A Comparison of Immunohistochemical Stain Quality in Conventional and Rapid Microwave Processed Tissues

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Abstract

Same-day turnaround of pathology specimens is desirable in this era of managed care, and rapid microwave tissue processing produces histologic features of a quality equivalent to overnight processing. We studied whether microwave-assisted rapid tissue processing adversely affects the quality of immunohistochemical staining. We selected 30 specimens (20 neoplastic and 10 nonneoplastic) from our routine surgical pathology workload. Paired large tissue blocks were made from each specimen type, one for microwave-assisted rapid processing and one for conventional processing. Two microarrays of 60 punches each were made from the donor blocks. The microarray blocks were examined for intensity and extent of staining by 44 commonly used antibodies. Slides were reviewed independently by 2 pathologists blinded to the type of processing used. In 5,280 tissue punches examined, we found a high degree of concordance in quality, as measured by intensity and extent of immunohistochemical staining, between microwave and routinely processed tissues. Our study demonstrates that quality of immunohistochemical staining is similar between rapid microwave and conventional processing. The potential need for immunohistochemical analysis is not a contraindication for microwave-assisted rapid tissue processing.

Histologic fixation by microwave technology was introduced by Mayers[1] in 1970. However, the lack of specifically designed microwave ovens, optimal reagents, and protocols hindered the widespread use of the method. However, these obstacles have been overcome.[2] During the last few decades, numerous reports have supported the use of microwave technology in a wide variety of applications in diagnostic surgical pathology, including transmission electron microscopy, histochemical staining, rapid immunoperoxidase staining of labile lymphocyte antigens, rapid fixation of large biopsy specimens, processing of renal biopsy specimens, microwave-stimulated fixation of reagents for cryostat sections, acceleration of decalcification, and improvement of sensitivity of immunohistochemical staining.[3-10]

Microwave-assisted rapid tissue processing as a substitute for, or an adjunct method to, conventional tissue processing in the daily workload of high-volume diagnostic surgical pathology laboratories was not accepted until the early to mid 1990s.[11] Although there has been resistance to substituting microwave processing for conventional tissue processing, microwave tissue processing provides many advantages for diagnostic surgical pathology.[12] Microwave-assisted processing allows for reduced turnaround times with same-day generation of a diagnostic report, which, in turn, results in reduced time to formulation of treatment plans, reduced patient anxiety, and reduced hospital bed costs.[12,13]

Leong[12] and Morales et al[14] recently reported their experience with the positive impact of microwave-assisted rapid tissue processing on turnaround times. Morales et al[14] noted that same-day review and reporting of cases improved from fewer than 1% of cases to approximately 55% of cases with the use of microwave-assisted processing. The mean

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turnaround time for conventional tissue processing was 21 hours in Leong's laboratory, whereas the mean turnaround time for microwave processing was 6.5 hours.[12] Morales et al[15] reported an even shorter turnaround time of 2 to 3 hours for specimens processed by a fully automated microwave method. In our own laboratory, we use microwave-assisted rapid tissue processing as the routine method for processing of small biopsy specimens such as gastrointestinal and transbronchial specimens. Same-day review is possible by this method, and when such specimens are submitted for rush analysis, as in the case of gastrointestinal biopsy specimens submitted for evaluation of graft-vs-host disease in bone marrow transplant recipients or transbronchial biopsy specimens for assessment of rejection in pulmonary transplant recipients, turnaround times are reduced to approximately 3 to 4 hours (L.L.E. et al, unpublished data, July-December 2000).

Rohr et al[13] documented the usefulness of microwave-assisted rapid processing in diagnostic surgical pathology by demonstrating comparable quality of H&E-stained microscopic sections produced from tissues processed by conventional means and those processed by the microwave method. As microwave-assisted tissue processing becomes more widely accepted in the diagnostic surgical pathology laboratory, the application of various diagnostic techniques to microwave-processed tissues will increase. Because of the prevalence of immunohistochemical analysis in the practice of diagnostic surgical pathology, we compared the quality of immunohistochemical staining in tissues processed by a rapid microwave-assisted method with that obtained by conventional overnight processing. To our knowledge, no such extensive comparative study has been published.

