

Paraform<sup>®</sup> sectionable cassette and there is no paraffin to trim around the cassette prior to sectioning. The Paraform<sup>®</sup> material has sectioning characteristics similar to paraffin. It allows the user to section right through the cassette bottom without artifacts and without damage to the blade. The Paraform<sup>®</sup> material does not pick up stain and does not interfere with microscopic examination.

There is no doubt that these two instruments, along with the Paraform<sup>®</sup> Cassette System, will have a profound impact on the operation of the histology laboratory. For the first time, specimens can be processed, embedded, sectioned, and stained within 2 hours, without sacrificing quality and with no changes in microscopic morphology. The ability to prepare specimens continuously and to use significantly less reagents will have a positive effect on turnaround times, efficiency, operating costs, and better patient care. Both instruments are currently going through clinical trials. Full market release is expected by late 2003.

**Reference**

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**Comparative Usefulness of Standard and Microwave-assisted Tissue Processing Methods**

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**Introduction**

The steps used to prepare specimens for histology are well known and illustrated in Fig. 1. Tissue processing, which includes dehydration, clearing,

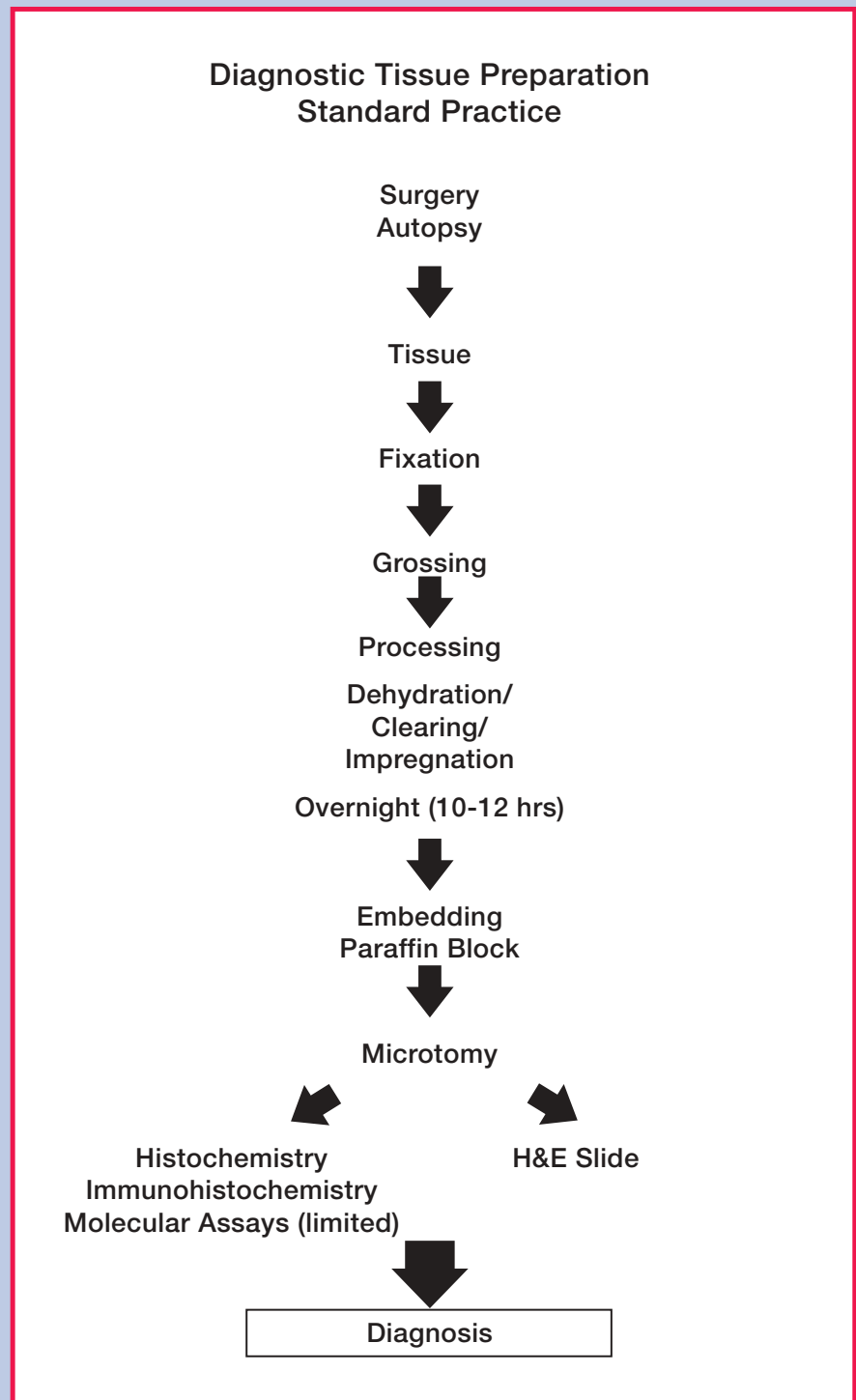


Fig. 1. This figure shows the steps required to prepare H&E stained slides from fresh tissue obtained following surgery or at the autopsy table.

and impregnation, is the lengthiest of these steps. It lasts 10 hours or longer, and is the major bottleneck in the workflow of histopathology laboratories. Typically after grossing, the samples are held in their respective cassettes for overnight

processing and embedded, sectioned, stained, and coverslipped in batches the next day. Batches of microscope slides are presented to the pathologist for review and diagnosis at the earliest, 1 day after grossing the specimen. This has been customary

