

Liquid dish wash solution – can it be an alternative in future for the expensive and hazardous xylene in hematoxylin and eosin staining of paraffin sections

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Abstract

Introduction: Xylene is the most commonly used clearing agent as it has excellent deparaffinising and clearing properties. Commercial xylene is a clear, colourless liquid that has a sweet and aromatic odour. However, xylene is not only expensive but also has detrimental effect on human health such as hepatitis, chemical pneumonitis, depression, anaemia, etc. Hence in this study we are replacing xylene with commercially available liquid dish wash solution(LDW) which is not only cheap but also non biohazardous. **Aims:** To assess the efficacy of LDW with xylene as a deparaffinising and clearing agent in routine hematoxylin and eosin staining (H&E). **Materials and Methods:** Blocks from 20 consecutively submitted samples at RL Jalappa Hospital between Jan – June 2015 each of breast, Cervix, Lymphnode, Fat, Salivary Gland, Muscle and Alimentary Tract was taken and two sections of 4-micron thickness were cut and deparaffinised using Liquid Dish Wash and Xylene respectively followed by H & E Staining. The 280 sections of the 140 blocks were evaluated and scored by two pathologists independently for nuclear staining, cytoplasmic staining, uniformity, clarity and crispness. **Results:** In the samples evaluated LDW seems to be on par with xylene cleared sections in terms of nuclear and cytoplasmic detail, clarity and crispness. However Xylene cleared sections showed slightly better uniform staining. However both sections were equally good for reporting. **Conclusion:** Liquid dish wash solution is an excellent alternative to xylene in H&E Staining. However more extensive studies need to be done on other types of tissues for validation

Key words: Xylene, Alternative, Liquid Dish wash, Staining, Xylene free staining.

Introduction

Histopathology is the gold standard for diagnosis in most surgical cases. Histopathology diagnosis requires tissues to be fixed and processed followed by section cutting and staining. Xylene is the most commonly used clearing agent as it has excellent deparaffinising and clearing properties. Commercial xylene is a clear, colourless liquid that has a sweet and aromatic odour.

However, xylene is not only expensive but also has detrimental effect on human health such as hepatitis, chemical pneumonitis, depression, anaemia, etc [1]. Western countries have strict guidelines regarding the exposure monitoring and maximum exposure limit of chemicals like xylene with strict chemical disposal rules

for laboratories. In developing countries like ours where cost of reports are kept to a minimum, monitoring and disposal of harmful chemicals like xylene may not be practical and hence safer alternatives for such chemicals are always being researched. Haematoxylin and Eosin (H&E) Staining has stood the test of time and is used most frequently in surgical pathology. In staining paraffin embedded sections, xylene is used which is hazardous to the working environment.

Many alternatives to xylene such as alcohols, kerosene, mineral oils and lemon water have been tried as an alternative, however standardisation of those reagents and easy availability haven't been possible. Liquid dish wash solution is eco-friendly, cost effective and time effective [2]. Liquid dish wash with its limonene properties and with boiling can dissolve wax. Xylene

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Original Research Article

free sections have been reported in earlier studies to be good as or better than conventional section [3-5]. Though xylene is an excellent deparaffinising and clearing agent. Lacunae in knowledge and need for study is to create a safe working environment in laboratory by finding cost effective, non toxic alternatives to chemicals like xylene which requires stringent concentration monitoring and safe disposal. Hence in this study xylene was replaced by 1.7% commercially available Vim liquid dish wash solution (LDW) which is not only cheap and effective but also non biohazardous.

Existing knowledge: It is well known that xylene though an excellent clearing and deparaffinising agent is hazardous to health and regulation deem it necessary to be monitored and disposed as per strict protocol. In developing countries where strict vapour monitoring, duration of work timings and safe disposal are not followed due to cost constraints.

Objectives

1. To assess the quality of staining in xylene free sections using 1.7% liquid dish wash solution.
2. To compare the quality of staining of xylene free staining versus the conventional staining using xylene

Materials and Methods

Place and Type of Study: It was a laboratory exploratory study conducted at Department of Pathology, R L Jalappa Hospital attached to Sri Devaraj Urs Medical College. Institutional ethical clearance was taken from ethical committee.

Sample Size: Sample size was estimated based on the values obtained from the study by Anuradha Ananthaneni et al, Diagnosis of lesion in conventional H & E was 100% and in Xylene free H & E was 95% [6]. Using this values sample size was estimated by using the formula of difference in proportions. Sample size of $190 + 90 = 380$ was obtained at $\alpha = 0.05$, β at 0.20 (Power at 80%).