Materials and Methods

A total of 30 paired specimens were 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 of specimens included in the present study. Each specimen was sectioned to obtain a tissue section 3 to 4 mm thick and up to 2 cm wide. Both members of each pair were fixed for a minimum of 1 hour in 10% neutral buffered formalin before processing. One member of each pair was processed by the conventional overnight method using a vacuum infiltration processor (E300 series, VIP Tissue-Tek, Torrance, CA) according to our laboratory's routine processing protocol for large specimens **Table 2**.The other member was processed according to the long microwave schedule **Table 3**.

The microwave processor (Model MWP 800, Energy Beam Sciences, East Granby, CT), as described earlier,[13] allows precisely controlled temperatures and processing times. Batch runs are performed in our laboratory at 6:00 AM, 12:00 PM, and 4:00 PM, but stat specimens may be run at any time during the day. The short and long microwave schedules (Table 3) are performed in an Energy Beam model MWP 800 temperature-controlled microwave processor (Energy Beam Sciences). The specimens in this study were processed on the long microwave schedule.

Table 2
Conventional Processing Schedules for Small and Large Specimens

Small Specimen Processing Times*	Large Specimen Processing Times*
Formalin, 60/40°C	Formalin, 60/40°C
Formalin, 60/40°C	Formalin, 60/40°C
70% alcohol, 20/40°C	70% alcohol, 45/40°C
95% alcohol, 30/40°C	95% alcohol, 45/40°C
95% alcohol, 30/40°C	95% alcohol, 60/40°C
100% alcohol, 30/40°C	100% alcohol, 45/40°C
100% alcohol, 30/40°C	100% alcohol, 45/40°C
100% alcohol, 30/40°C	100% alcohol, 60/40°C
Xylene, 30/40°C	Xylene, 60/40°C
Xylene, 30/40°C	Xylene, 60/40°C
Paraffin, 10/60°C	Paraffin, 30/60°C
Paraffin, 20/60°C	Paraffin, 30/60°C
Paraffin, 10/60°C	Paraffin, 30/60°C
Paraffin, 20/60°C	Paraffin, 30/60°C
Total time, 8 h*	Total time, 12 h*

* Times are given in minutes, followed by the processing temperature. Total times listed exceed the sums of the listed times for each schedule due to time required for reagent exchanges.

Table 1
Types of Specimens Processed

Nonneoplastic Tissue Specimens	Neoplastic Tissue Specimens	
Placenta	Metastatic colorectal adenocarcinoma	Ewing sarcoma
Prostate	Colorectal adenocarcinoma	Seminoma
Tonsil	Breast ductal adenocarcinoma	B-cell lymphoma
Thyroid	Alveolar soft part sarcoma	Papillary serous ovarian carcinoma
Kidney	Rhabdomyosarcoma	Metastatic papillary thyroid carcinoma
Breast	Synovial sarcoma	Renal oncocytoma
Cervix	Malignant melanoma	Granulosa cell tumor
Colon	Gastrointestinal stromal tumor	Non–small cell lung carcinoma
Endometrium	Pheochromocytoma	Sarcomatoid carcinoma
Stomach	Malignant mixed müllerian tumor	T-cell lymphoma

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Table 3
Microwave Processing Schedules

Short Microwave Schedule	Long Microwave Schedule
100% reagent-grade alcohol, 5 min, 67°C	100% reagent-grade alcohol, two 10-min cycles, 67°C
100% isopropanol, 5 min, 74°C	100% isopropanol, two 10-min cycles, 74°C
Paraffin, 5 min, 80°C (paraffin preheated to 75°C)	Paraffin, 10 min, 75°C (paraffin preheated in Lipshaw paraffin dispenser pot to 75°C and repeated for another 10 min, 80°C)
Total time, 15 min	Total time, 60 min

Microarray Technique

Tissue microarrays were made using the Manual Tissue Arrayer (model MTA-1, Beecher Instruments, Sun Prairie, WI). The 2-mm needle was chosen to punch samples from the donor tissue blocks to construct a tissue microarray block for this study. We started with 60 donor blocks (paired blocks from each of 30 different specimens). The microarrays were constructed so that each pair of blocks had 2 punches from each block. A microarray of 2-mm punches for the first 15 specimens, therefore, consisted of 60 punches (30 conventionally processed and 30 microwave-processed punches) **Image 1**. Microarray block 1 consisted of cases 1 through 15, and block 2 consisted of cases 16 through 30. The order of placement of the microwave-processed and conventionally processed punches in the microarray block was unknown to the 2 reviewing pathologists (L.L.E. and L.R.R.). A smaller 0.6-mm marker tissue was used as a location marker in the upper left corner of the block for orientation.