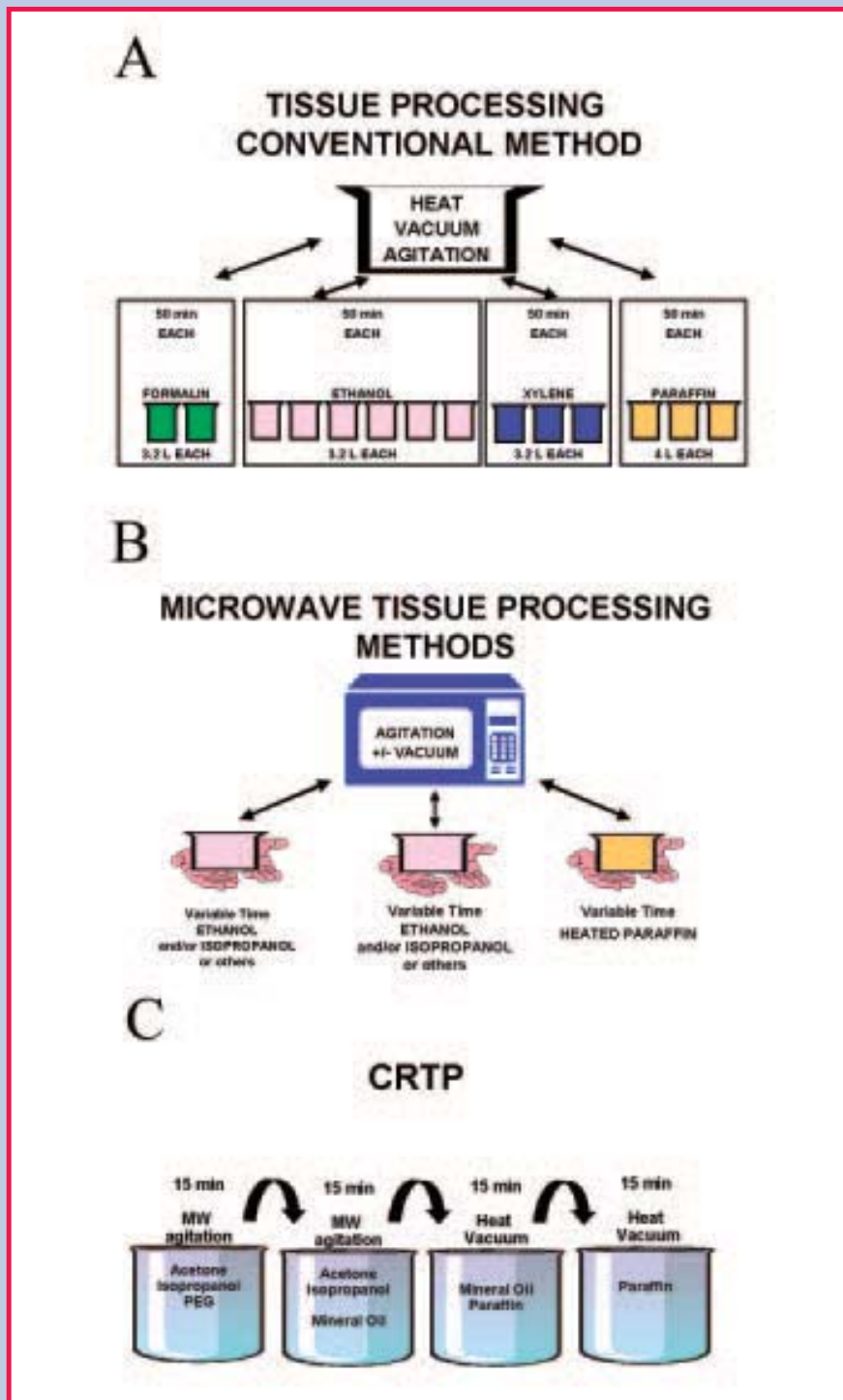


Fig. 2. Schematic representation of tissue processing methods. The major features in each method are highlighted: (A) standard; (B) common microwave-assisted; and (C) continuous rapid tissue processing.

for more than 5 decades. That practice is now increasingly challenged because of its shortcomings including its inability to meet the support required by current clinical demands and because there are now more efficient alternatives

such as microwave-based processing methods. The use of microwave technology in histology shortens tissue processing from hours to minutes, usually reducing by 1 day the time required to complete surgical pathology reports. In this

review, the utility of conventional and microwave-assisted tissue processing methods are compared.

### Processing Methods

To illustrate conventional processing methods and instrumentation, a closed system processor, such as the Sakura VIP, is schematicized in Fig. 2A. In this system the samples are placed in a retort where the solutions are pumped in and out. During the processing cycle the tissues are exposed to the reagents and subjected to heat, vacuum, and agitation. In this procedure, it is customary to use formaldehyde for fixation, followed by incubation in a series of increasing concentrations of ethanol for dehydration, then xylene for clearing tissue of alcohol, before impregnation in paraffin. Open processing systems or rotary processors utilize similar reagents and exposure steps but instead of a single retort, every reagent is kept in different containers and the samples are transferred from one solution to the next by mechanical devices. Whether open or closed, the processing cycle in any of these systems lasts 10 hours or longer and therefore, it is almost always carried out overnight.

A number of different microwave applicators, such as Milestone's and Energy Beam's have been in use for a number of years. Their introduction in histopathology resulted from the creative undertakings of visionaries in the field, notably Boon,<sup>1,2</sup> Kok,<sup>3,4</sup> Leong,<sup>5</sup> and a few others. The salient features of microwave-assisted processing methods are shown in Figs. 2B and 2C. As illustrated in Fig. 2B, the samples are manually carried in and out of the microwave chamber as many times as the steps require for the processing cycle. During each of these steps, the reagents bathe the samples for variable periods of time, usually depending upon the thickness of the sections. Reagents include an alcohol, either ethanol or isopropanol or both, or other undisclosed



Fig. 3. Shown in this photograph is the transfer of samples from a microwave chamber to a vacuum retort of the Continuous Rapid Tissue Processor. The cassettes with their respective tissue samples are shown in the basket, which is transported from one station to the next by a robotic arm.

reagent(s), and paraffin. Agitation, and in some systems vacuum, is applied during microwave irradiation of the tissue.

