Experience With an Automated Microwave-Assisted Rapid Tissue Processing Method

Validation of Histologic Quality and Impact on the Timeliness of Diagnostic Surgical Pathology

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Abstract

We studied the effect of a fully automated microwave-assisted rapid tissue processor (RTP) on histologic examination and on the turnaround time for surgical pathology reports. A quality assurance program reviewed the histologic sections obtained by the rapid processing method for the last 3 calendar years. In addition, the histologic results from this method were compared blindly with those obtained from the conventional overnight tissue processing (CTP) method by 9 pathologists with different levels of experience. The surgical pathology turnaround times for 1 year of use of the RTP were compared with the last year for CTP. We found that the RTP reproducibly yielded histologic material comparable in quality to CTP. The turnaround time for surgical pathology reports was improved substantially, and, in particular, same-day reporting was achieved in approximately 55% of cases compared with fewer than 1% before use of the RTP. Moreover, use of the RTP enhanced safety by eliminating formalin and xylene from the procedure.

Solid tissues need to be fixed and processed, to preserve their structures, and eventually impregnated with an appropriate hardening substance to permit making thin slices suitable for staining and microscopic evaluation. For almost 100 years, the steps followed to prepare tissues for microscopic review have remained practically unchanged.[1] Substantial shortcomings associated with that practice include at least a 1-day delay in providing the diagnosis,[2,3] reagent toxicity,[4] and degradation of nucleic acids.[5,6] Although microwave radiation seems to overcome some of the problems,[7-13] microwave-based processing methods have not gained widespread acceptance. The heretofore-manual nature of these methods might have been in great part responsible for their failure to replace the traditional overnight processing procedures.

Morales et al[13] reported a 1-hour processing method that permitted continuous high throughput of specimens with a considerably lower consumption of reagents than the conventional method. A fully automated version of that system has facilitated its use in our daily practice of surgical pathology. This report documents our experience with the new technique, including validation of histologic quality and its positive impact on the turnaround time of surgical pathology reports in a busy tertiary care hospital.

Materials and Methods

The study material consisted of surgical specimens examined in the Department of Pathology, University of Miami/Jackson Memorial Hospital, Miami, FL. Until September 1997, the tissues were fixed in 10% neutral formalin and processed by a standard overnight method in

automated tissue processors (conventional method) **Figure 1**. In September 1997, a microwave-assisted manual processing method was introduced.[13] This method was replaced by a prototype automated version in January 2001, and further improved with the development of the Tissue-Tek X-Press Rapid Tissue Processor (Sakura Finetek, Torrance, CA).

Instrument and Reagents

The robotic system is schematized in **Figure 2**. It consists of 4 stations or retorts. The first 2 of these retorts are microwave units, and the other 2 are vacuum retorts. The instrument can be loaded with 1 to 40 cassettes every 15 minutes in a continuous manner. The samples are held in each retort for 15 minutes and carried from one retort to the next by a robotic arm; thus, the entire process lasts 1 hour. In the first 2 retorts, the samples are submerged in a mixture of acetone and alcohol-based reagent while they are exposed to microwaves and agitated at 62°C. In the third retort, the tissue sample is heated at 65°C in a mixture of paraffin and mineral oil and subjected to vacuum. Finally, vacuum is applied to the samples in a bath of paraffin at 65°C in the fourth station. The microwave system used in the instrument was specially designed by Microwave Materials Technologies, Knoxville, TN. Its reaction chamber–retort dimension and shape were configured to supply microwave radiation uniformly at low wattage[14] **Image 1**.

Our histology laboratory uses a new set of reagents every day to handle a daily workload of approximately 450 samples. To properly assess the consumption of reagents by the method and automated processor, however, 1,000 slices of formalin-fixed liver tissue were processed with the same set of reagents. Regular tissue cassettes containing 1 liver section each were placed in the processor baskets, 40 cassettes per basket. The reagent bottles for the microwave retorts contain 3,800 mL each and the containers for the vacuum retorts, 3,300 mL each. We prepared H&E-stained slides from every 20th paraffin block to assess the quality of the material obtained from this reagent-processing study.