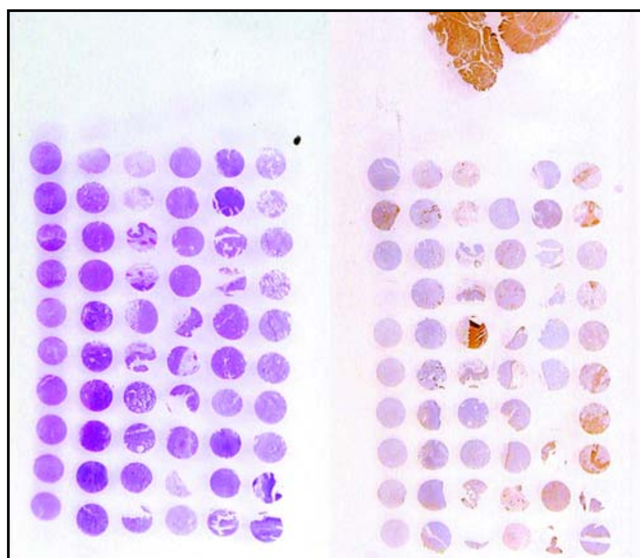

Image 1 Routine H&E and representative immunohistochemical stain (muscle-specific actin) produced from tissue microarray.

Immunohistochemical Technique

Once the blocks were completed, 4-μm sections were cut on Fisherbrand Superfrost/plus slides (Fisher Scientific, Pittsburgh, PA) and allowed to air dry overnight. Control samples for each antibody were placed at the top of the microarray slide cut from the first microarray block (cases 1-15). The slides were melted for 30 minutes in a 60°C oven, deparaffinized in 3 changes of xylene, and rehydrated in graded alcohols (100%, 95%, 70%). The slides were rinsed in deionized water, and antibodies were applied to the tissue according to the protocols for each antibody **Table 4**. The slides were placed on the Ventana automated immunostainer (ES, Ventana Medical Systems, Tucson, AZ). All steps performed on the immunostainer were at 40°C. The primary antibody dilution for each antibody is listed in Table 4. The detection kit used was the DAB Basic Kit (Ventana Medical Systems), which is a biotinylated goat antimouse/antirabbit secondary, streptavidin–horseradish peroxidase system using diaminobenzidine as the chromogen. The slides were counterstained with hematoxylin, dehydrated in graded alcohols (70%, 95%, and 100%), cleared in xylene, and coverslipped.

Immunostaining of the paired tissues was evaluated independently by 2 surgical pathologists (L.L.E. and L.R.R.) blinded to the method of tissue processing. Each pathologist assessed the intensity and extent of immunoreactivity for each antibody by the following rating scales: extent of staining, 0, no staining; 1, staining in 0% to 24%; 2, staining in 25% to 49%; 3, staining in 50% to 74%; and 4, staining in 75% to 100% of the cell population of interest; intensity of staining, 1+, weak; 2+, moderate; and 3+, strong **Image 2**.

A variation of 1 grade was considered acceptable interobserver variability. When the variation was greater than 1 grade, the discrepancy between the pathologists was resolved for each tissue punch by review of the slides at a double-headed microscope. Most disagreements of greater than 1 grade were caused by a difference in interpretation of the antibody as it applied to that particular tissue type. For example, one pathologist may have graded a colorectal carcinoma as having 3+ staining intensity in greater than 75% of the cells in evaluation of the CD34 immunostain, whereas the other pathologist graded the same tissue punch as having no staining in intensity or extent of staining because the latter pathologist was evaluating

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Table 4
Immunohistochemical Analysis

