

Continuous Throughput Rapid Tissue Processing Revolutionizes Histopathology Workflow

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■ Continuous throughput rapid tissue processing (CT-RTP) has the potential to introduce dramatic changes into the practice of modern histopathology, a field which has been relatively unchanged for many years. These changes can be grouped into 3 major categories: 1) where we work, 2) how we work, and 3) when we work.

■ The implementation of CT-RTP allows for rapid turn around of tissue biopsies, providing a standard hematoxylin and eosin stained slide, while preserving various macromolecules such as mRNA and proteins of interest for development of target directed therapies.

■ Despite initial resistance to these changes, we have successfully implemented CT-RTP by recognizing the necessary changes in our work patterns, including location, re-education in gross tissue sectioning techniques, fixation, and schedule adjustments in both technical and professional staffing.

Continuous throughput rapid tissue processing (CT-RTP) and laboratory automation have ushered in a new age in histopathology. In conventional histopathology laboratories, current practices are essentially unchanged for more than 50 years, and in fact probably for much longer. Routine use of formalin fixation, overnight dehydration and paraffin infiltration, and manual embedding and sectioning have served us well in producing relatively uniform, high-quality tissue sections for histopathologic examination. As we move into the 21st century, however, these methods are seriously deficient in prompt diagnoses and answers to questions clinicians and patients are now asking, particularly with respect to the identification of gene activation and detection of gene products such as messenger RNA (mRNA) and proteins.

Since 1997, we have been developing a tissue processing system which eliminated the use of formaldehyde and xylene, and used microwave energy to facilitate the diffusion of reagents and denaturation of proteins, as has been previously reported.¹ This system has been extensively studied and automated instruments have been developed which allow for continuous throughput, shortening the processing time to about 70 minutes. The method also reduced the volume of other chemicals used in processing. The resulting histologic preparations are at least comparable to traditional hematoxylin and eosin stained slides for older methods

(Image 1), and immunohistochemical staining is also comparable (Image 2) and in some cases enhanced (lower concentrations of primary antibodies needed). Other microwave-based tissue processes have recently been described with similar good results.²

Once the method was established and proven to be practical, issues of implementation had to be addressed. Histopathology is a field steeped in tradition (we've always done it that way), and overcoming resistance to change represented a major undertaking. I have divided these changes into several categories for consideration: **Where** we work, 2) **How** we work, and 3) **When** we work. The following are observations related to each of these categories.

Where We Work

Traditionally, pathologists have worked in offices located within a centralized pathology department separate from the histopathology laboratory, and when needed, would go to a small dedicated area near the operating room where a freezing microtome would be located. The need for rapid turnaround for transplantation patient biopsies, as well as for outpatients waiting in clinic reception areas for biopsy results, and in addition, the necessity for interacting directly with surgeons in immediate tissue

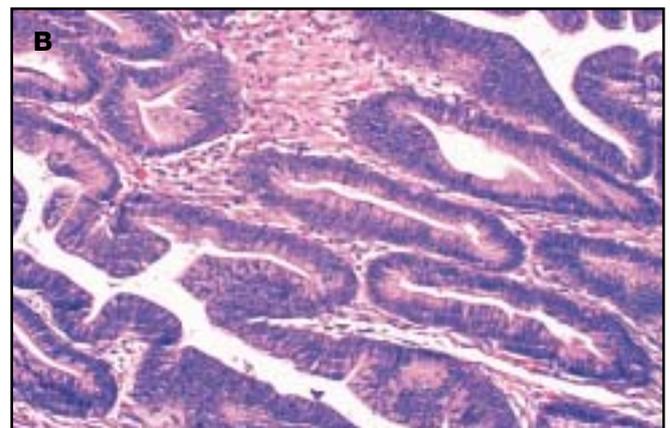
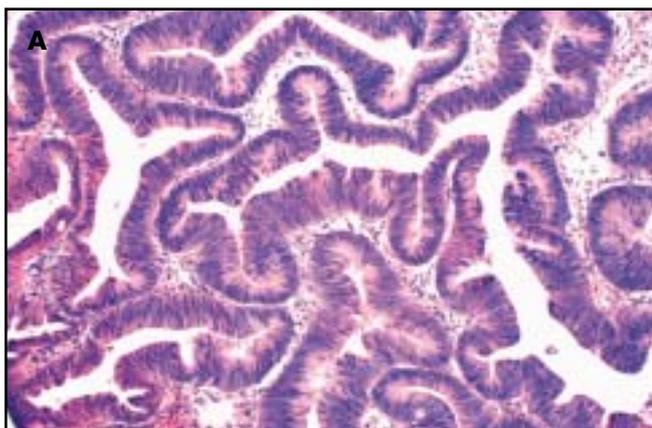


