THE UTILISATION OF A HEATING PLATE FOR LABORATORY-SCALE FIXATION OF HISTOLOGY SPECIMENS

Burhannudin[1*]
[1] Departement of Medical Laboratory Technology, Politeknik Kesehatan Kementerian Kesehatan Jakarta III, Indonesia
[*]*Corresponding author: burhannudin@kemkes.go.id*

ABSTRACT

Background: *The challenge of expediting the histology preparation process without compromising quality has led to exploring microwave technology. However, it is yet to be determined whether heating plates are effective. Investigating the potential of enhancing histology preparation processing is an area of interest.*
Methods: *This experimental study utilised a post-test-only control group design approach using forty-five specimens of Wistar white rat (Rattus norvegicus) hepatic tissues, which were divided into nine hotplate treatment groups. The groups were exposed to temperatures of 60°C (H1), 70°C (H2), and 80°C (H3) for 3 minutes (T1), 5 minutes (T2), and 10 minutes (T3), respectively. The tissues were fixed using FineFix solution and processed for routine staining, following the methodology described in the previous study. The quality of the preparation was assessed by scoring the results of Haematoxylin-Eosin staining for each treatment. The scores were analysed using the Kruskal-Wallis and Mann-Whitney tests to determine differences between the treatment groups.*
Results: *The treatment with a temperature of 80°C for 3 minutes (H3T1) showed the best results. Prolonged exposure of heat decrease the quality of histological spesimens*
Conclusion: *It is known that heat increases the fixation rate, yet, the temperature of 80°C for 3 minutes showed the best result in preparing the histology specimens.*

Keywords: *histological preparation, fixation, haematoxylin eosin, heating plate, time.*

INTRODUCTION

Histological preparations or preparations with haematoxylin and eosin staining are commonly used in anatomical pathology laboratories to work with tissue samples. Creating histological tissue preparations involves multiple stages: fixation, dehydration, clearing, infiltration, embedding, deparaffinisation, and staining[1]. These stages are complex and require strict adherence to protocols. The intricate process of manipulating tissue specimens in the Anatomical Pathology Laboratory is responsible for the extended duration necessary for assessing and communicating examination results. In some Indonesian medical centres, it can take up to 10 days to analyse tissue samples and for results to be provided to the patients[2,3]. The advancement of approaches to expedite the histological preparation procedure without compromising the standard of outcomes in the context of academic or clinical laboratories remains an ongoing field to develop. The fundamental challenge encountered in anatomical pathology laboratories is associated with the quality of tissue preparations[4].

The tissue processing quality is influenced by temperature, reagents, and duration of treatment[5]. Past studies have indicated that variations in tissue processing are highly dependent on the sample type and, therefore, cannot serve as a reference value for all samples[6]. Testing processing variations remains a prominent issue in histology slide preparation and staining, aimed at enhancing the speed and quality of findings[4-10]. However, the optimal variety of techniques and processing times is still a question that cannot be answered with certainty. Hence, the search for new methods and procedures continues to be developed.

A proposed method for expediting the production of histological specimens is regulating the heating temperature and the time reagents remain in contact. Microwave

heating temperature has been demonstrated to hasten the processing and staining of tissue[11-13]. Furthermore, similar treatment can maintain the quality of preparations and staining[7]. Additionally, a heating plate yields comparable results to microwave heating[14-16]. Nonetheless, the optimal temperature and time for heating plate use remain to be discovered. Hence, this study concentrates on the fixation stage to evaluate the quality of alternatives and optimise heating plate use in preparing histology specimens.

METHOD

Treatment groups

The experimental study compared the effects of various temperatures (60°C, 70°C, and 80°C) and treatment durations (3 minutes, 5 minutes, and 10 minutes) for tissue fixation using a heating plate. Previous studies[7,9,15-16] were used as a basis for this research.

Table 1. Research Treatment Group

Heating Treatment	Times (minutes)		
	3	5	10
60°C	H1T1	H1T2	H1T3
70°C	H2T1	H2T2	H2T3
80°C	H3T1	H3T2	H3T3

Source: Primary data (2023)

The nine treatment groups in this study comprise different combinations of heating plate treatment and time durations, documented in Table 1.

The study was conducted at Poltekkes Jakarta III's Cyto-histotechnology Laboratory from January to December 2023.

