International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Assessing the Effectiveness of Xylene-Free Deparaffinization in Hematoxylin and Eosin Staining

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Abstract: ***Xylene is the deparaffinizing agent used in Hematoxylin and Eosin staining. It is expensive, dangerous, and will contaminate the workplace. There's also the disposal issue. Therefore, using a low-cost and environment friendly deparaffinization replacement is highly beneficial. To assess how well xylene-free sections perform in terms of yielding sufficient H and E staining in a straightforward, economical, and faster manner in comparison to traditional H and E sections, forty paraffin blocks were taken into account, a piece was stained using the traditional H and E procedure and another using the H and E method without xylene. Slides were graded according to the following criteria: uniformity, clarity, crispness, nuclear and cytoplasmic staining.***

Keywords: Hematoxylin, Eosin, Xylene, Deparaffinization

1. Introduction

The foundation of routine pathology diagnostic work is the hematoxylin and eosin stain. The paraffin section, which is often stained with H&E 2, serves as the foundation for the majority of the daily diagnostic work in a pathology laboratory. As a universal stain, H&E is the main form of contrast used in medical biopsy specimen diagnosis. Staining with H and E is extremely durable. A wide variety of cytoplasmic, nuclear, and extracellular matrix characteristics are distinguished using it. This staining method hasn't changed in over 150 years [1]. Xylene and graded alcohols are the other ingredients in the H and E staining process, in addition to eosin and hematoxylin. During the staining process, these chemicals are employed to rehydrate and dehydrate tissue sections in between. However, there are still issues with this time-honored process, including toxicity, cost containment, the issue of disposing of dangerous chemicals like methanol and xylene, and a dirty working atmosphere.

In a laboratory, processing tissue, deparaffinizing tissue sections, cover slippage, cleaning tissue processors, and recycling all involve the possibility of xylene exposure. Methanol exposure happens when the tissue is processed and the slices are dewaxed prior to staining [2]. Many alternative chemicals, such as limonene reagents, aliphatic hydrocarbons, aromatic hydrocarbons, vegetable oils, olive oil, and mineral oil substitutes, have been used in the effort to end the use of xylene in laboratory settings.

While the largest exposure and handling of xylene occurs during the dewaxing of the tissue sections, these compounds were utilised to replace xylene as a clearing agent during regular processing.

The primary goal in all life science fields is to use environmentally friendly chemicals that are affordable, nontoxic, and less biohazardous. Cleaning dishes with liquid soap is a daily task in the home. It is a liquid detergent used in kitchens to clean oily kitchenware. The creative idea of replacing xylene with liquid dishwashing detergent to dewax the tissue sections. An essential component of a pathology lab is xylene. The histology laboratory's past usage of xylene is an illustration of a poor replacement.[3]

Initially, as the most secure substitute for hazardous substances like benzene and aniline oil.

When it came to toluene, dioxane, and chloroform in the 1950s, there were serious worries about their safety by the 1970s. Acute neurotoxicity, heart and kidney damage, deadly blood dyscrasias, skin erythema, dryness, scaling, and secondary infection are among the hazardous consequences of xylene [4]. Due to the awareness of xylene toxicity, 41% of US histology laboratories currently employ alternatives to xylene. Organisations such as the American Conference of Governmental Industrial Hygienists (ACCIH) and the Occupational Safety and Health Administration (OSHA) exist in nations like the US. These organisations have set guidelines for biological exposure thresholds, exposure monitoring, and controlling the recycling and disposal of xylene. However, we don't see these kinds of regulations in the majority of emerging nations, particularly in India. In developing nations, there are no standardised procedures for disposing of xylene in pathology labs, nor are there any mechanisms in place to monitor exposure. Thus, for diagnostic purposes as well as for maintaining a healthy laboratory environment, any technique that minimises the use of xylene by using non-biohazardous substitutes, shortens the staining time, and does not compromise the staining quality will be very valuable, thereby minimising the risk to the laboratory personnel. Therefore, I looked into using diluted liquid dishwashing solution as a deparaffinizing agent for the H&E staining process as a less harmful, affordable, and readily available way to improve the

working environment in a histology laboratory.

Sodium laureth sulphate, sodium dodecyl benzene sulfonate, cocamidopropyl betaine, and nonionic surfactants make up the liquid dishwashing solution. These ingredients are anionic surfactants, which are frequently found in shampoos and detergent soaps [5].

2. Method

For six months, the study was conducted in the pathology and MLT departments of the Govt. Medical College in Thiruvananthapuram. Blocks of paraffin were forty in number. A piece was stained using the traditional H and E procedure and another using the H and E method without xylene. Slides were graded according to the following criteria: uniformity, clarity, crispness, sharpness, nuclear and cytoplasmic staining.

