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AN OVERVIEW FROZEN SECTIONS (RAPID TECHNIQUE)

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Summary

Frozen section manner is a appreciated instrument utilized to formulate tissue slides quickly for explanation microscopically. Frozen section method is operated in various research and clinical situations. Pathologically, frozen sections are usually utilized for rapid diagnosis during surgery, presenting guidance for our surgical generations. Frequent applications of research depend on the frozen section procedure to make microscopic slides applying a host of molecular and immunohistochemical methods as well as stylish morphologic. Frozen section slides preparation is a complicated technical method needing an advanced refined technical service, in addition to the histology understanding, pathology and micro anatomy of the tissues preparing for examination.

INTRODUCTION

Literature review

Firstly, Lang apparently applied the usage of freezing for tissues hardening during the 19th century.[1] Made the revolutionary attempt of FS procedure usage for histopathological conclusion. Beside the progression of a recent dependable FS method, surgical complications might be referred soon. Excitingly, this advancement positioned the scientists of pathology base on the team of surgical theater where he started. Primary frozen section (FS) methods were hard, challenging, and essential as considerable art as science.[2] It is an honor to the technique was recognized absolutely.[3,4] The procedure announced in 1949 is interesting as compared with cryostat as recent events, in which the new sample was sited in an adhesive ten folds its capacity as well as increased its temperature until reached the point of boiling. The fixed (established) block is as well froze among dry ice pieces then dissection by

microtome knife utilization for creation of block with 10-15 um thickness. All sections are settled in a distilled water cup and consequently transferred by a glass type rod inside a cup containing toluidine blue solution in addition to carbol fuchsin. Following recurrent washing in a beaker series including purified water, the section is fixed onto a clean surface of glass slide and as soon as coated with 30% of cane sugar or sucrose and then cover slipped. This provision will last an approximately 60 minutes, and if the coverslip is perimetered with mount of flexible collodion, the section can be well-preserved for frequent days.[4]

In 1953 announced a slight change to the procedure, freezing the unfixed tissue immediately to a Spencer microtome and manipulating the section thickness average 10-15 um with a glass rod of stirring except for the first,[5,6] while in 1957, it fundamentally achieved the method by quickly inserting the unfixed example onto the microtome freezing condition step and subjecting it to occasional carbon dioxide gas liberation that caused rapid freezing, followed by tissue excising with 25um thickness and staining it with a droplet of aqueous thionine. Thionine in dye typically dissipates after 48 hours. For permanent sectioning,[6] recommended soaking the section in a limited drops of 95% concentration of alcohol and staining with the same haematoxylin and eosin (H&E) solution applied for paraffin implanted tissue sectioning. Slides created in this manner could be kept indefinitely.

The concept of frozen section

Occasionally, while performing surgical events, it is required to obtain an immediate conclusion of a pathologic method. The doctor of surgery may wish to determine whether the boundaries of his resection for a cancerous neoplasm are distinct prior to closure, or an unanticipated unhealthy procedure may be discovered and need a diagnostic evaluation in order to determine what to do subsequent, or it might be compulsory to decide whether the suitable tissue has been attained for additional checkup of a disease course. This may be achieved by implementing an FS. It must be seen that the method of FS is based on the study of morphological characteristics of the tissue utilizing customized H&E dyes and does not include any other supportive assessment methods which including special or immunohistochemical dyes that are routinely used in conclusion.

What is the cryostat

The cryostat is the critical instrument for cryosection which is essentially a microtome inside a freezer. The microtome is comparable to a highly precise "deli" slicer, having a capability

of slicing segments as slim as 1 micrometer thick. Typically, slices tissues are cut at a thickness of 5 to 10 micrometers.

Procedure

- Fast freezing cause converting of water to ice form in the tissue sample, which acts as an inserting medium, facilitating the sectioning of tissue. The tissue becomes firmer when the temperature is reduced, while temperature raising results in tissue softening. Several critical points to consider include the following:
- The cryostat machine's temperature range is typically 0C to -350C. The greater part of tissue could be sectioned properly ranged -150C and -250C. Water-containing tissue samples can be partitioned at elevated temperature, whereas more fatty tissues must be partitioned at decreased temperature. Inside the rotary microtome, the knife is immobilized as well as the movement of tissue happens with the rotary wheel assistance.
- Storing of tissue could be on its shelf on single border of the microtome. This assists in maintaining the samples in the temperature of freezing, as the temperature of tissue shelf is typically below than the temperature of cabinet. Small place available to make the knife and brush holder in position anterior for the machine of microtome.
- To ensure consistent burden along the entire length of the tissue samples being sectioned, the knife-edge should be secured with the knife angle to the holder maintained ranging between 5-7 degrees. In front of the knife, an antiroll plate is available to prevent the tissue from rolling during the partitioning process. The antiroll plate is typically made from glass material trapped in a metal structure.
- Additionally, a cool sable hair brush is available for unrolling tissue.
- The sample holder is available in a variety of sizes and shapes, based on the manufacturer.
- To hold the tissue in place, an ideal cutting temperature (OCT) composite like as water-soluble glycols or resin will be benefited as an implanting medium.

Sectioning

- Recognize the sample of tissue and requisition kind first.
- Examine the clinical data available, as it may aid in establishing probable differential diagnoses.
- Inspect the tissue's gross appearance in characterizing of consistency texture, color, and the sutures existence operated to denote the sample's position anatomically.

- Determine the resection planes and boundaries. Separate the medial and lateral edges with contrasting ink colors. Confirm the freshness of tissue, favorite to be dehydrated, without gauze, and staples or sutures before partitioning. After that, the tissue is sliced into several tiny fragments to aid in condition of freezing. Several tissue segments must be acquired to avoid mistake and to better understand the fundamental disease. Using mild pressure, partition the furthermost critical part utilizing a sharp blade.
- To embed tissue in the mold, place a small piece of tissue in the center and pour extra OCT over it. After that, a tissue holder is placed over the tissue.
- Put the tissue inside the chamber for sections of freezing. Cold spray might be used to expedite the procedure.
- Align the cutting knife properly when loading.
- Once frozen, the tissue will look yellowish. Put the frozen tissue in the microtome's bearer and trim for removal of any surplus OCT. This makes the cover of the tissue fit for sectioning.
- Using a chilled brush, gently excise the tissue and distribute it on the antiroll sheet.
- Firmly compress a glass material of slide above the section and immediately confirm for 1minute in methanol. Ethanol 95% concentration could as well be utilized for tissue repairing (for some seconds). Fast fixation process is required to avoid inflated cells and undistinguishable cell edges.

Staining

Typically, toluidine blue as well as hematoxylin and eosin (H&E) staining are used. The slide is cleaned in water tap and then immersed for 60 seconds in hematoxylin then subsequently washed for 5 seconds under water tap, tracked by 5 seconds again in Scott's tap of water. After dipping the slide in eosin for 20 seconds, it is immediately washed in tap water.

Controls

Controls are required in all fields of science. Because pathology is not a precise knowledge in addition to controls are not always accurate, nonetheless every effort is done to ensure the accuracy of our frozen partition. The frozen tissue is given for permanents with the designation "frozen section control." This tissue must be kept isolated from any other tissue succumbed for permanents. Thus, the pathologist has a restricted control over his frozen specimen. When anything on the permanents seems to be significantly different than on the frozen, the surgeon or physician caring for the patient should be alerted promptly.

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