Kerosene as a Potential Substitute for Xylene in Histopathology: A Prospective Comparative Study

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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 analyzed 60 tissue samples divided into three groups: xylene (Group 1), xylene–kerosene mix (Group 2), and kerosene alone (Group 3). Routine H&E staining was performed, and histological parameters—sectioning ease, cytoplasmic and nuclear staining quality, cell morphology, and clarity—were evaluated microscopically.  **Results:** Xylene outperformed the other groups across all histological parameters, showing the highest rates of good ribboning (80%), section quality (80%), ease of cutting (75%), and staining clarity and uniformity (70–80%). Kerosene alone yielded the poorest results, with up to 85% poor outcomes in ribboning, sectioning, and staining. The xylene–kerosene mix showed intermediate performance—better than kerosene but inferior to 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 vital role in histological section preparation. Xylene can efficiently deparaffinize and clear tissue samples, but the hazardous properties have raised health and environmental issues. Known for its carcinogenic potential, the latest researchers are searching 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. We assessed its impact 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**.

All tissue samples underwent clearing during routine processing using the respective clearing agents:

* **Xylene group (Group 1):** Cleared for **30 minutes** (standard protocol)
* **Xylene + Kerosene mixture (Group 2):** Cleared for **40 minutes** (increased time to compensate for reduced volatility of kerosene)
* **Kerosene group (Group 3):** Cleared for **60 minutes** (extended duration due to kerosene's lower clearing efficiency)

The longer clearing time for kerosene was necessary to ensure adequate tissue transparency, as it is less efficient than xylene in removing alcohol and infiltrating paraffin. These times were optimized based on prior studies and preliminary trials to balance tissue integrity with processing efficiency.

**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%). Regarding ribboning, high significant differences (p < 0.001) was noted with the Xylene group consistently outperforming the other groups

**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). Regarding thin section quality, high significant differences (p < 0.001) was noted with the Xylene group consistently outperforming the other groups

**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 favourable results, with 75% of samples graded 1, while Kerosene Group had 85% graded as difficult (grade 0). Regarding ease of section cutting, nuclear staining, high significant differences (p < 0.001) was noted with the Xylene group consistently outperforming the other groups

**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. Regarding nuclear staining, high significant differences (p < 0.001) was noted with the Xylene group consistently outperforming the other groups

**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. Regarding Cytoplasmic staining, significant differences (p = 0.006) was noted with Kerosene performing poorly, and the xylene–kerosene mixture yielding intermediate outcomes.

**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. Regarding differential staining, high significant differences (p < 0.001) was noted with the Xylene group consistently outperforming the other groups

**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. Regarding clarity, significant differences (p = 0.011), was noted with Kerosene performing poorly, and the xylene–kerosene mixture yielding intermediate outcomes.

**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). Regarding uniformity, significant differences (p = 0.008) was noted with Kerosene performing poorly, and the xylene–kerosene mixture yielding intermediate outcomes.

**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, the Kerosene Group showed the highest proportion of poor ribboning (85%), while the Xylene Group had the highest number of satisfactory ribboning outcomes (80%). Regarding the grade of thin sections, the xylene group again performed best, with 80% showing superior thin section quality (grade 1), while the kerosene group had mostly poor outcomes (80% graded 0). Regarding ease of cutting sections, the xylene group had the most favorable results, with 75% of samples graded 1, while the kerosene group had 85% graded as difficult (grade 0). The xylene group had the best nuclear staining, with 75% graded as good (grade 1), whereas the kerosene group had 80% with suboptimal staining. The xylene group showed the highest proportion of satisfactory cytoplasmic staining (75%), followed by the kerosene + xylene group (60%). The kerosene group had the lowest proportion (30%) of excellent cytoplasmic staining. Regarding differential staining, the xylene group again led with 80% graded as satisfactory, while the kerosene group and the kerosene + xylene group had better performance in grade 0. The xylene group showed the highest percentage of outstanding clarity (70%), while the kerosene group had the lowest (25%). The kerosene + xylene group had moderate performance. Regarding uniformity of staining, the xylene group had the highest percentage of consistent staining (75% graded 1), whereas the 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. Chiwar et al. [5] reported similar findings, observing partial limitations in kerosene-cleared samples for specific tissue types. However, the study by Singh M et al. [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 Shah et al. [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 Buesa et al. (2019) [8], who suggested it for specific tissue types to minimize such effects.

Comparable staining quality was achieved with kerosene and xylene, supporting the findings of Premalatha et al. [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 Ofusori 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 Niaz et al. [11]. Their study concluded that carefully adjusted tissue processing protocols maintain diagnostic reliability when using alternative clearing agents like kerosene and coconut oil. oil.

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

Clearing efficiency demonstrates adequate clearing properties, although it is less efficient than xylene. However, it is known for its toxicological risks. Kerosene was a cost-effective and less hazardous option compared to xylene. Regarding the tissue morphology, the histopathological examination will reveal 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 and maintained in kerosene-cleared tissues, allowing pathologists to identify histological features reliably. [12, 13]

                  Kerosene demonstrates promise as an alternative clearing agent in histopathology, offering a less toxic and more cost-effective solution compared to xylene. Although we noted minor differences in clearing efficiency and staining quality, they did not significantly affect tissue morphology or diagnostic accuracy. When using kerosene as a cost-saving alternative, it's crucial to extend the clearing duration. 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]

Kerosene underperforms as a clearing agent due to its lower solvent power, higher viscosity, and slower evaporation compared to xylene. These properties result in incomplete clearing, poor paraffin infiltration, and reduced section quality. Its lower refractive index and inefficient deparaffinization further compromise staining clarity and uniformity. Additionally, impurities in kerosene may interfere with staining, leading to suboptimal histological outcomes.

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

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

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.”

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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