Sampling Methods- Blocks from 20 consecutively submitted samples at RL Jalappa Hospital after 1st Jan 2015 each of breast, cervix, lymphnode, fat, salivary gland, muscle salivary gland, muscle, thyroid, skin, alimentary tract, liver and kidney was taken. Exclusion criteria was tissues not belonging to the above-mentioned specimen categories. Two sections of 4 micron thickness will be cut and deparaffinised using

1.7% commercially available Vim Liquid Dish Wash and Xylene respectively followed by H & E Staining. 1.7% Liquid Dishwash solution was prepared by diluting 25ml of Vim Liquid Dish wash in 1500ml of distilled water which was prepared freshly. The 400 sections of the 200 blocks will be evaluated and scored by two pathologists independently for nuclear staining, cytoplasmic staining, uniformity, clarity and crispness. Scoring system used was the one used by Ankle MR et al [4]. Procedure for staining using Liquid dish wash solution was from a study by Falkeholm et al [3].

Staining protocol has been mentioned below

Conventional Staining

1. Slide on hot plate / Incubator for 30 mins
2. 3 changes of Xylene – 3mins each
3. Absolute Alcohol – 2min
4. 90% Alcohol – 2min
5. 80% Alcohol – 2min
6. 70% Alcohol – 2min
7. Distilled Water – 5min
8. Staining with Hematoxylin and Eosin
9. 70% Alcohol – 2min
10. 80% Alcohol – 2min
11. 90% Alcohol – 2min
12. Absolute Alcohol – 2min
13. 3 changes of Xylene – 3minseach
14. Mount with DPX and cover slip

Xylene Free Staining Using Liquid Dish Wash Solution

1. Slide on hot plate / Incubator for 30 mins
2. 2 changes of 1.7% Liquid Dish wash Solution in Water bath at 90 C – 30 sec
3. Water at 90 C – 15 sec
4. 2 changes of tepid water– 15 sec each
5. Staining with Hematoxylin and Eosin
6. Dry in incubator at 60 C – 10 min
7. Mount with DPX and cover slip

Scoring System

Nuclear Staining: Adequate – Score 1 Inadequate – Score 0

Cytoplasmic Staining: Adequate – Score 1 Inadequate – Score 0

Clarity: Present – Score 1, Absent – Score 0

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Uniformity of Staining: Present – Score 1, Absent – Score 0

Crispness: Present – Score 1, Absent – Score 0

Total score of less than or equal to two was considered inadequate for diagnosis and 3 to 5 was considered adequate for diagnosis.

Statistical Analysis- Percentage for individual component scoring and sensitivity and specificity of Xylene free staining was calculated. P value was calculated. Agreement between scoring by two pathologists was assessed & Cohens Kappa was calculated.

Results

Table-1: Comparison of Hematoxylin and Eosin Staining Characteristics of various section after conventional staining and xylene free staining using liquid dish wash solution.

		Conventional Staining		Xylene Free Staining		P Value
		n	%	n	%	
Nuclear Staining (Percentage of cases)	Adequate	192	96	195	97.5	> 0.05
	Inadequate	8	4	5	2.5	
Cytoplasmic Staining (Percentage of cases)	Adequate	194	97	187	93.5	> 0.05
	Inadequate	6	3	13	6.5	
Clarity (Percentage of cases)	Adequate	184	92	188	94	> 0.05
	Inadequate	16	8	12	6	
Uniformity of staining (Percentage of cases)	Adequate	184	92	174	87	> 0.05
	Inadequate	16	8	26	13	
Crispness (Percentage of cases)	Adequate	168	84	164	82	> 0.05
	Inadequate	32	16	36	18	
Adequate for diagnosis (Score : 3-5)	Adequate	194	97	193	96.5	> 0.05
	Inadequate	6	3	7	3.5	