Animal acclimatization and handling

The study employed nine male Wistar rats (Rattus norvegicus) as the sample subjects. Rats underwent a 7-day acclimatisation period and were given access to drinking water ad libitum and 300 grams of standard feed per day. The light-dark cycle was set at 12 hours every day. The liver organ was the specific sample organ procured under previous research[6]. Preparations of tissue samples followed previous methods[4,6] with modifications applied to each treatment group. The histological preparation process involves several stages: fixation, dehydration, clearing, embedding, blocking, sectioning, staining, mounting, and identification[4,6-7].

The histological staining

The research focused on differences in heating plate fixation techniques, one of the steps in preparing tissue specimens. Fixation was done by immersing forty-five tissue specimens in FineFix solution (Milestone Medical) according to the group treatment set-up. Stirring was mandatory in this scenario. This was followed by dehydration by immersion in tubes containing graded alcohol solutions (70%, 80%, 96% and absolute alcohol). The clearing process was performed by immersion into xylol I and II solutions, then immersion into liquid paraffin solution at 81°C for 55 min[7,9]. The embedded paraffin block was then cut into 5-micron sections and placed on a microscope slide.

Statistical analysis

Next, the staining step was performed, and a pathologist analysed the results to obtain an assessment score of the staining quality data[17]. The data were tabulated and analysed bivariate using Kruskal-Wallis, as suggested by Mann-Whitney tests, to compare all scores from the treatment groups.

The ethical approval

The Universitas Muhammadiyah Purwokerto has approved all procedures and treatments under the ethics approval number KEPK/UMP/04/X/2023.

RESULTS AND DISCUSSION

The data for the study shown in Table 2 illustrates the division of the nine groups and the scoring results of Haematoxylin-Eosin staining by pathologists, which were scored based on a

previous study's reference[17]. The scoring values ranged from 0 to 3 and included nuclear morphology, cytoplasmic morphology, and overall morphology.

Fixation is the first step in preparing histological samples, aimed at preserving tissue consistency similar to its natural state in the body via a fixative solution[18]. Fixative solutions prevent enzyme activation, which can damage cells or tissues by denaturing and coagulating proteins and altering their shape, thus preserving tissue size and shape[19]. The fixation process is significantly affected by factors including temperature, time, pH, specimen dimensions, and the volume ratio of the fixative solution to the tissue[20]. Due to the lengthy fixation process, which takes roughly between 12 and 24 hours, past studies have endeavoured to regulate the temperature and heating time through various methods. Research on this topic is still ongoing to discover the most efficient value[7,18,21-24]. The fixation process demands utmost caution since the right time and temperature are crucial to avoid under-fixation or over-fixation.

Using a fixation temperature of 80°C for 3 minutes (H3T1 group) yielded the highest score according to the results, with a perfect score of 2 achieved by 100% of the samples (see Table 2).

Table 2. Percentage of scores for Haematoxylin-Eosin (HE) staining in the treatment group

Groups	N	Nuclear morphology (%)				Cytoplasm Morphology (%)				Overall morphology (%)			
		0	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
H1T1	5	0	20	80	0	0	20	80	0	0	20	80	0
H1T2	5	0	60	40	0	0	60	40	0	0	60	40	0
H1T3	5	0	60	40	0	60	20	20	0	60	20	20	0
H2T1	5	0	20	80	0	0	80	20	0	0	80	20	0
H2T2	5	40	40	20	0	60	20	20	0	60	20	20	0
H2T3	5	40	40	20	0	80	20	0	0	80	20	0	0
H3T1	5	0	0	100	0	0	0	100	0	0	0	100	0
H3T2	5	40	60	0	0	80	20	0	0	20	80	0	0
H3T3	5	40	60	0	0	80	20	0	0	80	20	0	0

However, as the prolonged duration of heating increased at 60°C, 70°C, and 80°C, the score results significantly deteriorated. This was evident from the increased number of slides that could not be diagnosed or scored 0. The Mann-Whitney test also produced similar results, indicating that the H3T1 group achieved the best outcomes when fixated in a heating solution at 80°C for 3 minutes (as shown in Table 3). Moreover, this group's results significantly differed from others ($p<0.05$). This shows that using high temperatures in a short time gives the best results compared to all treatments tested.

