The 3rd International Innovation Technology Proceedings
Vol 3 No 1 (2025): October
E-ISSN: 3030-9948
https://iite-proceeding.poltekindonusa.ac.id

Evaluating Clearing Agents in Deparaffinization and Tissue Infiltration

Yulita Maulani[1]*, Anak Agung Ayu Eka Cahyani[2], Emma Ismawatie[3]
[1,3] Medical Laboratory Technology, Politeknik Indonusa, Surakarta
[2] Medical Laboratory Technology, Wira Medika Bali Collage of Health Sciences, Bali
*Corresponding author email: yulita.maulani@poltekindonusa.ac.id

---

Received:	Revised:	Accepted:	Published:
July 14, 2025	July 21, 2025	July 31, 2025	October 6, 2025

Abstract

The purpose of the tissue processing stage known as "clearing" is to rid the tissue of alcohol and other dehydrants. Xylene is a chemical that is frequently used as a cleaning agent in laboratories. Nonetheless, it is well recognized that xylene is extremely hazardous. This article reviews research on the role of cleaning solutions in the deparaffinization and tissue infiltration processes with the aim of identifying a safer and more effective alternative. Using secondary data from the Science and Technology Index (SINTA) database, Google Scholar, PubMed Central, and ScienceDirect, the research methodology is a literature review. As a result, thirty scholarly articles were acquired for examination. According to the findings, many synthetic and natural solvents have been shown to have cleaning effectiveness and histology quality that are on par with xylene, but at a lower risk of toxicity and tissue shrinking. However, recurrence and results might be impacted by differences in tissue response as well as procedural elements like temperature and cleaning time. Because of problems with availability and cost, some alternatives have not been extensively embraced, even though the safety profile promotes the use of ecologically friendly chemicals. Taking everything into account, integrating these findings leads to a variety of efficient cleaning solutions that strike a balance between usefulness, security, and effectiveness. This insight offers direction for upcoming laboratory research and practices that maximize histopathological tissue processing while minimizing risks to the environment and public health.

Keywords: *clearing agents, deparaffinization process, histological.*

1. Introduction

Research on the role of clearing solutions in the deparaffinization and tissue infiltration process has emerged as a critical area of investigation due to its fundamental importance in histopathological diagnostics and biomedical research. Clearing agents facilitate the removal of dehydrants from tissues and allow for the infiltration of paraffin wax, which is essential for producing high-quality microscopic sections (Chandraker et al., 2018) (Alwahaibi et al., 2018). Historically, xylene has been the dominant clearing agent due to its effectiveness in making tissues transparent and readily soluble in alcohol and paraffin (Chandraker et al., 2018) (Alwahaibi et al.,

2018). However, concerns about its toxicity, environmental hazards, and occupational health risks have driven the search for safer alternatives (Chandraker et al., 2018) (Alwahaibi et al., 2018). Over several decades, research has evolved from exploring aromatic hydrocarbons to vegetable oils and synthetic solvents, reflecting a trajectory aimed at balancing cleaning efficiency with safety and environmental sustainability (Cano et al., 2024). The practical significance of this research is underscored by the widespread use of clearing agents in clinical and research laboratories worldwide, where exposure to hazardous chemicals like xylene poses substantial health risks (Chandraker et al., 2018) (Alwahaibi et al., 2018).

