HISTOLOGICAL TECHNIQUES

FROZEN SECTION TECHNIQUE

LECTURE 7

LEARNING OUTCOMES

Student should be able to:

- identify the equipment used to produce frozen sections.
- explain the procedures involve in frozen section technique.

1.0 FROZEN SECTION TECHNIQUE

Introduction

- Sections are prepared quickly for histological examination by freezing the tissue.
- The section should be thin, and without water crystals. Fast freezing to avoid ice crystal artifact.
- It is an important procedure for rapid diagnosis and surgical consultation.
- The cryostat is a key instrument to make frozen cut histological sections (cryosections).
- Many histochemical methods cannot be done without freezing microtomy because the steps involved in paraffin sectioning destroy or lose the material of the tissues.

1.0 FROZEN SECTION TECHNIQUE

Purposes of frozen section:

- Quick diagnosis
- Study the margins of cancer
- Enzyme histochemistry (ATPase, NADPH in muscle biopsy samples)
- Immunohistochemistry (target antigens in tissues)
- Detection of lipid
- Some molecular procedures

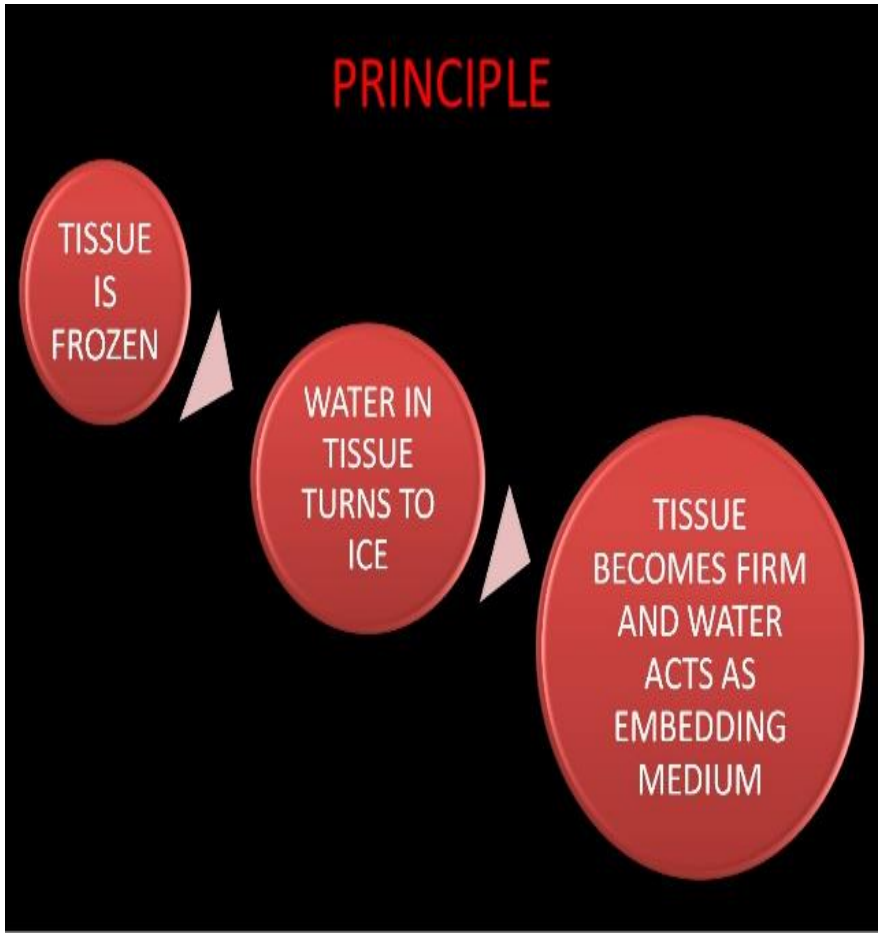
Disadvantages of frozen section :

