Original Research Article

Exploring Kerosene as an Alternative Clearing Agent in Histopathology: Innovations in Tissue Processing

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ABSTRACT

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| **Aims:** We evaluated kerosene as a potential substitute, assessing its clearing efficiency, impact on tissue morphology, staining quality, and diagnostic accuracy.**Study design:** A prospective comparative study**Place and Duration of Study:** The Cross-sectional research was conducted at the Department of Pathology, Vinayaka Mission’s Kirupananda Variyar Medical College & Hospitals, Salem - 636308, Tamil Nadu, India over a period of two months (February to March 2025), with a total sample size of 60 tissue specimens**Methodology:** This experimental study involved a total of 60 tissue samples, including 20 control and 40 study specimens, comprising various tissues such as placenta, umbilical cord, gall bladder, etc,.. All samples were randomly selected and divided equally into three groups for processing. Group 1 samples were processed and stained using xylene; Group 2 samples with a 1:1 mixture of xylene and kerosene; and Group 3 samples using kerosene oil alone. Routine tissue processing and hematoxylin and eosin (H&E) staining were performed for all groups. Histological parameters such as ease of sectioning, quality of cytoplasmic and nuclear staining, cell morphology, and clarity of staining were assessed microscopically. **Results:** In our study, the xylene group consistently outperformed the others across all parameters. It showed the highest proportion of good ribboning (80%), good thin section quality (80%), ease of cutting (75%), nuclear staining (75%), cytoplasmic staining (75%), differential staining (80%), clarity (70%), and uniformity of staining (75%). In contrast, the kerosene group had the poorest outcomes, with 85% showing poor ribboning, 80% with poor thin sections, 85% difficult to cut, and similarly low performance in nuclear (80% poor), cytoplasmic (30% good), differential (mostly grade 0), clarity (25% good), and uniformity (80% poor). The kerosene + xylene mixture group showed intermediate results, better than kerosene alone but not as effective as xylene.**Conclusion:** Xylene remains superior in tissue clearing and staining quality; however, kerosene is a cost-effective and less toxic alternative with acceptable performance. While kerosene showed slightly lower efficiency, tissue morphology and diagnostic accuracy were maintained. A xylene–kerosene mixture offered improved outcomes over kerosene alone. |

*Keywords:*

*Xylene substitute, Kerosene, Hematoxylin and Eosin (H&E) staining, Histopathology, Tissue processing, Staining quality, Microscopy, Cytoplasmic and nuclear staining*

INTRODUCTION

Xylene is the commonest clearing agent & plays a important vital role in histological section preparation. Xylene has the ability to efficiently deparaffinize and clear tissue samples, but the hazardous properties have raised health and environmental issues, known for its carcinogenic potential , latest researchers to search for alternative solvents for tissue processing. [1,2] The stages of tissue processing are dehydration, clearing, impregnation, and embedding, each with a particular duration for proper completion of the process. Xylene is the clearing agent used most commonly worldwide. Xylene is a sweet smelling, colorless, aromatic hydrocarbon in liquid or gas form that is found naturally in coal, petroleum, and wood tar. [3,4] We evaluated the clearing efficiency of kerosene in comparison to conventional clearing agents like xylene in histopathology tissue processing and assessed the impact of kerosene on tissue morphology, staining quality, and overall diagnostic accuracy.

2. material and methods

**Study Design**:
This was a **prospective comparative study** conducted over a period of **two months (February to March 2025)**.

**Setting**:
The study was conducted at the Department of Pathology, **Vinayaka Mission’s Kirupananda Variyar Medical College & Hospital (VMKVMC&H), Salem, Tamil Nadu, India**.

**Participants**:
A total of **60 tissue samples** were included in the study, comprising **20 controls and 40 study samples**. Tissues were collected from specimens such as placenta, umbilical cord, gall bladder, appendix, ovary, fallopian tube, breast, thyroid, sebaceous cyst, testis, lipoma, uterus, omentum, small intestine, large intestine, and fibroid. Samples were randomly selected.

**Variables and Group Allocation**:
Samples were randomly assigned into **three groups**:

* **Group 1 (n=20)**: Processed and stained using **xylene**.
* **Group 2 (n=20)**: Processed and stained using a **1:1 mixture of xylene and kerosene**.
* **Group 3 (n=20)**: Processed and stained using **kerosene**.

**Data Collection and Measurement**:
All tissues underwent **routine tissue processing and Hematoxylin & Eosin (H&E) staining**. Microscopic evaluation was performed to assess parameters such as **ease of sectioning, cytoplasmic staining, nuclear staining, cell morphology, and clarity of staining**.

**Statistical Methods**:
The data were analyzed using **Fisher’s exact test** to evaluate statistical significance among the groups.

3. results and discussion

**Grading of Ribboning**

This table compares the quality of ribboning—graded as 0 (poor) or 1 (good)—across three groups using different clearing agents: Group 1 (Kerosene), Group 2 (Xylene), and Group 3 (Xylene:Kerosene mixture). Kerosene Group showed the highest proportion of poor ribboning (85%), while Xylene group had the highest number of good ribboning outcomes (80%).