In the context of standard histopathological procedures, fixative solutions generally have a slow diffusion rate into the tissue section. However, the use of microwave heating has been shown to expedite this process through thermal conduction in previous studies[13,22-26]. The process of heating acts by speeding up the movement of reagents and promoting the physiochemical process of dyeing[26-27]. Additionally, microwave heating transfers energy directly to the tissue via molecular interaction, increasing molecular energy and accelerating the fixation process[28]. Nevertheless, laboratory microwaves were often not available, especially in low-cost settings. Consequently, a heating plate is more suitable.

The heating mechanisms of a heating plate and a microwave differ. A heating plate transfers heat from its surface to tissue via conduction[28]. Stirring was necessary to distribute heat evenly

throughout the tissue and solution, mimicking the microwave's heating process. Consequently, findings from a recent heating plate study were comparable to those of a previous study (see Table 3).

Table 3. Statistic test results based on treatment group

Groups	n	median (minimum – maximum)	
H1T1 (60°C for 3 minutes)	5	2 (1 – 2)^{a,b,c,d}	
H1T2 (60°C for 5 minutes)	5	1 (1 – 2)	
H1T3 (60°C for 10 minutes)	5	1 (1 – 2)	
H2T1 (70°C for 3 minutes)	5	2 (1 – 2)^{e,f,g,h}	
H2T2 (70°C for 5 minutes)	5	1 (0 – 2)^{a,e,i}	p<0,05
H2T3 (70°C for 10 minutes)	5	1 (0 – 2)^{b,f,j}	
H3T1 (80°C for 3 minutes)	5	2 (2 – 2)^{i,j,k,l}	
H3T2 (80°C for 5 minutes)	5	1 (0 – 1)^{c,g,k}	
H3T3 (80°C for 10 minutes)	5	1 (0 – 1)^{d,h,l}	

Notes: The presented data display median values with corresponding minimum and maximum ranges. Kruskal-Wallis's testing reveals significant results at a p-value of less than 0.05. Mann-Whitney test outcomes are depicted via capital letter notation. Capital letter notation in a single column indicates the statistical significance of the test results.

Source: Primary data (2023)

Achieving temperature stability and correct timing is essential for effective heating treatment. A recent investigation revealed that the optimal outcome was attained by warming up a heating plate to 80°C for three minutes (see Tables 2 and 3). This aligns with prior research indicating that high temperatures and short heating periods yielded the most favourable results[17]. Besides impeding solidification stemming from exposure to excess fixative solution, brief heating facilitates even distribution of the fixative. In addition to reducing the risk of tissue damage, this fast-heating method also upholds the sample's integrity for subsequent analysis. Additionally, it allows for shorter processing times, rendering it an appealing option for field studies.

It should be noted that prolonged heating reduced the quality of the staining results at all predetermined temperatures (Table 3). The staining results in the group with prolonged heating showed tears in the tissue. This indicates that extreme heating treatment has an optimal effect only when used for a short time, as shown in previous studies[17,29]. The tissue damage may be related to protein denaturation in the tissue due to extreme heating near the boiling point[30].

Heating treatment was applied in another fixative solution, such as NBF10%, which is considered the gold standard for histological specimens[7,17,29]. However, the heating was limited to 50°C. Although heating has been proven to expedite the fixation process, using lower temperatures and a slower heating process is recommended. Formaldehyde, contained in the NBF10%, has a low boiling point, so high temperatures and prolonged heating can cause the solution to evaporate. In addition, Kang et al. (2022) considered gaseous formaldehyde volatile and toxic[31]. Therefore, Finefix, an alcohol-based fixative, is the best substitute fixative solution in this scenario.

CONCLUSION

The effectiveness of using a heating plate to prepare histology slides has been demonstrated. However, high heat settings and fast times during the treatment are highly recommended. Additionally, the heat stability of the heating plate is crucial in the process, so it is highly recommended that a stable temperature be maintained. It is recommended to search for alternative heating methods that can maintain temperature stability, such as an oven or water bath, in addition to the microwave used.