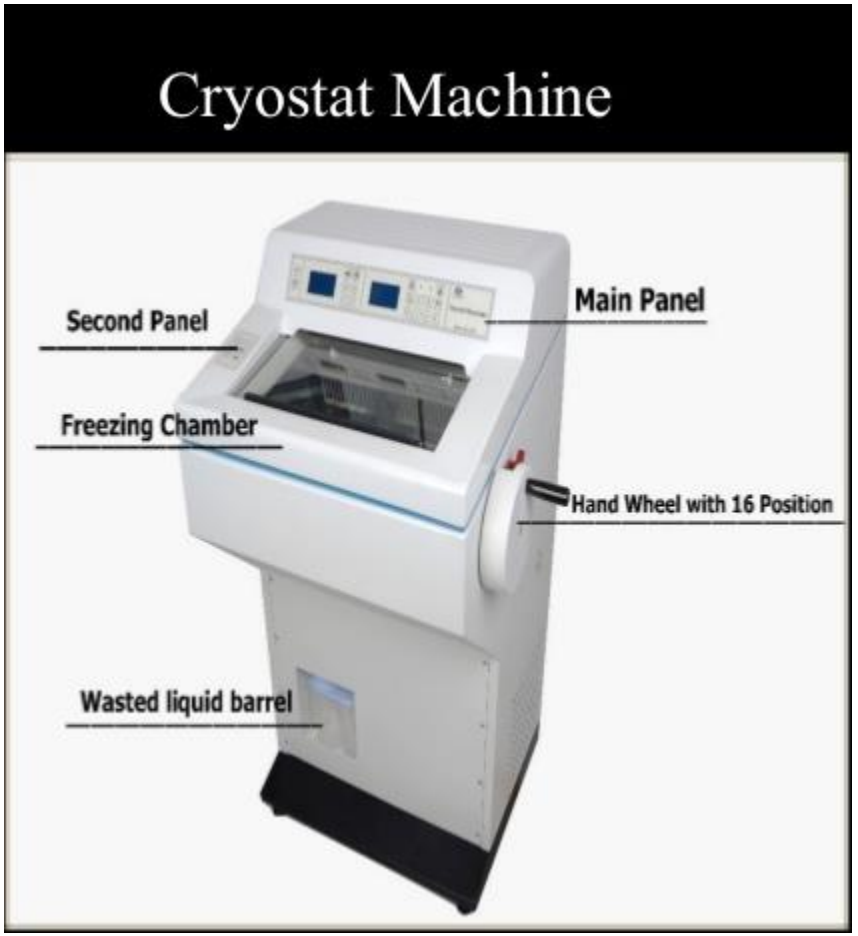
- The structural details are distorted because of the lack of embedding material.
- It is impossible to obtain serial sections.
- The staining is not satisfactory.
- Many freezing artifacts or ice crystals found in sections.
- Some of the finer details cannot be determined.

IMPORTANT

- Turnaround time (TAT) – Period for completing a *processcycle.* TAT is around 20 minutes for frozen section.
- TAT measured from the time pathologists received specimens to the time they communicated frozen section results to the surgeon.
- Specimen should be sent fresh; without formalin.

1.1 CRYOSTAT / FREEZING MICROTOME





FREEZING TECHNIQUES

- Liquid nitrogen (-190°C)
- Isopentane cooled by liquid nitrogen (-150°C)
- Dry ice (-70°C)
- Carbon dioxide gas (-70°C)
- Aerosol sprays (-50°C)

1.1 CRYOSTAT / FREEZING MICROTOME

- 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 - 20 to - 30°C.
- The freezer is either powered by electricity, or by a refrigerant like liquid nitrogen.
- To minimize unnecessary warming all necessary mechanical, movements of the microtome can be achieved by hand via a wheel mounted outside the chamber.

1.1.1 TISSUE PREPARATION

- Selection of tissue:

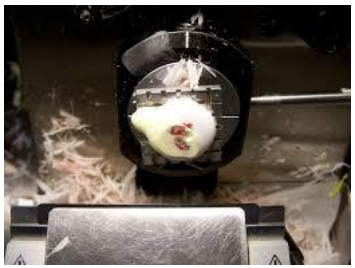
✓Tissue sample should represent the specimen

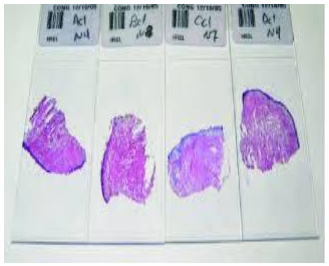
✓Tissue sample should not contain any necrotic area.

✓Block of fresh tissue is trimmed with a sharp scalpel.

✓Thickness of the tissue should be about 3mm to 4mm.

- Sample once collected should be frozen immediately for cryostat sectioning OR can be fixed with 10% formalin or formol alcohol.
- Boiled for 30 to 60 seconds along with the fixative in test tube or beaker.
- It is then washed in distilled water.





1.1.2 FREEZING THE TISSUE

- Place the tissue on the freezing stage/cryostat specimen disc/ chuck
- Cover the entire tissue block with cryo-embedding media.
- Air bubble should be removed

- a hole in a block

- Amount of compound

- block could be slipped and cause of unstable sectioning

- Size and position of tissue

- uneven staining, unable to secure blank place in a block

- Freezing spray is used to instantly cool or freeze samples.

