Jagua Publication

Journal of Applied Health Sciences and Medicine 3(1): 10 –20

Research Article

The Effect of Rapid Tissue Processing on Fixation Time Using Microwave Techniques

God'sman Chidugam Eberechukwu[1*], Amalachukwu O. Ike [1][ORCID], Gift C. Okwusike[1], and Ibiere Allwell Pepple [2]

[1]Department of Medical Laboratory Science, PAMO University of Medical Sciences, Port Harcourt, Nigeria
[2]Department of Medical Laboratory Science, Faculty of Science, Rivers State University, Port Harcourt, Nigeria.

www.jpub.org

Received:
December 15, 2022
Accepted:
January 14, 2023
Published:
January 28, 2023

How to cite:
Eberechukwu et al. 2023. "The Effect of Rapid Tissue Processing on Fixation Time Using Microwave Techniques". *Journal of Applied Health Sciences and Medicine*. 3(1): 10 – 20.
https://doi.org/10.58614/jahsm312

Corresponding author:
God'sman Chidugam Eberechukwu
meeteffective@gmail.com

Abstract: The quest for a faster means of tissue processing has been on the increase to accomplish the needs of medical scientists, especially for treating patients with critical health conditions. Specimens were processed using different schedules at different temperatures to ascertain the effect of rapid tissue processing on fixation time. A domestic microwave model of MW007GA-MG823 (W)/MW- MB0823MP, rated voltage: 230V5 0HZ was used for the study. A total of four (4) organs were used, each divided into two, one part processed with the microwave and the other processed with the conventional method. The rapid technique started without prior routine fixation, except a schedule that was fixed for nine (9) hours before the rapid process started at a temperature of 42°C following dehydration, clearing of tissues was carried out with isopropanol. Fixation times for the rapid schedules are 30 and 20 minutes. An independent t-test conducted between the rapid and conventional techniques showed Mean±SD = 30±2.00 mins, Mean±SD = 20±2.00 mins, and Mean±SD = 2880±2.00 mins respectively at p = 0.00 depicting statistical significance. The rapid technique produced sections of good quality without compromising the tissue architecture. Isopropanol could be used as a clearing agent instead of xylene as it is inflammable in the microwave.

Keywords: rapid tissue processing, fixation time, microwave technique, turnaround time, histological quality.

1. Introduction

Microwave mediated tissue processing is employed as an alternative means of providing treatment for patients. Conventional tissue processing and fixation cause delay in result generation and treatment for those living with critical health conditions (Rohr et al. 2021). According to (Pranab 2018), fixation is the process by which the cells in the tissue are fixed in a chemical and physical state, and all the biochemical and proteolytic activities within the cells are prevented so that the cells or tissues can resist any morphological change, distortion, or decomposition after subse- quent treatment with various reagents. Rapid fixation and processing with the microwave reduces turnaround time, giving way for faster pathologic diagnosis (Rohr et al. 2021). The time required for fixation, decalcification, processing and staining can be distinctly reduced via microwave irradiation. Increased microwave irradiation results