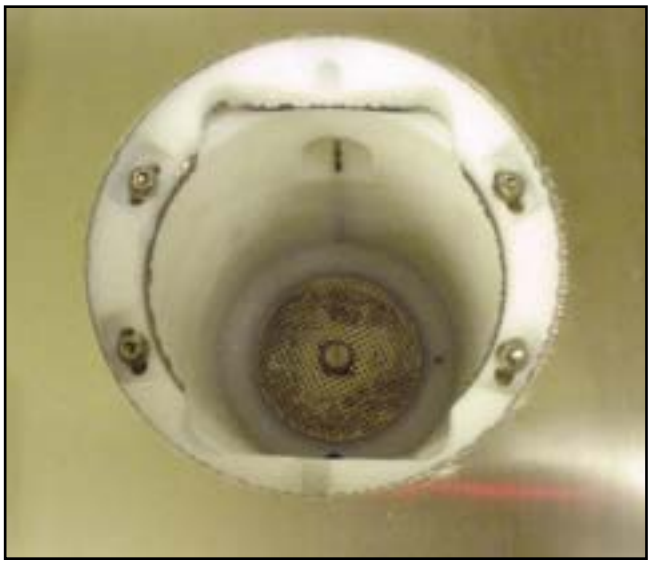

Image 1 The interior geometry of the microwave retort permits uniform distribution of microwave energy and heating of the contents.

Tissue Handling

The introduction of the microwave-assisted tissue processor required special attention to the gross dissection of specimens. Specifically, to accommodate the processing program, slices of tissue should not exceed 1.5 mm in thickness. This requirement was fulfilled with the assistance of gross dissection tools designed for that purpose.[15] Routinely, specimens are fixed in formalin or an alcohol-based fixative (UMFIX, Sakura Finetek)6 or used fresh. During gross dissection, slices of tissue are placed in their respective cassettes and dropped in a container with a solution similar to that used in the first microwave retort, where they are held until the entire specimen is dissected and then loaded in the processor, which accepts new samples every 15 minutes. On completion of the processing 1 hour later and every 15 minutes thereafter

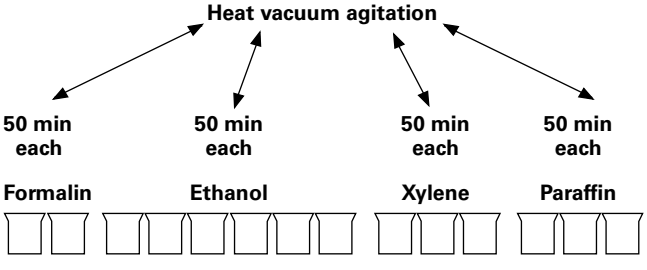

Figure 1 Schematic representation of the conventional overnight tissue processing method with a single retort and 14 stations that supply solutions to the retort. The exposure time and reagent volumes are shown.

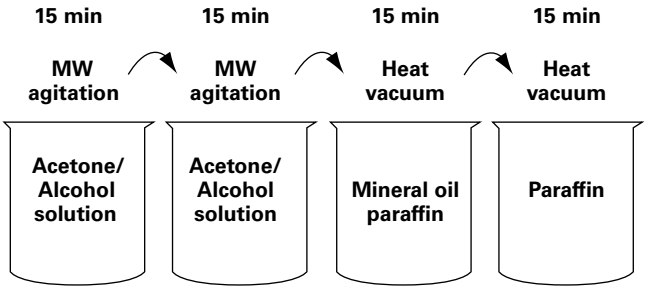

Figure 2 The rapid tissue processing method is relatively simple compared with the conventional overnight tissue processing method (Figure 1). MW, microwave.

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with new batches, processed tissue samples are unloaded from the processor, embedded in paraffin, sectioned and stained, and made available for review by pathologists. Specimens received before 4:00 PM undergo gross dissection and processing on the same day. Samples received after 4:00 PM are left for the following day instead of using traditional overnight processing.

Assessment of Histologic Specimen Quality

Two mechanisms were used to validate the quality of H&E-stained slides following tissue processing by the microwave-assisted method (rapid method). First, as part of the department's quality assurance (QA) and compliance program, approximately 1% of surgical pathology cases are randomly selected for QA by a surgical pathologist, who then completes a form concerning the quality of slides, concordance with the original diagnosis, and *Current Procedural Terminology* coding. The quality of slides is rated as satisfactory for diagnosis or suboptimal. Records of these reviews were tabulated.

The second approach consisted of a blind comparative study of 2 adjacent 1.5-mm-thick sections taken from formalin-fixed tissue samples from 100 surgical cases. One tissue section from each case was processed by the conventional method and a duplicate section by the rapid method. We double mounted 3-μm-thick sections from the 2 paraffin blocks in each case on a single glass slide in a random manner. Thus, in some cases, the conventionally processed specimens were placed toward the frosted end of the slides and the rapidly processed specimens toward the opposite end and vice-versa in other cases. H&E staining was performed in all 100 cases. Tissue samples from a variety of organs—skin, breast, testis, adenoids, pancreas, intestine, spleen, prostate, liver, thymus, lung, uterus, ovary, and parathyroid—were represented and included normal and diseased tissues. Nine pathologists with different levels of experience in the practice of surgical pathology assessed the slides. Observers attempted to determine which of the H&E-stained sections were processed by the conventional and which by the rapid method, and they were instructed to report their observations for every slide.

