Almaaqal University
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Department of Medical Laboratory Techniques

Lectures in Histology laboratories

By

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Lecture 1

Preparation of slides, method and materials used

- **For the preparation of histological sections, tissues were processed according to the following steps**

1-Fixation:

The specimens are fixed in Formalin solution 10% for 72hrs. After that, the specimens are washed in tap water until removed all formalin from the sections.

2- Dehydration:

- **The specimens are passed on several serial ascending concentration of alcohol (50%,70%,80%,90%,100%) to remove water from the tissues for2 hrs. each.**

3- Clearing:

The specimens are cleared in xylene to remove the ethanol alcohol from the tissues and to hide transparency of the tissue, then was replaced xylene by solvent miscible with paraffin wax. In this procedure, was used xylene twice passing for one hr. each.

4- Infiltration and Embedding:

It's also, called the saturation paraffin wax. The specimens are put in a mixture of clearing solution and pure paraffin wax. After that, are put in wax path at melting point (58 C $^{0)}$ Wax is replaced twice overnight, next the tissue is oriented and embedded in paraffin blocks.


StatLab

5-Trimming and Sectioning:

- **The specimens are trimmed and sectioned by Rotary microtome. The specimens are sectioned for (4- 5 µm) thickness.**

6- Mounting:

- **The paraffin sections are put in water path for 5 minutes. Then paraffin ribbons are transferred into glass slides that have been coated with Mayer's albumin , which was prepared by adding 50 ml of albumin (filtered by gauze) to 50 ml glycerin . The sections then are moved on warming hot plate for 24 hrs. to let them dry and they will adhere to the slides.**

7- Stains used in this study:

- Staining the slides by different stains, like H&E, Mallory, PAS, to differentiate their different components. After staining procedures the sections are examined under the microscope under magnification power (4 x, 10 x, 40 x and 100 x) and photographs are captured.

- *** Hematoxylin and Eosin Stain (H&E)**:

Use Hematoxylin (Harris) and Eosin as a routine histological stain has been used to demonstrate the general component of the tissue (Luna, 1968).

Staining Procedure:

- Hematoxylin is dissolved in alcohol, the alum in the water by the aid of heat. Removed from heat and mix the two solutions. Bring to a boil as rapidly as possible (Limit this heat to less than 1 minute and stir often). Remove from heat and add the mercuric oxide slowly.

- Reheat to a simmer until it becomes dark purple, remove from heat immediately and plunge the vessel into a basine of cold water until cool. The stain is ready for use as soon as it cools. Add 2- 4 ml of glacial acetic acid per 100 ml of the solution to increases the precision of the nuclear stain. Filter before use.

Thanks