in an increase in the oscillatory movement of the watermolecule, thereby increasing the temperature of the tissue (Katoh 2016). After the collision of the molecules, they absorb microwaves and the energy is converted into kinetic energy, consequently to thermal energy (Kok and Boon 1990). More so (Boon et al. 1995) revealed that microwave brings about heating by stimulating the rotation of molecules. This rotation causes heat energy. Heat decreases the viscosity of liquid substances, after that increasing the diffusion rate of reagents in and out of the tissue. Different from conventional heating, the heating occurs concurrently through the entire material subjected to internal heating (Boon et al. 1995). The entire process leads to an ample reduction in turnaround times and enables faster result generation for different tissue samples (Mathai et al. 2008). The resection of a histological sample is followed by fixation, dehydration, clearing, infiltration and embedding. This ensures that the sections produced after staining are of a diagnostic quality (Bancroft and Gamble 2008). Fixation is the basis of histological technique, and the outcome of subsequent events depends on the right choice of fixative. It then becomes pivotal to understand the action of fixatives on cell and tissue constituents (Baker and Silverton 1976) and the need for advanced techniques for fixation. Obtaining a quality slide is never an accident as it requires some necessary skills that are gained via constant practise and experience. As new instruments and techniques emerge, the role of the histology laboratory will continue to advance (Bancroft and Gamble 2008). Tissue fixation can be achieved by chemical and/or physical methods such as microwaving. The physical method of microwaving is not used in routine practice (Eltoum, Fredenburgh, and Grizzle 2001). Microwave can also be applied in fluorescence in situ hybridization analysis of paraffin sections (Kitayama, Igarashi, and Sugimura 2000). In a study by (Avci et al. 2006), it was reported that microwave irradiation has been applied for special staining and is employed in immune fluorescence staining processes (Katoh 2016). It was also reported that compared with routine procedures, microwave irradiation could reduce the duration of processing (Katoh 2016).

Moreover, microwaves have been used for antigen retrieval for immunohistochemistry (Katoh 2016). According to a method proposed by (Shi, Key, and Kalra 1991), microwaves altered the cross- linking effect of formaldehyde when employed in antigen retrieval for immunohistochemistry. In a study by (Munakata, james, and Hendricks 1993), it was reported that Good Ki-67 immuno-staining was observed for all heating times in tissue fixed for 4 hours. It was further reported that there was an improvement in staining intensity when the heating time lasted for a minimum of 14 minutes. Tissue processing poses the challenge of delay in diagnosis, thereby causing delayed treatment. The routine method of tissue processing is a laborious method and has been a concern in both hospitals and research centres. Some studies have made advances in tissue processing using a domestic microwave, but a paucity of data exists on the inclusion of the spleen and liver as observed from the review of literature. Besides, there is no gainsaying that histology laboratories are capital intensive, consequently the need for this study as its outcome would alleviate the physical and financial stress involved in tissue processing. Furthermore, much is yet to be done on the effect of the rapid tissue processing on fixation time. Hence, this study determined the effect of optimized rapid tissue processing on fixation time using microwave technique. Specifically, the study investigated and compared the fixation time of

tissues using the microwave and conventional tissue processors. Additionally, the study assessed the microscopic disparities of the tissue sections.

2. Materials and Method

This study was conducted in PAMO University of Medical Sciences. The study was based on the Effect of Optimized rapid tissue processing on fixation time using the microwave technique.

2.1. Sample Selection

A total of four (4) paired samples from Albino Rats were selected from the Animal House at PAMO University of Medical Sciences, Port-Harcourt. One member of each pair was processed with the microwave using different schedules at different temperature and time. The tissues subjected to fixation included the liver, kidney, spleen, and heart. The temperature ranges of the microwave radiation were determined using a laboratory thermometer before exposing the tissue samples to microwave irradiation. A representative part of the specimen was cut and transferred to a tissue cassette. Subsequently, the specimens were cut into equal halves to be processed by both routine and rapid (microwave) methods and afterwards stained with haematoxylin and eosin stain.

2.2. Administrative and Ethical Considerations

Approval was given by the chairman of Ethics Committee, PAMO University of Medical Sciences, Port- Harcourt.

2.3. Rapid Microwave Processing Method

A conventional Microwave oven was used for this study without modification. A microwave model of MW007GA-MG823 (W)/MW-MB0823MP, rated voltage: 230V5 0HZ was used for the study. For the reason that the structural quality of tissue components is determined by the type of reagent and the duration of exposure to the reagents, different schedules were tested. The first microwave schedule was carried out at a temperature of 50°C for 25 minutes for comparison with the conventional technique. Subsequently, a second schedule for the rapid technique was carried out at the temper- atures of 46°C, 50°C, and 42°C, for a duration of 30 minutes, 20 minutes and 20 minutes respectively. The longest schedule for fixation was carried out outside the microwave for 9 hours before the rapid technique was employed for 30 minutes (see table 1 and 2). The total rapid processing time is 1 hour 54 minutes for this schedule. The duration was shorter for other schedules because the tissues were not pre-fixed before the rapid technique began. The sections were then processed, sectioned, stained and photographs were taken for microscopic comparison between samples processed using the two methods (rapid and conventional). An independent t-test and ANOVA were carried out using the Statistical Package for Social Sciences (SPSS) to compare the mean differences in the fixation times for the rapid schedules and the routine method.