Histochemical and Immunohistochemical Analyses and In Situ Hybridization

The histochemical stains used in the study are listed in **Table 1**. Staining followed well-established procedures. Immunohistochemical analysis was performed by using the labeled streptavidin-biotin system in an automatic stainer (DakoCytomation, Carpinteria, CA). We used 31 monoclonal and polyclonal antibodies. **Table 2** lists the types, clones, the commercial sources of the antibodies, and whether they ordinarily required prior antigen retrieval. Antigen retrieval was performed using a 10-mmol/L concentration of citrate buffer in a vegetable steamer (DakoCytomation). The step-by-step immunohistochemical procedure has been described.[13] In situ hybridization for human papillomavirus and Epstein-Barr virus (DakoCytomation) and fluorescence (Vysis, Downers Grove, IL) and chromogenic (Zymed, South San Francisco, CA) in situ hybridization for HER-2 were performed on 112 cases. The histochemical and immunohistochemical staining results were evaluated for the pattern and the intensity of reactions and for the presence of nonspecific background staining.

Table 1
Histochemical Stains and Reaction Results on Tissue Samples Processed With the RTP Compared With Conventional Overnight Tissue Processing

Histochemical Stain	RTP Results
Acid fast (Ziehl-Neelsen)	No difference
Alcian blue	No difference
Argentaffin (Fontana-Masson)	No difference
Bile	No difference
Colloidal iron	No difference
Congo red (Benhold)	No difference
Elastic van Gieson (Verhoeff)	No difference
Iron (Gomori–Prussian blue)	No difference
Mucicarmine	No difference
Periodic acid–Schiff	No difference
Reticulin (Gordon Sweet)	No difference
Methenamine silver (Grocott)	Inferior
Trichrome (Masson)	Superior

RTP, rapid tissue processor.

Turnaround Time for Surgical Pathology Reports

The turnaround times for surgical pathology reports from January 1, 2002, to December 31, 2002, were extracted from the departmental records that track date and time, from the accessioning of specimens to the completion of reports. The turnaround time was calculated for all cases. No attempt was made to segregate cases according to their complexity or whether supplemental studies were performed. Because the rapid method was introduced in September 1997, the turnaround times for surgical pathology cases during 1996 also were obtained for comparison.

Results

Instrument and Reagents

The automated microwave system could be loaded with a new set of specimens every 15 to 17 minutes. The overall processing time was 67 minutes. The reagent consumption study showed that 1,000 samples of formalin-fixed slices of

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Table 2
Antibodies and Reaction Results for Tissue Samples Prepared by Using the RTP vs Conventionally Processed Specimens

Antibody	Type/Catalog No.	Source	Antigen Retrieval Required	Sensitivity of RTP vs Conventional Method
CD3	P/A0452	DakoCytomation, Carpinteria, CA	Yes	Same
CD20	M/M755	DakoCytomation	No	Same
CD30	M/M751	DakoCytomation	Yes	Same
CD31	M/M823	DakoCytomation	Yes	Higher
CD45	M/M701	DakoCytomation	No	Same
CD68	M/M814	DakoCytomation	No	Higher
Carcinoembryonic antigen	M/M7072	DakoCytomation	Yes	Same
Chromogranin	M/M869	DakoCytomation	No	Same
Keratin cocktail	M/M3515	DakoCytomation	Yes	Higher
Cytokeratin 7	M/M7018	DakoCytomation	Yes	Higher
Cytokeratin 20	M/M7019	DakoCytomation	Yes	Higher
High-molecular keratin	M /M630	DakoCytomation	Yes	Higher
Desmin	M/M760	DakoCytomation	Yes	Higher
Epithelial membrane antigen	M/M613	DakoCytomation	Yes	Same
Estrogen receptor(1D5)	M/M7047	DakoCytomation	Yes	Same
Hep Par 1	M/M7158	DakoCytomation	Yes	Same
HER-2	P/A485	DakoCytomation	No	Same
k chain	M/M827	DakoCytomation	Yes	Same
l chain	M/M614	DakoCytomation	Yes	Same
MIB-1 (Ki-67)	M/0505	Immunotech, Marseilles, France	Yes	Lower
Placental alkaline phosphatase	P/A268	DakoCytomation	No	Same
Progesterone receptor (636)	M/M3569	DakoCytomation	Yes	Same
Prostate-specific antigen	P/A562	DakoCytomation	No	Same
p53	M/P09-OP09L	Oncogene Science, Cambridge, MA	Yes	Same
Table RET	M/NCL-RET	Vector, Burlingame, CA	Yes	Higher
S-100 protein	P/Z311	DakoCytomation	No	Same
Synaptophysin	P/A010	DakoCytomation	Yes	Same
Thyroglobulin	P/A251	DakoCytomation	No	Same
Thyroid transcription factor-1	M/3575	DakoCytomation	Yes	Same
Trypsin	M/MAB1482	Chemicon, Temecula, CA	Yes	Same
HercepTest kit	—	DakoCytomation	Yes*	Same

M, monoclonal; P, polyclonal; RTP, rapid tissue processor.
* Following the kit's instructions.