Depicted in Fig. 2C is the system developed and presently practiced at the Department of Pathology of the University of Miami/Jackson Memorial Hospital (UM/JMH). It is a modification of a previously reported method.<sup>6</sup> It is a fully automated system that accepts specimens every 15 minutes. The tissues are processed through four stations. The specimens in their respective cassettes are placed in a basket and carried from one station to the next by a robotic arm (Fig. 3). Tissue samples are held in each station for 15 minutes, thus the entire processing cycle lasts 1 hour. In the first retort, the samples are immersed in a solution of acetone, isopropyl alcohol, and polyethylene glycol (PEG) and then subjected to microwave irradiation and agitation. A mixture of acetone, isopropyl alcohol, and mineral oil bathes the tissues in the second retort while they are irradiated with microwave

energy. In the third and fourth stations, the tissues are heated in a mixture of paraffin/mineral oil and paraffin, respectively, while subjected to heat and vacuum.

To examine the comparative usefulness of the above-mentioned processing systems, this review highlights the results, expediency, safety, cost, throughput, and versatility.

#### Quality of Results

Figs. 4A-C illustrate the quality of histology slides obtained by the various processing methods. These slides are composites of tissue processed by both the conventional and the UM/JMH microwave-assisted rapid tissue processing methods. The resulting sections were double-mounted in the same slide and stained with H&E, trichrome, and the estrogen receptor assay. Tissue structure and nuclear and cytoplasm characteristics are similar by these methods. Stronger staining in microwave methods is usually the case as demonstrated in the illustrations. This observation has

been made by other practitioners of microwave processing systems who report results with quality similar to those of conventional methods.

#### Expediency

Although a number of other benefits result from microwave-assisted tissue processing, its ability to save time led to its introduction into the histology laboratory. Those using microwave methods have all reported shortening the processing cycle to about 30 to 120 minutes.<sup>7-9</sup> None of these reports, however, provides comprehensive data on the improvement in turnaround time of surgical reports due to the implementation of the microwave technology. At the UM/JMH, we began to phase in a microwave processing method late in September 1997 as previously reported.<sup>6</sup> The original method, consisting of nine steps with three microwave stations, was simplified to four steps that include two microwave retorts, a practice that has been in effect for the last 2 years. With rare exceptions, all our surgical specimens are processed following this method. The impact on the turnaround time, shown in Fig. 5, was studied by reviewing the departmental records that track date and time of specimen accessioning and report completion. This turnaround time applies to all of the specimens examined. No attempt was made to segregate cases according to their complexity or whether immunohistochemistry or other studies supplemental to H&E staining were performed. The baseline to measure against was provided by the 1996 data, the year before the method started to be phased in. As Fig. 5 shows, there is major improvement in clinical responsiveness following implementation of our present practice. In fact, this turnaround time of surgical pathology reports is unmatched by those shown in published studies.<sup>10,11</sup>