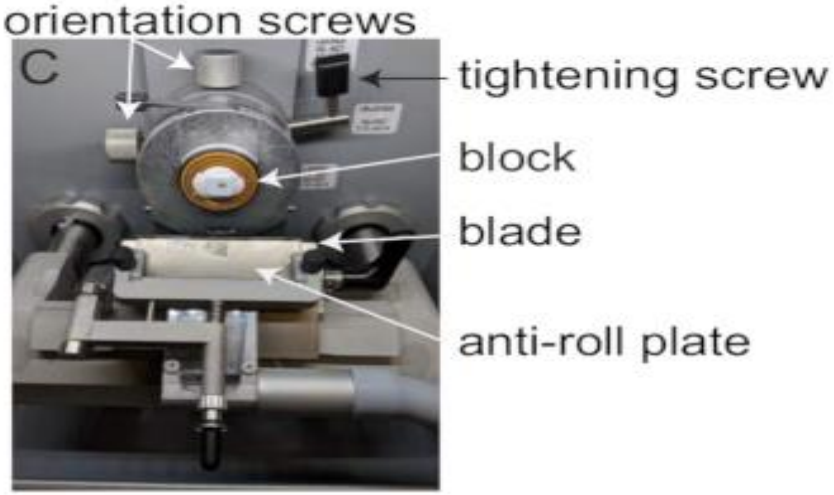
  Eg: Cryo Spray

Freezing spray

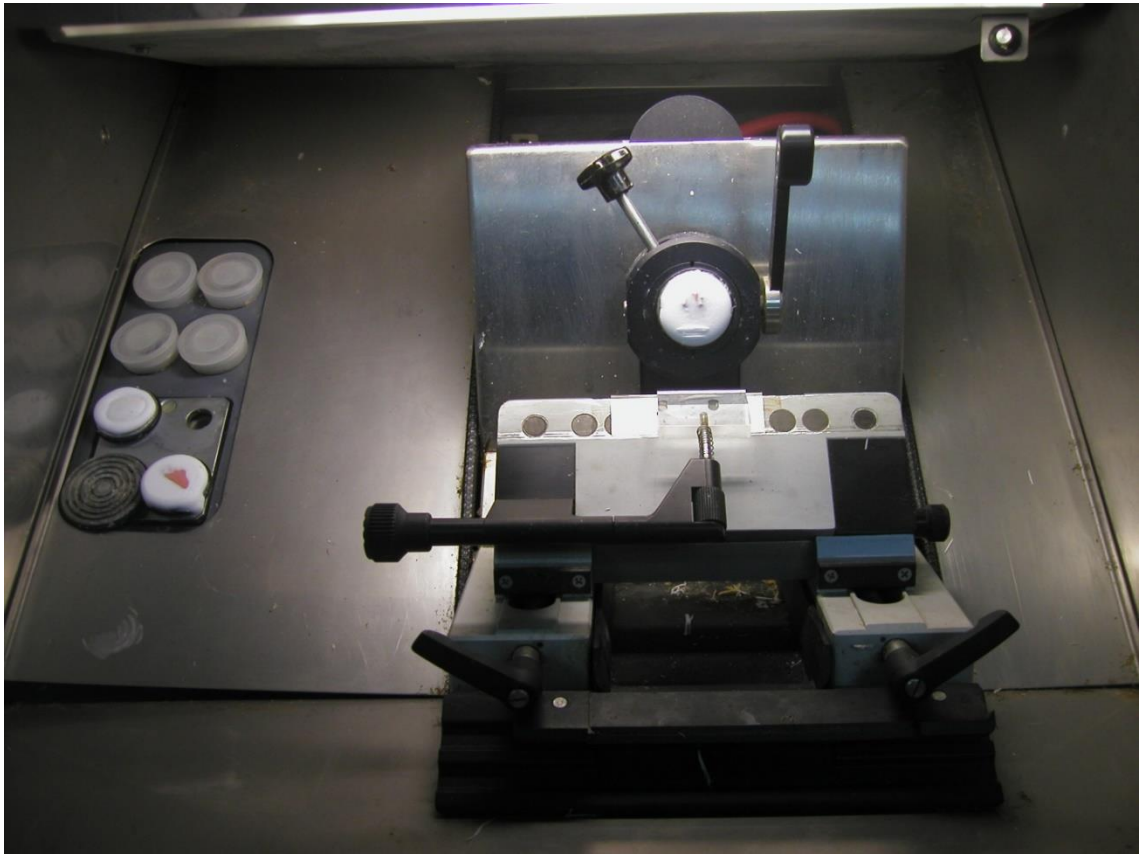
Cryo-embedding compound, Optimal cutting temperature (O.C.T) compound & Tissue freezing medium (TFM)

Cryostat specimen disc/chuck

- Orient the tilt and angle so that the face of the block is in the plane of sectioning and the block edge is parallel to that of the knife.
- Make appropriate adjustments and move the knife edge in a way that it just touches the block.
- Wipe off the surface of the knife, knife edge and the anti-roll plate and keep them clean.
- Position the antiroll plate. Tighten the clamp on the object holder securely.
- Set the feed mechanism to the desired thickness.
- Make sections.