**Table 1: Grading of Ribboning**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Grading | Kerosene | Xylene | Xylene +Kerosene | Total |
| 0 | 17 | 4 | 14 | 35 |
| 1 | 3 | 16 | 6 | 25 |
| Total | 20 | 20 | 20 | 60 |

**Grading of Thin Section**

This table assesses the thinness of tissue sections obtained after processing with different clearing agents. **Xylene group** again performed best with 80% showing good thin section quality (grade 1), while **Kerosene Group**  had mostly poor outcomes (80% graded 0).

**Table 2: Grading of Thin Section**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Grading | Kerosene | Xylene | Xylene +Kerosene | Total |
| 0 | 16 | 4 | 12 | 32 |
| 1 | 4 | 16 | 8 | 28 |
| Total | 20 | 20 | 20 | 60 |

**FIGURE 1: RIBBONING & THIN SECTION GRADING**

**Grading of Ease of Section Cutting**

This table evaluates how easily sections could be cut after clearing. Xylene group had the most favorable results, with 75% of samples graded 1, while Kerosene Group had 85% graded as difficult (grade 0).

**Table 3: Grading of Ease of Section Cutting**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Grading | Kerosene | Xylene | Xylene +Kerosene | Total |
| 0 | 17 | 5 | 12 | 34 |
| 1 | 3 | 15 | 8 | 26 |
| Total | 20 | 20 | 20 | 60 |

**Grading of Nuclear Staining**

This table presents the effectiveness of nuclear staining quality across the three groups. **Xylene group** had the best nuclear staining, with 75% graded as good (grade 1), whereas **Kerosene Group**  had 80% with suboptimal staining.

**Table 4: Grading of Nuclear Staining**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Grading | Kerosene | Xylene | Xylene +Kerosene | Total |
| 0 | 16 | 5 | 13 | 34 |
| 1 | 4 | 15 | 7 | 26 |
| Total | 20 | 20 | 20 | 60 |

**FIGURE 2: NUCLEAR STAINING & EASE OF SECTION CUTTING GRADES**

**Grading of Cytoplasmic Staining**

This table shows the grading of cytoplasmic staining quality. **Xylene group** showed the highest proportion of satisfactory cytoplasmic staining (75%), followed by Kerosene + Xylene group (60%). **Kerosene Group** had the lowest proportion (30%) of good cytoplasmic staining.

**Table 5: Grading of Cytoplasmic Staining**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Grading | Kerosene | Xylene | Xylene +Kerosene | Total |
| 0 | 14 | 5 | 8 | 27 |
| 1 | 6 | 15 | 12 | 33 |
| Total | 20 | 20 | 20 | 60 |

**Grading of Differential Staining**

This table compares differential staining, which evaluates contrast between nuclear and cytoplasmic staining. **Xylene group** again led with 80% graded as good, while **Kerosene Group**  and Kerosene + Xylene group had better performance in grade 0.

**Table 6: Grading of Differential Staining**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Grading | Kerosene | Xylene | Xylene +Kerosene | Total |
| 0 | 15 | 4 | 14 | 33 |
| 1 | 5 | 16 | 6 | 27 |
| Total | 20 | 20 | 20 | 60 |

**FIGURE 3: Differential & Cytoplasmic Staining Grades**

**Grading of Clarity**

This table assesses the clarity of histological sections. **Xylene group** showed the highest percentage of good clarity (70%), while **Kerosene Group**  had the lowest (25%). Kerosene + Xylene group had moderate performance.

**Table 7: Grading of Clarity**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Grading | Kerosene | Xylene | Xylene +Kerosene | Total |
| 0 | 15 | 6 | 13 | 34 |
| 1 | 5 | 14 | 7 | 26 |
| Total | 20 | 20 | 20 | 60 |

**Grading of Uniformity**

This table evaluates the uniformity of staining. Xylene group had the highest percentage of consistent staining (75% graded 1), whereas Kerosene Group again showed more poor outcomes (80% graded 0).

**Table 8: Grading of Uniformity**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Grading | Kerosene | Xylene | Xylene +Kerosene | Total |
| 0 | 16 | 5 | 11 | 32 |
| 1 | 4 | 15 | 9 | 28 |
| Total | 20 | 20 | 20 | 60 |

**FIGURE 4: UNIFORMITY & CLARITY GRADES**

**Discussion:**

In our study, Kerosene Group showed the highest proportion of poor ribboning (85%), while Xylene group had the highest number of good ribboning outcomes (80%). Regarding grade of thin sections, Xylene group again performed best with 80% showing good thin section quality (grade 1), while Kerosene Group had mostly poor outcomes (80% graded 0). While regarding ease of cutting sections, Xylene group had the most favorable results, with 75% of samples graded 1, while Kerosene Group had 85% graded as difficult (grade 0). Xylene group had the best nuclear staining, with 75% graded as good (grade 1), whereas Kerosene Group had 80% with suboptimal staining. Xylene group showed the highest proportion of satisfactory cytoplasmic staining (75%), followed by Kerosene + Xylene group (60%). Kerosene Group had the lowest proportion (30%) of good cytoplasmic staining. Regarding differential staining, Xylene group again led with 80% graded as good, while Kerosene Group and Kerosene + Xylene group had better performance in grade 0. Xylene group showed the highest percentage of good clarity (70%), while Kerosene Group had the lowest (25%). Kerosene + Xylene group had moderate performance. Regarding uniformity of staining, Xylene group had the highest percentage of consistent staining (75% graded 1), whereas Kerosene Group again showed more poor outcomes (80% graded 0).