#### Safety

Those working in the field pursue reduction, or preferably elimination,

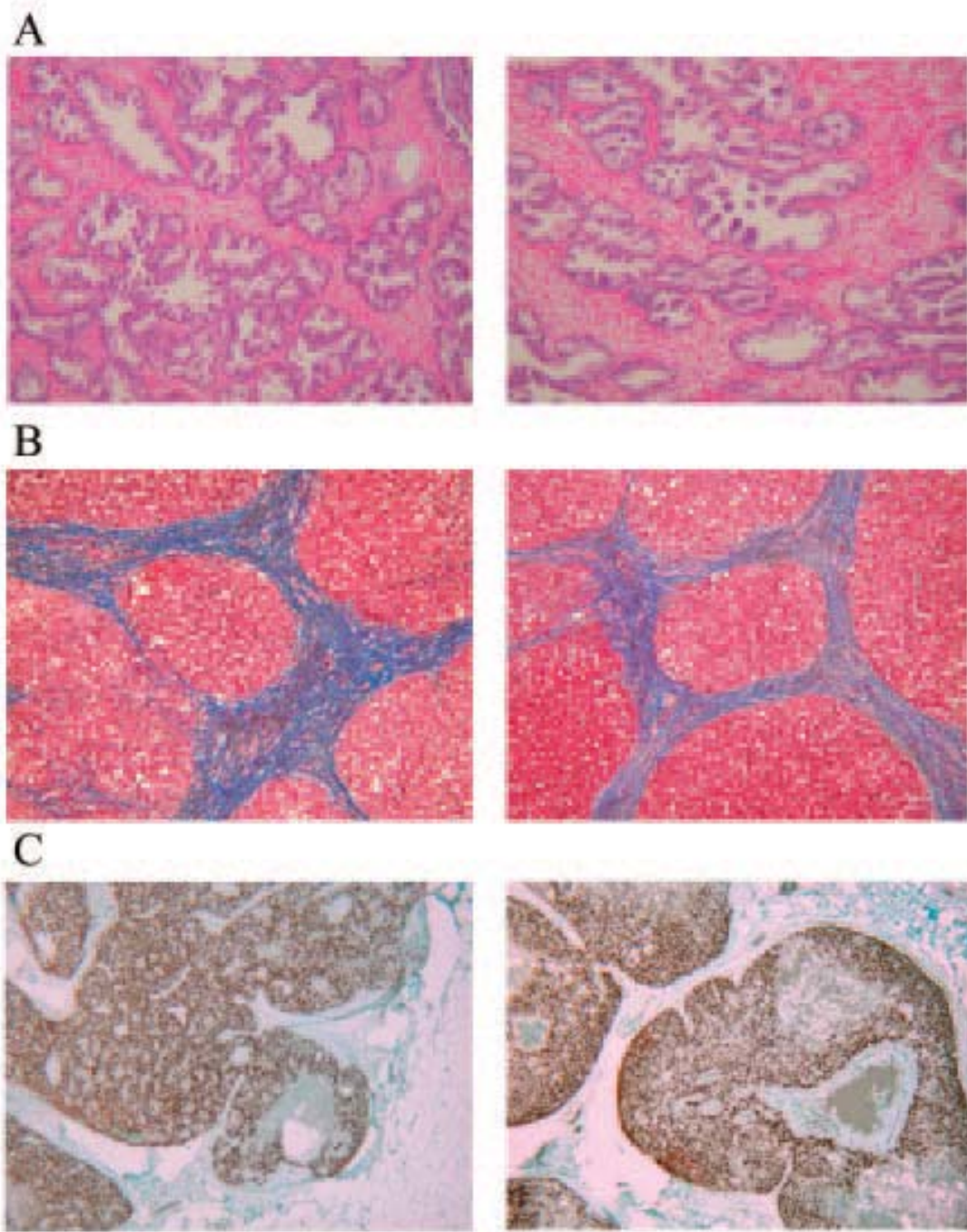


Fig. 4. (A) H&E stained slides of prostate hyperplasia, (B) cirrhosis of the liver stained with Masson's trichrome, and (C) estrogen receptor immunoreactivity in carcinoma of the breast. Sections on the right-hand side of this composite panel were processed by the conventional method and on the left, by the rapid tissue processing method. As can be appreciated, the CRTP-processed tissues have a slightly stronger affinity for the stains.