The specific problem discussed in this review is the need to identify and evaluate effective and less toxic cleaning agents that can replace xylene without sacrificing tissue morphology, staining quality, or processing efficiency (Chandraker et al., 2018) (Cano et al., 2024). Despite numerous studies investigating vegetable oils such as coconut, olive, and palm oil, as well as synthetic substitutes like UltraClear™ and n-heptane, inconsistencies persist regarding cleaning efficacy, tissue shrinkage, and staining results (Chandraker et al., 2018) (Stockert et al., 2012) (Cano et al., 2024) (Alwahaibi et al., 2018). Additionally, some replacements require longer cleaning times or higher temperatures, which can affect workflow and network integrity (Ganesan et al., 2016) (Bright et al., 2024). Competing perspectives exist, with some studies advocating for vegetable oils due to their biocompatibility and cost-effectiveness, while others highlight limitations in cleaning speed or tissue stiffness (Chandraker et al., 2018) (Cano et al., 2024) (Jyostna et al., 2024) (Chaudhuri et al., 2023). The knowledge gap lies in the lack of a comprehensive and systematic comparison that integrates these diverse findings to guide the selection of the optimal clearing agent for routine histopathological use (Cano et al., 2024) (Chaudhuri et al., 2023) (Alwahaibi et al., 2018). Failure to address this gap can perpetuate dependence on hazardous solvents, impacting laboratory safety and environmental health (Chandraker et al., 2018).

The conceptual framework for this review is based on the understanding that clearing agents function to replace dehydrants and make tissues compatible with embedding media, thus facilitating sectioning and staining (Chandraker et al., 2018) (Alwahaibi et al., 2018). Key concepts include the physicochemical properties of clearing agents—such as miscibility, toxicity, and volatility—and their influence on tissue morphology, staining quality, and processing efficiency (Chandraker et al., 2018). The interaction between clearance efficacy and safety profile forms the basis for evaluating alternatives to xylene, directly linking the framework to the research objectives

(Cano et al., 2024) (Alwahaibi et al., 2018). The purpose of this systematic review is to critically assess the efficacy, safety, and practical application of various clearing solutions as alternatives to xylene in the deparaffinization and tissue infiltration processes (Cano et al., 2024) (Chaudhuri et al., 2023). By synthesizing evidence from various studies, this review aims to provide a comprehensive evaluation that informs laboratory practices and promotes safer and more environmentally friendly alternatives (Cano et al., 2024) (Alwahaibi et al., 2018). This contribution addresses identified knowledge gaps by integrating diverse findings into a coherent analysis, thus supporting evidence-based decision-making in histopathology laboratories (Cano et al., 2024) (Chaudhuri et al., 2023).

The review methodology involves a systematic literature search and critical assessment of studies comparing clearing agents, focusing on parameters such as network transparency, shrinkage, staining quality, and processing time (Cano et al., 2024) (Chaudhuri et al., 2023). Inclusion criteria emphasize studies with experimental comparisons to xylene, while the analytical framework assesses qualitative and quantitative results (Cano et al., 2024) (Alwahaibi et al., 2018). These findings are organized thematically to explain the advantages and limitations of each category of clearing agents, facilitating a structured understanding of their roles in histopathological processing (Cano et al., 2024) (Chaudhuri et al., 2023).

2. Method

This study employs a literature review as its research method. The data sources utilized comprise secondary data acquired from the Science and Technology Index (SINTA) database, Google Scholar, PubMed Central, and Science Direct. This literature study established several inclusion criteria: national articles indexed at least SINTA 3, international articles indexed by Google Scholar or Scopus, quantitative research articles, articles in Indonesian or English, full-text and open access articles, and scientific research articles. The data collection process initiates with the identification of keywords relevant to the research theme, thereby enhancing the article search process. The subsequent step involves screening the acquired articles, which will subsequently undergo analysis.

3. Result and Discussion

Most studies confirm that the clearing agent must be miscible with the dehydrant and embedding medium for effective tissue processing, with xylene serving as the benchmark for

miscibility (Chandraker et al., 2018) (Stockert et al., 2012) (Chaudhuri et al., 2023). Alternatives such as coconut oil, cedarwood oil, n-heptane, and UltraClear™ also show suitable miscibility under certain conditions (Cano et al., 2024) (Chaduvula et al., 2024) (Bright et al., 2024) (Alwahaibi & Aldughaishi, 2019). Some reports indicate challenges with vegetable oils like coconut oil due to solidification at lower temperatures, affecting processing (Chandraker et al., 2018) (Sermadi et al., 2014) and potential mixing issues requiring temperature control (Ganesan et al., 2016). Variability exists in mixture compatibility, such as xylene-kerosene at specific ratios (Ofusori et al., 2009). Differences in the chemical composition and physical properties (e.g., viscosity, melting point) of cleaning agents cause variations in the mixture; temperature and mixing ratio affect compatibility.