Table 3
Results of Comparative Study of 100 H&E-Stained Slides From Tissues Processed in Parallel by Conventional and Rapid Tissue Processing Methods*

Pathologist No.	Unable to Determine	Chose Correct Method	Chose Incorrect Method
1	49	40	11
2	46	19	35
3	34	48	18
4	67	25	8
5	98	1	1
6	49	40	11
7	1	41	58
8	0	73	27
9	78	16	6
Total No. (%)	422 (46.9)	303 (33.7)	175 (19.4)

* Each pathologist was asked to review the 100 slides and indicate the processing method that had been used.

tissue could be processed with the same set of reagents. No noticeable difference was found in the quality of H&E-stained slides prepared from every 20th paraffin block in the study. One reagent set for the microwave processor required a total volume of 14.2 L of a mixture of acetone and alcohol-based reagent, mineral oil, and paraffin.

Histomorphologic Results

The QA records showed that 717 cases were reviewed during the calendar years 2000 to 2002. The histologic quality was satisfactory for diagnosis in all cases, and no instance of suboptimal quality was found. No similar program was available for comparison with the conventional method. **Table 3** provides the results of the review of 100 slides by 9 pathologists. There was considerable disparity among reviewers in determining the type of processing method used. No clear correlation was found between the correct prediction of the method and the experience of the pathologists in surgical pathology. In 46.9% of slides, the reviewers could not determine which processing method was used, in 33.7% the correct method was chosen, while in 19.4% the assumption was incorrect. Complete concordance among all pathologists was found in only 14 cases. In 7 of these cases, pathologists correctly established the method, whereas in the other 7 cases, they were incorrect. **Image 2** illustrates the quality of histologic sections produced by the rapid and conventional methods.

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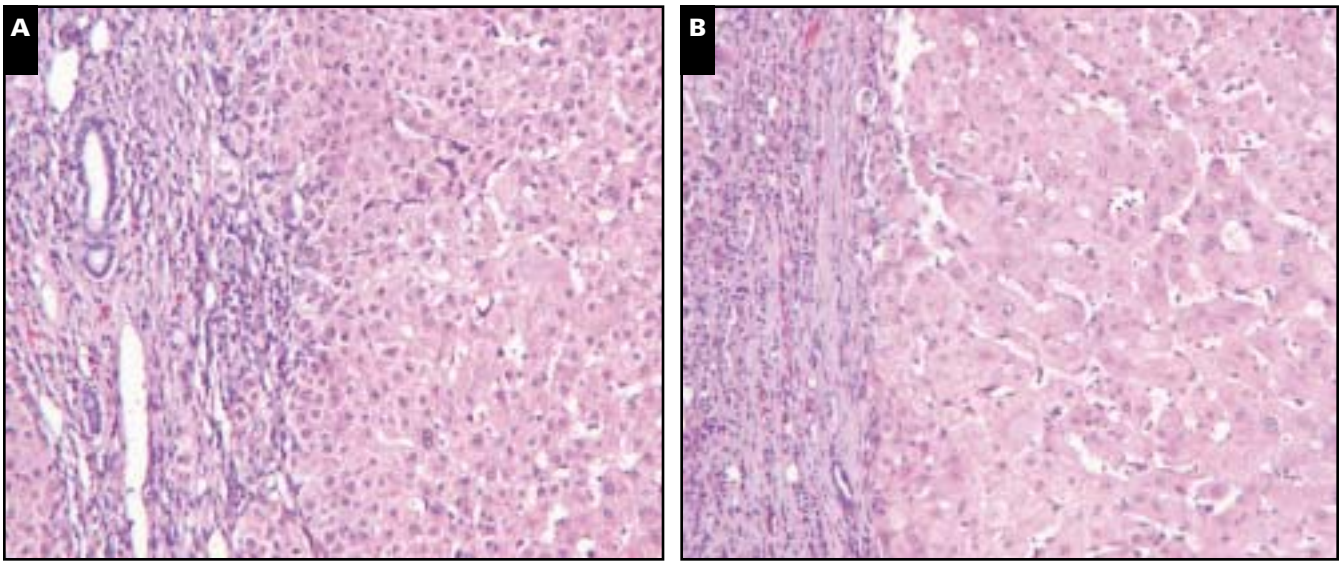

Image 2 Sections of cirrhosis of the liver processed by the conventional overnight (**A**) and the rapid (**B**) tissue processing methods showing similar histomorphologic features (**A** and **B**, H&E, ×200).

