

Tissue Processing, Our Experience in the Lab

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Abstract: All the techniques employed for the study of histology bring an insight into the details inside the cell with the use of microscope. The simplest and most common method in the study of histology is preparation of sections. Tissues are exposed to the series of reagents that fix, dehydrate, clear, infiltrate with final embedding in a medium which provides support for the tissue. The quality of structural preservation is determined by the choice of reagents and exposure times to the reagents during processing. Each step in the tissue processing is important from the procurement of the specimen, selection of sample, determining the appropriate protocols and reagents to be used. This paper compares certain methods of tissue processing and the results obtained through them.

Key words: Tissue, processing, staining, fixing, dehydration, clearing.

I. Introduction

Aim of tissue processing is to remove water from the tissue and replace it with the medium that solidifies to allow thin sections to be cut. Biological tissues must be supported in a hard matrix to allow sufficiently thin sections to be cut, typically 5µm thick for light microscopy. For light microscopy paraffin wax is most frequently used. Since it is insoluble in water, the main constituent of biological tissue, water must first be removed in the process of dehydration. Samples are transferred through baths of progressively more concentrated ethanol to remove the water. This is followed by hydrophobic clearing agent such as xylene to remove the alcohol and finally molten paraffin wax the infiltration agent, which removes the xylene. After the block is obtained, sectioning can be done using a steel knife mounted in a microtome to cut 4µm thick tissue sections which are finally mounted on a glass slide. Biological tissue has little inherent contrast in either light or electron microscope. Staining is employed to give both contrast to the tissues as well as high lighting particular features of interest. Haematoxylin and eosin are the most commonly used light microscopical stain in histology and histopathology. Haematoxylin, a basic dye stains nuclei blue due to affinity to nucleic acid. Eosine, an acidic dye stains cytoplasm pink¹.

Aims and objectives

To compare three different methods of tissue processing and to decipher which method yields a good slide.

II. Materials

Liver and lung tissue from white mice
10% formalin
Propanol of different grades (30%, 50%, 70%, 90%, absolute)
Xylene
Wax bath
Paraffin wax with ceresin
Rotary Microtome
Floatation bath
Glass slides
Egg albumin
Harris haematoxylin and eosine stain
Cover slips

Methods

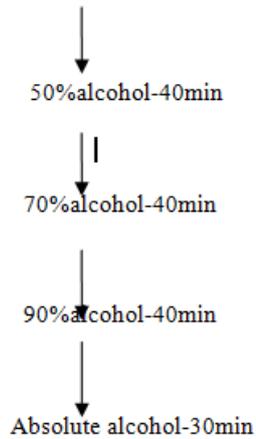
Three different methods of tissue processing were used to compare the outcome of H and E staining on the lung and liver tissue.

After obtaining lung and liver tissues from the white mice, they were thoroughly washed in running tap water and were cut into small pieces of 3 to 4 mm thickness and immersed in 10% formaline for 2 days.

Method 1

Fixation: 10% formalin for two days

Dehydration : 30% alcohol-30min



Clearing:

Mixture1-(25ml xylene:75ml absolute alcohol)-30min

Mixture 2-(50ml xylene:50ml absolute alcohol)-30min

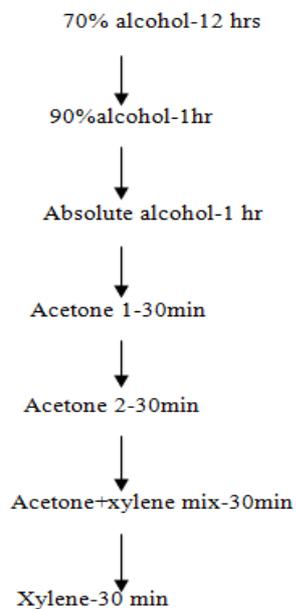
Mixture3-(75ml xylene:25ml absolute alcohol)-30min

Pure clearing agent-till tissue becomes transparent ⁽²⁾

Method 2

Fixation: 10% formaline for two days

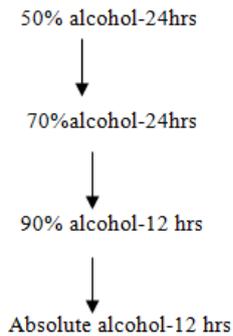
Dehydration and clearing



Method 3

Fixation: 10% formaline for two days

Dehydration:



Clearing

Xylene-till the tissue becomes transparent

III. Embedding

Once the tissues become transparent ,they are transferred to the paraffin wax contained in the wax bath for embedding(temperature 60°C). There are four vessels containing the paraffin wax in which tissues are transferred successively.