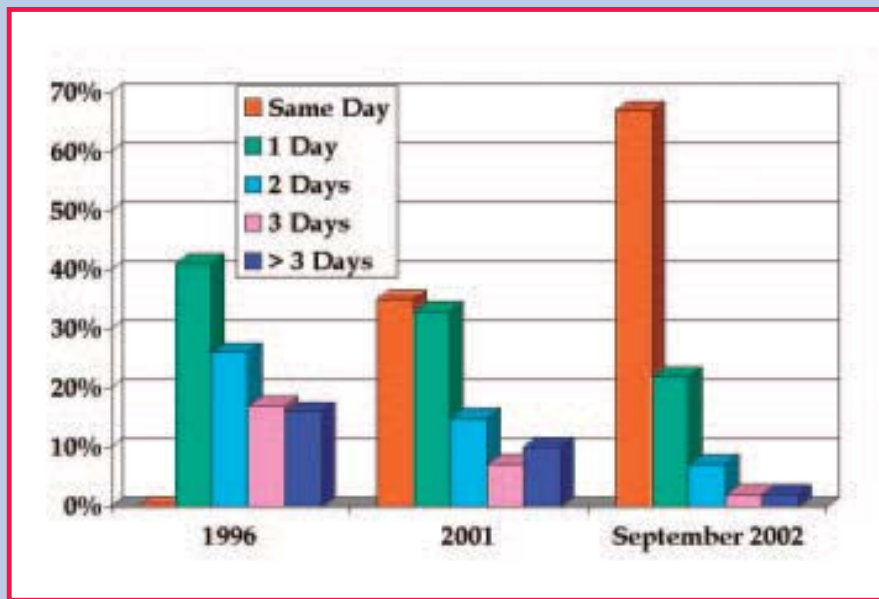


Fig. 5. This illustration shows the remarkable improvement in turnaround time of surgical reports following implementation of the rapid tissue processing method at the UM/JMH.

of toxic reagents from histopathology. Microwave procedures are conducive to that aim, as the volume of reagents used and the toxic exposure are significantly lower. In particular, replacing xylene with mineral oil<sup>6</sup> or isopropyl alcohol for clearing,<sup>1,4,9</sup> and excluding formaldehyde<sup>1,4,6,7,9</sup> from processing via microwave methods is a welcome improvement in histology.

### Cost

Microwave procedures utilize considerably smaller volumes of reagents than conventional methods, resulting in the reduction of purchase, storage, and disposal costs, which practically eliminates the need for recycling these chemicals. Published studies discuss this point but no quantitative data have been provided to substantiate the reagent savings provided by microwave methods. It is helpful to compare reagent consumption by examining a processing cycle in the VIP and the method that we are currently using, shown in Tables 1A and 1B. Every day we start with new solutions for each of the 4 retorts of the automated Continuous Rapid Tissue Processor (CRTP). These solutions are used during the entire day.

Because of the throughput of the instrument, it is possible to process up to 900 samples in an 8-hour shift with the same reagents. As can be appreciated in the tables, the savings of reagents vary according to the number of samples processed.

A significant factor affecting cost is automation, or the lack thereof. Conventional processing is carried out in automated devices requiring only the loading of specimens and reagent changes. Microwave processors, on the other hand, are labor intensive, as the samples need to be handled manually to proceed from one step to the next of the processing cycle. The single exception is the CRTP, developed by Sakura Finetek Inc., which utilizes microwave applicators originated at Microwave Materials Technology, Knoxville, TN. As previously described, this is a fully automated system where the samples are carried by a robotic arm. Reagents are placed in the instrument with ease and no technical attention during processing is required other than to load and unload the samples.