Antibody	Manufacturer	Pretreatment	Dilution/Time (min)*
AE1/3	Boehringer Mannheim, Indianapolis, IN	Protease 2, 8 min	1:2,800/32
C-NEU	Oncogene, Boston, MA	Microwave retrieval	1:1,600/32
CA 125	Signet, Dedham, MA	Microwave retrieval	1:200/32
Calcitonin	DAKO, Carpinteria, CA	None	1:500/10 (room temperature)
Calretinin	Zymed, San Francisco, CA	Microwave retrieval	1:160/32
CAM 5.2 (Cyto.8/18)	Novocastra, Newcastle upon Tyne, England	Protease 1, 2 min	1:40/32
CD1a	Immunotech, Marseille, France	Microwave retrieval	Prediluted/25
CD3	Novocastra	Pressure cooker retrieval, BORG buffer, pH 9.5 (Biocare Medical, Walnut Creek, CA)	1:100/32
CD15	Becton Dickinson, San Jose, CA	Pressure cooker retrieval, citrate buffer, pH 6.0	1:20/32 (with amplification kit)
CD20	DAKO	Microwave retrieval	1:2,000/32
CD30	DAKO	Pressure cooker retrieval, citrate buffer, pH 6.0	1:200/32 (with amplification kit)
CD31	DAKO	Microwave retrieval	1:40/32
CD34	BioSource, Camarillo, CA	Microwave retrieval	1:200/32
CD45	DAKO	Microwave retrieval	1:1,000/32
CD45RO	Zymed	Microwave retrieval	1:2,000/32
CD79a	DAKO	Pressure cooker retrieval, citrate buffer, pH 6.0	1:160/32
CD99 (O13)	Signet	None	1:200/32
CEA (polyclonal)	DAKO	None	1:800/32
Chromogranin	Novocastra	Microwave retrieval	1:100/32
CK5/6	Chemicon, Temecula, CA	Pressure cooker retrieval, citrate buffer, pH 6.0; protease 2, 2 min	1:160/32
CK7	DAKO	Microwave retrieval	1:400/32
CK20	DAKO	Microwave retrieval	1:200/32
Desmin	DAKO	Microwave retrieval	1:200/32
EMA	DAKO	Microwave retrieval	1:200/32
Estrogen receptor	Ventana Medical Systems, Tucson, AZ	Pressure cooker retrieval, citrate buffer, pH 6.0	Prediluted/32
FVIII	DAKO	Microwave retrieval	1:1,600/32
GFAP	DAKO	Microwave retrieval	1:400/32
HCG	DAKO	Microwave retrieval	1:3,000/10 (room temperature)
HPL	DAKO	Microwave retrieval	1:12,800/10 (room temperature)
HMB45	DAKO	Protease 2, 6 min	1:100/32
Inhibin	Serotec, Raleigh, NC	Pressure cooker retrieval, citrate buffer, pH 6.0	1:25/overnight (room temperature; with amplification kit)
Keratin903	Enzo, Farmingdale, NY	Microwave retrieval	1:40/32 (with amplification kit)
Melan A	DAKO	Pressure cooker retrieval, citrate buffer, pH 6.0	1:50/32 (with amplification kit)
Muramidase (lysozyme)	DAKO	Protease 2, 8 min	1:3,200/32
MSA	DAKO	None	1:100/32
PLAP	DAKO	Microwave retrieval	1:200/32
Progesterone receptor	Ventana Medical Systems	Pressure cooker retrieval, citrate buffer, pH 6.0	Prediluted/32
PAP	DAKO	Microwave retrieval	1:1,600/32
S-100	DAKO	Microwave retrieval	1:3,000/32
SMA	DAKO	None	1:200/32
Synaptophysin	DAKO	Microwave retrieval	1:200/32
Thyroglobulin	DAKO	Pressure cooker retrieval, citrate buffer, pH 6.0	1:500/32
Vimentin	DAKO	Microwave retrieval	1:300/32

CEA, carcinoembryonic antigen; CK, cytokeratin; EMA, epithelial membrane antigen; FVIII, factor VIII; GFAP, glial fibrillary acidic protein; HCG, human chorionic gonadotropin; HPL, human placental lactogen; MSA, muscle-specific actin; PAP, prostatic acid phosphatase; PLAP, placental alkaline phosphatase; SMA, smooth muscle actin.
* Unless otherwise specified.

only the neoplastic cells rather than the vessels supplying the tumor. These disagreements were resolved easily. Following completion of evaluation and consensus review on disparate cases, the processing code was broken, and the results were analyzed.

