

A Comparison of Routine and Rapid Microwave Tissue Processing in a Surgical Pathology Laboratory

Quality of Histologic Sections and Advantages of Microwave Processing

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Key Words: Microwave; Radiation; Fixation; Processing

Abstract

Rapid processing of histopathologic material is becoming increasingly desirable to fulfill the needs of clinicians treating acutely ill patients. Traditional techniques for rapid processing of paraffin-embedded tissues require 4 to 5 hours, delaying treatment for some critically ill patients and requiring additional shifts of technologists in the laboratory. Microwave processing further shortens this time, allowing even more rapid histopathologic diagnosis. Few data exist comparing quality of microwave-processed tissue with that processed by more traditional techniques.

We randomly selected 158 paired specimens from 111 patients. One member of the pair was processed routinely overnight, while the other was processed by the rapid microwave technique. The slides then were compared for quality of histologic preparation in a blinded fashion by 2 pathologists.

Eight routinely processed specimens were judged as suboptimal, while 6 microwave-processed specimens were judged as suboptimal and 1 was considered unsatisfactory for evaluation. In the remaining cases, the material obtained by the 2 techniques was considered of identical quality. Microwave processing considerably shortens the preparation time for permanent histologic sections without a demonstrable decrease in section quality or "readability."

Turnaround time has been an important issue for many years and has become increasingly important in this age of managed care and commitments to overall reduction of costs for health care services. Initially, efforts were directed toward reducing specimen processing time for intraoperative consultations and rush specimens when a clinical requirement existed for a rapid diagnosis and initiation of emergency therapy based on the histopathologic findings. The development of the frozen section technique allowed a rapid processing time for intraoperative histopathologic evaluation, but this technique is associated with difficulty preparing and cutting certain types of specimens (fatty tissues), as well as certain morphologic changes that make histopathologic interpretation more difficult. These limitations have inhibited the use of frozen sections from becoming the predominant tissue-processing method for routine specimens in North America.

Modifications of routine processing techniques have allowed the processing of small biopsy specimens through paraffin with production of H&E-stained slides in approximately 4 hours. While this represents a considerable time savings in contrast with the overnight procedures routinely used, it is relatively labor intensive and applicable predominantly to small biopsy specimens. Hence, its use has been restricted largely to the processing of small biopsy specimens (eg, transbronchial, liver, and some gastrointestinal) from patients for whom a rapid histopathologic diagnosis is required to initiate life-saving antimicrobial, immunosuppressant or chemotherapeutic intervention. The demands of these methods have not permitted their wide acceptance for routine tissue preparation for H&E slides.

During the last 30 years, microwave-assisted tissue processing has been studied. The technique has achieved increasing acceptance in the last decade.¹⁻³ In 1993, Leong¹

reviewed microwave techniques described for diagnostic laboratories. Since that report, the increased popularity of microwave-assisted tissue processing has led to the production of commercially available microwave ovens specifically designed to ensure uniform rapid tissue processing under precisely controlled specimen temperatures. These machines also precisely control the on-off cycling of the heating. Such commercial units have facilitated accomplishment of tissue processing and diagnosis on the same day in which the specimen was obtained.

While a number of authors have reviewed the techniques and results of microwave-facilitated tissue fixation and processing,¹⁻⁷ we are unaware of any previous studies comparing the quality of microwave-processed and routinely processed tissues from matched specimens procured from the workload of an ordinary surgical pathology laboratory using a commercially available microwave oven. The purposes of the present study were to document the usefulness of microwave-assisted tissue processing and to determine whether it can replace standard formalin fixation and paraffin-embedded overnight processing as the routine technique for tissue preparation. Changing the standard technique for tissue fixation and preparation from the currently used overnight processing to same-day microwave tissue preparation could substantially reduce turnaround times, permitting same-day diagnosis that would facilitate patient diagnosis and management on a 1-day basis. This improvement in turnaround time could reduce costs associated with diagnosis and patient dissatisfaction and increase the rapidity with which neoplastic diseases are diagnosed and therapy is initiated.