Table 1. Schedule for Fixation

Technique	Temperature	Time (minutes)
Microwave (MW1)	50°C	30
Microwave (MW2)	46°C	30
Microwave (MW3)	50°C	20
Microwave (MW4)	42°C	560
Control	25°C	2880

Table 2 below shows a summary of microwave tissue processing. The samples were first placed in 10% formal saline for 9 hours before undergoing the rapid schedule at 42°C. Following microtomy, sections were stained with the traditional haematoxylin and eosin staining method, using the protocol established by (Carleton and Drury 1957; Cullings, Allison, and Barr 1985; Bancroft and Gamble 2008). The sections were evaluated using the format: satisfactory or unsatisfactory. After the evaluation the result was analysed considering the fixation time.

3. Results

This study investigated the effect of rapid tissue processing on fixation time. The methodology comprised the routine method (control) and the microwave technique scheduled into (SCH1, SCH2, SCH3, SCH4). The study findings revealed the following results: Table 3 shows an independent t-test (mean comparison) between the control and different rapid schedules: the control group (Mean±SD = 2880±2.00) compared with schedule 1 (Mean±SD = 30±2.00) had a t-value of 1745.26 with p = 0.00 at 95% confidence interval. More so, comparing the control with schedule 2, which was irradiated for 30 minutes recorded a t-value of 916.65 with p = 0.00. Also, schedule 3 revealed a.

Table 2. Microwave tissue processing

Reagent	Temperature	Time
10% formal saline	25°C	9 hours
10% formal (microwave) saline	42°C	20 minutes
70%alcohol	42°C	15 minutes
90% alcohol	42°C	15 minutes
Absolute alcohol	42°C	15 minutes
Paraffin 1	50°C	20 minutes
Time consumed		1 hour 54 minutes

standard deviation of 2.00, t-value = 1751.30, and p = 0.00. The final schedule (sch4) with the control group had t = 1746.19 with p = 0.00 indicating statistical significance. Here, the study failed to retain the null hypothesis and accept the alternative

hypothesis that there are statistically significant differences between the means of the rapid techniques and the control group.

Table 3. Independent t-test (Mean comparison) of Fixation Time between the Control and Rapid Schedules(Minutes)

Method	Mean±SD	t-test	p-value	Remark
Control (ctrl)	2880±2.00	1745.26	0.00	Sig
Schedule (sch1)	30±2.00			
Control (ctrl)	2880±2.00	916.65	0.00	Sig
Schedule (sch2)	30±5.00			
Control (ctrl)	2880±2.00	1751.39	0.00	Sig
Schedule (sch3)	20±2.00			
Control (ctrl)	2880±2.00	746.19	0.00	Sig
Schedule (sch4)	560±5.00			

Sig= significance

One way ANOVA was performed to compare the effect of the different techniques on fixation time. A one way ANOVA revealed that there was a statistically significant difference in the mean score among the schedules. See Table 4 for details.

Turkey's HSD Test for multiple comparisons found that the mean value of fixation time was sig- nificantly different between the different schedules. This implies a statistically significant difference in the mean fixation time between the schedules ($p < 0.05$). See Table 5 for details. Qualitatively, after staining with haematoxylin and eosin, tissue architecture was satisfactorily preserved for some.

