**Tissue processing**

**\* Histology:**

It is the branch of science which deals with the gross &microscopic study of normal tissue

**\*Histopathology:**

It is the branch of science which deals with the gross & microscopic study of tissue affected by disease.

**Histotechniquse :**

The techniques for processing the tissue , whether biopsies,larger specimen removed at surgery, or tissue from autopsy so as at to enable the pathologist to study them under the microscope .

**Protocols followed in Histotechniquse**

**1- Recept & Identification**

**2- labeling of the specimen with numbering.**

**3- fixation .**

**4- Washing .**

**5- dehydration .**

**6- clearing .**

**7- impregnation .**

**8- Embedding :**

**9- section cutting .**

**10- staining .**

**11- mounting .**

**protocols followed in Histotechniquse**

* **1- Recept & Identification**

Tissue specimen received in the surgical pathology laboratory have a request form that list the patient information and history along with a description of the site of origin .

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**2-labeling of the specimen with numbering.**

The specimen are accessioned by giving them a number that will identify each specimen for each patient .



**3- fixation .**

**\*** It is a process in which aspecimen is treated by exposing it to afixative for aparticular period of time in order to facilitate the succeeding step .

**\*** The puropose of fixation is to preserve tissue permanently in as life-like astate as possible .

The fixative should be 15-20 times more in volume then the specimen **\***

**\*** Mechanism of action **.** it forms cross linke between amino acide of proteins thereby making them insoluble .

**\*** The bite should of size of approximately 2x2 cm & 4-6 micrometer in thickness for optimum fixation to take place .

**\*** Tiny biopsies or small specimen can be wrapped in filter paper and then put in a cassette & fixed .

**Aim of fixation**

1- It should prevent autolysis & putrefaction of the cell .

2- It should penetrate evenly and rapidly .

3- It should harden the tissues .

4- Increase the optical density .

5- Should not cause shrinkage or swelling of the cells .

6- Must not react with the receptor sites & thus must not interefere with the staining procedure .

7- It must be cheap and easily avaible .

**Properties of an ldeal fixative**

1- Prevents autolysis and bacterial decomposition .

2-Preserves tissue in their natural state and fix all components .

3- Make the cellular components insoluble to reagent used in tissue processing .

4- Preserves tissue volume .

5- Avoid excessive hardness of tissue .

6- Allows enhanced staining of tissue .

7- Should be non toxic and non-allergic for user .

8- should not be very expensive .

**Classification of fixatives**

A) Physical fixatives : heat , freezing …… etc .

B) Chemical fixatives : 1- simple fixatives .

2- compound fixative .

**1-simple fixatives :**

The most commonly used fixative is Formalin . it is prepared by mixing 40% formaldehyde gas in 100 w/v of distilled water . the resultant mixture is 100% formalin .routinely , 10% formalin is used which is prepared by mixing 10 ml of 100% formalin in 90 ml of distilled water .

**Other simple fixative :**

1**-**Picric acid .

2-Osmic acid .

3- Mercuric chloride.

|  |  |
| --- | --- |
| DIS ADVANTAGES FORMALIN | ADVANTAGES FORMALIN |
| 1-Irritant to the nose ,the eyes and mucous membranes . 2- Formation of precipitate of para –formaldehyde which can be prevented by adding 11-16% methanol .3- Formation of black formalin pigment ,Acid formaldehyde hematin.  | 1- Rapid penetration 2- Easy availability&cheap 3- Dose not overharden tissue4- Fix lipids for frozen section5- Ideal for mailing   |

**2- Compound fixatives :**

**a) Microanatomical fixatives :**these are used to preserve the anatomy of the tissue like,10%formalin saline, Zenker's fluid ,Bouin's fluid .