Image 1_(A) Section of colonic carcinoma produced using the continuous throughput processing method, compared with **(B)** similar section processed by conventional methods (Hematoxylin and eosin stain, original magnification 200x).

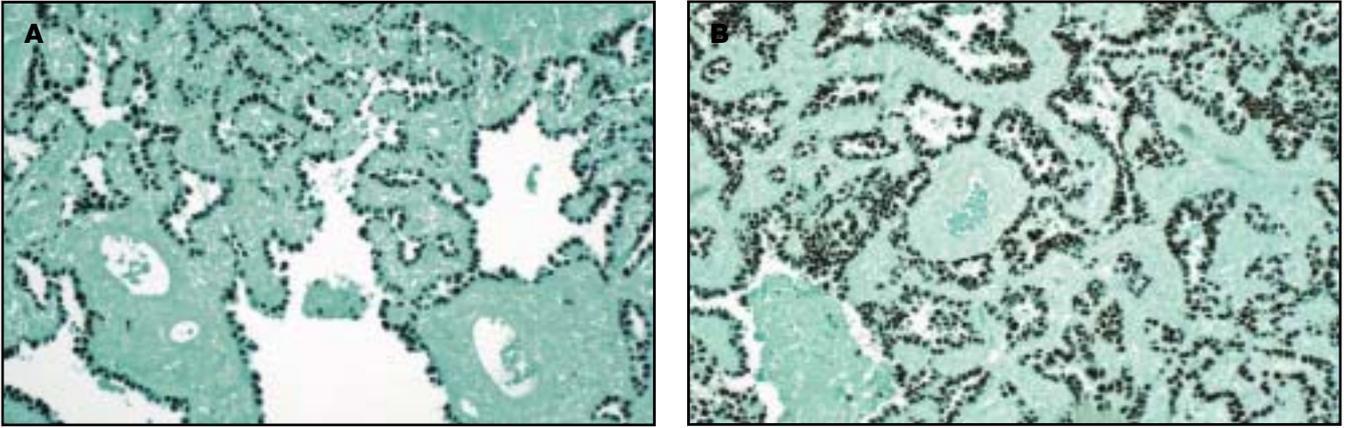


Image 2_ (A) Immunohistochemical stain for TTF-1 on lung carcinoma processed using the continuous throughput processing method, compared with results **(B)** using conventional methods. (Immunoperoxidase for TTF-1, diaminobenzidine and light green counterstain, original magnification 200x)

procurement, all suggested the need for a tissue laboratory located adjacent to the surgical suites. We have opened such a laboratory, which also facilitates communications between surgeons and pathologists. This laboratory contains materials and instruments needed for frozen section examinations, as well as all the elements of a routine histopathology laboratory (tissue processor, embedding station, microtomes, staining, and coverslipping equipment); special stains and other procedures are performed at another site.

Rapid fixation in a molecular friendly fixative has been shown to protect macromolecules. A molecular friendly tissue fixative was developed and has been shown to be useful in recovery of macromolecules such as long mRNA from paraffin embedded blocks, obviating the need for rapid freezing and frozen tissue storage.³

Prompt fixation is necessary for the most optimal recovery of macromolecules. The on-site pathologist performs frozen sections and also is available to go into the operating room to select tissue for immediate fixation in molecular friendly fixative. Preliminary work underway in our laboratory indicated that this same universal molecular fixative is also useful as a fixative for frozen section slides, eliminating the formol-alcohol currently used in most frozen section procedures.⁴

How We Work

Traditional histopathological techniques have evolved over long periods, typically on an empirical basis. Current practices include routine overnight fixation for large specimens, followed by dissection and preparation of tissue slices up to a maximum of about 3 mm in thickness. Smaller specimens may be dissected the same day followed by overnight processing, and very small biopsies can be rush processed the same day they are received using abbreviated protocols available on modern tissue processors. For the most part, however, tissues are routinely fixed and processed overnight, followed by manual embedding, sectioning, staining, and coverslipping.

