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Rapid-Response, Molecular-Friendly Surgical Pathology: A Radical Departure from the Century-Old Routine Practice

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BACKGROUND: Currently, surgeons have to wait for at least 1 day to receive the pathology report of a biopsy or other surgical excision. This delay is mandated by the overnight tissue-processing methods that have been in use for more than a century. Patient anxiety and delay in treatment are consequences of this practice. Here we report the impact of a tissue-processing system on the turnaround time of surgical pathology reporting and its potential effect on overall patient management. This technique provides the feasibility for performing molecular assays on the same sample used for pathologic diagnosis.

STUDY DESIGN: Biopsies and other surgically removed specimens from patients treated at the University of Miami, Jackson Memorial Hospital during calendar year 2005 were processed by an automated, microwave-assisted rapid tissue-processing method. Turnaround time for surgical pathology reports was calculated and compared with that of year 1996, the last year before the new technology was phased in.

RESULTS: Total tissue-processing time was reduced from 8 to 10 hours to 67 minutes, resulting in the availability of slides in less than 3 hours. In 80% of the patients, diagnoses were reported on the same day they were received in the laboratory. The 1-day turnaround for the reports in 1996 was < 1%. Histology of rapidly processed tissues and their histochemical and immunohistochemical properties were comparable with those of the traditionally prepared material.

CONCLUSIONS: The rapid turnaround capability of the new tissue-processing system has allowed the pathology laboratory to render the final report in the majority of specimens on the day they are received. The feasibility of preserving macromolecules in the same clinical samples used for diagnosis is a timely advantage in the era of molecular medicine. (J Am Coll Surg 2008;207:320–325. © 2008 by the American College of Surgeons)

Surgical samples need to be fixed to prevent autolysis and to allow thin slicing for microscopy. It is necessary to harden the tissue so that a few-micron–thick slice can be cut by a microtome. This thin slice of tissue is mounted on a glass slide and stained for microscopic review. The longest step in preparation of samples for microscopy is tissue processing, which hardens the specimen by dehydration and impregnation in paraffin. The processing step usually takes 10 or more hours by conventional methods and is carried out overnight in automated instruments. This century-old tissue preparation system is attendant with numerous shortcomings, including a minimum 1-day delay in generating the pathology report, impeded work flow in the histology laboratory by the requisite batch processing of samples, and the toxicity of reagents used, necessitating complicated and expensive measures to protect technical personnel.[1] In addition, conventional tissue-handling and preparation methods cause irreversible damage to the structure of nucleic acids and proteins, a serious impediment to the application of molecular techniques for diagnosis and research.[2,3]

Development of a continuous-flow, rapid tissue-processing (CRTP) system radically changed the traditional practice by eliminating the disadvantages related here.[4] The CRTP system employs microwave energy along with a vacuum and a combination of common histologic reagents—minus formalin and xylene—and permits preparation of paraffin blocks from either fresh or prefixed tis-

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sue in approximately 1 hour. In this article, we examine the impact of the CRTP on the timeliness of surgical pathology reports and overall patient management at the University of Miami, Jackson Memorial Hospital.

METHODS

Specimens

A total of 27,341 surgical pathology samples were received in the Department of Pathology, University of Miami, Jackson Memorial Hospital in 2005. Of those, 2,477 samples were nontissue material, such as foreign bodies and inserted medical devices, and did not require processing. Of the remaining 24,864 specimens, 92% (n = 22,875) were processed by the CRTP system (Tissue-Tek Xpress; Sakura Finetek USA). These included 17,367 biopsies and small specimens and 5,508 surgically excised larger samples. Overall, 81,386 blocks of tissue were processed by the CRTP. Biopsies and small specimens were all received in a fixative solution, either in formalin (n = 14,292; 82%) or in a molecular fixative (n = 3,075; 18%). Large specimens were received either fresh or immersed in formalin. A breakdown of the type and number of various specimens is listed in Tables 1 (large specimens) and 2 (biopsies and small specimens). The molecular fixative (UMFIX) is a combination of methanol and polyethylene glycol that has been shown to protect tissue macromolecules at ambient temperature.[5]

Specimens were collected from the operating rooms and clinics every hour, with the final pickup at 4:00 PM. These samples were processed the same day. A few larger specimens received from the operating rooms after 4:00 PM were processed the next day. When received in the histology laboratory, specimens were grossly described and, if needed, thinly cut. Blocks of tissues were then submitted for processing. The system could be loaded with new specimen cassettes every 15 minutes, and this eliminated the common practice of "batching" of samples before and after processing. Routine hematoxylin and eosin staining was performed on all slides.