**b) Cytological fixatives :** these are used to fix intracellular structures it two type

|  |  |
| --- | --- |
| **Cytoplasmic fixative** | **Nuclear fixative** |
| Champy's fluid , Regaud's fluid | Carnoy's fluid , Clarke's fluid |

**C) Histochemical fixatives :**These are used to demonstrate the chemical constituents of the celllike Cold acetone , Ethanol

**4- Dehydration .**

It is the process in which the water content in the tissue to be processed is completely reduced by passing the tissue through increasing concentration of dehydrating agents .

The various dehydration agents used are : Ethyl alcohol , Acetone , Isopropyl alcohol ,Dioxane .

The duration of the procedure can be noted down as :

1-30% alcohol – 1 hour .

2- 50% alcohol – 1 hour.

3-70% alcohol – 1 hour .

4-70% alcohol – 1 hour

5- 90% alcohol – 1 hour .

6- 95 % alcohol – 1 hour .

7-Absolute alcohol – 1 hour .

8- Absolute alcohol – 1 hour .

Dehydration is done so that the wax i.e Paraffin wax , which is used for impregnation, can be easily miscible as it is immiscible with water.

**5- Clearing (Dealcoholization)** .

It is the procedure where in the alcohol in the tissue is replaced by fluid which will dissolve the wax used for impregnating the tissues .

 **The various clearing agents used are :**

\* Cedar wood oil : the best agent but is expensive .

 Benzene : it is carcinogenic .\*

 Xylene : it is most commonly used .\*

 chloroform : tixic and expensive .\*

 **6**- **Impregnation** :

# In this the tissue is kept in a wax bath containing molten paraffin wax foe 6-8 hours .the wax is infiltrated in the interices of the tissue which increases the optical differentiation & hardens the tissue & helps in easy sectioning of the tissue .

The various waxes which used are : (paraffin wax ,paraplast , Gelatin )

**Embedding : 7-**

It is done by transferring the tissue which has been cleared of the alcohol to a mould filled with molten wax & is allowed to cool & solidify .after solidification , awax block is obtained which is then sectioned to obtain ribbons .

 \* **Type of moulds : A) Leuckhart's Moulds :** L-shaped brass pieces which is placed in opposing positions & can be manipulated to increase or decrease the size of the block be prepared .

B) Glass or Metal petri dishes

C) Watch glass .

 D) Paper boats .

**8- Section cutting**

It is the procedure in which the blocks which have been prepared are cut or sectioned and thin strips of varying thickness are prepared .

The instrument by which this is done is called as a Microtome

**Type of Microtome** : sliding , rotary , rocking , freezing , base sledge

**Rotary microtome :**

It is the most commonly used . Also known as Minnot, s Rotary microtom

In this the Blok holder moves up and down while the knife remains fixed

It is suitable for cutting of small tissues & serial sections can be taken on it.

**Parts of a Microtome (Rotary)**

A. Block holder .

 B. Knife clamp screws

C. Knife clamps

D. Block adjustment

E. Thickness gauge

F. Angle of tilt adjustment

G. Operating handle





**Tissue floatation bath**

It is thermostatically controlled water both with the inside colored black.

 It is maintained at a temperature maintained 5-6 degree paraffin wax.



**9- Staining :**

Is process by which we give colour to asection , there are hundred of stains available , and can be classification to: Acid stains , Basic stains , Neutral stains .

**Acid stains :**In an acid dye the basic component is colored and the acid component is colorless , Acid dyes stain basic components e.g. eosin stains cytoplasm , the color imparted is shade of red .

 **Basic stains :** In an basic dye the acide component is colored and the basic component is colorless , Basic dyes stain acidic components e.g. basic fuchsin stains nucleus , the color imparted is shade of blue .

 **Neutral stains :** When an acid dye is combined with abasic dye aneutral dye is formed , As it contains both colored radicals , it gives different colors to cytoplasm and nucleus simultaneously .this is the basis of Leishman stain .

**10- Mounting :**

Adhesives used for fixing the sections on the slides , the adhesives like : Albumin solution(mayor's egg albumin) , Starch paste , Gelatin .

Mountants permanent agent : Canada balsam , Dpx , Terpene resin ,