Materials and Methods

A total of 158 paired specimens from 111 patients were randomly selected from the routine workload of the Surgical Pathology Laboratory, University of Utah Health Sciences Center Hospital, Salt Lake City. **Table 1** lists the types and numbers of specimens included in the present study. One member of each pair was processed routinely overnight according to 1 of 2 schedules, that is, a small or a large biopsy specimen schedule **Table 2** using a vacuum tissue processor. The other member of each pair was processed according to 1 of 2 microwave schedules, a short schedule for small biopsy specimens less than 2 mm thick and less than 10 mm in diameter, and a long schedule for biopsy specimens more than 2 mm thick or containing abundant blood, mucus, or both **Table 3**. In both procedures, the specimen was initially fixed in 10% neutral buffered formalin for a minimum of 1 hour.

The short microwave biopsy schedule (Table 3) was performed in a temperature-controlled microwave processor

Table 1
Types and Numbers of Specimens Processed

Type	Number
Large specimens	
Colon	20
Gallbladder	1
Uterus	13
Small intestine	2
Breast	10
Prostate	3
Lymph node	4
Small specimens	
Gastrointestinal	52
Liver	7
Endometrial	6
Prostate	34
Cervix (LEEP)	6

LEEP, loop electrosurgical excision procedure.

Table 2
Traditional Processing for Small and Large Specimens

	Fixation Times (min)	
	Small Specimens	Large Specimens
40°C		
Formalin	60	60
Formalin	60	60
70% alcohol	20	45
95% alcohol	30	45
95% alcohol	30	60
100% alcohol	30	45
100% alcohol	30	45
100% alcohol	30	60
Xylene	30	60
Xylene	30	60
60°C		
Paraffin	10	30
Paraffin	20	30
Paraffin	10	30
Paraffin	20	30
Total time*	8 h	12 h

* Total times are slightly more than sums of listed times owing to time required for changeover of fluids in vacuum tissue processors.

(Energy Beam, model H2800, Energy Beam Sciences, Agawam, MA). The long microwave biopsy schedule (Table 3) used the same temperature-controlled microwave processor. Total microwave processing times were 15 minutes for small biopsy specimens and 60 minutes for larger biopsy specimens. At the histologist's discretion, the long biopsy schedule was lengthened to 90 minutes (15 minutes in each solution instead of 10 minutes) for exceptionally fatty specimens such as skin, breast, and bowel, particularly when the tissue appeared less than well fixed. These times compare favorably with traditional processing times of 8 hours for biopsy specimens and 12 hours for large and fatty specimens.

The Energy Beam microwave oven allows precisely controlled temperatures and processing times. Its external

Table 3
Microwave Processing Schedules

Short Schedule	Long Schedule
100% reagent grade alcohol, 5 min at 67°C	100% reagent grade alcohol, 10 min at 67°C
100% isopropanol, 5 min at 74°C	100% reagent grade alcohol, 10 min at 67°C
Paraffin, 5 min at 80°C (paraffin preheated at 75°C)	100% isopropanol, 10 min at 74°C
—	100% isopropanol, 10 min at 74°C
—	Paraffin, 10 min at 75°C (paraffin preheated to temperature and repeated for another 10 min at 80°C)
Total time, 15 min	Total time, 60 min*

* On some large, fatty skin, bowel, and breast specimens, the time in each solution was lengthened to 15 minutes (total time, 90 min) at the discretion of the histologist, depending on how well fixed the tissue was at the start of processing.

dimensions are 19" deep × 20" high **Image 1**. It has no internal plumbing; the histotechnologists easily change solutions by hand. The oven comes with a vent and does not require placement in a hood, although there obviously must be an exhaust to which the oven vent may be connected. The oven's cassette tray holds 24 cassettes, but a single cassette may be processed in the case of unique stat specimens. There is only 1 temperature probe, so it is recommended to use only a single cassette tray with 24 cassettes per run. The oven may operate continuously throughout the day without "cooldown" time. At the study site, we use a single microwave oven for biopsy specimens only and batch the runs at 12 noon, 4:00 PM, and 6:00 AM; stat specimens are run at any time. We still use routine processing for most of our large resection specimens (eg, bowel, mastectomy). Our satellite histology laboratory at Associated Regional and University Pathologists Laboratory, Salt Lake City, operates 2 microwave ovens continuously throughout the day to process 16,000 specimens annually, mostly veterinary biopsy specimens.