Microscopic evaluation

All histologic preparations were reviewed by board-certified pathologists, with professional experiences ranging from 4 to 41 years. Evaluation of samples for detection of specific disease processes included overall tissue architecture, cell and nuclear morphology, and the tinctorial reaction in routine hematoxylin and eosin stain.

Ancillary techniques (histochemistry, immunohistochemistry, and in situ hybridization)

There were a total of 5,328 histochemical, 6,576 immunohistochemical, and 653 in situ hybridization tests per-

Table 1. Type and Number of Large Specimens

Specimen type	n
Adrenal gland, resection	26
Amputation, leg	284
Bladder, resection	86
Bone, resection	154
Breast, mastectomy, partial/simple	74
Breast, mastectomy, with lymph nodes	43
Cervix, cone	73
Colon, resection	45
Heart, explant	12
Heart, resection	7
Hip, resection	3
Ileum, resection	2
Kidney, partial or total nephrectomy	176
Larynx, partial or total resection	4
Leiomyoma, myomectomy	6
Lip, wedge resection	4
Liver, complete resection	35
Liver, partial resection	113
Lung, total/lobe/segment resection	34
Mandible, mandibulectomy	11
Maxilla, maxillectomy	8
Multivisceral resection/organ block	1
Neck, radical resection	76
Omentum, resection	26
Ovary, biopsy/wedge resection	4
Ovary, resection	49
Pancreas, resection	39
Parathyroid gland	57
Penis, resection	4
Placenta	1,921
Prostate, nonradical resection	1
Prostate, radical resection	98
Retroperitoneum mass, resection	4
Salivary gland, resection	2
Small intestine, resection	12
Soft tissue, extensive resection	1
Spleen	99
Stomach, sub/total resection	4
Testis, castration	16
Testis, other	4
Thymus, thymectomy	22
Thyroid, lobectomy	86
Thyroid, thyroidectomy	54
Tonsil	440
Ureter, resection	57
Uterus with or without tubes/ovaries, for prolapse	58
Uterus with or without tubes/ovaries, neoplastic	62
Uterus with or without tubes, other	46
Vulva, total resection	1
Whipple resection	64

Table 2. Type and Number of Small Specimens and Biopsies

Type of specimen	n
Artery, biopsy	12
Biopsy, GI	342
Biopsy, mediastinal	2
Biopsy, NOS	2,639
Biopsy, oral	127
Biopsy, orbital	5
Biopsy, retroperitoneal	5
Bladder, biopsy	267
Bone, biopsy	72
Breast, biopsy	230
Cervix, biopsy	1,814
Colon, biopsy	361
Colonic polyp	815
Duodenal biopsy	304
Endobronchial biopsy	41
Endocervix, curettage	1,341
Endometrium, biopsy	1,313
Endomyocardium, biopsy	202
Esophageal biopsy	428
Gastric biopsy	345
Ileum, biopsy	476
Jejunum, biopsy	35
Kidney, biopsy	120
Laryngeal biopsy	111
Liver biopsy	318
Lung biopsy	209
Lymph node, biopsy	1,458
Nasal mucosa, biopsy	227
Nasopharyngeal biopsy	76
Nerve, biopsy	31
Omentum, biopsy	90
Pancreas, biopsy	56
Penis, biopsy	8
Pericardium, biopsy	4
Peritoneum, biopsy	22
Pleura, biopsy	13
Prostate, needle biopsy	740
Prostate, TUR	73
Rectal biopsy	237
Salivary gland, biopsy	6
Sinus, biopsy	116
Skin, biopsy	2,857
Soft tissue mass, biopsy	22
Spinal biopsy	71
Synovium, cyst	55
Testis, biopsy	8
Thymus, biopsy	14
Thyroid, biopsy	21
Tongue, biopsy	106
Tonsil, biopsy	17
Trachea, biopsy	68
Transplant biopsy, GI	568
Transplant biopsy, heart	223
Transplant biopsy, kidney	144
Transplant biopsy, liver	234
Transplant biopsy, lung	34
Ureter, biopsy	167
Urethral biopsy	68
Vaginal biopsy	198
Vulvar biopsy	67