Results

The microwave- and conventionally processed tissues stained with antibody to factor XIII showed diffuse background staining in all tissues, and, therefore, this antibody was

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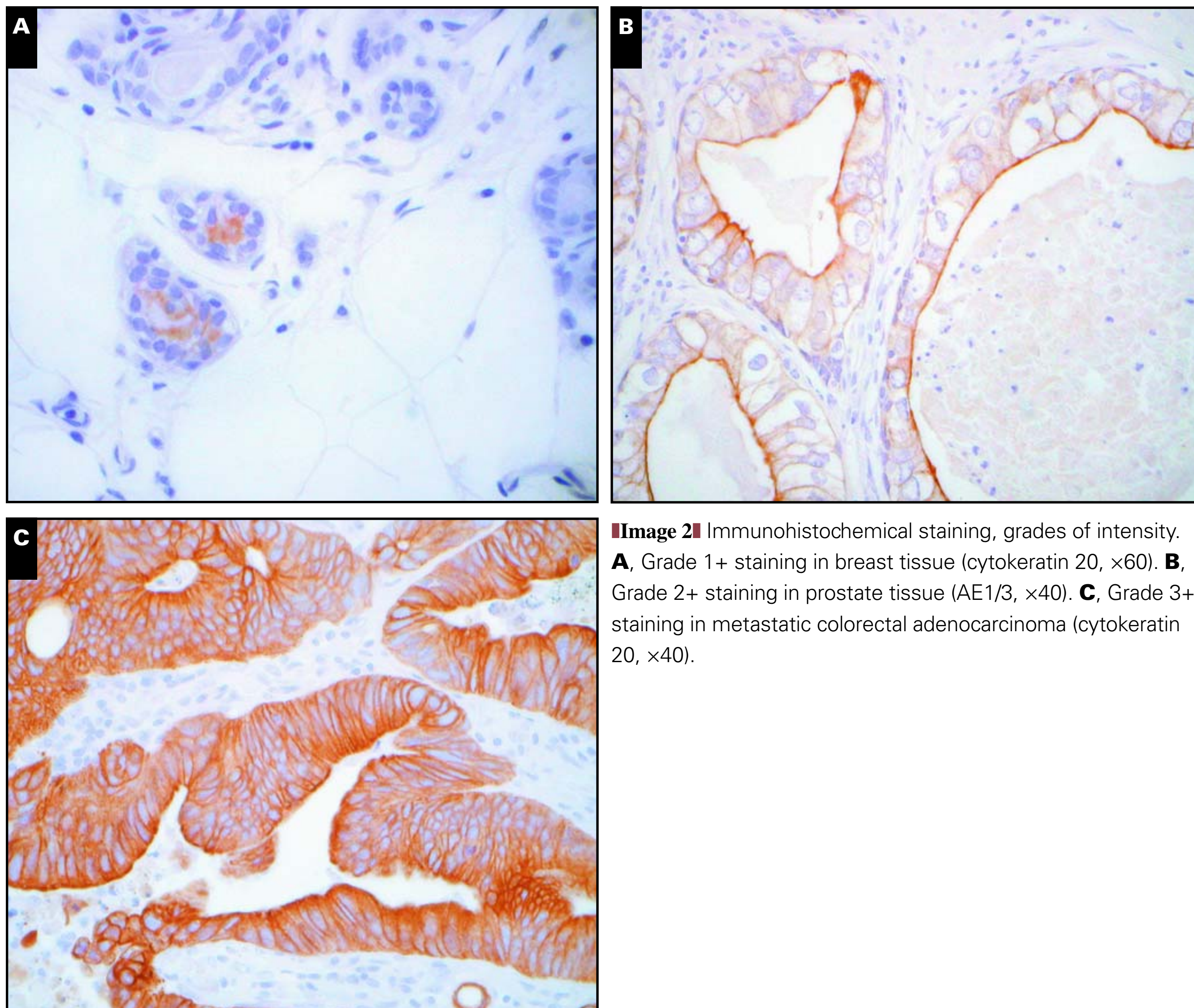

❚Image 2❚ Immunohistochemical staining, grades of intensity. **A**, Grade 1+ staining in breast tissue (cytokeratin 20, ×60). **B**, Grade 2+ staining in prostate tissue (AE1/3, ×40). **C**, Grade 3+ staining in metastatic colorectal adenocarcinoma (cytokeratin 20, ×40).