Image 1 Microwave tissue processor used in our laboratory.

The paired tissues were evaluated independently by 2 experienced surgical pathologists (L.R.R. and L.J.L.) without knowledge of the type of processing used. Each pathologist evaluated the tissues according to the following scheme: satisfactory, suboptimal but satisfactory for diagnosis, and unsatisfactory. There was virtually complete concurrence between the 2 pathologists' independent evaluations of the adequacy of the tissue. Minor differences were resolved by joint evaluation at a double-headed microscope. Following completion of evaluation, the processing code was broken, and the results were analyzed.

Results

The consensus judgments of the 2 reviewing surgical pathologists are described in **Table 4** and **Table 5**. A representative prostate biopsy specimen processed by routine and rapid microwave techniques is shown in **Image 2**. There were 6 suboptimal microwave specimens and 1 unsatisfactory microwave specimen, and 8 routinely processed specimens were judged as suboptimal.

One unsatisfactory microwave specimen was a full-thickness section of uterine wall that was judged unsatisfactory owing to inadequate fixation. All suboptimal microwave specimens were large specimens in which there was less than optimal nuclear and cytoplasmic detail or there was fatty tissue dropout.

One of the routinely processed suboptimal specimens was a colon resection sample that was judged suboptimal owing to inadequate fixation or dehydration. A second suboptimal specimen was a lymph node with less than optimal nuclear and cytoplasmic detail. Three of the remaining 6 specimens judged suboptimal were prostate needle biopsy specimens judged to be cut too thick, 2 were liver biopsy specimens with less than optimal nuclear and cytoplasmic detail and clarity, and 1 was a gastrointestinal biopsy specimen with excessive fragmentation.

Overall, the quality of microscopic tissues from the traditional processing and the microwave-processing

Table 4
Problems With Microwave-Processed Specimens

Specimen Type	Specimen Quality	Problem Encountered
Uterus	Unsatisfactory	Poor fixation
Uterus	Suboptimal	Darkened smudged center of tissue suggestive of less penetration
Uterus	Suboptimal	Suboptimal fixation, compromised cellular detail
Breast	Suboptimal	Not optimally fixed, fatty tissue dropout
Lymph node	Suboptimal	Suboptimal fixation, fatty tissue dropout
Lymph node	Suboptimal	Suboptimal fixation, fatty tissue dropout
Colon resection	Suboptimal	Suboptimal cellular detail

methods was extremely similar. It was not possible to distinguish between the 2 techniques by studying the tissue sections.

Discussion

Since the advent of modern histopathology, paraffin wax has been used as the embedding medium of choice for histologic processing. Its physical properties are ideal for an embedding medium in that it has a liquid form when heated and a solid but easily cut form when cooled to room temperature. Its melting point is such that it can be liquefied at a temperature that does little damage to tissue. Before tissue can be embedded within paraffin, it must be completely fixed. This procedure requires the removal of the formalin-based fixative, followed by complete dehydration using a

Table 5
Problems With Routinely Processed Specimens

Specimen Type	Specimen Quality	Problem Encountered
Gastrointestinal biopsy	Suboptimal	Fragmented tissue
Liver biopsy	Suboptimal	Suboptimal clarity and cellular detail
Liver biopsy	Suboptimal	Suboptimal clarity and cellular detail
Lymph node	Suboptimal	Suboptimal cellular detail
Prostate biopsy	Suboptimal	Sections too thick
Prostate biopsy	Suboptimal	Sections too thick
Prostate biopsy	Suboptimal	Sections too thick
Colon resection	Suboptimal	Suboptimal fixation/penetration

series of graduated alcohols that have been cleared with xylene; the tissue is then infiltrated with the melted paraffin wax. This traditional processing technique requires an overnight schedule, although abbreviated versions require 4 to 5 hours by omitting various alcohol baths and using vacuum infiltration for the paraffin-permeation stages.

