The evaluation of an alternative clearing agent to xylene

Introduction

The aim of tissue processing is to infiltrate the tissue with a medium which, when solid, will be firm enough to support the tissue and enable thin sections to be cut. The most satisfactory embedding material in routine use is paraffin wax. Since paraffin wax is not miscible with aqueous-based fixatives, the tissue must be processed to allow impregnation with this medium. This is achieved by the dehydration of the tissue to remove fixative and water and replacing them with dehydrating fluid. The next stage, namely clearing, replaces the dehydrating fluids with a fluid that is miscible with both the dehydrating fluid and the embedding media. The final stage replaces the clearing agent with the embedding medium.

Xylene is the commonly used clearing agent, although it poses several disadvantages to the user. It is a flammable liquid and exposure by inhalation or skin contact is harmful. Indeed, the working exposure limit for xylene is 100ppm. Additionally, the toxic nature of xylene prohibits disposal via the drain. It must be collected and disposed of in a specialised way which imposes an extra cost on the user.

The study reported here aimed to test the Sakura Tissue-Clear preparation as an alternative to xylene. Developed to overcome many of the disadvantages associated with the use of xylene and other commonly used clearing agents, Tissue-Clear is non-toxic, non-carcinogenic, non-flammable, virtually odourless and it is fully biodegradable.

Method

450 pieces of tissue were selected from a broad range of tissue types (figure 1) and processed to paraffin wax using the Tissue-Tek Vacuum Infiltration Processor (VIP). The size of the tissue samples varied from 5mm to 25mm in maximum dimension. The different tissue types were chosen to provide a good range with which the ability of Tissue-Clear to deal with varying tissue consistencies could be tested. Some tissue types commonly cause difficulties during processing, for example fat in breast tissue and dense muscle in the uterus.

Each day for 5 days, 1 block of the 15 different tissue types was processed using xylene in the three stations on the VIP designated as clearing stages. Additionally, 5 blocks from the 15 different tissue types were processed using Tissue-Clear in these stations. The Tissue-Clear solution was not renewed between the processing of these 5 blocks, to assess the build-up of contamination.

2µm sections were then cut using a rotary microtome and subsequently stained using a variety of special stains, including immunoperoxidase methods. Half the stained slides were mounted using the Tissue-Tek coverslip and the other half were hand mounted using Eukitt mounting media. As Tissue-Clear is incompatible with some proprietary mounting media, the hand-mounted sections were treated with Histoclear to remove all traces of Tissue-Clear prior to mounting.

Each slide was then examined microscopically to assess the quality of the processing and staining of the tissue. The blocks were also checked every few days for signs of tissue shrinkage.

TISSUE TYPES PROCESSED

Days	Tissue type	No. of blocks processed in TISSUE-CLEAR	No. of blocks processed in Xylene
1 – 5	Testis	5	1
1 – 5	Skin	5	1
1 – 5	Colon	5	1
1 – 5	Prostate	5	1
1 – 5	Uterus	5	1
1 – 5	Placenta	5	1
1 – 5	Liver	5	1
1 – 5	Breast	5	1
1 – 5	Lung	5	1
1 – 5	Lymph Node	5	1
1 – 5	Thyroid	5	1
1 – 5	Kidney	5	1
1 – 5	Spleen	5	1
1 – 5	Appendix	5	1
1 - 5	Gall Bladder	5	1
Total Number of Blocks		375	75

Figure 1. Tissue types and processing procedure used to evaluate Tissue-Clear as an alternative to xylene.

Results

Both the speed of removal of the dehydrating agent and the ease of removal of Tissue-Clear by the embedding medium, were as good as that of xylene. Indeed, there was no obvious difference between the blocks processed using xylene and those processed with Tissue-Clear. Upon regular follow-up examination there was no visible shrinkage or distortion of the tissue.

Microscopic examination showed no discernible difference between the sections processed via the different methods. Artefacts observed normally in tissue processed using xylene were the same as those using Tissue-Clear. No new artefacts were observed which is important as laboratories choosing to use Tissue-Clear would not have to re-train the pathologists to recognise new artefacts. In addition, all tissue components stained were equally well demonstrated, with no noticeable effect on section morphology and the specificity and sensitivity of any antigens demonstrated was not affected either.

After 5 processing runs, the Tissue-Clear in the clearing reagent stations on the VIP visually appeared relatively clean and contaminent free. This implies that Tissue-Clear will require less frequent changing than xylene, thereby reducing the quantity of clearing agent used. It was not possible to perform more than 5 processing runs during this study, hence the time after which unsatisfactory levels of contamination occur has yet to be determined.

Conclusion

The results of this study indicate that Tissue-Clear is a viable alternative clearing agent to xylene. Tissue-Clear is safer to use and may prove to be more cost effective, due to less frequent replacement of the clearing solution. The use of Histoclear to remove the Tissue-Clear prior to mounting in Eukitt, does reduce some of the health and safety advantages of using Tissue-Clear as Histoclear is flammable and cannot be disposed of via the drain. However, Tissue-Clear is not incompatible with all proprietary mountants so a change of mounting medium would overcome this problem.

Sakura Finetek Europe BV,
Hoge Rijndijk 48A,
2382 AT Zoeterwoude,
The Netherlands,
Tel. +31-71-5898300
Fax +31-71-5898488

This article appeared in the December 2000 - January 2001 issue of Clinical Laboratory International