Table 4. Intra-group comparison between the different microwave fixation schedules

Method	Mean±SD
Schedule 1	30±2.00
Schedule 2	30±5.00
Schedule 3	20±2.45
Schedule 4	560±5.00
P-value	<0.05
Remark	Sig

Sig=significant

Table 5. Intra-group comparison between the different microwave fixation schedules

Method	Mean±SD	t-test	p-value	Remark
Schedule 1	30±2.00	6.12	0.04	Sig
Schedule 3	20±2.00			
Schedule 1	30±2.00	-170.47	0.00	Sig
Schedule 4	560±5.00			
Schedule 2	30±5.00	3.216	0.032	Sig
Schedule 3	20±2.45			
Schedule 2	30±2.00	-129.82	0.00	Sig
Schedule 4	560±5.00			

Schedule 3	20±2.00	-173.68	0.00	Sig
Schedule 4	560±5.00			

Sig=significant

schedules. Typically, a rapid schedule, which had good presentation is the schedule performed after pre-fixation for 9 hours before employing the rapid method of fixation for 30 minutes. In tissues fixed for only 30 minutes without prior fixation, unsatisfactory tissue architecture was observed. Following fixation, tissue macroscopy showed some firm tissues, but not hard enough. Darkening of tissues was moderate on microscopy and details were sustained.

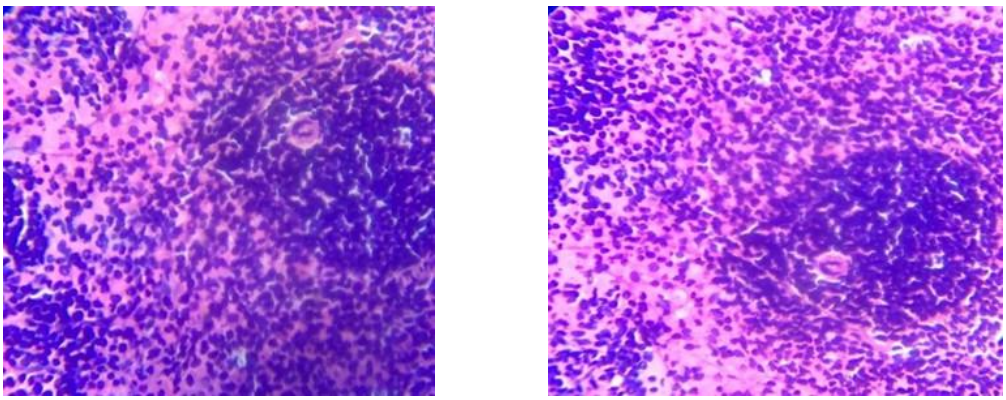
1A, Photomicrograph of the spleen processed with the conventional method. 1B, spleen pro- cessed with the domestic microwave after H & E staining (×10 objectives). B shows slight darkening of the section on microscopy, but the red pulp and white pulp were visible.

2A, Photomicrograph of the liver processed with the conventional method after H & E staining (×10 objective). 2B, Photomicrograph of the liver processed with the microwave after H & E staining (×10 objective).

A, Photomicrograph of the liver processed with the conventional method after H & E staining (×40 objective). B, Photomicrograph of the liver processed with the microwave after H & E staining (×40 objectives).