GI, gastrointestinal; NOS, not otherwise specified.

formed in 2005. All these tests were performed according to routine protocols—the same that were used for the traditional overnight processed tissues.[6,7]

Molecular techniques

Procedures for evaluation of high molecular-weight DNA, RNA, and intact proteins from UMFIX- and formalin-fixed tissues have been described previously.[5,8]

RESULTS

Formalin-fixed, CRTP processing

Routine histology

Quality of all processed tissue was adequate for rendering a specific diagnosis. Of a total of 81,386 samples processed by the CRTP, not a single histology block was lost or severely damaged by the new system. There were 84 (0.1%) samples that required reprocessing, usually because of poor paraffin penetration in inappropriately thick tissue sections submitted for processing. This number was considerably lower than the samples that had to be reprocessed by the 1996 overnight system (n = 1,692 or 1.7%).

Ancillary tests

All histochemical stains produced tinctorial reactions that were indistinguishable from the conventional overnight processing. Similarly, the sensitivity and specificity of all immunohistochemical reactions on formalin-fixed tissues were exactly the same as those of overnight processing. No modification of staining protocols was necessary for diagnostic or predictive immunohistochemical markers. In situ hybridization by fluorescent or chromogenic tags of the tissues processed by the CRTP were comparable with overnight processing in both signal intensity and resolution.

UMFIX, CRTP processing

Morphologic features, including tissue architecture and tinctorial reaction of UMFIX CRTP samples, were comparable with formalin-fixed CRTP-processed tissue. The im-

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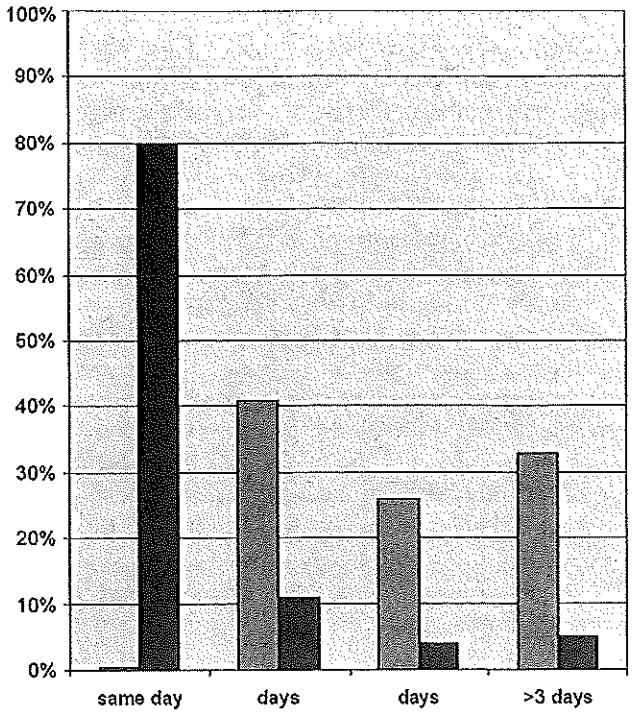

Figure 1. Comparison of surgical pathology turnaround time before (red bar, 1996), and after (blue bar, 2005) implementation of rapid tissue processing.

munohistochemical staining of samples fixed in the molecular fixative has been extensively documented before. In general, the signal is shown to be stronger with some antibodies and the procedure required modification, as described previously.[7]

Molecular assays

Quality of DNA and RNA in the UMFIX-exposed, CRTP-processed, paraffin-embedded tissue was similar to that obtained from freshly frozen samples.[8] This was not the case with formalin-fixed material, where there was considerable degradation of nucleic acids. Similarly, the protein profile of UMFIX/CRTP samples detected by two-dimensional gel electrophoresis, Western blot, and spectrometry revealed a pattern indistinguishable from their fresh counterparts, but formalin-fixed tissues did not yield intact protein.[8]