Histochemical and Immunohistochemical Analyses and In Situ Hybridization

Formalin-fixed tissue samples that were processed by the rapid method showed similar histochemical **Image 3** and immunohistochemical **Image 4** reactions compared with the same cases processed by the conventional method. There was an increase in immunohistochemical sensitivity with a few antibodies by the rapid method, particularly with antibodies against intermediate filaments. A lower staining sensitivity was observed with Ki-67 in tissue samples processed by the rapid method, but comparable reaction intensity could be achieved by minor adjustments of the working concentrations of antibodies. In situ hybridization studies done in 112 cases yielded excellent gene visualization **Image 5**.

Impact on Turnaround Time

Figure 3 shows the comparison of turnaround times of surgical pathology cases reported during 2002, which were processed with the rapid method, with those for the year 1996, which were processed by the conventional method. As noted in Figure 3, there was an increase of more than 6,500 cases in the annual workload of the surgical pathology unit from 1996 to 2002. The number of surgical pathologists did

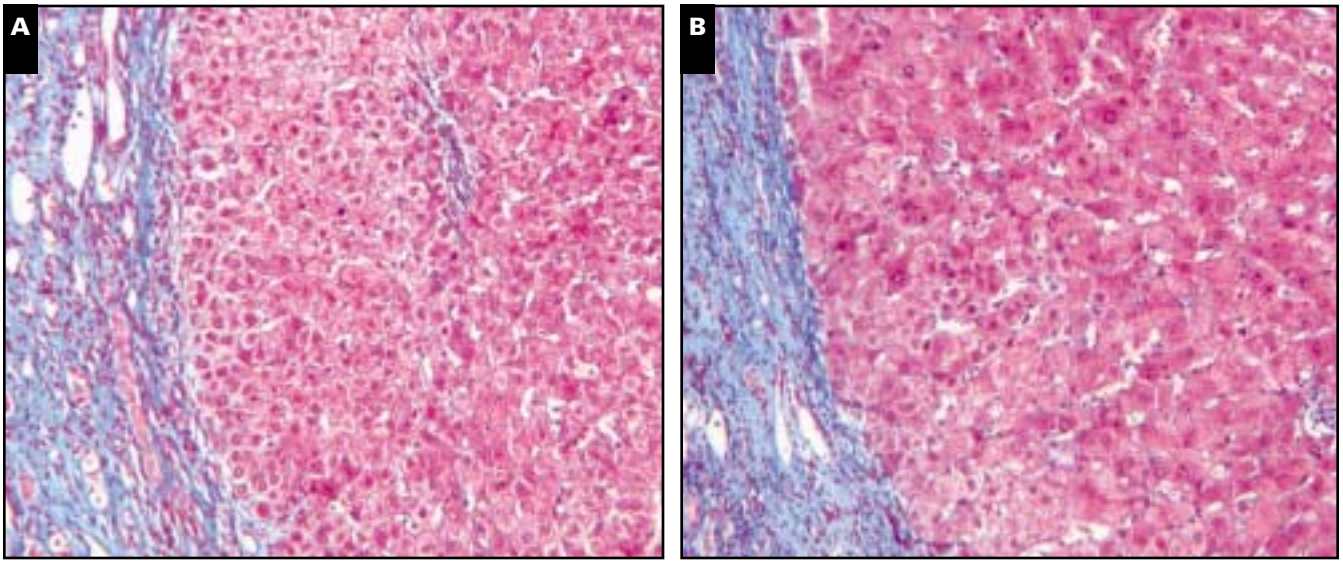

Image 3 Identical results of histochemical examination for samples processed by the conventional overnight (**A**) and rapid (**B**) methods are shown in the same case as in Image 2 (**A** and **B**, Masson trichrome, ×200).