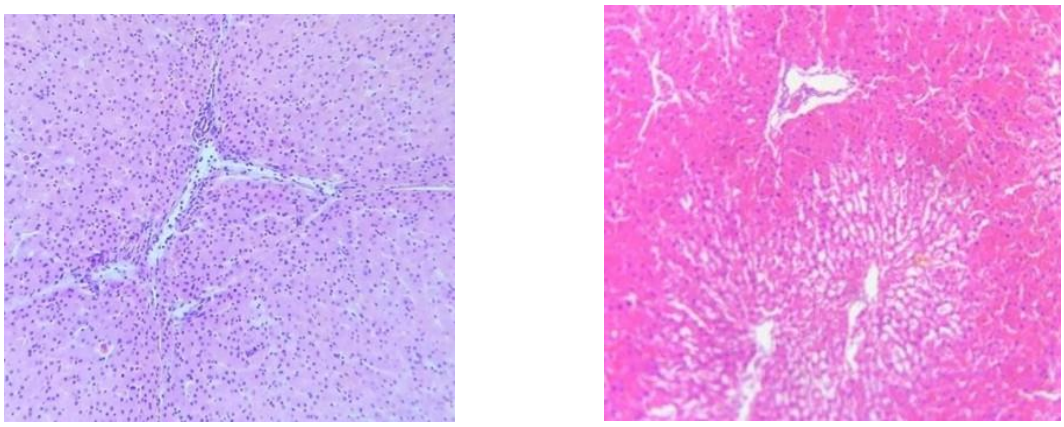
3.1. Summary of Finding

The study confirmed that the rapid tissue processing could reduce the time required for fixation. Schedule IV gave the best result of overall tissue morphology within a shorter duration (560 minutes) compared with the control group (2880 minutes), The second best result was obtained from the schedule II where tissue was immediately subjected to rapid fixation for 30 minutes without prior- fixation.



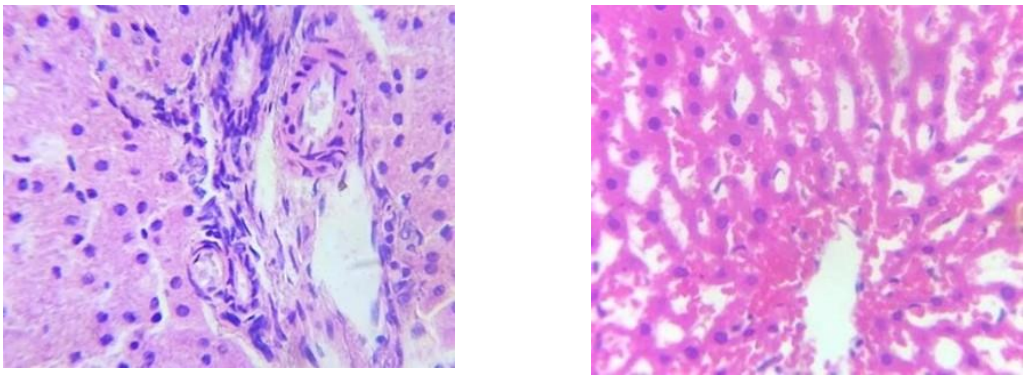
(a) (b)

Figure 1. This is a caption for the entire figure



(a) (b)

Figure 2. Liver sections from the same rat (×10 objective)



(a) (b)

Figure 3. Liver sections from the same rat (x40 objective)

4. Discussion

Fixation as an indispensable step in anatomical pathology helps maintain tissues in a lifelike state. The concept of fixation provides information on the cell structure, thus, the quest for a faster means of achieving this important histological step. Fixation and processing are time - consuming processes. It takes days and the whole process depends on the thickness and size of the tissues. From the viewpoint of the finished product, microwave technique reduces the time for medical diagnosis. For objective one, the fixation time of the tissues was reduced to a few minutes in the rapid schedules. Also an experience shared by (Rohr et al. 2021), confirmed that fixation, processing sectioning and staining time was reduced to 3 hours for small tissue specimens without affecting the histological quality. A study by (Rohr et al. 2021), showed that the quality of tissues processed routinely and the rapid methods were similar such that it was impossible to differentiate the sections. The outcome of the study revealed that no qualitative difference existed between the two techniques (Rohr

