

COMPARING THE EFFICACY OF DISHWASH SOLUTION, DILUTED LEMON WATER, COCONUT OIL AND XYLENE AS DEPARAFFINIZING AGENTS FOR HEMATOXYLIN AND EOSIN STAINING PROCEDURE

Aparna. B^{*1}, Manjunath.AB², Ahmed Mujib B.R³, Arun Kumar. N⁴.

^{*1} Post Graduate Student, Department of Oral Pathology and Microbiology, Bapuji Dental College and Hospital, Davangere, Karnataka, India.

² Senior Lecturer, Department of Oral Pathology and Microbiology, Bapuji Dental College and Hospital, Davangere, Karnataka, India.

³ Professor and Head of the Department, Department Of Oral Pathology and Microbiology, Bapuji Dental College and Hospital, Davangere, Karnataka, India.

⁴ PhD Biostatistician, Bapuji Dental College And Hospital, Davangere, Karnataka, India.

ABSTRACT

Aim: To compare the efficacy of dish wash solution, diluted lemon water, coconut oil and xylene as deparaffinizing agents for hematoxylin and eosin staining procedure.

Objective: The objective is to find eco-friendly deparaffinizing agents like dish wash solution, diluted lemon water and coconut oil as substitute to xylene and comparing the staining characteristics of each individual deparaffinizing agent with Xylene.

Materials and Methods: The study comprised of paraffin embedded 45 blocks of various tissues. Each block of four sections of 5 microns thickness was prepared. They were considered in four different groups like A, B, C and D. Tissue sections in Group A were stained with H & E method where xylene was used as deparaffinizing agent. The other three sections were stained with H & E where dish wash solution, diluted lemon water and coconut oil were used as deparaffinizing agent's alternative to Xylene. Staining characteristics were compared with xylene and scoring was given. The total score of 3–5 was regarded as satisfactory for diagnosis and less than that is insufficient for diagnosis.

Statistical Analysis: Chi square test was used.

Results: Adequacy of staining characteristics such as nuclear, cytoplasm, uniformity, clarity and crispiness of staining for diagnosis was greater with dish wash solution followed by diluted lemon water, coconut oil and xylene.

Conclusion: The Eco-Friendly deparaffinizing agents such as dish wash solution, diluted lemon water, and coconut oil can be used as alternatives to xylene.

KEY WORDS: Eco-friendly Xylene Substitutes, Xylene Free Deparaffinization, Hematoxylin and Eosin Staining Procedure.

Address for Correspondence: Dr. Aparna. B, Post Graduate Student, Department of Oral Pathology and Microbiology, Bapuji Dental College and Hospital, Davangere- 577004, Karnataka, India. Tel phone No: 7892783252 E-Mail: aparnalalitha@gmail.com

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INTRODUCTION

In dentistry, xylene is used in histological laboratories for tissue processing, staining and mounting and also in endodontic retreatment as a guttapercha solvent. Its high solvency factor allows maximum displacement of alcohol and renders the tissue transparent, enhancing paraffin infiltration. In staining procedures, its excellent dewaxing and clearing capabilities contribute to brilliantly stained slides. Although it is extremely useful, it leads to various health hazards and toxicity to skin, eyes, nose, nervous system, and musculoskeletal system [1-3].

Exposure to xylene can occur via inhalation, ingestion, and eye or skin contact. The National Institute for Occupational Safety and Health recommended exposure limits for xylene at 100 ppm as a Time-Weighted Average for up to a 10-h work shift and a 40-h work week and 200 ppm for 10 min as a short-term limit. Elimination of xylene from tissue processing cuts costs, saves time, and improves the laboratory environment [1,2].

The objective of the study is to find eco-friendly deparaffinizing agents such as dish wash solution (DWS), diluted lemon water and coconut oil as an alternative to xylene by comparing the staining characteristics such as nuclear staining, cytoplasmic staining, clarity, uniformity and crispness of staining of each individual deparaffinizing agent with xylene.

MATERIALS AND METHODS

The study comprised of 45 paraffin embedded blocks of various tissues. Four sections of 5 microns thickness were prepared from each block. They were stained with hematoxylin and eosin and considered in to 4 groups where xylene (Group A), 1.5% dish wash solution (Group B), 95% diluted lemon water (Group C) and coconut oil (Group D) were used as deparaffinizing agents.

The stained sections are graded based on the parameters of

- Nuclear staining
- Cytoplasmic staining
- Clarity of staining
- Uniformity of staining
- Crispness of staining

Morphometric analysis: After reviewing, the sections were further subjected to morphometric analysis. The images were captured using a three chip CCD camera attached to a trinocular research microscope with a 100X objective. The final image captured on the monitor had a magnification of 1000X. For each specimen, distinct cellular and nuclear outlines were seen, avoiding overlapping to know the uniformity, clarity and crispness of staining.