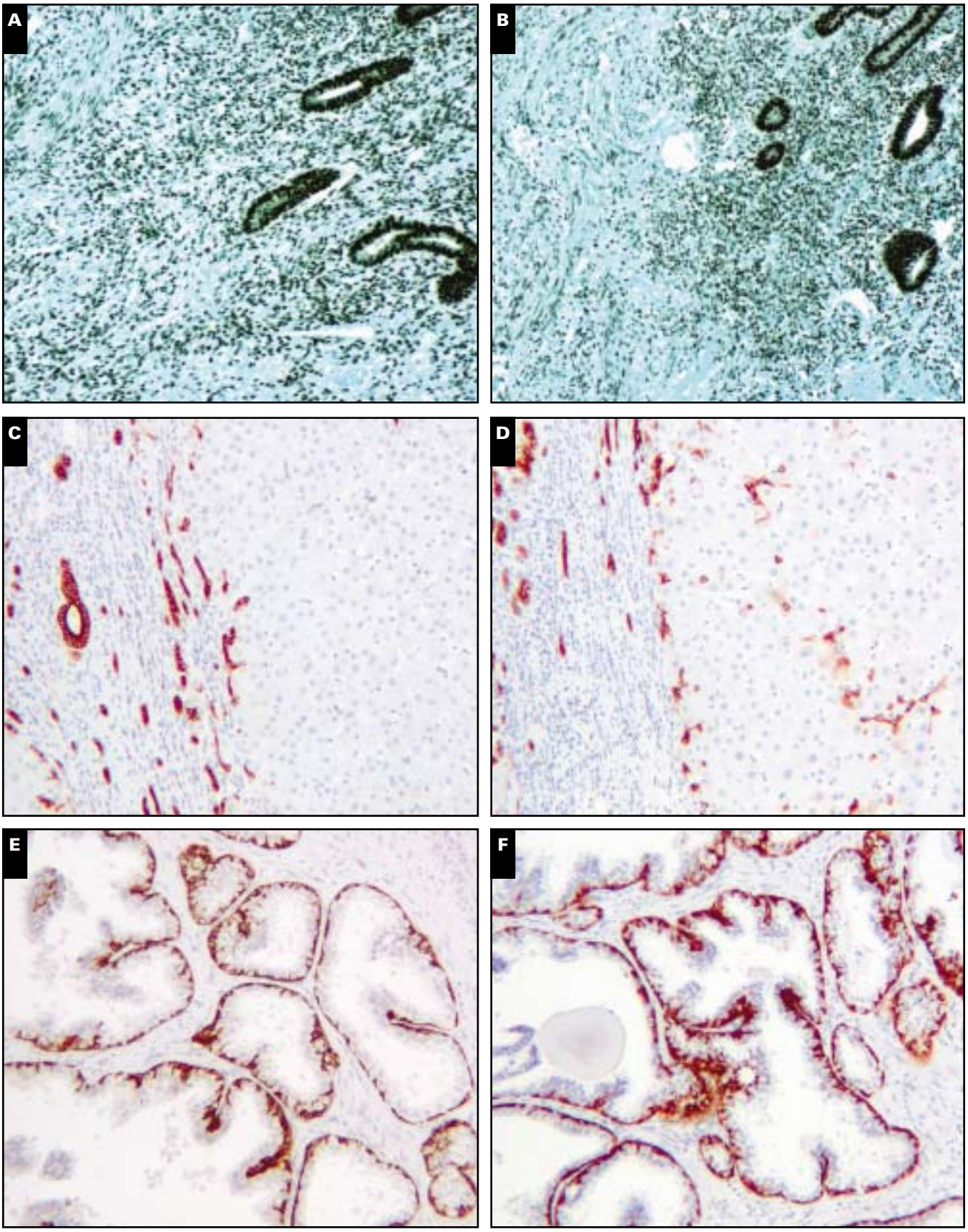

Image 4 Immunohistochemical stains of tissue samples processed by the conventional (**A**, **C**, **E**) and rapid (**B**, **D**, **F**) methods. **A** and **B**, Immunoreactivity in endometrial glands and myometrium (estrogen receptor, ×200). **C** and **D**, bile duct epithelium of a cirrhotic liver (cytokeratin 7, ×200). **E** and **F**, Basal cells in prostatic hyperplasia (high-molecular keratin, ×200).