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considered unsatisfactory for evaluation, excluding 120 tissue punch sections. The oncocytoma of kidney had a background blush with all of the antibodies and, therefore, another 172 tissue punch sections were excluded from analysis. Seventy conventionally processed specimens (1.3%) and 377 microwave-processed specimens (7.1%) were judged to be suboptimal and uninterpretable owing to technical difficulties in adherence of tissue to the slides during the immunostaining process. After exclusion of all uninterpretable tissues, 4,541 tissue punch sections remained for interpretation. Although any tissue microarray that had less than half of the tissue present for evaluation was indeed considered a technical problem, data from these tissues were still used in these cases if both pathologists deemed the tissue to be satisfactory for interpretation. We had 100 microwave-processed tissues (1.9%) and 69 conventionally processed tissues (1.3%) in this category. Of the 4,541 interpretable tissue punch sections, the pathologists varied by more than 1 grade in assessment of intensity in 918 tissue punch sections (20.2%) and in extent of staining in 973 tissue punch sections (21.4%).

Analysis of Concordance

For intensity and extent of staining, the ratings given by each of the reviewing pathologists were averaged for both sites from the donor tissue specimen to create a composite rating. This composite rating was the basis for the analysis. We formed 1,132 composite ratings using nonmissing values. This number is smaller than the 1,320 attempted measurements owing to the technical issues described in the previous section. The composite ratings for extent of staining were compared for both processing methods.

Identical extent of staining composite ratings were categorized as total concordance (85.8%). Those differing by no more than 1 rating point, more than 1 but no more than 2 rating

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points, more than 2 but no more than 3 rating points, and more than 3 but no more than 4 rating points were categorized as mild, moderate, major, and severe discordance, respectively. The percentages of composite observations in the discordant categories are 8.3%, 3.3%, 1.5%, and 1.1%, respectively **Figure 1**.

The same procedure was used to analyze the intensity of staining with the maximum difference being 3 rating points rather than 4 owing to the nature of the rating scale previously described. Identical intensity of staining composite ratings were categorized as total concordance (86.5%). Those differing by no more than 1 rating point, more than 1 but no more than 2 rating points, and more than 2 but no more than 3 rating points were categorized as mild, moderate, and severe discordance, respectively. The percentages of composite observations in the discordant categories were 8.8%, 3.4%, and 1.3%, respectively **Figure 2**.

Discussion

The potential application of microwave technology to histotechnology was first recognized in 1970 by Mayers.[1] Since then, many investigators have studied the application of microwave technology to clinical medicine, and evidence has accumulated to support its use in various aspects of diagnostic surgical pathology, including transmission electron microscopy, histochemical staining, rapid immunoperoxidase staining of labile lymphocyte antigens, rapid fixation of large biopsy specimens, processing of renal biopsy specimens, microwave-stimulated fixation of reagents for cryostat sections, and acceleration of decalcification and immunohistochemical staining.[3-10]

One of its most valuable applications has been in the area of diagnostic surgical pathology where it is used to reduce turnaround times. A waiting period of up to 1 day for diagnoses is customary in most surgical pathology laboratories. If the specimen is complex, requiring special diagnostic studies such as immunohistochemical analysis, diagnoses may take even longer. Use of microwave methods considerably reduces the processing time with 1-step dehydration and 1-step clearing before paraffin infiltration.[16] Microwave-assisted tissue processing, therefore, can reduce turnaround time to 2 to 6 hours, depending on the method used.[13-15] This reduction in turnaround time has a potential for considerable clinical impact by reducing the time to diagnosis, time to initiation of treatment, patient anxiety, and potentially costs associated with length of hospital stay.[12,13]

A previous report confirmed the comparable quality of microwave-processed tissues and conventionally processed tissues in routine H&E stain preparations.[13] The microwave processing schedules in our laboratory have remained unchanged since that report, and we continue to successfully use this method for the rapid processing of small biopsy specimens, including gastrointestinal, transbronchial, and renal specimens. We have found that microwave-assisted processing greatly facilitates reduction of turnaround time for batches of these types of specimens and, in the case of stat specimens, often aids in establishing a diagnosis within hours of biopsy for critically ill patients (L.L.E. et al, unpublished data, July-December 2000).