- Paraffin I-1hr
- Paraffin II-30min
- Paraffin III-30min
- Paraffin IV-30min

Block preparation and tissue cutting

Once the tissues are embedded with the paraffin wax ,tissue blocks are prepared with the help of brass moulds and section cutting is done with the help of rotary microtome. Thin ribbons are transferred to floatation bath. Glass slides coated with egg albumin are used to take out the thin sections from the floatation bath. These sections are stained using following staining method.

Staining procedure(H and E staining) ³

For deparaffinising the tissue -Xylol-5 to 10min

Absolute alcohol-2min

For hydration-

- 90% alcohol-2min
- 70%alcohol-2min
- 50% alcohol-2min
- Distilled water-10min

For staining

- Harris Haematoxylin-5 min
- Running tap water-10min
- Eosin-1min
- 90%-just dip

For full dehydration

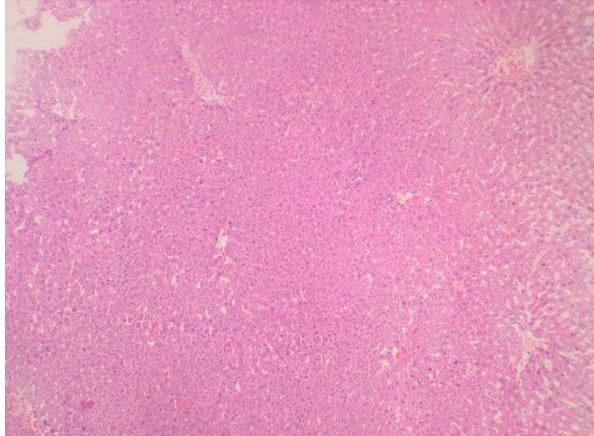
- Absolute alcohol-2min

Xylol-5 to 10min(until section appears absolutely clear)

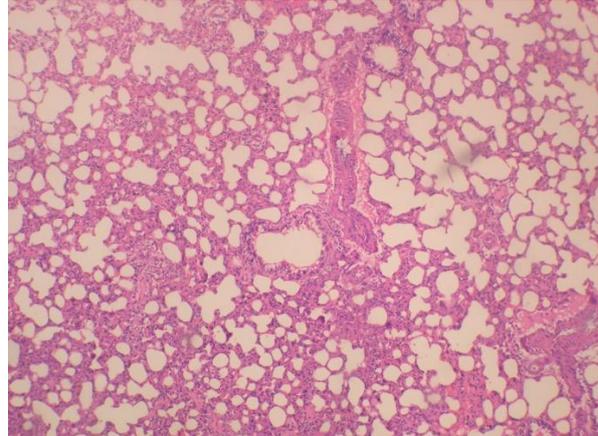
Mount in D.P.X.