et al. 2021). To buttress the observations in this study, the disparity recorded between the microwave and conventional techniques indicates that the microwave technique could speed up the fixation process. A satisfactory result was obtained when sections of the rapid technique and the routine method were place side by side. The study demonstrated a reduction in fixation time from days to a few hours with optimum retention of the general tissue structure. Similarly (Babu, Malathi, and Agesh 2011), disclosed that slides were ready within 75 minutes using the rapid technique. It is a fact that performing an effective fixation and obtaining sections of good quality are important parameters in histological techniques. On this note this study established no ample dissimilarity in the overall quality of tissue sections prepared using the rapid microwave technique and the conventional method. Among the four (4) paired specimens, products of the rapid method that were considered as satisfactory were slightly comparable to those of the conventional. Concerning the rapid schedule that gave the best result, a few sections were judged unsatisfactory. Similarly, an adequate result was also accounted for by (Mac-Moune et al. 1987), on kidney biopsy after processing with the microwave. More so, in describing the microscopic appearance of the kidney (Mayers 1970), as the first person to suggest the possible application of microwave energy on fixation, observed that the distal and proximal tubules and glomeruli were well preserved. Also, sections were evenly stained, shrinkage was minor and artefacts were negligible. Mayers further established that the nuclear and cytoplasmic structures were fairly preserved. Conversely, just as Mayers reported, on macroscopy specimens were not hard, this could be linked to the fact that xylene was not used at all. This rapid technique, according to (Boon et al. 1988), was also used to fix the whole brain after which sections were produced within 24 hours. Other studies revealed that the technique can be employed in the fixation of prostate glands (Ruijter et al., 1997), and whole eyes (Margo, Saxe, and Grossniklaus 1992). The liver was observed to be poorly fixed (as shown in figure 3 above) alongside the lungs, and cellular clarity was suboptimal. Tissues were somewhat dark in some cases, possibly because of less penetration relative to the size; a similar report was also made by (Mayers 1970). In most of the cases, there was not much qualitative difference between the test and control groups. Schedule 4 (sch4) of this study was used as a standard for the rapid schedules tested because it gave a better microscopic presentation. This may be linked to the fact that fixation was conducted at room temperature for 9 hours. Furthermore, a study by (Giberson, Demaree, and Nordhausen 1997), showed no considerable difference in the quality of tissue preservation as seen under transmission electron microscope in comparison between the rapid microwave technique and the routine method. In spite of the difficulty encountered in the fixation of fatty tissues, (Katoh 2016)Kazuo, (2016) could achieve fixation of thymus gland with microwave within a short time. Using different criteria for judgment (Babu, Malathi, and Agesh 2011), recorded no statistical significance in the individual scores allotted by observers. Generally, the study showed that the features of the tissues processed in the microwave were better than the routinely processed slides (Babu, Malathi, and Agesh 2011). The study revealed that microwave fixation and processing technique significantly allows speedy result generation. For rapid tissue processing using a microwave, different studies employed different schedules (Chaudhari, Chattopadhyay, and Dutta 2000). In this study, isopropanol was

used for clearing order than xylene, which is routinely used. In one of the rapid schedules, which underwent Pre-fixation with 10%formal saline for nine (9) hours before the start of the rapid schedule, it was observed that a better result was obtained due to the prior fixation compared to the other schedules carried out immediately after tissue resection. Also, as observed in this study, it is recommended that the whole process should be monitored closely to avoid laboratory hazards that could be caused by the flammability of some reagents. Similarly, (Devi et al. 2013), reported that plastics should be used for microwave irradiation, as metallic materials could cause the danger of explosion. About formalin fixation (Chaudhari, Chattopadhyay, and Dutta 2000), also recorded a satisfactory result of 80% cases. Also, on clearing with isopropanol, 80% cases gave a satisfactory result as claimed by (Chaudhari, Chattopadhyay, and Dutta 2000). Additionally, just as recommended by (Rao et al. 2020), laboratory scientists should work toward achieving histopathology results without being exposed to harmful chemical substances. A good result was achieved even as the level of exposure to chemicals was minimal. It was specifically suggested that xylene should be replaced with isopropanol for clearing and where possible formaldehyde to be ruled out for fixation while using the microwave technique (Rao et al. 2020). We deduced that routinely fixed tissues may be processed with a domestic microwave to produce sections of similar quality to those processed conventionally. Subsequently, adequate fixation is recommended before rapid processing. We believe that rapid microwave tissue processing is advantageous, reducing fixation and processing time, and allowing medical laboratory Scientist to provide same day diagnosis. This study showed that the alternative method if managed properly can reduce fixation time; hence, turnaround time in anatomical pathology can be reduced with no substantial loss of section quality. Incorporating the domestic microwave method into routine laboratory practise can be achieved by designing (determining) the temperature of each function/power.