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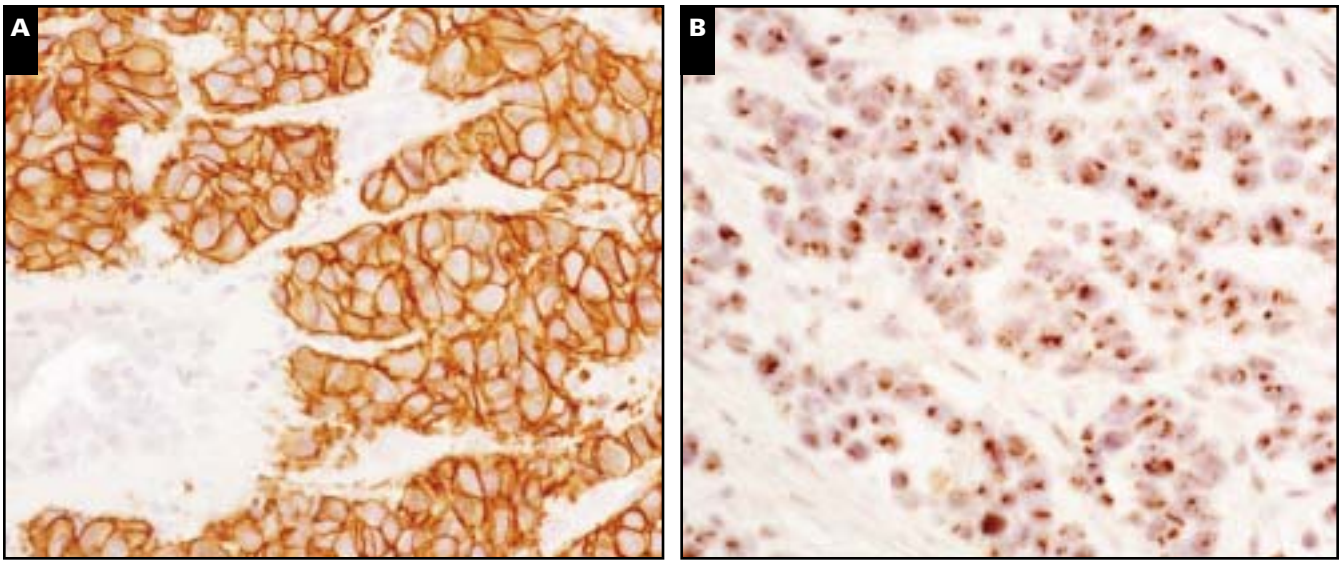

Image 5 **A**, Infiltrating ductal carcinoma of breast. Strong (3+) membrane staining of the cells but not of normal duct (right) (HercepTest [DakoCytomation, Carpinteria, CA], ×200). **B**, Chromogenic in situ hybridization (Zymed, South San Francisco, CA) of the same case showing amplification of the HER-2/*neu* gene (×200).

not change during that time. Same-day review and reporting of cases improved from fewer than 1% of cases to approximately 55% of all cases.

Discussion

The practice of surgical pathology during the last 5 decades has been enriched by advances in our knowledge of the morphologic expression of disease and by new technologies such as immunohistochemical and molecular assays. The handling of tissue samples from surgical removal to the preparation of H&E-stained slides, however, has remained impervious to scientific advances. In particular, formalin fixation followed by currently used conventional processing methods has been the standard for almost 100 years.[1]

A 1-day minimum delay in the preparation of diagnostic slides,[2,3] toxicity of reagents used,[4] and degradation of nucleic acids[5,6] are a few of the important shortcomings associated with that practice. The introduction of microwave technology into the histology laboratory provides a way to overcome many of these problems.[7-13] The practice of microwave-assisted tissue processing brought about a reassessment of traditional concepts.

The paradigm of numerous sequential steps of tissue fixation, dehydration, clearing, and impregnation ingrained as a tradition of conventional methods has been greatly simplified in all reported microwave methods. These procedures reduce the processing steps to 4 as reported herein, 3,[11] or 2 steps,[10] with considerably shortened times in each step and, consequently, in the entire processing cycle. Microwave

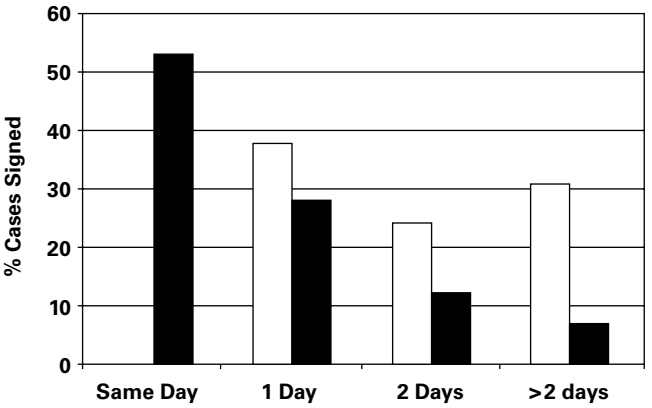

Figure 3 Shown is the substantial impact of rapid tissue processing on turnaround time for surgical pathology reports for 1996 (white bars; n = 23,349) and 2002 (black bars; n = 29,878). The annual workload increased by more than 6,500 cases for the surgical pathology unit from 1996 to 2002, and the number of surgical pathologists did not change. Same day review and reporting of cases improved from fewer than 1% to approximately 55% of all cases.

energy accounts for most of this expediency. The excitation of polar fluids, which is the mechanism responsible for heating, also might enhance the diffusion of liquids into and out of the cells.