Microwave processing changes the procedure considerably; it permits a more rapid completion of fixation before the initiation of histologic processing. Dehydration is achieved in 1 step instead of multiple graded solutions of alcohol, and paraffin impregnation occurs at a higher temperature, speeding the process. In principle, microwave processing uses previously fixed tissue that is dehydrated rapidly using microwave energy to heat the reagent alcohol to just below its boiling point. Isopropanol further dehydrates the tissue and prepares it for paraffin infiltration. The residual isopropanol is effectively “boiled out” by using microwave energy to heat

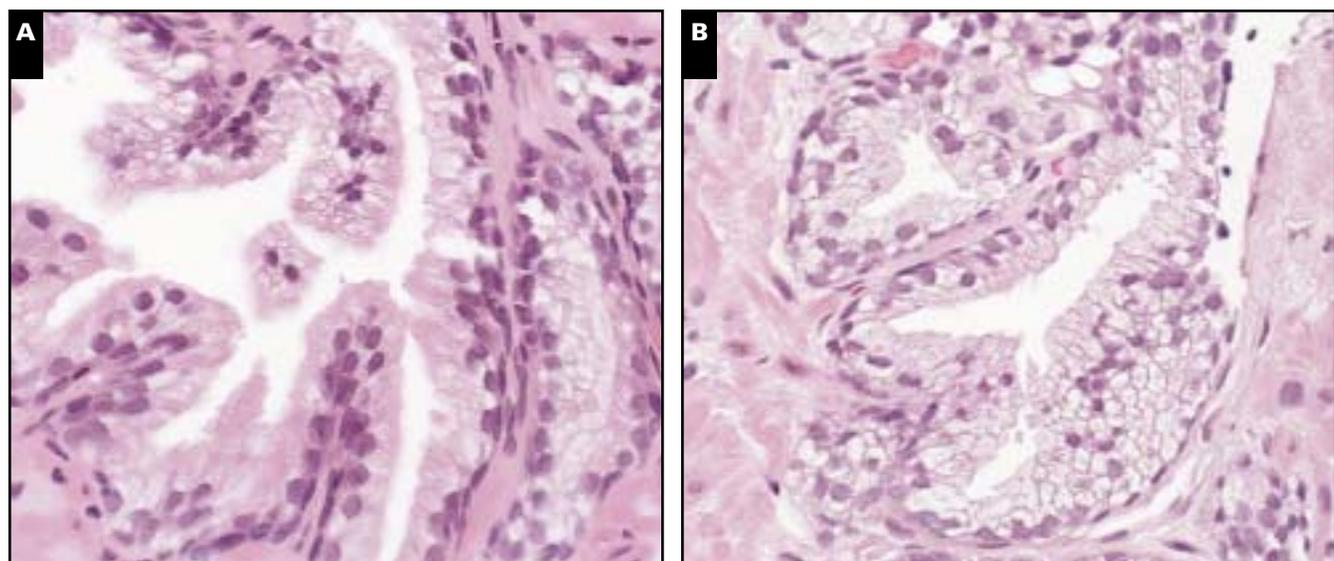


Image 2 Prostate specimens from the same patient. **A**, Routinely processed specimen. **B**, Microwave-processed specimen. (H&E, original magnification x400)

liquid paraffin above the boiling point of isopropanol. This procedure substantially shortens the processing time without degradation of histomorphologic features.

