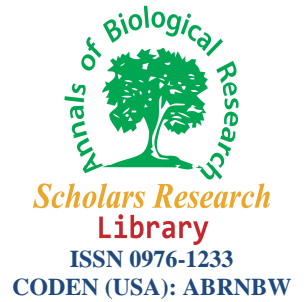




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Tissue processing: An Overview

Hamed ganjali

Ph.D Student of Histology, Veterinarian Department, Ferdowsi university of Mashhad, Iran

ABSTRACT

The present study is about Histology is the study of the microscopic anatomy of cells and tissues of plants and animals. It is commonly performed by examining cells and tissues by sectioning and staining, followed by examination under a light microscope or electron microscope. Tissues from the body taken for diagnosis of disease processes must be processed in the histology laboratory to produce microscopic slides that are viewed under the microscope by pathologists. The techniques for processing the tissues, whether biopsies, larger specimens removed at surgery, or tissues from autopsy, are described below. The persons who do the tissue processing and make the glass microscopic slides are histotechnologists.

Key words: Histology, biopsies, autopsy, tissue processing, histotechnologists.

INTRODUCTION

Tissue Processing

If tissue is completely fixed, processing problems are less likely to occur.

Once the tissue has been fixed, it must be processed into a form in which it can be made in to thin microscopic sections. The usual way this is done is with paraffin. Tissues embedded in paraffin, which is similar in density to tissue, can be sectioned at anywhere from 3 to 10 microns, usually 6-8 routinely. The technique of getting fixed tissue into paraffin is called tissue processing. The main steps in this process are dehydration and clearing.

First, the water from the tissues must be removed by dehydration. This is usually done with a series of alcohols, say 70% to 95% to 100%. Sometimes the first step is a mixture of formalin and alcohol. Other dehydrants can be used, but have major disadvantages.

The next step is called "clearing" and consists of removal of the dehydrant with a substance that will be miscible with the embedding medium (paraffin). The commonest clearing agent is xylene. Toluene works well, and is more tolerant of small amounts of water left in the tissues. Chloroform used to be used, but is a health hazard, and is slow. Finally, the tissue is infiltrated with the embedding agent, almost always paraffin. Paraffins can be purchased that differ in melting point, for various hardness, depending upon the way the histotechnologist likes them and upon the climate (warm vs. cold). A vacuum can be applied inside the tissue processor to assist penetration of the embedding agent.

Fixation

Fixation is the single most influential factor in the long sequence of steps between procurement of the specimen and cover slipping the stained slide; nearly any other step can be reversed to ameliorate a problem.

The purpose of fixation is to preserve tissues permanently in as life-like a state as possible (Hopwood, 1996). Fixation should be carried out as soon as possible after removal of the tissues (the case of surgical pathology) or soon after death (with autopsy) to prevent autolysis (Eltoum et al., 2001).

There is no perfect fixative, though formaldehyde comes the closest. Therefore, a variety of fixatives are available for use, depending on the type of tissue present and features to be demonstrated (Carson, 1997). There are five major groups of fixatives, classified according to mechanism of action:

- Aldehydes
- Mercurials
- Alcohols
- Oxidizing agents
- Picrates (Eltoum et al., 2001).

Sectioning

Sectioning can be done in limited ways. Vertical sectioning perpendicular to the surface of the tissue is the usual method. Horizontal sectioning is often done in the evaluation of the hair follicles and pilo sebaceous unit.

Tissues are sectioned using a microtome. Turn on the water bath and check that the temp is 35-37°C. Once the tissues have been embedded, they must be cut into sections that can be placed on a slide. This is done with a microtome. The microtome is nothing more than a knife with a mechanism for advancing a paraffin block standard distances across it. There are three important necessities for proper sectioning: (1) a very sharp knife, (2) a very sharp knife and (3) a very sharp knife.

A glass knife can section down to about 1 micron. Thin sections for electron microscopy (1/4 micron) are best. Microtomes have a mechanism for advancing the block across the knife. Usually this distance can be set, for most paraffin embedded tissues at 6 to 8 microns.

It is important to have a properly fixed and embedded block much arte fact can be introduced in the sectioning. Common arte facts include tearing, ripping, "Venetian blinds", holes, folding, etc

The glass slides are then placed in a warm oven for about 15 minutes to help the section adhere to the slide (Bancroft, 2002).

