SEP 18, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.3byl4q9r2vo5/v1

Protocol Citation: Taufik Suryadi . 2023. Protocol of histopathology preparation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4q9r2vo5/v1

Protocol status: Working
We use this protocol and it's working

Created: Sep 04, 2023

Last Modified: Sep 18, 2023

Protocol of histopathology preparation

Taufik Suryadi
.[1]

[1]Universitas Syiah Kuala

Taufik Suryadi .: Thank you

T Taufik Suryadi .

ABSTRACT

This protocol is a step by step from research entitled: Comparison between pre-mortem histopathological findings in rats with and without traumatic brain injury: prospective application in forensic medicine

Step by step description of histopathology preparation

1 Each group's tissue was taken for observation on histopathological preparations with a series of manufacturing processes with several stages including euthanasia, fixation, trimming with subsequent stages in the form of dehydration, clarification, paraffin infiltration, blocking, cutting, staining, gluing, and labeling.

2 Fixation

Fixation is a process of preserving protoplasm so that the structure of the tissue remains stable and does not undergo post-mortem changes such as the process of autolysis caused by proteolytic enzymes and putrefaction by putrefactive germs from outside the body. The fixation solution used was 10% formalin (a mixture of 10 cc of 40% formaldehyde with 90 cc of distilled water). The brain organs are immersed in a neutral buffer solution of 10% formalin, which serves to provide a hard consistency so that the tissue can be sliced thin and provide staining and optical differentiation. The tissue was fixed for 24 hours.

3 Cutting (Trimming)

Trimming is cutting organ samples into smaller sizes with the aim of facilitating the next preparation stage. Tissues that had been fixed for 24 hours were then drained on a sieve and cut crosswise using a scalpel knife with a thickness of 1x1x1 mm and arranged into cassettes and labeled using a pencil. Labels are provided on the outside and inside of the cassette.

4 Dehydration

Dehydration is the stage of immersing the tissue in several ethanol solutions with graded concentrations. The dehydration process is carried out by soaking the tissue (which has been arranged in a cassette) in graded alcohol starting from 70% ethanol I, II, for two hours each. Next, with the same treatment, soaking was carried out again with 90% ethanol, 96% ethanol and absolute ethanol twice for 2 hours each.

5 Clearing

Clearing is the stage of making the network clear and transparent using xylol solvent. The cleaning process was done by immersing the tissue in a solution of xylene I and II for two hours. Aims to remove alcohol from the network and replaced with paraffin.

6 Paraffin Infiltration (Embedding)

Paraffin infiltration is soaking the tissue in melted paraffin at a temperature of 58 - 60°C for 30 minutes to 6 hours, to remove the clearing agent from the tissue so as to make the tissue resistant to cutting. The purpose of using paraffin is to prevent the retention of a number of clarifying substances in the tissue, because it will make the tissue soft and difficult to incise.

7 Blocking

Blocking is the process of freezing a preparation so that it can be cut with a microtome. This

process is carried out by pouring a small amount of paraffin into a metal cube-shaped mold, then inserting the tissue with heated tweezers and then covering it with a cassette. Liquid paraffin is poured again until it covers the entire mold. Next, it is cooled, so that the surface of the tissue hardens and forms a tissue block (paraffin block).

8 Cutting (Sectioning)

Sectioning is cutting the block of preparations using a microtome. The paraffin block was then cut using a rotary microtome with a thickness of 5µm. The ribbon-shaped pieces are placed in a water bath at 45°C to loosen the paraffin and the tissue to solidify. Pieces of slides were taken with an object glass smeared with albumin mayers (egg white: glycerol = 1:1) and then placed in a slide warmer at 37°C for 24 hours, which would then be stained.

9 Evaluation of Preparations

The next process is evaluating the preparation after cutting to see whether the tissue preparation is good or not. The tissue preparation is placed on a glass object and then observed with a microscope to see whether there is any damage. When it is good then dry it and proceed to coloring.

10 Coloring (Staining)

Staining is the process of giving tissue color. Before staining with Hematoxylin Eosin (HE), to prevent it from dissolving in water, tissue containing paraffin must be removed using xylol, followed by hydration using 100% and 95% concentrated alcohol 2 times each for 5 minutes and rinsed with running water for 3 minutes. The slide preparations were dipped in hematoxylin staining solution for 3-5 minutes, then washed with running water for 3 minutes, docolorized by dipping in alcohol for 2 seconds, then washed again with running water for 3 minutes and then dipped in eosin solution for 2-5 minutes and rinsed with running water for 30 seconds. The next process is dehydration, namely the tissue slide is dipped back into alcohol with a concentration of 95% and absolute for 2 minutes (each done 2 times). Cleaning was carried out by soaking the sample in xylol for 2 minutes, drying it and dripping it with 1 drop of adhesive per mount, then covering it with a cover glass and leaving it to dry. Histopathological preparations were observed using a light microscope with 400x magnification