IV. Result

Method 1

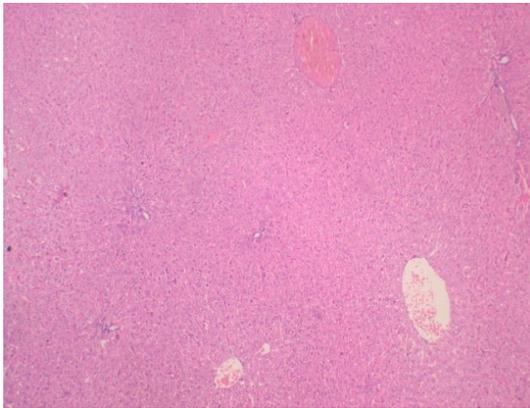


Liver

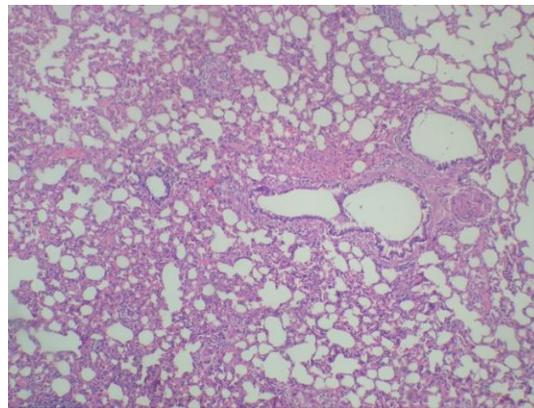


Lung

Method 2

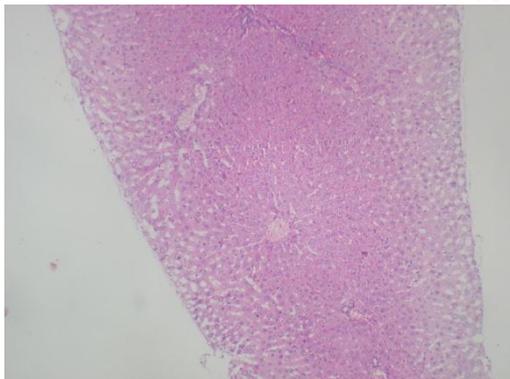


Liver

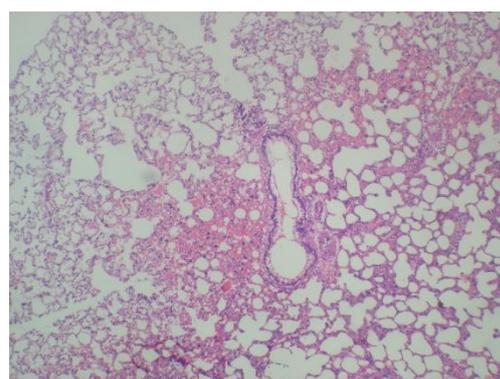


Lung

Method 3



Liver



Lung

The best result was obtained in **method 1** in terms of cellular architecture and staining by H&E.

V. Discussion

Fixation in all the three methods (method 1,2 and 3) was obtained by immersing the tissue in 10% formalin for two days. The difference was apparent in the procedure of dehydration, where in, in method 1, slow and gradual dehydration was done by transferring the tissue through graded concentrations of alcohol(30% alcohol for 30min,50% alcohol for 40min,70%alcohol for 40min,90%alcohol for 40min,absolute alcohol for 30min).Gradual dehydration has the advantage of preventing shrinkage of tissues⁴. In method three although gradual dehydration was attempted, we omitted keeping the tissue in 30% alcohol and proceeded directly, to 50% alcohol.(50% alcohol for 24hrs,70%alcohol for 24hrs,90% alcohol for 24hrs ,absolute alcohol for

12hrs). Keeping the tissues too long in higher dilutions of alcohol makes the tissues brittle and difficult to cut subsequently, also keeping the tissue in lower dilutions of alcohol should be avoided as it will macerate the tissue.⁵ This could be the reason why method 3 did not give good result. The common practice of taking out the tissue out of alcohol and then placing the tissue in the clearing agent is faulty. The reason being that alcohol escapes from the surface of tissue into the air quicker than the clearing agent can get into it. This causes tissue shrinkage, which could have happened in method 2 and 3.⁶

VI. Conclusion

To conclude although the method of fixation was common in all the three methods, dehydration, clearing and embedding differed in the three methods. All the three methods of tissue processing ended with the common staining of haematoxylin and eosin. The best result was obtained through method 1 probably because of slow dehydration and appropriate method of clearing.

References

- [1]. Culling C.F Handbook of histopathological and histochemical techniques. 3rd Ed; (1974) Butterworth and Co Ltd.
- [2]. D.R.Singh Principles & Techniques in Histology Microscopy and Photomicrography. 1st Ed ; (2003) CBS Publications.
- [3]. Lena T. Spencer and John D Bancroft. Tissue Processing Theory and Practice of Histological Techniques. 6th Ed. Elsevier Publications.
- [4]. M.I.Cox. Experimental Molecular Pathology. 2006. Vol 80; 183.
- [5]. Carlson F.L.. Histotechnology A self instrumental text. 3rd Ed 2007. Chicago: ASCP Press
- [6]. Stehen Gallik. Tissue Processing & Histological stains 2009 (Internet) Available from histologyyolm.stevegalik.org