Microwave-assisted tissue processing has been studied for a variety of applications since 1970. It has achieved widespread acceptance as a processing technique for paraffinized tissue placed on slides for immunohistochemical staining. However, microwave technology was first used in the processing of tissue for routine histologic preparation. Despite this acceptance as the preferred method for antigen retrieval and slide preparation for immunohistochemical staining, microwave processing has not become a widely accepted method for the routine processing of surgical pathology specimens. Since 1986, a number of articles have been published, predominantly from outside the United States, describing the use of microwave ovens in various areas of tissue processing.¹⁻⁸

Microwave-based techniques have been used to accomplish rapid fixation of large specimens without formalin, including colon specimens, lymphadenectomy specimens, and mastectomy specimens, thereby hardening the tissue for paraffinization and sectioning without exposure of laboratory staff to noxious formalin fumes.² Mac-Moune et al³ reported satisfactory results with immunofluorescence in kidney biopsy specimens fixed in a saline medium by microwave irradiation. Kok et al⁴ reported frozen sections of superior quality produced by a method using microwave radiation to assist in fixation of tissue sections on slides covered with a few drops of solution containing ethyl alcohol and polyethylene glycol. This technique improved section appearance without increasing the time to diagnosis. Microwave irradiation has been used to fix whole brain specimens and produce microscopic slides of brain specimens within 24 hours.⁵ Microwave irradiation alone or microwave-augmented fixation has been used successfully to rapidly fix whole prostate glands,⁶ whole eyes,⁷ and a variety of other tissue specimens.⁸

Microwave irradiation has several advantages over routine methods from the perspective of laboratory personnel. It also has certain environmental advantages. It eliminates the need for xylene in tissue processing. It may reduce or eliminate the need for formalin, as determined by the laboratory. From the perspective of the final product, microwave irradiation substantially shortens the time from specimen reception to diagnosis. In our experience, this reduced preparation time (2-3 hours, including fixation, processing, microtomy, and staining) allows same-day tissue processing and diagnosis of small biopsy specimens without compromising the overall quality of the histologic section. This rapidity of tissue preparation has a number of advantages. Many patients seen at our institution travel long distances for diagnostic studies and definitive cancer therapy.

In some instances, it is desirable to perform a biopsy and definitive surgery on the same day, thereby decreasing patient expense and the requirement for multiple trips between home and the referral center. Reliance on frozen section with its attendant difficulties of interpretation and greater expense can be reduced substantially. For instance, a patient with a Papanicolaou smear of the cervix showing squamous cell carcinoma may undergo a loop electrosurgical excision procedure (LEEP) biopsy of the cervix at 8:00 AM in the clinic to determine the presence and extent of invasion and undergo definitive gynecologic surgery at 2:00 PM the same day with the advantage of quality permanent section analysis of the entire cone biopsy specimen. The avoidance of frozen section also decreases anesthesia time. Urgent biopsies for transplant patients may be performed and diagnosed the same day, an attractive advantage preceding long weekends, because the overtime expenses for histotechnologists and pathologists can be decreased.

Our study demonstrated no substantial differences in overall quality or diagnosability of tissue sections prepared by microwave tissue processing vs standard overnight formalin fixation and paraffin embedding. Of the 158 paired specimens, the number judged as suboptimal was similar for the 2 techniques (6 microwave-processed specimens, 8 routinely processed specimens). Only 1 microwave-processed specimen was judged unsatisfactory for diagnosis. In almost all cases, there was no qualitative difference between the 2 techniques. Although we did not specifically study effects of microwave processing on immunohistochemistry, we have used this technique for more than 3 years on biopsy specimens and have not noticed adverse effects on the quality or expected results of immunohistochemistry. We plan to examine this more specifically in a future study.

We believe that routinely received, formalin-fixed, small and large tissue specimens may be processed rapidly by microwave irradiation and sectioned and stained without compromising quality of the histologic sections. We also believe that this microwave processing technique can serve as the routine method for tissue processing and preparation of histologic specimens in an active university hospital histology laboratory. This technique has been used routinely in our daily workload for the past 3 years. The use of a commercially available microwave oven (see "Materials and Methods") for the routine rapid processing of small biopsy specimens has not been associated with problems in tissue quality, as evidenced by our quality assurance and quality control records. We believe that rapid microwave-assisted tissue processing is the optimal method for substantially reducing turnaround time and permitting the histopathology laboratory to consistently provide same-day diagnosis for a variety of types of tissue biopsy specimens.

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