A variety of ovens have been used for tissue processing and for other functions in histology laboratories.[11,12] The configurations of these microwave ovens have been similar to those used in a kitchen. A well-recognized deficiency of

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these ovens is that the microwave energy or radiation density is quite nonuniform because of reflections, interference, and resonance within the chamber. In fact, the kitchen microwave is not optimized for any given situation, as it is designed to perform equally well for a wide variety of materials and masses. The microwave oven used in our automated instrument was designed specifically so that the chamber serves as a retort for the processing fluid, and, at the same time, its cylindrical geometry is configured to provide uniform distribution of microwave energy. The design of this oven also permitted it to be incorporated into a robotic processor. Through the use of nonaqueous mixtures of acetone and alcohol-based reagents in these microwave ovens, the tissue samples are fixed, dehydrated, and prepared for impregnation with molten paraffin. The latter is accomplished in 2 simple steps at 65°C degrees with negative pressure. Although prior fixation of tissue is not required by this system, equally satisfactory results can be obtained from tissues that are prefixed in formalin or in an alcohol-based fixative.

The use of the microwave-assisted tissue processing system schematized in Figure 2 has permitted the study and report of about two thirds of our surgical pathology cases on the day the specimen in received from surgery. The quality of the histologic sections was not compromised by the expediency of the system, as substantiated in several ways. First, histologic examination, part of our daily practice that produces about 450 H&E-stained slides every day, demonstrated the adequacy of the material. Second, review of histologic slides selected at random as a component of our QA program demonstrated no deficiencies. Third, the quality of histologic preparations processed by the new method was judged by a panel of pathologists to be comparable to the quality of preparations processed (in parallel) with the conventional method (Table 3).

Routine histochemical stains by either method produced comparable results, including the distribution and intensity of color reactions. Likewise, when compared with conventional overnight processing, the rapid method preserved most antigens in formalin-fixed tissue samples in a similar or superior manner. It seems that the introduction of microwave energy in tissue processing, as is also the case during fixation,[16] might by itself improve the integrity of immunohistochemical markers. Furthermore, because the system is formalin-free, there is no masking or modification of antigens by formalin during the processing steps. The enhanced sensitivity made the use of antigen retrieval unnecessary for a number of antibodies. Even with the use of antigen retrieval, none of the markers produced undesirable or nonspecific background staining, and minor adjustment in antibody concentrations produced results comparable to those obtained by using the conventional method.

As reported elsewhere,[6,13] rapid tissue processing by this method permits improvement in the quality of extracted DNA and RNA, particularly when formalin is not used for prior fixation. When tissue samples are prefixed in formalin and conventionally processed, RNA consistently is degraded, as indicated by complete absence of 28S and 18S ribosomal bands, although small amplicons of less than 200 base pairs still can be obtained by reverse transcriptase–polymerase chain reaction. On the other hand, RNA from microwave-processed tissue samples, although degraded, contains partially intact 18S ribosomal bands, permitting amplification of larger segments of nucleic acid.[13] Furthermore, the quality of the tissue RNA can be improved substantially if the specimen is obtained immediately at surgery and is not exposed to formalin before microwave processing.[6]

Appropriate sample preparation is paramount to the successful use of the rapid method. With conventional processing, and more so with microwave-assisted methods, proper trimming of the samples during gross dissection is an essential step to attain good histologic sections. Microwave processing methods, other than the method described herein, adjust the time of exposure to microwave irradiation and the length of the entire processing cycle according to the thickness of the tissue slices.[7-12] That approach complicates automation of microwave-assisted tissue processing, so we standardized the thickness of tissue samples to a maximum of 1.5 mm. This is attained easily with the assistance of specially designed gross dissection tools that permit obtaining sections of the desired thickness.[13,15] It also is possible, however, to process samples thicker than 1.5 mm if the immersion times in the retorts are increased (A.R.M., personal observation).

Improvement in the workflow of the histology laboratory also attended the introduction of the microwave-assisted system. Conventional tissue processing requires gross dissection of tissue samples during the day and overnight batch processing, whereas continuous rapid processing occurs throughout the day. As the specimens undergo gross dissection, they are placed immediately in the processor, and 1 hour later they are embedded, sectioned, stained, and made available to the pathologists in a continuous flow. Furthermore, the histotechnologists are not required to work at odd hours of the morning or on weekends, when surgical procedures are not normally performed.

The method reported herein reproducibly yields histologic material of similar or superior quality to that provided by time-honored conventional processing. It has many advantages, including expediency, safety, potential for preservation of molecular integrity of specimens that might be used in subsequent studies, and improvement in the workflow of the laboratory, permitting the preparation of diagnostic material during the day at family-friendly hours.

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