3. Results

Table 1: The overall staining character of the five parameters

Distribution of adequate staining character	Modified		Conventional		McNemar test
	N	%	N	%	p
Nuclear Staining	39	97.5	36	90.0	0.375
Cytoplasmic Staining	36	90.0	33	82.5	0.508
Uniformity	35	87.5	33	82.5	0.687
Clarity	35	87.5	34	85.0	1.000
Crispness	38	95.0	34	85.0	0.219

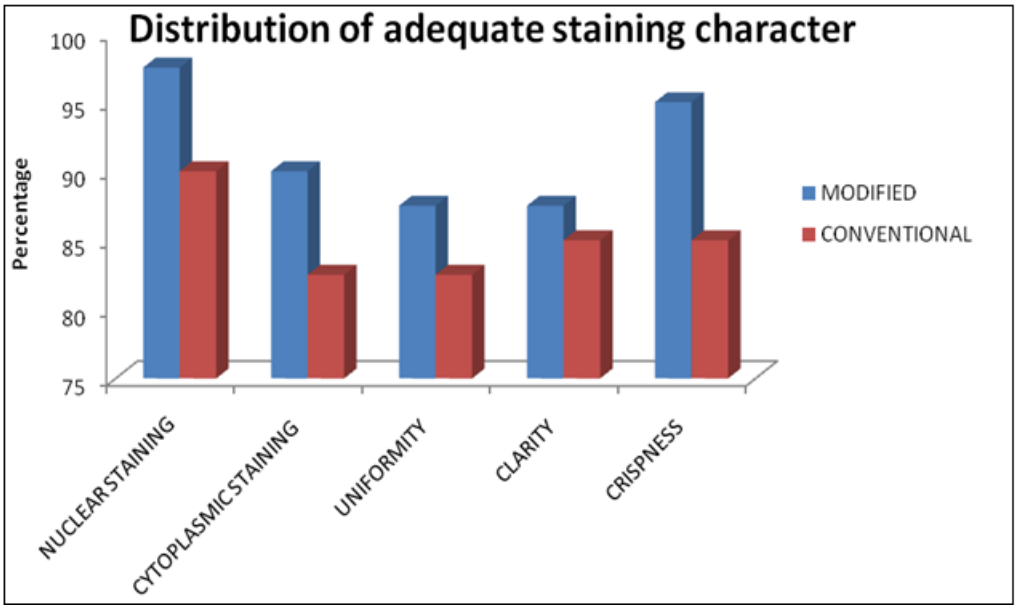

Figure 1

Table 2: Total scores for each slides

Total score		CONVENTIONAL			Total
		3	4	5	
MODIFIED	3	1	1	0	2
	4	1	10	2	13
	5	2	11	12	25
Total		4	22	14	40

McNemar test P=0.041

Based on the total scores obtained for each slides the observed P value is 0.041 and there is significant difference between modified method and conventional method in the aspect of total scores for each slides.

Graphical representation of total scores of each sides in two different methods

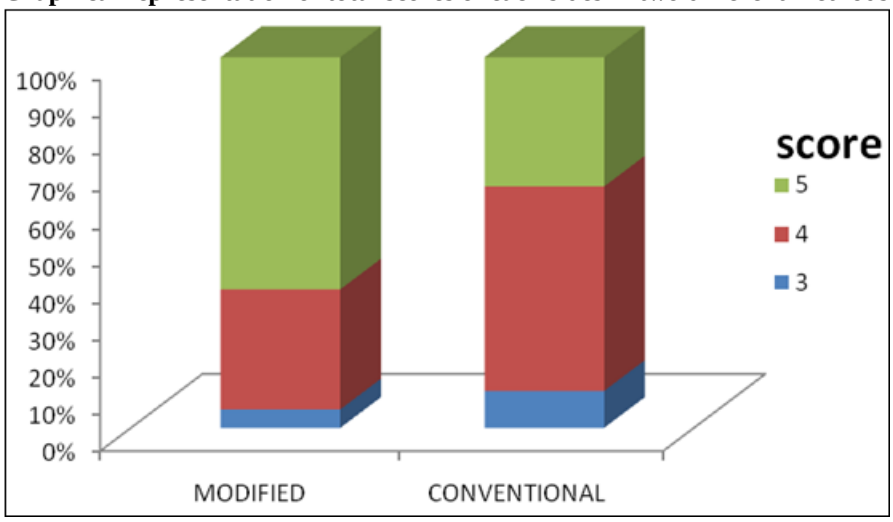

Figure 2

4. Conclusion

The xylene-free H and E staining procedure carried out using a simple diluted dish washing soap solution gave excellent positive results and is at par with the conventional H and E procedure. It produces a quality staining with sufficient clarity and a crisp nuclear and cytoplasmic staining. It also has added advantages of being nontoxic, economical, nonflammable, nonhazardous, no problem of disposal, reduces staining time and is easy to handle.

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