



7

TISSUE PROCESSING

7.1 INTRODUCTION

The technique of getting fixed tissues into paraffin is called tissue processing. This describes the steps required to take animal and human tissues from fixation to the state where it is completely infiltrated with a suitable wax i.e. paraffin wax and can be embedded and ready for section cutting on microtome.



OBJECTIVES

After reading this lesson, you will be able to:

- define tissue processing
- describe its aim and method of processing.

Aim: To process the fixed tissue into a form in which it can be made into thin microscopic sections.

Processing: The steps in this process are dehydration and clearing.

Dehydration: It is the process of removing water from tissues. It is important because paraffin is not miscible with water. Dehydration is usually complete when less than 3-4% of water remains in the tissues. Time required for this depends on:

1. Permeability of tissues
2. Continuous rotation of fluid to prevent stagnation of fluid around tissues
3. Temperature
4. Vacuum applied

Dehydrants: Ethyl alcohol, Methyl alcohol, Butyl alcohol and Isopropyl alcohol.

The most commonly used dehydrant is ethyl alcohol.



Notes

Alcohol Method: The tissues are passed through a series of progressively more concentrated alcohol baths. Concentration of first alcohol bath depends on the fixative and size and type of the tissue, e.g. delicate tissue needs lower concentration of alcohol and smaller interval between two strengths of alcohol.

Usually 70% alcohol is employed as the first solution and 100% as the last solution. After about 40 tissues have passed through the first change of alcohol, it is discarded and all the other changes are brought one step lower. Absolute alcohol at the end is always fresh.

Usually tissues are kept in each solution for 40 to 60 minutes.

Use of copper sulphate in final alcohol: A layer of anhydrous CuSO_4 is placed at the bottom of a dehydrating bottle or beaker and is covered with 2-3 filter paper of approximate size to prevent staining of the tissue. Anhydrous CuSO_4 removes water from alcohol as it in turn removes it from tissues.

Anhydrous CuSO_4 is white in colour while the hydrated form is blue. Therefore, it acts as an indicator for the presence of water.

Advantage of CuSO_4 -

1. Rapid dehydration
2. Prolongs life of alcohol
3. Blue colouration of CuSO_4 indicates that both alcohol and CuSO_4 should be changed.

Acetone - Acetone is clear colourless inflammable fluid which is miscible with water, ethanol. It is used for complete dehydration. Four changes of acetone of half an hour or two changes of one hour are given to achieve complete dehydration of tissues.

Advantages

- Rapid action
- Easily removed by most clearing agents
- Less expensive

Disadvantages

- Highly volatile
- Causes shrinkage and brittleness of tissues
- Dissolves lipid more than ethanol

Clearing – 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

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been removed. If the refractive index of the clearing agent is similar to the protein of tissue the tissue becomes transparent. 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.

Clearing Agents

- Xylene - It is colourless and most commonly used. Two changes of one hour each are given to get the end point. Prolonged treatment hardens the tissues. It is not preferred for brain tissue.

Other Clearing Agents

- Toluene
- Dioxane
- Cedarwood oil
- Chloroform
- Benzene
- Carbol-xylene - clears rapidly, it is kept reserved for material difficult to clear.

7.2 INFILTRATION AND IMPREGNATION

After clearing, tissues are transferred to molten paraffin wax for filtration and impregnation. During this process clearing agent diffuses out and molten wax is infiltrated. The wax which has infiltrated in the tissue gets deposited. This process is called impregnation. Routinely two changes are given in the wax to get proper impregnation. The duration and number of changes required for thorough impregnation of tissue depends on -

1. Size and type of tissues-Longer time is required for thicker tissues. Vacuum reduces the time required for complete impregnation.
2. Clearing agent employed
3. Use of vacuum imbedding

Tissue processing may be performed manually or with the help of automated tissue processor. Routinely 12 containers containing different solutions are used for processing in the following order

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Histology and Cytology



Notes

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Notes

- 10% formalin – container no 1, 2
- 50% alcohol – container no 3
- 90% alcohol – container no 4 & 5
- Absolute Alcohol – container no 6
- Acetone – container no 7 & 8
- Xylene – container no 9 & 10
- Paraffin Wax – container no 11 & 12