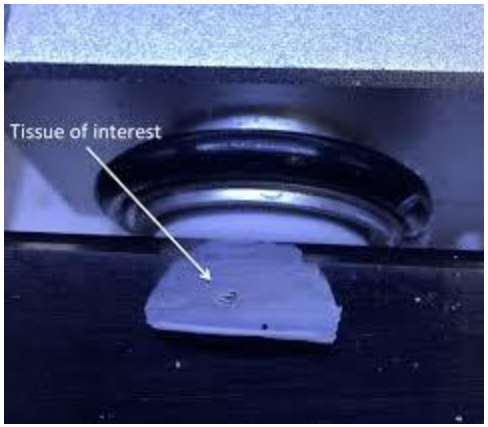
Anti-roll plate



Inside cryostat chamber.
The optimal temperature for cryostat sectioning depends on the nature of the tissue and on whether the tissues have been freshly frozen or pre-fixed with subsequent cryoprotection.

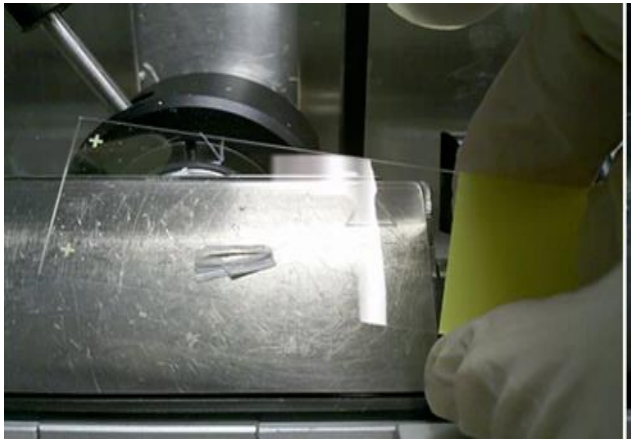
1.1.3 SECTIONING

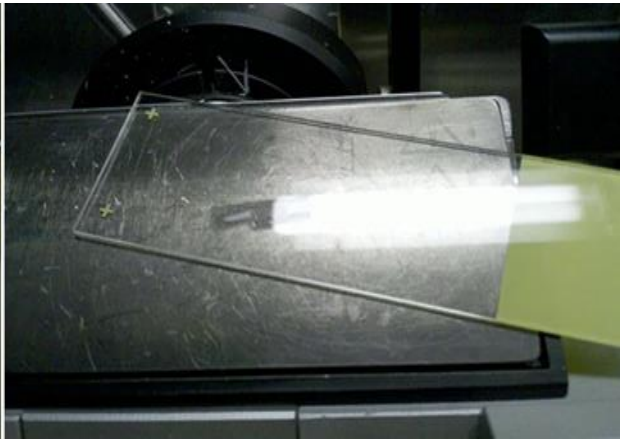
- The cryostat cuts individual sections unlike the ribbons of sections with the paraffin preparations.
- Sections should be cut with a slow and even motion at about 10 to 15 u thickness.
- The section will glide smoothly and flat beneath the antiroll plate.
- The cutting of a frozen section requires experience and touch.
- Do not freeze the tissue too hard or the sections may shatter.
- If it has become too soft the sections will also shatter or fracture.



1.1.4 PICKING UP OF SECTION

- Frozen section should handle carefully.
- The antiroll plate is flipped back after the section is cut.
- The glass slide is lowered gently, the section will automatically transfer from the knife to a warm (room temperature) clear glass slide, where it will instantaneously melt and adhere.
- Never press the slide down on the section.
- No adhesive is needed to stick the section of unfixed tissue on the slide. Air dry the section for about 30 to 60 sec before staining.
- The cryostat section quality is poorer as compared to fixed tissue sections.
- In case of a fixed section, use albumin as the adhesive.
  - The section may be dried before staining (56°C for 30 to 60 minutes or at 37°C overnight).





After the tissue section is laid flat using the paintbrush(es), tap the glass slide (face down) against the tissue section. The section sort of melts onto the slide.