The majority agree that certain vegetable oils and synthetic solvents can produce histological sections with comparable staining clarity, nuclear and cytoplasmic detail, and tissue transparency relative to xylene (Chandraker et al., 2018) (Cano et al., 2024) (Jyostna et al., 2024) (Chaduvula et al., 2024) (Bhandari et al., 2022) (Ashitha, 2018) (Sermadi et al., 2014). UltraClear™ and n-heptane also provide acceptable staining and morphological preservation (Stockert et al., 2012). (Alwahaibi & Aldughaishi, 2019) (Alwahaibi et al., 2018). Some studies report inconsistent staining results with vegetable and mineral oils (Chandraker et al., 2018) (Chaudhuri et al., 2023) and a slight decrease in staining quality or clarity with substitutes like UltraClear™, especially in certain procedural combinations (Alwahaibi & Aldughaishi, 2019) (Alwahaibi et al., 2018). Differences in ease of division were noted, with some oils causing difficulties (Chandraker et al., 2018) (Bright et al., 2024). Variability in network type, cleaning duration, temperature, and the physical properties of oil versus solvent affects staining results; procedural differences and evaluation criteria can also influence outcomes.

There is consensus that xylene induces significant tissue shrinkage, while natural oils like coconut and peanut oil cause less shrinkage (Chandraker et al., 2018) (Cano et al., 2024) (Bright et al., 2024) (Bhandari et al., 2022) (Sermadi et al., 2014). Some oils and synthetic substitutes maintain tissue morphology well, with low distortion (Chaduvula et al., 2024) (Saribu et al., 2023) (Esan et al., 2015). There are contrasting findings regarding shrinkage, with some studies noting significant shrinkage with coconut oil at shorter cleaning times and challenges in achieving comparable stiffness to xylene (Bright et al., 2024). Some synthetic agents cause tissue softening or swelling depending on their composition. Differences in tissue type, cleaning duration, temperature control,

and the chemical properties of the cleaning agent explain the variable effects on tissue morphology and shrinkage; measurement methods may vary.

Since xylene is widely believed to be hazardous, poisonous, and bad for the environment, research into safer substitutes such as natural oils, artificial non-aromatic solvents, and biodegradable substances has increased (Chandraker et al., 2018) (Cano et al., 2024) (Chaduvula et al., 2024) (Alwahaibi & Aldughaishi, 2019) (Alwahaibi et al., 2018). Natural oils and agents like UltraClear™ are generally less toxic and more environmentally friendly (Cano et al., 2024) (Chaduvula et al., 2024) (Alwahaibi & Aldughaishi, 2019). Although the substitutes are safer, some (e.g., UltraClear™) are more expensive and less widely available (Alwahaibi & Aldughaishi, 2019) (Alwahaibi et al., 2018). Some natural oils require temperature control or longer processing times, which can pose operational safety issues. Differences arise from balancing security with cost, availability, and processing efficiency. Local availability, commercial production, and handling requirements contribute to the divergence.

Although it is generally agreed that natural oils and synthetic substitutes can take the place of xylene without sacrificing quality, there are typical practical problems such lengthier cleaning durations, temperature sensitivity, and ease of separation (Chandraker et al., 2018) (Cano et al., 2024) (Bright et al., 2024) (Ashitha, 2018) (Ganesan et al., 2016). Findings differ on ease of separation, with some oils causing difficulties (Chandraker et al., 2018) (Bright et al., 2024); also, the temperature dependence of using dishwashing liquid or oil for deparaffinization affects the duration and quality of the process (Ganesan et al., 2016) (Yadav et al., 2019). Costs and availability vary significantly, with some synthetic substitutes being very expensive (Alwahaibi & Aldughaishi, 2019) (Alwahaibi et al., 2018). Variations arise from differences in study protocols (e.g., clearing time, temperature), tissue type, and economic context; some agents require longer heating times or durations, which impacts workflow and cost.