Statistical Analysis: Chi square test was used.

Fig. 1: H & E stained tissue section deparaffinized with xylene.



Fig. 2: H & E stained tissue section deparaffinized with dish wash solution.

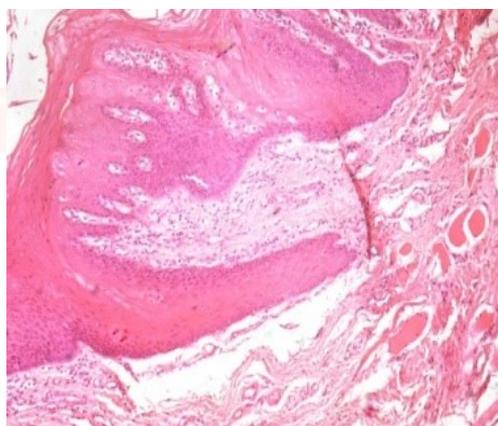


Fig. 3: H & E stained tissue section deparaffinized with diluted lemon water.

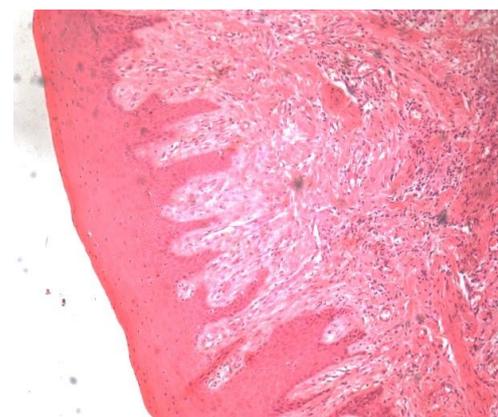
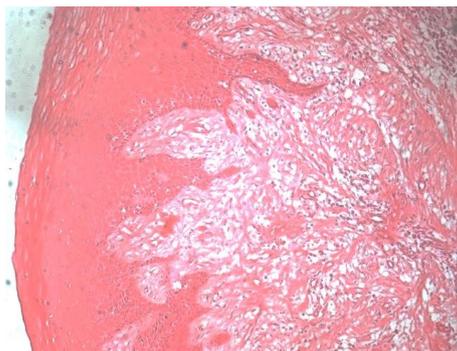


Fig. 4: H & E stained tissue section deparaffinized with coconut oil.



RESULTS

Adequacy of staining characteristics such as nuclear, cytoplasm, uniformity, clarity and crispness of staining for diagnosis was greater with dish wash solution followed by diluted lemon water, coconut oil and xylene. (Graph 1)

DISCUSSION

Xylene is used in histological laboratories though there are a large number of clearing agents available. Xylene is a stable fluid which rapidly removes the dehydrating agent, easily removes the molten wax, causes minimum tissue damage and is cost effective. Histopathological technicians who routinely come in contact with xylene-contaminated solvents in the workplace are the population most likely to be exposed to high levels of xylene. Xylene causes health effects from both acute (<14 days) and also chronic (>365 days) exposure. The type and severity of health effects depends on several factors, including the amount of chemical and the length of time of exposure [1].

However, many substitute chemicals like limonene reagents, aliphatic and aromatic hydrocarbons, and vegetable oil mixtures are being used to substitute xylene as a clearing agent during tissue processing.

Liquid dish wash solution is highly foaming mixture of surfactants principally made up of alkylbenzenesulfonates with low skin irritation and is principally used for hand washing of cutlery, glasses, cooking utensils, and plates. In earlier studies, it was successfully demonstrated as an alternate for xylene in deparaffinizing tissue sections [5-7].

Lemon juice is customarily used to brighten up copper cookware, as sanitary kitchen deodor-

izer, and to remove grease, polish, and wood cleaner, and so forth. The concept of using diluted lemon water as deparaffinizing agent was from its solvent property used to dissolve wax [5]. The underlying principle is that the high temperature of 90 to 94°C will help in removing the wax and dish wash by its surfactant property reduces the surface tension whereas lemon water by its solvent property prevents the wax from resticking onto the slides, thus helping in deparaffinizing these sections [5].

Coconut oil is non hazardous, less expensive and causes less shrinkage of the tissue. It can be used as a deparaffinizing agent in the histopathological laboratory, without losing the quality of the histological details [8].