REFERENCE

1. Isaac, U. E., Oyo-Ita, E., Igwe, N. P. & Ije, E. L. 2023. Preparation of Histology Slides and Photomicrographs:

Indispensable Techniques in Anatomic Education. Anatomy Journal of Africa, 12(1): 2252–62. https://doi.org/10.4314/aja.v12i1.1.
2. RSUDAM. 2018. Standar Pelayanan Laboratorium Patologi Anatomi RSUD Dr. H. Abdul Moeloek Provinsi Lampung. RSUDAM, Bandar Lampung.
3. RSUD Muntilan. 2018. Standar Pelayanan Publik pada Rumah Sakit Umum Daerah Muntilan Kabupaten Magelang, Standar Pelayanan Instalasi Laboratorium Patologi Anatomi. RSUD Muntilan, Magelang.
4. Maheswari, N., Behera, S., Mahapatro, A. & Ray, S. 2024. A Prospective Observational Study on The Role of Special Stains in Physiological and Histopathological Study of Liver. Fortune Journal of Health Science. 7: 192–196. https://doi.org/10.26502/fjhs.177.
5. 17.Muthiawati, S., Wiryanti, W., Durachim, A., & Mulia, Y. S. 2023. Optimasi Waktu Dan Suhu Fiksasi Spesimen Terhadap Kualitas Preparat Jaringan. Jurnal Kesehatan Siliwangi, 4(1), 479-484. https://doi.org/10.34011/jks.v4i1.1508.
6. Ellyawati. 2018. Penentuan Waktu yang Tepat pada Proses Staining dalam Pembuatan Preparat Histologis Hati. Jurnal Temapela 1(1): 28 - 30. http://dx.doi.org/10.25077/temapela.1.1.28-30.2018.
7. Ariyadi, T. & Suryono, H. 2017. Kualitas Sediaan Jaringan Kulit Metode Microwave dan Conventional Histoprocessing Pewarnaan Hematoxylin Eosin. Jurnal Labora Medika 1(1): 7 - 11.
8. Howat, W. J. & Wilson, B. A. 2014. Tissue Fixation and The Effect of Molecular Fixatives on Downstream Staining Procedures. Methods 70(1): 12 - 19. https://doi.org/10.1016/j.ymeth.2014.01.022.
9. Leong, A. S. 2004. Microwaves and Turnaround Times in Histoprocessing: Is This a New Era in Histotechnology? American Society for Clinical Pathology 121(4): 460 - 462. https://doi.org/10.1309/PLQ523DENNH8R00Q.
10. Kango, P. G. & Deshmukh, R. S. 2011. Microwave Processing: A Boon for Oral Pathologists. Journal of Oral and Maxillofacial Pathology 15(1): 6 - 13. https://doi.org/10.4103%2F0973-029X.80031.
11. Kumar, G. L., Kiernan, J. A., Gill, G. W., Badve, S., Floyd, A. D., Dapson, R. W., et al. 2010. Education Guide: Special Stains and H&E Second Edition. Dako North America, Carpinteria, California.
12. Mukunda, A, Narayan, T. V., Shreedhar, B., Shashidara, R., Mohanty, L. & Shenoy, S. 2015. Accelerated staining technique using kitchen microwave oven. Indian J Pathol Microbiol 58:316–322. https://doi.org/10.4103/0377-4929.162863.
13. Katoh, K. 2016. Microwave-Assisted Tissue Preparation for Rapid Fixation, Decalcification, Antigen Retrieval, Cryosectioning, and Immunostaining. International Journal of Cell Biology 2016: 1–9. https://doi.org/10.1155/2016/7076910.
14. Vinod, K. R., Jones, D. & Udupa, V. 2016. A simple and effective heat induced antigen retrieval method. MethodsX 3(2016):315–319. https://doi.org/10.1016/j.mex.2016.04.001.
15. Burhannudin & Herbyananda, A. D. 2024. Use of a Hotplate at 80°C for 3 minutes to help Fixation of Histological Preparations. Biomedika 16(2): 59-64.
16. Burhannudin, Warida & Puspita, I.

2023. Penggunaan Hotplate Suhu 60ºC Selama 10 Menit Sebagai Alternatif Pemanasan Proses Fiksasi Sediaan Histologi. Jurnal Bahana Kesehatan Masyarakat 7(2): 89-94.