Our current tissue processing method uses formalin fixation, and, therefore, we have not adopted microwave processing for large surgical specimens. We are, however, studying the possibility of making the transition to a continuous-throughput

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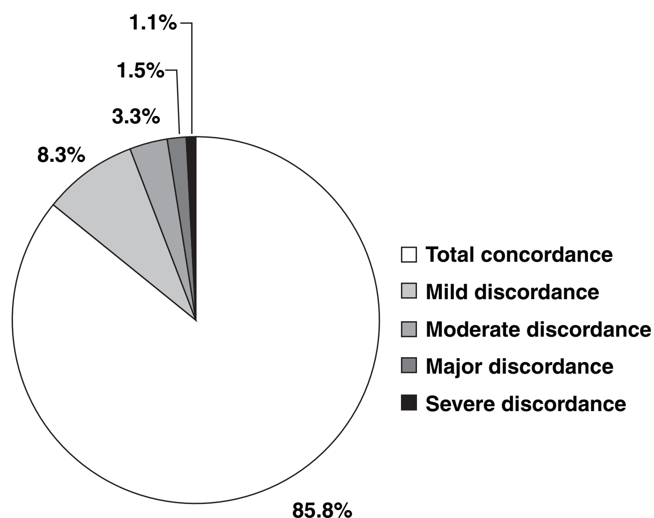

Figure 1 Percentages of cases differing in extent of staining.

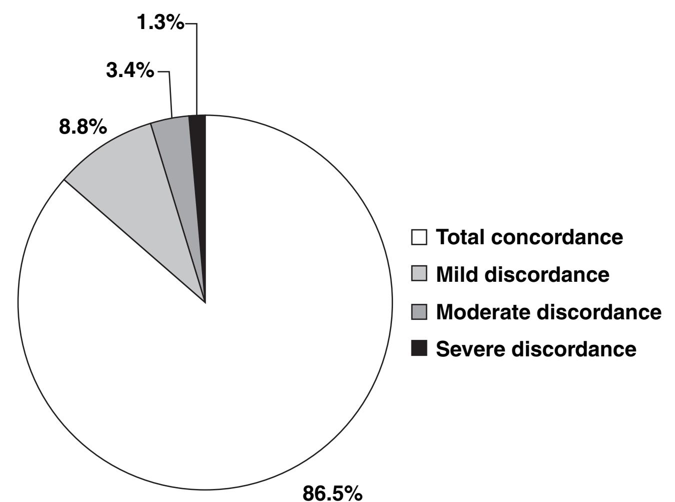

Figure 2 Percentages of cases differing in intensity of staining.

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microwave processing method as described by Morales et al.[15] This method entirely eliminates the use of formalin fixation and the xylene associated with conventional processing and allows continuous processing of specimens throughout the day. Even with the admitted benefit of such a method, its widespread acceptance has been slow.[15] Leong[12] discusses this resistance to change and observes that this resistance may be due partly to concerns about the impact on established work practices in the surgical pathology laboratory. Admittedly, our own resistance may have been due in large part to a reluctance to address the necessary changes in histotechnologist scheduling, gross room staffing, and training inherent in such a transition.

The potential effects of modifications in processing technique on the quality and reliability of ancillary tests, including immunohistochemical analysis, has led to reluctance among pathologists in accepting the routine use of microwave processing. Concerns exist among surgical pathologists that microwave processing may alter the quality, sensitivity, or specificity of immunohistochemical staining by antibodies commonly used in the routine practice of surgical pathology. We studied the impact of microwave processing of tissue on the quality of immunohistochemical staining for a variety of commonly used antibodies.

We noted that the microwave technique seemed to reduce tissue adherence to slides during the immunostaining process. While only 70 conventionally processed specimens (1.3%) showed technical difficulties associated with adherence of tissue to slides during the staining process, 377 microwave-processed specimens (7.1%) had this problem. This difference in specimen adherence during immunostaining may be a technical issue affecting the usefulness of microwave processing when immunohistochemical staining is likely to be required for diagnosis; however, it should be noted that there is some variability among the various microwave tissue processors in the amount of heat produced, and high-energy processors such as the one used in our study may create tissue-adherence difficulties.