The use of 10% neutral buffered formalin as the fixative of choice is a time-honored tradition in histopathology. Formalin is well-recognized as an irritant and is also considered toxic, and requirements for efficient ventilation and fume concentration monitoring add to the expense and complexity of the laboratory. The development and use of a universal molecular fixative together with the elimination of xylene and formalin from the tissue processor precludes the need for atmospheric monitoring of these chemicals.

Rapid tissue processing in our laboratory requires thinner tissue sections than those typically prepared by pathologists for routine processing. For this reason, new dissecting boards have been developed, consisting of a metal plate mounted on dissecting board, with shallow wells or chambers in which partially trimmed tissues are placed (**Image 3**). A special guide allows the insertion of a blade, which when moved along the chamber results in a section of uniform 1.5 mm thickness. A few ancillary tools to aid in dissection have also been developed. These ideal thin sections are then processed using the microwave based method.

When We Work

In traditional histopathology practice, there is an expectation of at least a 1 day (overnight) wait for pathologic diagnosis. The schedules of pathologists, histotechnologists, and ancillary personnel have evolved to accommodate this delay, but with CT-RTP the vast majority of cases can be interpreted and reported on the same day as the surgical procedure. This is especially useful for small biopsies, including those from transplantation cases⁵ in which immediate adjustments in therapy may follow, as well as for clinic patients who may be waiting in a holding or reception area. An example would be our breast disease center at JMH/UM where diagnosis is provided within 3 hours of receipt



Image 3_ Specially designed dissecting board, with recessed areas and scalpel blade guides for trimming tissues to desired thickness.

of the specimen. This rapid turnaround time allows for discussion and scheduling of follow-up visits before the patient leaves the facility. Since the inception of CT-RTP, we have dramatically reduced the turnaround time for surgical pathology specimens (Figure 1).

In most traditional pathology laboratories using overnight processing, pathologists typically receive the slides for interpretation early in the day, and technical staff having reported to work hours earlier to embed, section, and stain materials prepared the previous afternoon. Reports are generally available to physicians by the afternoon. CT-RTP dramatically changes this traditional work flow, since cases from the previous day have, for the most part, already been reported. Thus, histotechnologists typically begin working later during the day, and using a staggered arrival time, staff the laboratory throughout the day as cases arrive from the surgical suites. The process precludes “batching” of specimens and slides, as there is a continuous output of the final products (blocks and slides) throughout the day. Likewise the pathologists typically have few cases to review in the early morning hours, but as the day progresses, more cases become available for immediate interpretation, as shown in Figure 2. Since some cases inevitably arrive later in the afternoon, it follows that a pathologist needs to be available to interpret these “late” cases. Figure 3 illustrates the work patterns for histotechnologists prior to and after the inception of CT-RTP. The resultant schedule is more “family-friendly” and avoids the need for extremely early arrival times for technical staff. Pathologists cover the “late” cases on a rotational basis, thus assuring the same-day availability of results and improved patient management. An added advantage is that since cases are signed out late Friday afternoon, there is no need for Saturday morning histotechnologist and pathologist staffing.

