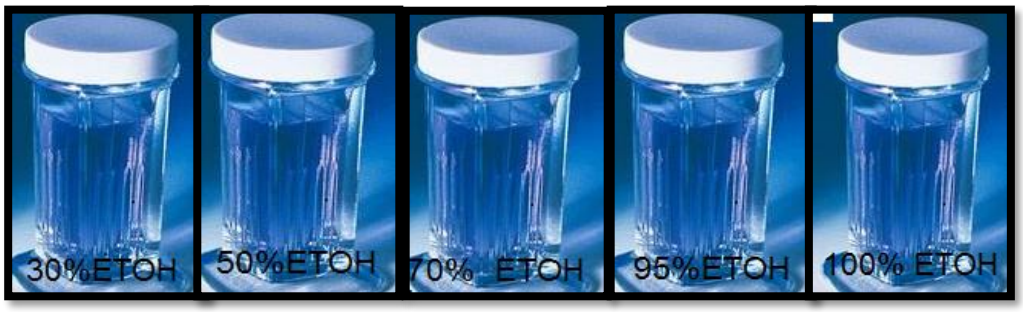
- Saturated picric acid (1.2 gm/ 100 ml) - 750 ml
- Formaldehyde (40%) - 250 ml
- Glacial acetic acid - 50 ml

Dehydration

The process by which the water present in the tissue are removed and replaced by alcohol is called **dehydration**. The dehydration is achieved by passing the tissue through gradually increasing percentage of alcohol.

Because of the negative effects of water on the processes after fixation it's necessary to remove water in the samples and replace it with other solutions by dehydration.

Dehydration is achieved by passing the tissue through increasing concentrations of alcohol (in dehydration, use a series of graded ethanol in ascending concentrations, common dehydrating fluids is ethanol).



The dehydrating agents (dehydrants)

1. **Isopropyl alcohol**
   - is a favored reagent because it is miscible in paraffin.
   - It does not harden the tissue like ethanol.
   - It is expensive.

2. **Ethanol**
   - is a routine dehydrant.
   - Not expensive

3. **Acetone**
   - It is clear, colorless volatile inflammable fluid.
   - It has a rapid action in dehydrating the tissue but produces shrinkage and distortion and subsequent brittleness to the tissue.
   - Low cost is also an advantage.

4. **Dioxane**
   - It dehydrates and clears at the same time. It is miscible with paraffin and with water and alcohol, tissue from dioxane can be transferred straight to paraffin.
   - There is less shrinkage of tissues.
   - Tissues can be left in dioxane without danger of hardening for longer period of time.
   - Disadvantage: It is more expensive than alcohol.
   - It is toxic to man.

Clearing

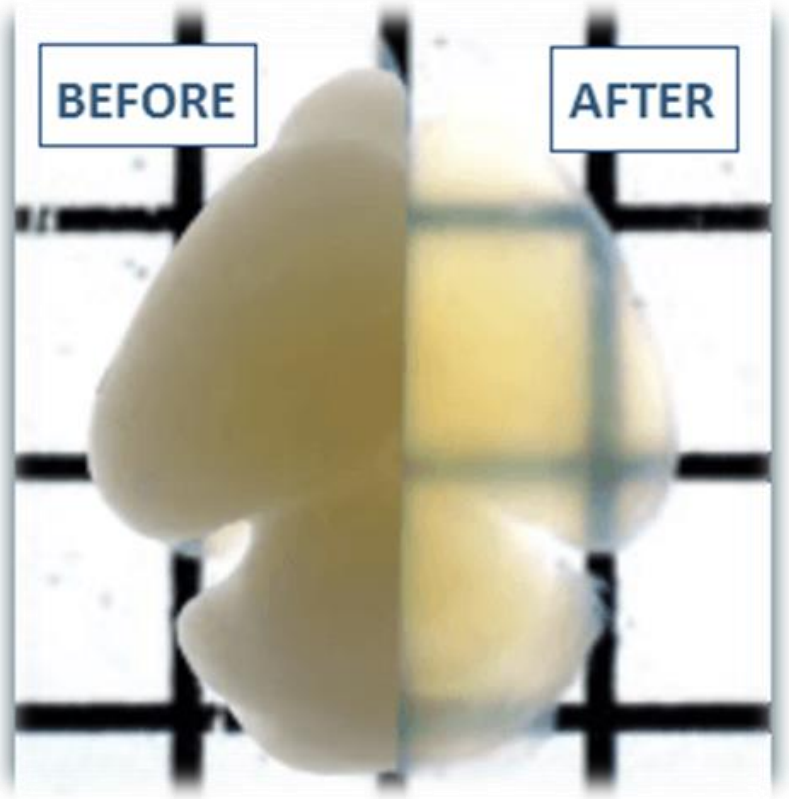
Clearing is the substitution of dehydrating agent by the solvent called clearing agent. The term clearing is also used because of the fact that the solvent or clearing agent imparts transparency to tissue.