INTEXT QUESTIONS 7.1

1. In tissue processing the tissues are impregnated into
2. Steps in tissue processing are &
3. Process of removing water from tissue is
4. Most commonly used dehydrant in tissue processing is
5. Usually alcohol is employed as first solution
6. is used as an indicator for the presence of water
7. Clearing is the process which leaves the tissues &
8. Clearing serves two purposes namely &
9. Most commonly used clearing agent is
10. The process by which the infiltrated tissue gets deposited is called

7.3 TISSUE PROCESSING (PRACTICAL-1)

Tissue processing can be performed either manually or through automated tissue processor. The device can handle larger number of tissues, process more quickly and produces better quality outcome.

Two types of devices are available

- Tissue transfer or dip dunk
- Fluid transfer or enclosed

Advantages of automated tissue processor - Saves time, decreases human error, effective fluid circulation, Temperature can be adjusted and vacuum/pressure can also be incorporated.

Tissue Transfer Type

The machine consists of a time clock, a circular superstructure that contains basket carrier, a receptacle basket and receptacles (stainless steel or plastic capsules), and a circular deck which holds the reagent beakers and plastic baths. Small blocks of tissue are enclosed in the perforated capsules. These capsules are placed in the basket which in turn is attached to one of the yokes in the superstructure, while it is in the raised position. When the superstructure descends the basket is immersed in the first solution and other reagent beakers are covered preventing evaporation of reagents. To move the basket from one reagent to the next the entire superstructure ascends and descends at scheduled intervals controlled by the time clock. During immersion the basket rotates so the infiltration of fluid into the tissues is optimum. The entire process takes about 16 hours. The machine is started in the evening so that the process is complete in the morning, and embedding is done.



Notes



Fig. 7.1: Automated Tissue Processor

Enclosed Type

In this type of tissue processor the tissues remain in one container but reagents get changed at scheduled interval.

Manual

In this process the tissue is changed from one container of reagent to another by hand.



Notes



Fig. 7.2: Enclosed Type Tissue Processor

Advantages

- Can be used when the number of tissue blocks is limited
- Non-availability of automated tissue processor

Disadvantages

- Difficult to use when large number of tissue blocks are to be processed
- Proper agitation of reagent not achieved
- More evaporation of reagents
- Process is tedious and requires constant attention

Precautions

1. Labels should be written with graphite pencil, India ink or typed.
2. The fluid used in complete dehydration and clearing tend to become contaminated with fluid carried over from previous vat by the tissue. Every alternate day daily the last solution in the series are replaced by fresh solution of 100% alcohol, acetone and xylene and the previously used one moved forward while the first one is discarded. Other reagents are changed twice a week or earlier with an average work load. It is far better to change the reagents a day earlier than to have a precious surgical specimen improperly infiltrated.



WHAT HAVE YOU LEARNT

- The technique of getting tissues fixed into paraffin is called tissue processing so that thin microscopic sections can be achieved.

Tissue Processing

- The main steps in processing are dehydration and clearing
- Dehydration is the process of removing water from tissues and time required depends on permeability of tissues, temperature, vacuum applied and continuous rotation of fluid to prevent stagnation of fluid around tissues.
- Most commonly used dehydrant is ethyl alcohol
- Anhydrous copper sulphate removes water from alcohol as it inturn removes it from tissues and acts as a indicator for the presence of water
- Acetone is used for complete dehydration
- Clearing is the process which leaves the tissues clear and transparent
- Clearing serves two purpose as it removes alcohol to make paraffin impregnation complete and acts as a solvent for mounting media
- Xylene is the most commonly used clearing agent
- Impregnation is the process by which the infiltrated wax gets deposited



TERMINAL QUESTIONS

1. Explain dehydration process of tissue processing
2. Explain clearing process of tissue processing
3. List the advantages of copper sulphate



ANSWERS TO INTEXT QUESTIONS

7.1

1. Paraffin wax
2. Dehydration and clearing
3. Dehydration
4. Ethyl alcohol
5. 70%
6. Anhydrous copper sulphate
7. Clear & Transparent
8. Removes alcohol and acts as a solvent
9. Xylene
10. Impregnation

MODULE

Histology and Cytology



Notes