### Throughput

Because commercially available tissue processors cannot be accessed with

additional samples until their processing cycle is completed, their throughput is limited. The number of samples that can be processed by any of these methods, conventional or microwave-assisted, is dependent upon the load permitted by their containers and retorts and the length of the processing cycle. Consequently, the throughput of the Sakura VIP-300 is one batch of 300 samples or less every 10 hours. For practical purposes, the samples are processed overnight and therefore, its throughput is limited to one batch per day. Shortening the processing cycle with microwave methods should theoretically increase the throughput. Unfortunately, there are no available data reported to support this notion. Visinoni et al reported a processing cycle load of up to 60 samples that can be processed in 30 to 120 minutes.<sup>7</sup> The period of time required to process these samples is adjusted according to the thickness of the slices of tissue, a common practice in reported microwave methods. Moreover, throughput with these microwave methods is further limited by the requirement of tissue fixation prior to processing. Although at variance with these methods, we elected to standardize grossing and slicing of tissue samples no thicker than 1.5 mm. This may be considered a daunting task, but we have developed appropriate tools that make this easily possible. One of these tools was described in a previous publication.<sup>6</sup> We believe that another distinct advantage of the system in practice at our institution is that both fixed and fresh tissues are amenable to processing. Further, the ability to introduce additional samples every 15 minutes allows for a continuous flow of specimens at a rate of 120 samples per hour. In fact, instead of accumulating tissue samples in batches, the system is available to process along with the grossing pace.

### Versatility

The potential applications and versatility of microwave processing

**Table 1. These tables illustrate the difference in volume of reagents consumed by the conventional and rapid tissue processing methods**

| <b>Table 1A</b>                                       |        |          |
|---|--------|----------|
| <b>Reagent Volume (liter) per 300 Samples or Less</b> |        |          |
|   | 1 VIP* | 1 CRTP** |
| Formalin  | 4      |          |
| Alcohol 80%   | 4      |          |
| Alcohol 95%   | 4      |          |
| Alcohol 100%  | 4      |          |
| Isopropyl ALC, Acetone & PEG                          |        | 6.8      |
| Xylene  | 4      |          |
| Mineral Oil   |        | 1.8      |
| Paraffin  | 4      | 6.1      |
| Total   | 24     | 14.7     |

| <b>Table 1B</b>                               |        |          |
|---|--------|----------|
| <b>Reagent Volume (liter) per 900 Samples</b> |        |          |
|   | 3 VIP* | 1 CRTP** |
| Formalin                                      | 12     |          |
| Alcohol 80%                                   | 12     |          |
| Alcohol 95%                                   | 12     |          |
| Alcohol 100%                                  | 12     |          |
| Isopropyl ALC, Acetone & PEG                  |        | 6.8      |
| Xylene  | 12     |          |
| Mineral Oil                                   |        | 1.8      |
| Paraffin                                      | 12     | 6.1      |
| Total   | 72     | 14.7     |

\* Sakura VIP-300 tissue processor.

\*\* Sakura Continuous Rapid Tissue Processor.

methods are unattainable with conventional procedures. Rapid and continuous tissue processing as we presently practice at the UM/JMH, literally means no batching samples, no holding specimens, and stat capabilities. Processing is done in real time or concurrent with other histopathology activities, eliminating the need for midnight or other odd-hour shifts, or weekend duties. This method is ideal for surgical suites—we have done this in our institution, where specimens are brought directly from the patient to the

grossing station and the tissue processor, creating point-of-care surgical pathology. This practice seems ideal for surgicenters, and because of the high throughput, is convenient for both centralized hospital laboratories and independent reference laboratories with a high specimen volume. More importantly, it puts the laboratory in a unique position to study macromolecules directly from the paraffin block. Contrary to formalin fixation and conventional tissue processing, which destroy most macromolecules,

our preliminary studies show the feasibility of performing molecular assays on tissue processed by our microwave method.

### Summary

Microwave-assisted tissue processing has brought a revolutionary improvement to histopathology. It is responsive to the patient and physician needs; improves utilization of reagents, while reducing or eliminating their toxicity; creates a personnel-friendly workflow; and places the laboratory in a better position to meet the demands of the rapidly expanding field of molecular medicine.

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### Erratum

In the article by Joyce Hrad appearing in the Spring 2002 issue of *HistoLogic* entitled "Use of Commercially Clarified Methyl Green in the Methyl Green-Pyronin Stain," Figures 2 and 3 were reversed in error. We regret any inconvenience this may have caused the reader.