



PERSPECTIVE



Note on Types of Tissue Formation Methods

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Description

Histopathology is the microscopic examination of biological tissues to observe the appearance of sick cells. A biopsy, which is a process that includes obtaining a tiny sample of tissue and is usually performed by a pathologist who is an expert in disease diagnosis, is common in histopathology.

The methods necessary to take an animal or human tissue from fixation to the point where it is entirely infiltrated with an appropriate histological wax and can be imbedded ready for section cutting on the microtome are referred to as "tissue processing". Tissue processing can be done manually, however it is far more easy and efficient to use an automated tissue processing machine when dealing with several specimens. These devices have been around since the 1940s and have gradually improved to be safer to operate, handle bigger specimen numbers, process faster, and generate higher-quality results. Tissue-transfer machines, in which specimens are transported from container to container to be processed, and fluid-transfer machines, in which specimens are maintained in a single process chamber or retort and fluids are pumped in and out as needed, are the two basic types of processors. To improve processing and shorten processing times, most current fluid-transfer processors use higher temperatures, better fluid circulation, and vacuum/pressure cycles.

Importance of tissue processing

The necessity of tissue processing is stressed by most laboratory supervisors to their personnel. It is important to emphasize that using an incorrect processing schedule or making a fundamental error can result in the generation of tissue specimens that cannot be sectioned and hence will not provide any valuable microscopic information. When dealing with human diagnostic tissue, where the entire specimen has been processed, this can

be disastrous. There isn't any extra tissue. There is no such thing as a diagnosis. However, there is one patient to whom an explanation is required.

Obtaining a recently collected specimen

Fresh tissue samples will be collected from various locations. It's worth noting that when they're removed from a patient or an experimental animal, they're easily injured. They must be treated with care and correctly repaired as soon as possible following dissection. Fixation should preferably take place at the time of removal, such as in the operating room, or as soon as possible after transport to the laboratory if that is not possible.

Fixation

A liquid fixing agent, such as formaldehyde solution, is used to fix the specimen. This will gradually enter the tissue, creating chemical and physical changes that will harden, preserve, and protect the tissue from further processing. There are just a few reagents that can be used for fixing since they must have specific qualities that make them suited for the job. The most common fixative for preserving tissues that will be processed to generate paraffin slices is formalin, which is normally in the form of a phosphate-buffered solution. Ideally, specimens should be fixed for long enough for the fixative to penetrate every portion of the tissue, and then for another period to allow the fixation chemical reactions to reach equilibrium.

Dehydration

Because melted paraffin wax is hydrophobic (insoluble in water), the majority of the water in a specimen must be eliminated before wax can be infiltrated. Immersing specimens in a series of ethanol (alcohol) solutions of increasing concentration until pure, water-free alcohol is obtained is a typical method. Ethanol is miscible with water in all quantities; therefore the alcohol gradually replaces the water in the specimen. To minimize signif-

icant tissue distortion, a succession of escalating concentration is used.

Clearing

Despite the fact that the tissue is now essentially water-free, we are still unable to permeate it with wax due to the fact that wax and ethanol are highly incompatible. This step is known as “clearing,” and the reagent used is known as a “clearing agent.” The word “clearing” was adopted because many clearing agents, due to their relatively high refractive index, provide optical clarity or transparency to the tissue.

Infiltration of wax

A suitable histological wax can now be injected into the tissue. The paraffin wax-based histology waxes are the most popular, despite the fact that many alternative reagents have been investigated and employed for this purpose throughout the years. At 60°C, a common wax is liquid and can be infiltrated into tissue, then cooled to 20°C, where it solidifies to a consistency that permits pieces to be cut regularly. These waxes are made out of pure paraffin wax and a variety of additives, such as styrene or polyethylene resins.