5. Conclusion/Recommendation

The microscopic results for the various specimens were close to those processed in the conven- tional method. The fixation time was considerably reduced. In this study, it was deduced that rapid tissue processing using a microwave significantly reduced the time for section preparation without a serious decrease in section quality. The microscopic features of products from the two techniques were similar and the distinguishing qualities were minimal. Consequently, from our study, we would recommend that microwave tissue processing can be incorporated in routine laboratory practice. This study was conducted using the tissues of laboratory animals. Organs from humans and other larger mammals, and pathological specimens can be processed too. The study is restricted to the effect on fixation time and tissue architecture. Other histochemical methods can be used to verify the effect on histochemistry. Moreover, effort was not made to study the effects of microwave on dye molecules, we hope to investigate these more specifically in a subsequent study. Further investigation in the study is needed to develop more sophisticated microwave tissue processors to enable same day diagnosis.

Study Limitations

(a) In the study, four (4) tissue types of Albino Wister rat; kidney, liver, heart and spleen were anal- ysed. A large sample size would give a more detailed conclusion.

(b) The experiment was conducted with the domestic microwave, hence, our recording of tempera- ture may not be perfect.

Acknowledgment

The authors are grateful to the Chief of Haematology unit, PAMO University of Medical Sciences, Port-Harcourt, Ms. Ihua Nnena for her support and encouragement through the period of study.

References

[1] Avci, B., Z. Kahveci, N. Kahveci, and S. Sirmali. 2006. ""Using Microwave Irradiation in Marchi's Method for Demonstrating Degenerated Myelin,"." Biotechnic and Histochemistry 81 (2-3): 63–69.

[2] Babu, T., N. Malathi, and K. Agesh. 2011. "Comparative Study on Microwave and Routine Tissue Processing." Indian Journal of Dental Research 22:50–55.

[3] Baker, F., and R. Silverton. 1976. Introduction to Medical Laboratory Technology 5th edition: Butterworth Co Publishers. .

[4] Bancroft, J., and M. Gamble. 2008. "Theory and Practice of Histological Techniques. 6th ed." Missouri: Harcourt Publishers.

[5] Boon, M., E. Marani, P. Adriolo, J. Steffelaar, G. Bots, and L. Kok. 1988. "Microwave Irradiation of Human Brain Tissue: Production of Microscopic Slides Within One Day." Journal of clinical pathology 41 (5): 590–593. https://doi.org/10.1136/jcp.41.5.590.

[6] Boon, M., C. Wals-Paap, F. Visinoni, and L. Kok. 1995. "The Two-Step Vacuum-Microwave Method. Microwave Newsletter 13." Eur J Morphol 33:349–58.

[7] Carleton, H., and R. Drury. 1957. Histological Technique for Normal and Pathological Tissues and the Identifica- tion' of Parasites (3rd ed). Oxford university press. .

[8] Chaudhari, K., A. Chattopadhyay, and S. Dutta. 2000. "Microwave Technique in Histopathology and its Compar- ison with the Conventional Technique." Indian journal of pathology microbiology 43 (4): 387–394.

[9] Cullings, C., R. Allison, and W. Barr. 1985. "Cullings Histological Technique Processing. 4th ed. London: Butter- worths and Co-publishers," 51–77.

[10] Devi, R., A. Subhashree, P. Parameaswari, and B. Parijatham. 2013. "Domestic Microwave Versus Conventional Tissue Processing: A Quantitative and Qualitative Analysis." Journal of Clinical and Diagnostic Research 7 (5): 835–839. https://doi.org/10.7860/JCDR/2013/5630.2953.