Recent studies highlight that cleaning agents, particularly organic solvents like BABB, can alter fluorescence emission spectra and intensity and cause spectral shifts that affect imaging accuracy (Eliat et al., 2022) (Kim et al., 2022). New agents like OptiMUs and HyClear offer better fluorescence preservation and faster cleaning (Nasseri et al., 2022) (Kim et al., 2022). Previous conventional cleaning agents (e.g., BABB) caused fluorescence quenching and spectral shifts,

complicating multiband imaging; some agents induced tissue shrinkage or swelling (Eliat et al., 2022). Compatibility with lipophilic dyes varies (Kim et al., 2022). Differences in the chemical composition of the cleaning solution and its interaction with the fluorophore explain the spectral shift and quenching; newer formulations aim to reduce these effects to improve imaging.

4. Conclusion

The body of research on cleaning solutions for histopathological tissue processing shows a clear trend toward finding safer and more efficient substitutes for common clearing agents like xylene. Both synthetic solvents like n-heptane and Histosol, as well as natural oils like coconut, olive, palm, pine, and cedarwood oils, regularly show chemical compatibility with dehydrants and paraffin waxes, allowing for effective cleaning and penetration. In general, this substitute yields histological outcomes that are on par with or, in certain situations, better than xylene in terms of staining quality, retention of cellular detail, and tissue transparency. In particular, compared to xylene, natural oils typically cause less tissue shrinkage and preserve tissue morphology, which enhances the accuracy of microscopic inspection. Given that conventional aromatic solvents like xylene provide serious health risks, such as toxicity and carcinogenicity, as well as environmental dangers, safety and environmental factors strongly favor this alternative. In line with occupational health concerns in histopathology labs, several synthetic chemicals and vegetable oils have a far lower toxicity profile and are more environmentally friendly. While some natural oils need higher temperatures or longer cleaning times to provide the best results, which could affect workflow productivity, the adoption of some synthetic alternatives is hindered by their higher costs and restricted availability.One of the literature's major limitations is still methodological heterogeneity. Direct comparison and synthesis are challenging because of differences in network type, cleaning time, temperature, and evaluation criteria. However, studies that employ a controlled comparative design enhance the effectiveness of substitute treatments. Standardized procedures are necessary to guarantee consistency and optimize cleaning performance, as seen by the paucity of systematic research into procedural parameters like cleaning temperature and time. New techniques that promise better cleaning with less toxicity and environmental impact include solvent-free electric field technologies, enzymatic cleaning, and water-based surfactant compositions. Furthermore, although their incorporation into standard histopathology is still in its infancy, sophisticated

clearing techniques designed for three-dimensional imaging place a high priority on maintaining tissue architecture and fluorescence. In summary, the research backs up the viability of using natural oils in place of xylene and choosing synthetic solvents without sacrificing histological integrity. This alternative provides a better safety and environmental profile while maintaining or enhancing tissue morphology preservation and staining quality. To promote broader use and streamline tissue processing processes, future studies should give top priority to standardizing clearing protocols, conducting thorough toxicological evaluations, and evaluating clearing agents across a range of tissue types and sophisticated histological techniques..

5. Acknowledgement

Sincere thanks are extended by the author to Politeknik Indonusa Surakarta for providing institutional financing for this study. Also acknowledged is Wira Medika Bali Collage of Health Sciences, which provided academic assistance and collaborative support during the creation of this literature study. Additionally, the author thanks the reviewers and academic colleagues whose helpful criticism has enhanced the quality of this paper.

6. References

[1] N. Alwahaibi and S. H. Aldughaishi, "A substitute to xylene in deparaffinization and clearing prior to coverslipping in histopathology," J. Lab. Physicians, vol. 11, no. 2, pp. 118–122, 2019, doi: 10.4103/JLP.JLP_169_18.