Significant reduction in staining time was noted between conventional and xylene free staining. Conventional staining using xylene for deparaffinisation and clearing took around 55-60 minutes whereas Xylene free staining using LDW took only 25-30 minutes. Hence using xylene free staining reduces the deparaffinisation time by 30 minutes. Average time taken for conventional staining was 64 minutes and for xylene free staining was 28 minutes. 96.5% cases were adequate for diagnosis (Total Score: 3-5) and 3.5% was inadequate for diagnosis in xylene free staining versus 97% cases adequate for diagnosis and 3% inadequate for diagnosis conventional staining. Nuclear staining, cytoplasmic staining, crispness, uniformity of staining and clarity was 96% and 97.5%, 97% and 93.5%, 92% and 94%, 92% and 87% and 84% and 82 % in Conventional and Xylene Free Staining respectively. The sensitivity and specificity was more than 95 % for xylene free staining. P value was > 0.05 showing no significant difference in the two staining techniques. Most parameters showed comparable results between conventional and xylene free staining. However xylene free staining had lower score in uniformity of staining which was usually due to incomplete deparaffinisation which was either due to lower temperature or shorter duration of sections in water bath with liquid dish wash solution. Cohen's Kappa measure of agreement was used to calculate the agreement between the two pathologists in scoring and a value of 0.74 was obtained indicating substantial (good) agreement.

Discussion

Xylene is used extensively in histopathology laboratories since decades. However concerns are being raised due to xylene substitutes. Xylene is hazardous to the health of health care professionals exposed to it and the severity of health hazards depends amount and duration of exposure to the chemical. New regulations in countries like US by Occupational Safety and Health Administrations and American Conference of Governmental Industrial Hygiene have given limits for exposure and disposal of hazardous chemicals like xylene [7]. Xylene on exposure can cause reactions between mild skin irritation to neurotoxicity [9]. As such measures like chemical monitoring and disposal are not practical in cost cutting in developing countries like ours and hence ecofriendly alternatives have been looked for to be an alternate for xylene. Falkeholm et al was the first to use liquid dish wash an alternative to the hazardous xylene [3]. Liquid dish wash solution is used in our kitchen to clean utensils due to its detergent and mild acidic action. Liquid dishwash solution is non-biohazardous, ecofriendly and cheap but also a good deparaffinising agent acting by its detergent action by reducing the

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surface tension. It has many advantages such as it is non toxic, not combustible, has an aromatic odor, reduces turn around time and is cost effective. There was almost no difference in the staining using conventional and xylene free techniques when adequacy for diagnosis was considered. Similar results were seen in other studies.

Table-2: Comparison of hematoxylin and eosin staining characteristics of various studies with present study

		Present Study	Ankle M R et al	Anuradha A et al	Ramulu S et al	Pinki Pandey et al
Nuclear Staining (Percentage of cases)	Conventional Staining	96	96.6	100	94	83
	Xylene Free Staining	97.5	98.3	95	96	90
Cytoplasmic Staining (Percentage of cases)	Conventional Staining	97	93.3	100	92	76
	Xylene Free Staining	93.5	83.3	100	86	90
Clarity (Percentage of cases)	Conventional Staining	92	85	100	94	86
	Xylene Free Staining	94	88.3	100	96	90
Uniformity of staining (Percentage of cases)	Conventional Staining	92	70	65	92	88
	Xylene Free Staining	87	50	75	80	72
Crispness (Percentage of cases)	Conventional Staining	84	76.6	100	96	74
	Xylene Free Staining	82	83.3	95	88	95
Adequate for diagnosis (Score : 3-5)	Conventional Staining	97	88.3	100	94	84
	Xylene Free Staining	96.5	90	95	90	86

Nuclear staining is very important in routine staining as it helps to distinguish between benign and malignant lesions as identifying features such as nucleoli, hyperchromasia, irregular nuclear membrane and mitotic figures favour a diagnosis of malignancy. In our study comparable or better nuclear staining was noted in xylene free staining compared to conventional staining. Cytoplasmic staining, clarity and crispness of staining was comparable between the two staining techniques. Uniformity of staining was slightly better in conventional staining compared to xylene free staining. This has been observed in previous studies by Ramulu S et al, Pinky Pandey et al, Anuradha A et al and Ankle MR et al [2,4,5,6].

This non – uniformity in staining could be attributed to inadequate deparaffinisation, section tears, thick sections and moisture in sections [8]. Other studies have also had similar shortcomings in the xylene free technique where if temperature and timings of deparaffinisation if not followed may lead to incomplete deparaffinisation leading foci of unstained areas on the slide. Few studies used progressive staining, however we used the regressive staining like few other studies using Harris hematoxylin [2]. Few studies noted a bluish tinge in xylene free, however the present study did not show any such findings. Xylene free staining provided satisfactory results as can be seen by all the studies [9]. It not only provides a safe working environment for laboratory but also decreases the turn around time. However xylene free technique is a temperature sensitive procedure and hence extreme care should be taken regarding the temperature and duration of sections in liquid dish wash solution. In cases of increased time in water bath with liquid dish wash solution the sections would float off whereas reduced time would lead to incomplete dewaxing. There have been occasional reports of mild allergic reactions of skin with use of liquid dish wash solution in laboratory personnel [11]. There have been similar mild reactions with other xylene substitutes that have been used [12]. At the end of the study a feedback was taken from the technicians regarding the use of xylene free staining. Though initially hesitant to use the xylene free technique after knowing the health hazards of xylene and the reduced turn around time and comparable staining characteristics of xylene free technique, the technicians were not only willing but wanted to adopt the xylene free technique for routine histopathology.