17. Chafin, D., Theiss, A., Roberts, E., Borlee, G., Otter, M. & Baird, G. S. 2013. Rapid Two-Temperature Formalin Fixation. PLoS ONE 8(1): e54138. https://doi.org/10.1371/journal.pone.0054138.
18. Bancroft, J. D., Suvarna, K. S., & Layton, C. 2019. Bancroft's Theory and Practice of Histological Techniques (8th Edition). Elsevier, China.
19. Kumar, H., Kalkal, P., Buch, A., Chandanwale, S. S., Bamanikar, S. & Jain, A. 2014. Role of microwaves in rapid processing of tissue for histopathology. Med J DY Patil Univ 7(4): 458–462.
20. Khristian, E. & Inderiati, D. 2017. Sitohistoteknologi. Pusat Pendidikan Sumber Daya Manusia Kesehatan, Jakarta.
21. Tripathi, M., Bansal, R., Gupta, M., & Bharat, V. 2013. Comparison of routine fixation of tissues with rapid tissue fixation. Journal of Clinical and Diagnostic Research, 7(12): 2768–2773. https://doi.org/10.7860%2FJCDR%2F2013%2F6233.3754.
22. Rao, M., Pai, S., Khanagar, S., Siddeeqh, S., Devang, D., & Naik, S. 2020. Microwave-assisted tissue processing, fixation and staining in tissues of different thicknesses: A comparative study. Journal of Oral and Maxillofacial Pathology, 24(1): 186. https://doi.org/10.4103%2Fjomfp.JOMFP_290_19.
23. Adam, M., Iris, A., Ali, A., & Khalid, I. O. 2022. Evaluation of the Effect of Time of Fixation and Microwave Treatment on Quality of Fatty Tissue Fixation in Breast Cancer Specimens, SPR, 2(1): 402–408. https://doi.org/10.52152/apr/2022.166.
24. Annisa, A. S., & Sofyanita, E. N. 2023. Pengaruh Penggunaan Minyak Zaitun dengan Pemanasan sebagai Larutan Penjernih (Clearing) Terhadap Kualitas Sediaan Jaringan Hepar Mencit. Jurnal Labora Medika, 7(1): 6–12. https://doi.org/10.26714/jlabmed.7.1.2023.6-12.
25. Tisserat, B., O'kuru, R. H., Hwang, H., Mohamed, A. A. & Holser, R. 2012. heating was found to significantly accelerate chemical reaction processes, resulting in higher chemical conversions compared to conventional heating methods. Journal of Applied Polymer Science 125(5): 3429–3437. http://dx.doi.org/10.1002/app.36669.
26. Priya, A. H. H., Venkatanarasu, B., Chellaswamy, S., Jeyaraj, M. Francis, S. F. & Rajasekaran, S. 2020. Evaluation of Efficacy of Microwave Staining over Conventional Staining in Replicating Tissue Architecture: A Prospective Study. J Pharm Bioallied Sci 12(Suppl 1): S283–S288. https://doi.org/10.4103/jpbs.JPBS_86_20.
27. Shirbhate, P. N., Deshmukh, A. V. & Shivkumar, V. B. 2022. Rapid Microwave Tissue Processing and Staining Method Using a Kitchen Microwave Oven in Histopathology Laboratory: A Comparative Study with Routine Histoprocessing Method. Med J Babylon 19:383–390. http://dx.doi.org/10.4103/MJBL.MJBL_47_22.
28. Akbari, S., Nour, A. H., Jamari, S. S. & Rajabi, A. Q. 2016. Demulsification of Water-in-Crude Oil Emulsion via Conventional Heating and Microwave Heating Technology in their Optimum Conditions. Australian Journal of Basic and Applied Sciences, 10(4): 66–74.

29. Bauer, D. R., Leibold, T. & Chafin, D. R. 2021. Making a science out of preanalytics: An analytical method to determine optimal tissue fixation in real-time. PLoS ONE, 16(10): e0258495. https://doi.org/10.1371/journal.pone.0258495.
30. Tsuboyama, K., Osaki, T., Matsuura-Suzuki, E., Kozuka-Hata, H., Okada, Y., Oyama, M., Ikeuchi, Y., Iwasaki, S. & Tomari, Y. A widespread family of heat-resistant obscure (Hero) proteins protect against protein instability and aggregation. PLoS Biol, 18(3): e3000632. https://doi.org/10.1371/journal.pbio.3000632.
31. Kang, Y., Jo, H., Jang, M., Ma, X., Jeon, Y., Oh, K. & Park, J. 2022. A Brief Review of Formaldehyde Removal through Activated Carbon Adsorption. Appl. Sci. 2022, 12, 5025. https://doi.org/10.3390/app12105025.