Our study demonstrated very high concordance between intensity and extent of immunostaining between processing methods. It should be recognized, however, that sensitivity and specificity as they relate to immunohistochemical analysis usually are a function of fixation type and duration rather than processing method. Many studies have shown that microwave fixation is comparable if not superior to formalin fixation in terms of antigen preservation.[5,17-19] In addition, underfixation and overfixation with formalin may cause problems with immunostaining results owing to autolysis and excessive cross-linking of proteins, respectively.[16,20] The minor observed differences in staining seen in our study, therefore, may have been due to formalin fixation issues because formalin prefixation times varied widely and were not controlled tightly. Short formalin fixation times can cause incomplete cross-linking of proteins and thereby affect immunohistochemical staining.[20] The conventional method may have compensated somewhat in such cases of underfixation with an extra 2 hours of formalin fixation (Tables 2 and 3), whereas the microwave-assisted method could not have compensated in a like manner.

We did not specifically study the potential for increased sensitivity resulting from microwave tissue processing, nor did we formally examine the sensitivity of each of the antibodies as applied to the various tissue types. An additional issue not entirely addressed by the present study is that of antibody specificity. Microwave antigen retrieval is known to improve antigen recognition by a variety of antisera, and antigen retrieval by microwave processing is a widely used technique to improve the sensitivity of immunohistochemical staining for many antibodies used in clinical practice. However, in occasional clinical situations, antigen retrieval is detrimental to the specificity and clinical usefulness of immunostaining. Immunohistochemical staining for c-kit is an example.

C-kit is used widely for the diagnosis of gastrointestinal stromal tumors and seems to predict the response to imatinib therapy. When antigen retrieval is used, immunohistochemical staining of intra-abdominal spindle cell neoplasms seems to be less specific and does not have the same predictive value for imatinib response. Some authorities (C. Fletcher, MD, verbal communication, May 2003) have recommended elimination of antigen retrieval for c-kit immunohistochemical analysis in the diagnosis of gastrointestinal stromal tumors. Our data do not address the effect of microwave tissue processing on the specificity of c-kit immunohistochemical analysis, and we did not formally study the sensitivity of each of the antibodies as applied to the various tissue types. Nevertheless, our data indicate that immunohistochemical analysis as applied to microwave-processed tissue has a high degree of concordance in quality with tissue processed by the conventional method for a wide variety of commonly used antibodies applied to a large number of tissue types.

Various aspects of the quality of immunohistochemical analysis following the use of microwave technology have been studied. Margo et al[21] reported that immunoperoxidase staining for cytokeratins, S-100 protein, and glial fibrillary acidic protein was positive after microwave-stimulated chemical fixation of whole eyes. Arana-Chavez and Nanci[17] studied high-resolution immunocytochemical analysis in noncollagenous matrix proteins of rat mandibles processed by microwave irradiation and found that immunoreactivities were generally more intense, particularly at early stages of tooth formation. McCluggage et al[22] examined immunohistochemical staining in sections cut for plastic-embedded bone marrow trephine biopsies after microwave heating compared with paraffin-embedded trephine biopsies after decalcification and found

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that with few exceptions, the results were satisfactory. Moran et al[19] studied preservation of extracellular matrix antigens, including collagen III, collagen IV, fibronectin, and laminin, and found good preservation of antigenicity after microwave fixation of specimens.

Our study was structured to examine the quality of immunohistochemical analysis in tissues processed by microwave-assisted rapid processing after formalin fixation, as commonly performed in our laboratory on small specimens, compared with that of tissues processed by the conventional overnight method. We examined 44 antibodies applied to 5,280 microarray tissue punches and found a high degree of concordance in quality of immunohistochemical staining between the methods.

Our data show that immunohistochemical analysis performed on tissue processed by microwave-assisted rapid tissue processing of previously formalin-fixed large surgical specimens is of comparable quality to that performed on tissue processed by conventional methods for a large battery of antibodies commonly used in diagnostic surgical pathology. These findings support the growing evidence that microwave-assisted rapid tissue processing is a desirable and reliable alternative to conventional tissue processing in the diagnostic surgical pathology laboratory.

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