All of the above-described changes in the pathology laboratory have been evolving as CT-RTP has been instituted. While the more immediate behavioral changes, including preparation of thin (1.5mm) sections and introduction of molecular friendly fixative, are more readily achieved, changes in work location, and also in the schedules of histotechnologists and pathologists, can be more difficult to implement. By our nature, we prefer to stay with what has served us well in the past, yet, as we enter the 21st century we know that we simply cannot continue business as usual. The evolution of modern histopathology practices must

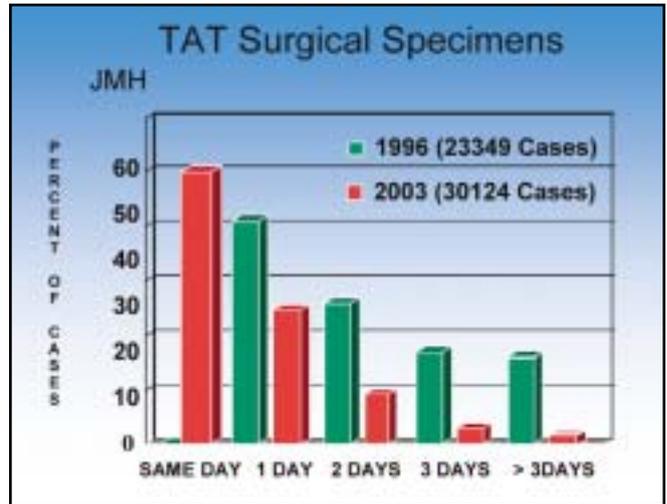


Figure 1 Turnaround times (TAT) at Jackson Memorial Hospital before (1996) and after (2003) the inception of CT-RTP.

move forward as molecular analyses and target-directed therapies augment our reliance on traditional histopathologic diagnoses. CT-RTP has set the stage for this radical departure from reliance on morphology alone to a synthesis of histopathology, immunohistochemistry, and molecular analysis. LM

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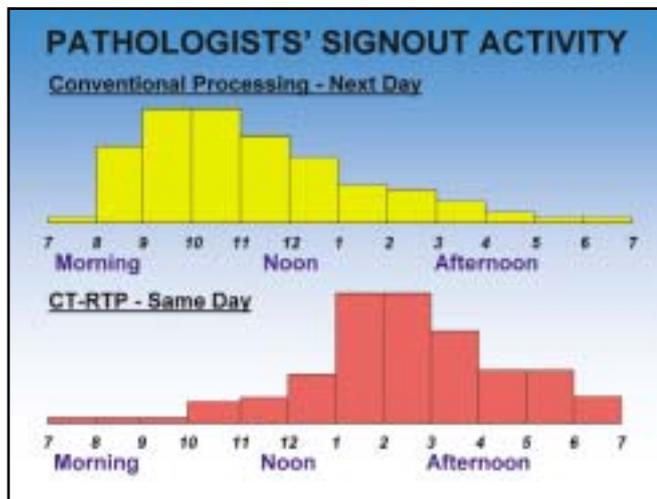


Figure 2 Graphic representation of pathologist sign-out activities with conventional overnight tissue processing and CT-RTP.

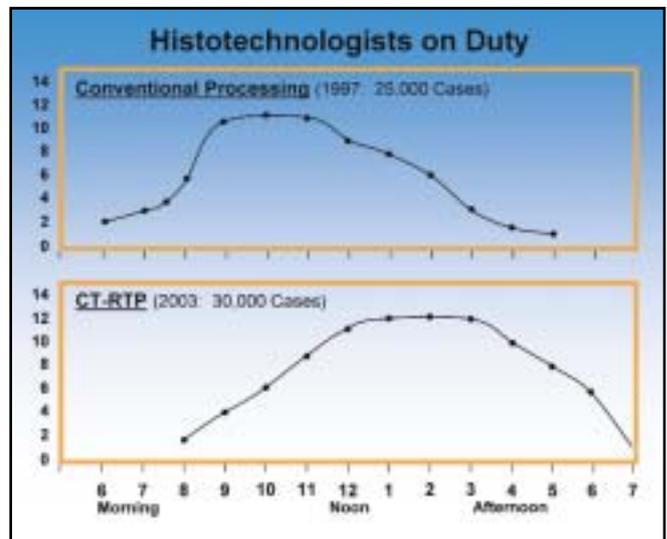


Figure 3 Schedule for histotechnologists before and after the inception of CT-RTP. In general the bulk of work is done during the middle of the work day as cases are received from surgeries.