1.2 STAINING

- The staining technique for frozen sections is basically the same as for paraffin sections.
- Since the major use of the cryostat is for rapid surgical diagnosis, time may be saved by using unfixed tissue.
- The tissue will be attached easily to the glass slide without the need for any adhesive mixture.
- The advantage of using unfixed frozen sections is that the cellular enzymes and other substances that may be studied by histochemical techniques are preserved.
- If the tissues are fixed, they should be washed thoroughly before staining.
- For rapid surgical diagnosis two methods are widely used :

i. Haematoxylin & eosin

ii. Polychrome methylene blue

1.2.1 HAEMATOXYLIN & EOSIN STAIN

- The routine H and E staining method can be applied.

Staining procedure

1. Fix in pure acetone for 15 to 20 seconds.
2. Place in water until no longer greasy or cloudy.
3. Place in Harris's haematoxylin stain for 1 to 2 minutes.
4. Wash in running water for 5 to 10 seconds.
5. Dip in 0.5% sodium borate until blue.
6. Place in 70% ethanol for 5 seconds.
7. Counterstain in 1% alcoholic eosin, 1 to 2 quick dips.
8. Wash well in running water.
9. Dehydrate, clear and mount.

1.2.1 …CONTINUE

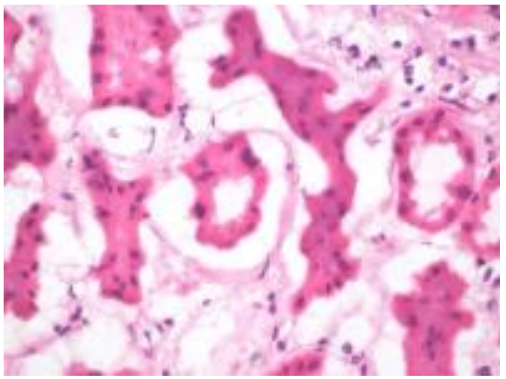
- Alternatively :

1. Stain with Harris's haematoxylin for 3 to 10 minutes.
2. Wash in running tap water.
3. Differentiate in 1% acid alcohol.
4. Dip in 2% ammonia water the sections will change to a blue colour.
5. Wash and counterstain with eosin.
6. Dehydrate, clear and mount.

FROZEN SECTION H & E STAIN

Compression Artifacts

- Cellular tissues will be compressed by expanding ice bubbles.
- Cause: slow freezing of tissue
- Solution: fast (flash/snap) freezing This is most evident in edematous tissues.
- Eg: kidney parenchyma
- Picture A shows the renal tubules being compressed by the clear ice crystals.
- Picture B shows tissue which was never frozen



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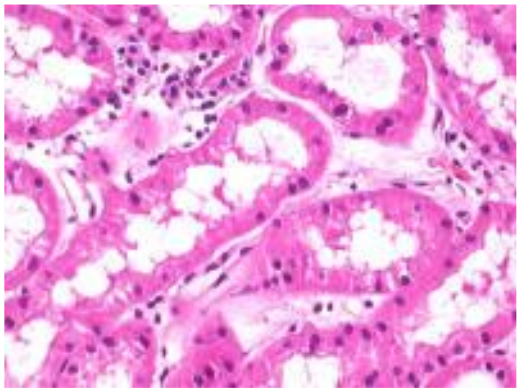


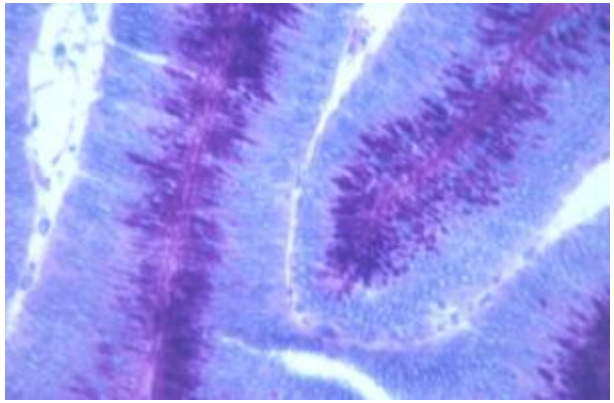
B

1.2.2 POLYCHROME METHYLENE BLUE STAIN

- The method of polychrome methylene blue staining is recommended for rapid diagnosis with frozen sections.

Staining procedure

1. Stain the section with polychrome. methylene blue for 30 to 60 seconds.
2. Rinse with water.
3. Dehydrate, clear and mount.



LAB PRACTICAL

- Frozen Sectioning