The results showed that of the 180 tissue sections, 91.1% of both Group A and Group B tissue sections showed adequate nuclear staining as compared with 75% of Group C and 84% of Group D tissue sections. A statistically significant upgradation of nuclear staining was noted in both Group A and B. (Graph 1)

Adequate cytoplasmic staining was seen in all the tissue sections of both Group A and Group D of about 100%. These groups had superior cytoplasmic staining over Group B and Group C. (Graph 1)

82.2% of Group B and Group C tissue sections showed adequate clarity of staining compared with 71.1% tissue sections of Group A and Group D. Group B and Group C had showed superior clarity of staining than Group A and Group D. (Graph 1)

84.4% of Group B and Group D tissue sections showed adequate uniformity compared with 80% of tissue sections of Group A and 75.5% tissue sections of Group C. The Groups B and Group D had superior uniformity of staining over Group A and Group C. (Graph 1)

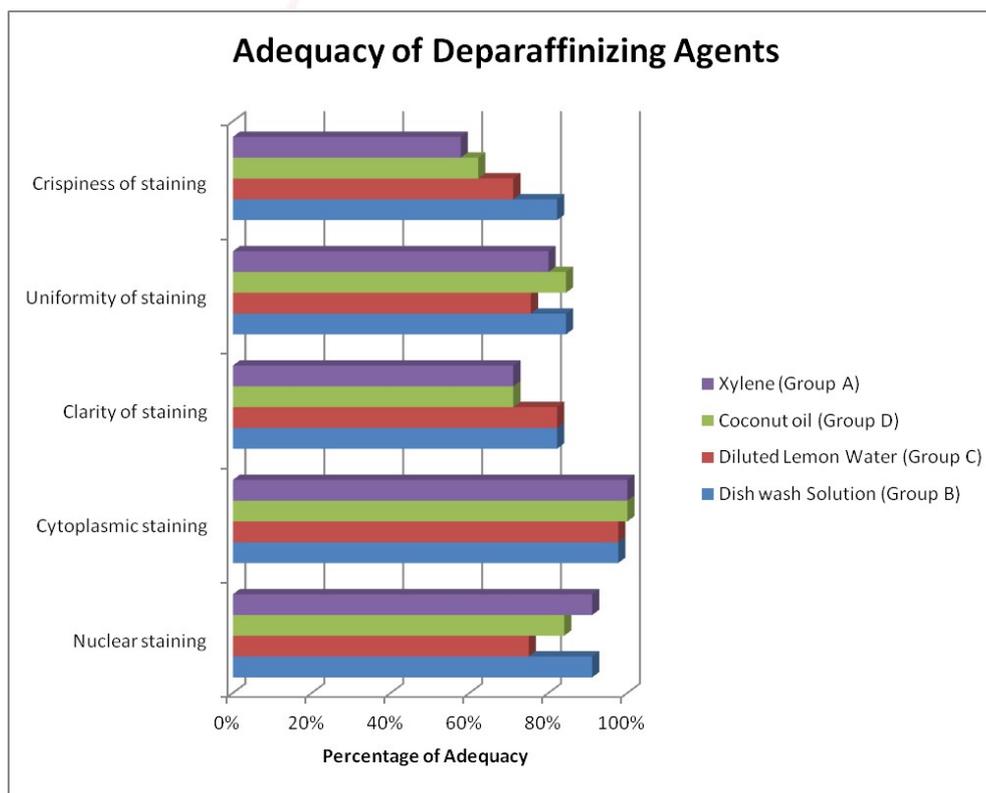
82.2% of tissue sections of Group B showed adequate Crispiness of Staining over 57% of tissue sections of Group A, 71.1% of Group C and 62.2% of Group D. The Group B had superior crispiness of staining over other groups. (Graph 1)

Considering the adequacy of diagnosis of stained sections obtained by four staining methods on the basis of scores obtained, it was

Table 1: Properties of xylene and ecofriendly deparaffinizing agents [3-5].

Sl.no.	Properties	Xylene	Dish wash solution	Diluted lemon water	Coconut oil
1	Cost	Rs 900	Rs 20	Rs 5	Rs 30
2	Time	50-55 min	25-30 min	54 min	25-30 min
3	Toxicity	Yes	No	No	No
4	Bioharzadous	Yes	No	No	No
5	Inflammable	Present	Absent	Absent	Absent
6	Disposal	Difficult	Easy	Easy	Easy
7	Preparation	Synthetic	Synthetic	Naturally available	Naturally available

Graph I: Adequacy of Deparaffinising Agents.



evident that 87.52% of the tissue sections of Group B stained slides were found to be adequate for diagnosis as compared to 79.84% of Group A, 82.30% of Group C and 80.34% of Group D stained slides. The results showed that Group B had showed superior staining properties in all the parameters followed by Group C, Group D and Group A. (Graph 1)

CONCLUSION

Being a Pathologist, it is essential to decrease the price and hazardous of unsafe chemical agents used in histopathological laboratories. The quality of eco-friendly deparaffinizing agents is more efficient than xylene in H & E staining procedure. In addition to it, these are harmless, quicker and price effective. The knowledge of

using dishwash solution, diluted lemon water and coconut oil as natural replacements to xylene is a small step to the future xylene free histopathological laboratories.

Conflicts of Interests: None

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