[11] Eltoum, I., J. Fredenburgh, and W. Grizzle. 2001. "Advanced Concepts in Fixation:Effects of Fixation on Immunohisto- chemistry and Histochemistry, Reversibility of Fixation and Recovery of Proteins, Nucleic Acids, and other Molecules from Fixed and Processed Tissues, Special Methods of Fixation." Journal of Histotechnology 24:201–210.

[12] Giberson, T., S. Demaree, and W. Nordhausen. 1997. "Four-Hour Processing of Clinical/Diagnostic Specimens for Electron Microscopy using Microwave Technique." J Vet Diagn igation 9 (1): 61–67.

[13] Katoh, K. 2016. "Microwave-Assisted Tissue Preparation for Rapid Fixation, Decalcification, Antigen Retrieval, Cryosectioning, and Immunostaining." International Journal of Cell Biology Volume 2016 2016:ID 7076910. http://dx.doi.org/10.1155/2016/7076910.

[14] Kitayama, Y., H. Igarashi, and H. Sugimura. 2000. ""Initial Intermittent Microwave Irradiation for Fluorescence In situ Hybridization Analysis in Paraffin-Embedded Tissue Sections of Gastrointestinal Neoplasia,"." Laboratory Investigation 80 (5): 779–781.
[15] Kok, L., and E. Boon. 1990. "Microwaves for Electron Microscopy." International Journal of Cell Biology Volume 2016, 217–222. https://doi.org/10.1016/0739-6260(90)90121-U.
[16] Mac-Moune, L., K. Lai, E. Chew, and J. Lee. 1987. "Microwave Fixation in Diagnostic Renal Pathology." Pathology 19 (1): 19:17–21. https://%20doi.org/10.3109/003130287090651.
[17] Margo, C., S. Saxe, and H. Grossniklaus. 1992. "Microwave-Stimulated Chemical Fixation of Whole Eyes." Ophthalmology 99 (7): 1117–1122. https://doi.org/10.1016/S0161-6420(92)31863-9.
[18] Mathai, A., R. Naik, M. Pai, S. Rai, and P. Baliga. 2008. "Microwave Histoprocessing Versus Conventional Histo- processing." Indian Journal of Pathology and Microbiology 51:12–16. https://www.ijpmonline.org/text. asp?2008/51/1/12/40383.
[19] Mayers, C. 1970. "Histological Fixation by Microwave Heating." Journal of Clinical Pathology 23 (3): 273–275. https://doi.org/Doi:10.1136/jcp.23.3.273.
[20] Munakata, S., B. james, and Hendricks. 1993. "Effect of Fixation Time and Microwave Oven Heating Time on Retrieval of the Ki-67 Antigen from Paraffin-embedded Tissue." The Journal of Histochemistry and Cytochemistry 41 (8): 1241–1246.
[21] Pranab, D. 2018. Basic and Advanced Laboratory Techniques in Histopathology and Cytology ISBN 978-981-10- 8251-1. Springer. ISBN 978- 981-10-8252-8. https://doi.org/10.1007/978-981-10-8252-8.
[22] Rao, M., S. Pai, S. Khanagar, S. Siddeeqh, D. Devang, and S. Naik. 2020. "Microwave-Assisted Tissue Processing, Fixation and Staining in Tissues of Different Thicknesses: A Comparative Study." Journal of oral and maxillofacial pathology: JOMFP 24 (1): 186. https://doi.org/10.4103/jomfp.JOMFP_290_19.
[23] Rohr, L., L. Layfield, D. Wallin, and D. Hardy. 2021. "A Comparison of Routine and Rapid Microwave Tissue Pro- cessing in a Surgical Pathology Laboratory. Quality of Histologic Sections and Advantages of Microwave Processing." Aerican Journal of Clinical Pathology 115:703–708.
[24] Shi, S., M. Key, and K. Kalra. 1991. "Antigen Retrieval in Formalin-Fixed, Paraffin-Embedded Tissues: an Enhancement Method for Immunohistochemical Staining Based on Microwave Oven Heating of Tissue Sections." J Histochem Cytochem 39:741.