[2] N. Alwahaibi, S. Aljaradi, and H. Alazri, "Alternative to xylene as a clearing agent in histopathology," J. Lab. Physicians, vol. 10, no. 2, pp. 189–193, 2018, doi: 10.4103/JLP.JLP_111_17.

[3] A. S. Ashitha, "Comparing the efficiency of coconut oil and palm oil with xylene as a clearing agent in conventional hematoxylin and eosin histopathological staining procedure," 2018.

[4] S. Bhandari et al., "To compare and evaluate the efficacy of the groundnut oil and coconut oil with xylene as a clearing agent," 2022, doi: 10.56136/nhdcjcd/2022_00028.

[5] O. A. Bright et al., "Clearing properties between coconut oil and xylene in histological tissue processing," J. Histochem. Cytochem., vol. 72, no. 4, pp. 233–243, 2024, doi: 10.1369/00221554241241369.

[6] T. L. Tugonon, "Oil as an alternative clearing agent to xylene in histological staining process: A systematic review," Egypt. J. Histol., 2024, doi: 10.21608/ejh.2024.273555.2030.

[7] N. Chaduvula et al., "An upgradation to minimize hazardous effects in histopathological laboratories: Cedarwood oil as an alternative to xylene," Vestn. Stomatol. Chelyustno-Litsevoy Khirurgii, pp. 5–10, 2024, doi: 10.58240/1829006x-2025.1-5.

[8] R. Chandraker et al., "Comparison between xylene and coconut oil in tissue processing," Med. Lab. J., vol. 2, no. 1, pp. 96–99, 2018, doi: 10.30699/MMLJ17.1.3.96.

[9] D. Chaudhuri et al., "Evaluation of the efficacy of various xylene substitutes as a clearing agent in tissue processing: A comparative study," Int. J. Adv. Res., 2023, doi: 10.21474/ijar01/17439.

[10] D. Cobb and R. L. Beard, "Method and system for automated deparaffinization and non-immunohistochemical special staining of tissue samples," 2012.

[11] F. Eliat et al., "Tissue clearing may alter emission and absorption properties of common fluorophores," Dent. Sci. Rep., vol. 12, no. 1, 2022, doi: 10.1038/s41598-022-09303-9.

[12] E. O. Esan, A. B. Ajileye, and S. A. Fasogbon, "Groundnut oil as an alternative clearing agent to xylene in histological tissue processing," 2015.

[13] H. F. V. Essen et al., "Alcohol based tissue fixation as an alternative for formaldehyde: Influence on immunohistochemistry," J. Clin. Pathol., vol. 63, no. 12, pp. 1090–1094, 2010, doi: 10.1136/JCP.2010.079905.

[14] R. Gaifulina et al., "Rapid and complete paraffin removal from human tissue sections delivers enhanced raman spectroscopic and histopathological analysis," Analyst, vol. 145, no. 4, pp. 1499–1510, 2020, doi: 10.1039/C9AN01030K.

[15] G. Ganesan, S. Ananth, and D. Boopathy, "Substitutes for xylene in histopathology and the role of temperature," 2016, doi: 10.17511/JOPM.2016.I02.04.

[16] A. Gomez-Samper et al., "Histological analysis of an alkaline based tissue clearing technique for fetal musculoskeletal tissue," Int. J. Morphol., vol. 40, no. 3, pp. 557–561, 2022, doi: 10.4067/s0717-95022022000300557.

[17] C. Huang et al., "Rapid clearing and imaging of mohs and melanoma surgical margins using a low-cost tissue processor," Biomed. Opt. Express, 2023, doi: 10.1364/boe.510132.

[18] E. M. Humphries et al., "Quantitative comparison of deparaffinization, rehydration, and extraction methods for ffpe tissue proteomics and phosphoproteomics," Anal. Chem., 2024, doi: 10.1021/acs.analchem.3c04479.

