Czech
Journal of Multidisciplinary Innovations
Volume 5, May, 2022.
ISSN (E): 2788-0389
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

Techniques for Preparation of Histologics

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Abstract: The main purpose of the article is to study the technique of preparation of histological micropreparations, the structure and operation of light and electron microscopes, that is, the correct use of the microscope to distinguish the structural properties of tissues and cells in drugs, depending on their specific color.

Keywords: preparation, cutting, deparaffinization, washing, lighting, fixation, transfusion, dehydration, thickening, dyeing, finishing, cell, tissue, biopsy.

Temporary and permanent drugs are usually prepared from biological objects for histological examination. Preparation of regular drugs consists of several stages.

1. Receipt of materials;
2. Fixation;
3. Washing;
4. Dehydration-compaction;
5. Pouring;
6. Cutting;
7. Deparaffinization;
8. Painting;
9. Lighting;
10. Conclusion.

Obtaining materials. The material is examined in small pieces during autopsy or by biopsy of live animals and humans. Materials obtained from mammals (dogs, cats, rabbits, rats) are used for the preparation of drugs. To do this, the animals are killed by injecting air into their blood vessels (embolism) or slaughtering (decapitation), and immediately fragments are taken from their organs to prepare the drug.

Fixation. The resulting material should be fixed immediately. The purpose of fixation is to preserve the vital structure of the tissues. The essence of fixation is the freezing of the cell cytoplasmic protein. This prevents the protein from decomposing, which means that the structure of cells and tissues is preserved.

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Washing. When the fixation is complete, the fixative is drained and the particles are washed with water. After some fixatives (alcohol, carnua, etc.) the tissue is transferred directly to the next stage of dehydration without walking.

Dehydration and compaction. After washing, the class is carried out in alcohols with increasing temperature of the particles, starting from 50%, 60, 70, 80, 90, 96 and finally 100%, ie in absolute alcohols. In alcohols, the particles become dehydrated and condensed.

Infusion. Although the particles are somewhat concentrated in alcohols, they are not yet hard enough to make fine cuts, so they are then impregnated with special substances. Only then can the pieces have the same density and be cut into thin sections. Tissues to be examined: celloidin, paraffin or gelatin.

Cutting. Celloidin or paraffin slices are cut under a microtome. Special steel knives are used. The thickness of paraffin cuts is 5-7 microns. In the preparation of drugs for electron microscopy, ultra-thin sections of synthetic resins are used to make ultra-thin incisions using diamond or glass blades. The cuts are made in special nets with a thickness of 200-300 A.

Painting. Microscopic examinations often use stained sections. Because most tissues are colorless blanking (except in rare cases), studying them under a light microscope without staining is ineffective.

Lighting. Once the sections have been dehydrated, they should be illuminated to allow light to pass through or be clear. The following lighting materials are used for this purpose:

1. Carbol-xylene.
2. Xylene or toluene.
3. Clove oil and others. The incisions are held in the illuminating material for 0.5–1 min.

Completion. Canadian or kefir balm is used for finishing. It not only closes the incision, but also helps to lighten the drug. The objects are removed from the illuminating substance by cutting the incisions attached to the glass, a drop of balm is dripped on it, and it is covered with a closed glass. Only then is it used for viewing.

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