Conclusion

As a pathologist as important as it is to give a correct diagnosis, it is to provide a safe working environment to healthcare professionals working at the laboratory.

Liquid dish wash solution is an excellent alternative to xylene free sections as it is not only ecofriendly and

non-biohazardous but also effective deparaffinising agent and less time consuming as shown by the results above. However care has to be taken temperature maintenance in water bath is very important as lower temperatures lead to inadequate deparaffinisation and non-uniformity of staining.

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It is well known that xylene is not only expensive but hazardous to health of laboratory personnel who are exposed to it. Several alternatives have been tried and documented but either the procedure is not standardized for all types of tissues or the section quality hasn't been satisfactory when attempted in routine histopathology. This study provides the standardized procedure and documents satisfactory quality of staining of variety of tissues so that the procedure can be adopted in all laboratories easily.

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References

1. Kandyala R, Raghavendra SP, Rajasekharan ST. Xylene: An overview of its health hazards and preventive measures. *J Oral Maxillofac Pathol.* 2010 Jan; 14 (1):1-5. doi: 10.4103/0973-029X.64299.
2. Ramulu S, Koneru A, Ravikumar S, Sharma P, Ramesh D, Patil R. Liquid dish washing soap: an excellent substitute for xylene and alcohol in hematoxylin and eosin staining procedure. *J Orofac Sci* 2012; 4 (1): 37-42. doi: 10.4103/0975-8844.99890.
3. Falkeholm L, Grant CA, Magnusson A, Möller E. Xylene-free method for histological preparation: a multi-centre evaluation. *Lab Invest.* 2001 Sep; 81(9):1213-21.
4. Ankle MR, Joshi PS. A study to evaluate the efficacy of xylene free hematoxylin and eosin staining procedure as compared to the conventional hematoxylin and eosin staining: An experimental study. *JOMFP* 2011; 15(2): 161-167. doi: 10.4103/0973-029X.84482.

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5. Pinki Pandey, Alok Dixit, Aparna Tanwar, Anuradha Sharma, Sanjeev Mittal. A comparative study to evaluate liquid dish washing soap as an alternative to xylene and alcohol in deparaffinization and hematoxylin and eosin staining. *J Lab Physicians* 2014; 6 (2):84-90. doi: 10.4103/0974-2727.141504.

6. Anuradha Ananthaneni, Srilekha Namala, Vijay Srinivas Guduru, et al., Efficacy of 1.5% Dish Washing Solution and 95% Lemon Water in Substituting Perilous Xylene as a Deparaffinizing Agent for Routine H and E Staining Procedure: A Short Study. *Scientifica.* 2014; 2014: 11.doi.org/10.1155/2014/707310.

7. Buesa RJ, Peshkov MV. Histology without xylene. *Ann Diagn Pathol.* 2009 Aug; 13(4): 246-56. doi: 10.1016/j.anndiagpath.2008.12.005. Epub 2009 Feb 5.

8. Hamil JR, Spencer JM, Morgan MB. Histotechnique and staining troubleshooting. *On Atlas of Mohs and Frozen Section Cutaneous Pathology.* Ch. 20. New York: Springer Link; 2009.

9. Chen CY, He T, Mao XL, Friis TE, Qin RH, Jian YT. A novel xylene substitute for histotechnology and histochemistry. *Biotech Histochem.* 2010 Aug; 85 (4): 231-40. doi: 10.3109/10520290903235445.

10. Miller M, Miller D, Driscoll PE. Biodegradable, effective substitute for xylene in the ehrlich indole procedure. *J Clin Microbiol* 1994; 32:2028-30.

11. Foti C, Zamboni CG, Conserva A, et al. Occupational contact dermatitis to a limonene-based in a histopathology technician. *Contact Dermat* 2007; 56 (2): 109-12.

12. Tarbet F, Anderson J. Toxin reduction in Histology. A look at advantages, disadvantages of xylene substitutes. *Advance AdmLab* 2005; 14(4):42.