Clearing is the transition step between dehydration and infiltration with the embedding medium. Clearing is a process which leaves the tissues clear and transparent.

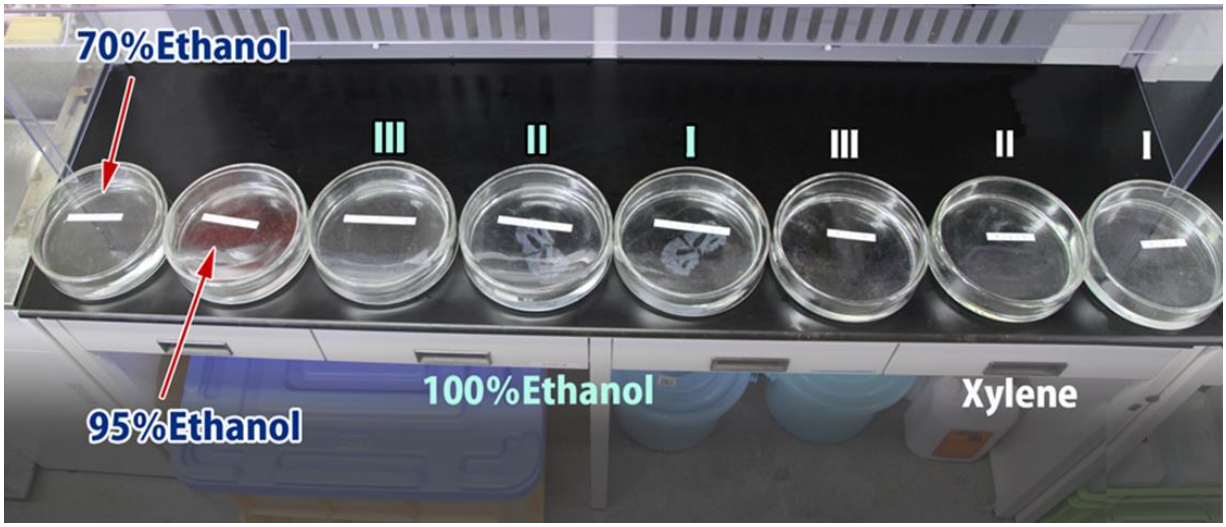
This term relates to the appearance of the tissues after the dehydrating agent has been removed. The end point of clearing can be noted by the transparent appearance of the tissue.

Thus, clearing serves two purposes:

1. Removes alcohol to make paraffin impregnation complete.
2. Acts as solvent for the mounting media which renders the tissues transparent and improves the refractive index, making microscopic examination easier.



Xylene is used in the last dehydration phase instead of alcohol - this is because the wax used in the next stage is soluble in xylene where it is not in alcohol allowing wax to permeate (infiltrate) the specimen.



Commonly used clearing agents are as follows:

1. **Xylene**: Immersion time must not be prolonged otherwise the tissue become brittle.
2. **Toluene and Benzene** are similar in properties to xylene but are less damaging to the tissues on prolonged exposure.
3. **Chloroform** - It is slower in action and it causes less brittleness therefore tissue can be left in it overnight.
   It is expensive.
   It is inflammable.
4. **Carbon tetrachloride** - It has similar properties to chloroform but is cheaper.