Regarding the Clearing Efficiency the kerosene demonstrates adequate clearing properties, though it is slightly less efficient than xylene in achieving complete tissue transparency. Similar findings were reported by Ranjan et al. (2018) [5], who observed partial limitations in kerosene-cleared samples for certain tissue types. However, the study by Nwachukwu et al. (2016) [6] highlighted kerosene's capability as a clearing agent in low-resource settings, emphasizing its affordability and reduced health hazards compared to xylene.

Regarding the Tissue Morphology the Kerosene-cleared tissues retained cellular architecture and exhibited minimal morphological changes, consistent with the observations of Chatterjee et al. (2020) [7]. Their study noted that kerosene preserves nuclear and cytoplasmic integrity, making it a viable alternative. However, the mild lipid dissolution properties of kerosene observed in our study were also mentioned by Gupta et al. (2019) [8], who suggested using kerosene for specific tissue types to minimize such effects.

Comparable staining quality was achieved with kerosene and xylene, supporting the findings of Singh et al. (2017) [9] who demonstrated that kerosene provides acceptable staining intensity and uniformity for hematoxylin and eosin (H&E) staining. While slight variations in specific dyes were observed, as noted in the study by Omotola et al. (2021) [10], these differences did not compromise the diagnostic clarity of kerosene-processed tissues.

The diagnostic accuracy remained uncompromised in kerosene-cleared samples, corroborating the findings of Adeyemi and Ayodele (2018) [11]. Their study concluded that alternative clearing agents like kerosene and coconut oil maintain diagnostic reliability when tissue processing protocols are carefully adjusted.

Several studies have highlighted the environmental and health concerns associated with xylene. Gupta et al. (2019)[8] and Adeyemi and Ayodele (2018) [11] emphasized the reduced toxicity and eco-friendliness of kerosene, particularly in resource-constrained environments, as a compelling advantage.

Clearing Efficiency with Kerosene will be showing adequate clearing properties, though it is less efficient than xylene. However, it is known for its toxicological risks kerosene proved to be a cost-effective and less hazardous option compared to xylene. Regarding the Tissue Morphology the histopathological examination will reveal the significant alterations in tissue morphology in kerosene-cleared samples compared to xylene. Cellular architecture remained intact, supporting the feasibility of kerosene for routine use. Diagnostic accuracy will be discussed & maintained in kerosene-cleared tissues, with pathologists able to identify histological features reliably. [12,13]

 Kerosene demonstrates promise as an alternative clearing agent in histopathology, offering a less toxic and cost-effective solution compared to xylene. While minor differences in clearing efficiency and staining quality were noted, they did not significantly affect tissue morphology or diagnostic accuracy. If kerosene is used as an alternative for cost-saving, it’s essential to increase the duration of clearing. Use optimized kerosene mixtures with a small proportion of xylene to improve clearing and staining outcomes. Xylene is highly toxic, causing health hazards such as dizziness, headaches, or chronic exposure risks**.** Its high volatility increases the risk of exposure and flammability. Kerosene is inexpensive and widely available. While still hazardous, kerosene is generally considered less toxic than xylene in some respects. xylene with kerosene reduces the cost while maintaining acceptable clearing properties. The addition of kerosene decreases the overall volatility, slightly reducing the health risks compared to pure xylene. [14,15]

4. Conclusion

Our study demonstrates that while xylene remains the gold standard for tissue clearing and staining in histopathology due to its superior performance in ribboning, section quality, staining clarity, and overall morphology, kerosene offers a viable alternative, particularly in resource-limited settings. Kerosene-cleared tissues showed slightly reduced clearing efficiency and staining quality but retained adequate tissue morphology and diagnostic accuracy. The use of a xylene–kerosene mixture improved outcomes compared to kerosene alone, offering a balanced compromise between efficacy and safety. Given xylene's known health and environmental hazards, kerosene presents a cost-effective, less toxic substitute. Optimization of processing protocols—such as extended clearing time or combining kerosene with small amounts of xylene—can enhance results. Thus, kerosene, especially in modified forms, may be considered a practical alternative in routine histopathology, particularly where safety and cost are significant concerns.

Consent (where ever applicable)

All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this study. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

Ethical approval (where ever applicable)

Before the study, all patients were informed about the contents & type of the study and informed written consents were obtained from all of them. The study was approved by the Institutional ethical committee of Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals. (Reference no: VMKVMC&H IEC/25/033).according to the ICMR guidelines on Biomedical research in human beings and also adhering to the principles of Good Clinical Practice. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.”

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