Staining

Biological tissue has little inherent contrast in either the light or electron microscope. Staining is employed to give both contrast to the tissue as well as highlighting particular features of interest. Where the underlying mechanistic chemistry of staining is understood, the term histo chemistry is used.

The embedding process must be reversed in order to get the paraffin wax out of the tissue and allow water soluble dyes to penetrate the sections. Therefore, before any staining can be done, the slides are "deparaffinized" by running them through xylenes (or substitutes) to alcohols to water. The staining process makes use of a variety of dyes that have been chosen for their ability to stain various cellular components of tissue. The routine stain is that of hematoxylin and eosin (H and E). Other stains are referred to as "special stains" because they are employed in specific situations. Therefore, Hematoxylin and eosin (H&E stain) is the most commonly used light microscopical stain in histology and histopathology. Hematoxylin, a basic dye, stains nuclei blue due to an affinity to nucleic acids in the cell nucleus; eosin, an acidic dye, stains the cytoplasm pink (Kiernan, 1981).

H and E staining

(Histological Staining - Haematoxylin & Eosin)

A modified H&E is employed. The reduced differentiation increases contrast of the sections, allowing easier identification of vacuolar pathology. Hematoxylin is the oxidized product of the logwood tree known as hematein. Since this tree is very rare nowadays, most hematein is of the synthetic variety. Hematoxylin will not directly stain tissues, but needs a "mordant" or link to the tissues.

This is provided by a metal cation such as iron, aluminum, or tungsten. (Kiernan, 2008). Eosin is an acidic dye with an affinity for cytoplasmic components of the cell. There are a variety of eosins that can be synthesized for use, varying in their hue, but they all work about the same (Godwin, 2011). Eosin is much more forgiving than hematoxylin and is less of a problem for the lab. About the only problem you will see is over staining, especially with decalcified tissues. (tabel 1).

Tabel 1-Common laboratory stains

Stain	Common use	Nucleus	Cytoplasm	Red blood cell (RBC)	Collagen fibers	Specifically stains
Haematoxylin	General staining when paired with eosin (i.e. H&E)	Blue	N/A	N/A	N/A	Nucleic acids—blue ER (endoplasmic reticulum)—blue
Eosin	General staining when paired with haematoxylin (i.e. H&E)	N/A	Pink	Orange/red	Pink	Elastic fibers—pink Collagen fibers—pink Reticular fibers—pink
Toluidine blue	General staining	Blue	Blue	Blue	Blue	Mast cells granules—purple
Masson's trichrome stain	Connective tissue	Black	Red/pink	Red	Blue/green	Cartilage—blue/green Muscle fibers—red
Mallory's trichrome stain	Connective tissue	Red	Pale red	Orange	Deep blue	Keratin—orange Cartilage—blue Bone matrix—deep blue Muscle fibers—red
Weigert's elastic stain	Elastic fibers	Blue/black	N/A	N/A	N/A	Elastic fibers—blue/black
Heidenhain's AZAN trichrome stain	Distinguishing cells from extracellular components	Red/purple	Pink	Red	Blue	Muscle fibers—red Cartilage—blue Bone matrix—blue
Silver stain	Reticular fibers, nerve fibers, fungi	N/A	N/A	N/A	N/A	Reticular fibers—brown/black Nerve fibers—brown/black Fungi—black
Wright's stain	Blood cells	Bluish/purple	Bluish/gray	Red/pink	N/A	Neutrophil granules—purple/pink Eosinophil granules—bright red/orange Basophil granules—deep purple/violet Platelet granules—red/purple
Orcein stain	Elastic fibres	Deep blue	N/A	Bright red	Pink	Elastic fibres—dark brown Mast cells granules—purple Smooth muscle—light blue
Periodic acid-Schiff stain (PAS)	Basement membrane, localizing carbohydrates	Blue	N/A	N/A	Pink	Glycogen and other carbohydrates—magenta

Decalcification

Some tissues contain calcium deposits which are extremely firm and which will not section properly with paraffin embedding owing to the difference in densities between calcium and paraffin. Bone specimens are the most likely type here, but other tissues may contain calcified areas as well. This calcium must be removed prior to embedding to allow sectioning. A variety of agents or techniques have been used to decalcify tissue and none of them work perfectly. Mineral acids, organic acids, EDTA, and electrolysis have all been

used. Strong mineral acids such as nitric and hydrochloric acids are used with dense cortical bone because they will remove large quantities of calcium at a rapid rate. Organic acids such as acetic and formic acid are better suited to bone marrow, since they are not as harsh. Formic acid in a 10% concentration is the best all-around decalcifier. Some commercial solutions are available that combine formic acid with formalin to fix and decalcify tissues at the same time.