[19] N. Jyostna, D. Bhargava, and P. Bansal, "Use of carrot oil, olive oil, pine oil, rose oil in comparison to xylene as clearing agent during tissue processing: An in vitro study," Biosci. Biotechnol. Res. Asia, vol. 21, no. 3, pp. 1111–1120, 2024, doi: 10.13005/bbra/3290.

[20] K. Kim et al., "Optimized single-step optical clearing solution for 3d volume imaging of biological structures," 2022, doi: 10.1038/s42003-022-03388-8.

[21] V. Lim et al., "Deparaffinization of tissue by electric field generation and ionization," 2017.

[22] Z. Lin, J. Chi, and P. Cohen, "A clearing method for three-dimensional imaging of adipose tissue," 2022, doi: 10.1007/978-1-0716-2087-8_4.

[23] Q. Luong et al., "Development of an optimized clearing protocol to examine adipocyte subpopulations in white adipose tissue," Methods Protoc., vol. 4, no. 2, 2021, doi: 10.3390/MPS4020039.

[24] M. Mamay, G. N. Mutmaina, and I. A. Nurahma, "Utilization dishwashing soap as a substitute of xylol in the deparaffinization process of hematoxylin-eosin dye: Review article," Medicra J. Med. Lab. Sci. Technol., vol. 5, no. 1, pp. 47–55, 2022, doi: 10.21070/medicra.v5i1.1629.

[25] L. Mathot, K. Hartman, and T. Sjöblom, "Deparaffinization of ffpe tissue samples," 2016.

[26] T. S. Mazzoni and I. Quagio-Grassiotto, "In totum deparaffinization of biological samples and re-embedded in historesin for better diagnostic," Microsc. Res. Tech., vol. 84, no. 7, pp. 1362–1367, 2021, doi: 10.1002/JEMT.23695.

[27] M. Li et al., "Improved clearing of lipid droplet-rich tissues for three-dimensional structural elucidation," Acta Biochim. Biophys. Sin., vol. 49, no. 5, pp. 465–467, 2017, doi: 10.1093/ABBS/GMX018.

[28] S. S. Nasseri, E. M. J. Siren, J. N. Kizhakkedathu, and K. C. Cheung, "Hyclear: A novel tissue clearing solution for one-step clearing of microtissues," Cells, vol. 11, no. 23, p. 3854, 2022, doi: 10.3390/cells11233854.

[29] D. A. Ofusori et al., "Mixture of kerosene and xylene: A contribution to clearing agents," 2009.

[30] —, "Replacement of xylene with extra virgin coconut oil in the clearing step of the histological routine," 2023, doi: 10.56238/devopinterscie-133.

[31] R. L. C. D. Saribu et al., "The potency of olive oil, coconut oil, and bleached palm oil as clearing agent in histological preparation," Berk. Ilm. Biol., vol. 14, no. 2, pp. 48–54, 2023, doi: 10.22146/bib.v14i2.6528.

[32] W. Sermadi et al., "Comparing the efficacy of coconut oil and xylene as a clearing agent in the histopathology laboratory," J. Oral Maxillofac. Pathol., vol. 18, no. 4, 2014, doi: 10.4103/0973-029X.141348.

[33] S. Shuai and L. Xun, "Application of y clearing agent, a substitute of xylene, in pathological section preparation," 2012, doi: 10.3969/j.issn.2095-4344.2012.28.014.

[34] M. Singh, A. Singh, and J. Kaur, "Comparing the efficacy of xylene and kerosene oil as a clearing agent during processing and staining in histopathology," Indian J. Pathol. Oncol., vol. 9, no. 3, pp. 253–260, 2022, doi: 10.18231/j.ijpo.2022.059.

[35] J. C. Stockert et al., "Replacing xylene with n-heptane for paraffin embedding," Biotech. Histochem., vol. 87, no. 7, pp. 464–467, 2012, doi: 10.3109/10520295.2012.701764.