

MODULE

Histology and Cytology



Notes

15

CRYOSTAT AND FROZEN SECTION

15.1 INTRODUCTION

Sections are prepared quickly for histological examination by freezing the tissue. The section should be thin, and without water crystals. It is an important procedure for quick diagnosis.



OBJECTIVES

After reading this lesson, you will be able to:

- enlist the indications of frozen section
- explain the disadvantages of frozen section
- describe cryostat.

15.2 PURPOSES OF FROZEN SECTION

Frozen sections are used for following purpose

- Quick diagnosis
- Study the margins of cancer
- Enzyme histochemistry
- Immunohistochemistry
- Detection of lipid
- Some molecular procedures

Disadvantages

- Morphology is distorted
- Cellular details are not well seen,
- Staining is not very good
- Some special stains cannot be performed.

Handling of specimen

Tissue must reach histopathology laboratory immediately. To avoid tissue being dried it should be kept in saline. The size of the tissue should be small thin, so that good smooth sections can be obtained and freezing is quick. Thickness of the tissue should be about 3mm to 4mm. The tissue can directly be taken to cryostat or can be fixed with 10% formalin or formol –alcohol

Embedding media

Sucrose (20%) or a drop of water may be applied on the chuck. Optimum Cooling temperature (OCT) compounds or 20% sucrose gives good result. Other embedding media are available with cryostat. Completion of freezing is observed by the change of color of tissue which turns glossy white. Freezing should be done fast. This will prevent ice crystal formation. The morphology is better preserved and artifacts are less.

Different freezing substances are used depending upon the availability and feasibility.

Carbon Dioxide gas is most commonly used with freezing microtome. This gives good results. Liquid Nitrogen is another substance used for freezing the tissue. An expertise is required while using liquid nitrogen to get uniform freezing. Aerosol sprays are also used for this purpose.

Cryostat: Cryostat is used in medicine to cut histological sections. They are usually used in a process called frozen section histology. The cryostat is essentially an ultrafine “deli-slicer”, called a microtome, placed in a freezer. The cryostat is usually a stationary upright freezer, with an external wheel for rotating the microtome. The temperature can be varied, depending on the tissue being cut - usually from minus 20 to minus 30 degree Celsius. The freezer is either powered by electricity, or by a refrigerant like liquid nitrogen. Small portable cryostats are available and can run off generators or vehicle inverters. To minimize unnecessary warming all necessary mechanical movements of the microtome can be achieved by hand via a wheel mounted outside the chamber. Newer microtomes have electric push button advancement of the tissue. The



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precision of the cutting is in micrometres. Tissue are sectioned as thin as 1 micrometre. Usual histology slides are mounted with a thickness of about 7 micrometres.

Specimens that are soft at room temperature are mounted on a cutting medium (often made of egg white) on a metal “chuck”, and frozen to cutting temperature (for example at -20 degrees C). Once frozen, the specimen on the chuck is mounted on the microtome. The crank is rotated and the specimen advances toward the cutting blade. Once the specimen is cut to a satisfactory quality, it is mounted on a warm (room temperature) clear glass slide, where it will instantaneously melt and adhere. The glass slide and specimen are air dried, and stained. The entire process from mounting to reading the slide takes from 10 to 20 minutes, allowing rapid diagnosis in the operating room, for the surgical excision of cancer. The cryostat section quality is poorer as compared to fixed tissue sections.



INTEXT QUESTIONS 15.1

1. To avoid drying, the tissue should be kept in
2. Tissues can be fixed with
3. or is used as embedding media
4. gas is most commonly used with freezing microtome



WHAT HAVE YOU LEARNT

- Sections are prepared quickly for histological examination by freezing the tissue
- Frozen section is used for quick diagnosis, studying margins of cancer, enzyme histochemistry, Immunohistochemistry
- Tissues must reach the laboratory immediately and to prevent drying of tissue it should be kept in saline
- The tissue can be fixed with 10% formalin or formal alcohol
- Optimum cooling temperature or 20% sucrose is used as embedding medium
- Ice crystal formation may be prevented by freezing the specimen fast
- Carbon dioxide gas is most commonly used with freezing microtome
- Cryostat is used for cutting histological frozen sections



TERMINAL QUESTIONS

1. What is a cryosection?
2. Write two indications of cryosections.
3. What is a cryostat?
4. What are the substances used in cryostat to cool the device?
5. Write three embedding media used for cryosections.



Notes



ANSWERS TO INTEXT QUESTIONS

15.1

1. Saline
2. 10% formalin
3. Optimum cooling temperature or 20% sucrose
4. Carbon dioxide