EDTA can remove calcium and is not harsh (it is not an acid) but it penetrates tissue poorly and works slowly and is expensive in large amounts. Electrolysis has been tried in experimental situations where calcium had to be removed with the least tissue damage

Smears

Histotechnicians sometimes perform special stains on cytology smears, blood films and cy to preps from other departments within the laboratory. Increasingly, the commonly received cytoprep is that of the "thin prep." These smears are wet-fixed in 95% ethanol immediately after preparation to preserve the fine structure of the chromatin and help in the evaluation of nuclear changes.

Arte facts in Histologic Sections

Arte fact is any irregularity on an image that is not caused by the proper shadowing of tissue by the primary beam. A number of arte facts that appear in stained slides may result from improper fixation, from the type of fixative, from poor dehydration and paraffin infiltration, improper reagents, and poor microtome sectioning. The presence of a fine black precipitate on the slides, often with no relationship to the tissue (i.e., the precipitate appears adjacent to tissues or within interstices or vessels) suggests formalin-hem e pigment has formed. This can be confirmed by polarized light microscopy, because this pigment will polarize a bright white (and the slide will look like many stars in the sky). Tissues such as spleen and lymph node are particularly prone to this arte fact. Making thin sections and using enough neutral-buffered formalin (10 to 1 ratio of fixative to tissue) will help. If the fixative solution in which the tissues are sitting is grossly murky brown to red, then place the tissues in new fixative. Tissues that are insufficiently dehydrated prior to clearing and infiltration with paraffin wax will be hard to section on the microtome, with tearing arte facts and holes in the sections.

Tissue processor cycles should allow sufficient time for dehydration, and final ethanol dehydrant solution should be at 100% concentration. (Brown,2009).

Though alcohols such as ethanol make excellent fixatives for cy to logic smears, they tend to make tissue sections brittle, resulting in microtome sectioning arte facts with chattering and a "venetian blind" appearance.

Safety in the Lab

The lab should be well-ventilated. There are regulations governing formalin and hydro car bond s such as xylem e and toluene.

Every chemical compound used in the laboratory should have a materials safety data sheet on file that specifies the nature, toxicity, and safety precautions to be taken when handling the compound.

The laboratory must have a method for disposal of hazardous wastes. Health care facilities processing tissues often contract this to a waste management company.. Tissues that are collected should be stored in formalin and may be disposed by incineration or by putting them through a "tissue grinder" attached to a large sink (similar to a large garbage disposal unit).

Every instrument used in the laboratory should meet electrical safety.

Flammable materials may only be stored in approved rooms and only in storage cabinets that are designed for this purpose.

Fire safety procedures are to be posted. Safety equipment including fire extinguishers, fire blankets, and fire alarms should be within easy acces.

Specific hazards that you should know about include:

- 1- Bouin's solution is made with picric acid. This acid is only sold in the aqueous state .When it dries out, it becomes explosive.
- 2- Ben zidine, benzene, anthrax cene, and nap thol containing compounds are carcinogens and should not be used.
- 3- Mercury-containing solutions (Zenker's or B-5) should always be discarded into proper containers. Mercury, if poured down a drain, will form amalgams with the metal that build up and cannot be removed.

CONCLUSION

The aim of Tissue Processing is to remove water from tissues and replace with a medium that solidifies to allow thin sections to be cut. Biological tissue must be supported in a hard matrix to allow sufficiently thin sections to be cut, typically 5 μm (Micro metres; 1000 micro metres = 1 mm) thick for light microscopy and 80-100 nm (nanometre; 1,000,000 nanometres = 1 mm) thick for electron microscopy. For light microscopy, paraffin wax is most frequently used. Since it is immiscible with water, the main constituent of biological tissue, water must first be removed in the process of dehydration.

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