Surgical pathology turnaround time

Of the 22,875 surgical specimens processed by the CRTP in 2005, there were 18,300 (80%) processed, read, and reported on the same day they were received in the laboratory (Fig. 1). For small specimens and biopsies, 1-day turnaround time was 84% (14,588 of 17,367). Conversely, the same-day turnaround time for surgical pathology reports in 1997 was < 1% (27 cases), and that was accomplished by shortening the cycle of the overnight processor for a few transplant biopsies. Factors that influenced the longer turnaround time of both small and large specimens included incomplete patient information, requirement for specimen orientation, inadequate clinical history, need for histochemical or immunohistochemical stains, and additional time needed for decalcifying bony tissue.

Impact on patient management

Although the CRTP system was originally developed to improve the efficiency and functionality of the pathology laboratory, it quickly became apparent that there were additional real benefits for the patient and clinician. For example, the new system allowed for outpatient small biopsies to be read before the patients were discharged home, and the surgeons had the opportunity to discuss treatment options with patients on the same day, based on a final pathology report. Historically, after major operations, patients were sometimes discharged home before the final pathology report was available. In this era of instant gratification, that is often very dissatisfying to the patient and family. The shortened pathology turnaround time resulted in a final diagnosis being available early in the postoperative period, allowing sharing of this information with the patient before discharge. With cancer patients, knowing the final diagnosis allowed an early discussion by the surgeon with the patient and family, eliminating the disquieting wait for the patient to come back for his postoperative visit. Planning for any requisite adjuvant therapy, and introduction of appropriate consultants, including medical and radiation oncologists, could now be conveniently done as a group while the patient was still in the hospital. One interesting, additional, unanticipated consequence of the rapid turnaround was that surgeons stopped doing as many frozen sections just so that they could have information to discuss with the family. A survey of surgeons in our institution revealed that approximately 15% to 20% of frozen-section requests are primarily for informing family members. When it became clear that a final diagnosis would routinely be available in less than a day, surgeons preferred to avoid sharing the sometimes misleading information derived from a frozen section and the rate dropped to < 5%.

DISCUSSION

Today, most histopathology laboratories use tissue fixation and processing technologies that are more than a century old. This practice is now being challenged by the trend in patient management that requires timely pathologic diagnosis and the increasing demand for molecular assays on surgical samples.[9-11] In addition, most diagnostic and ther-

apeutic decisions are currently based on evaluation of small tissue biopsies, and laboratories are expected to use the same precious small volume of tissue for both histologic diagnosis and molecular tests. It is not unreasonable to predict that in this rapidly advancing era of personalized medicine, molecular methods could eventually replace the current morphologic approaches or, at the very least, will be used in conjunction with them. The histopathology laboratory of the 21st century must devise tissue-handling systems that allow for both to be expediently carried out on the same tissue sample.[8] The value of using microwave energy for histopathology in general, and for tissue processing in particular, has been well established. In most previous undertakings, conventional microwave ovens were used, including some that were adapted for histology purposes.[12,13] In the system reported by Morales and colleagues,[4] a specially designed cylindrical, low-energy microwave was used. Unlike regular microwaves, this modified version distributed the energy uniformly throughout the chamber, avoiding creation of hot and cold spots commonly observed in the conventional microwave processing. Complete automation was an expected natural evolution of such a system and is comprised of well-defined steps, time intervals, and temperatures. The result was introduction of an automated CRTP system. An external panel of pathologists judged the quality of routine histology and ancillary testing on material prepared by CRTP as comparable with that obtained by the overnight system.[6] From the beginning, use of this system resulted in a dramatic increase in the number of same-day surgical pathology reports at the authors' institution. This rapid turnaround time (TAT) was partly a result of shortening the processing time from 8 to 10 hours to 67 minutes and partly because of the continuous flow-through nature of the system.

There is great disparity in turnaround times for surgical pathology reports among the world's institutions.[14] Although a number of factors external to the laboratory can influence the timeliness of the reports, a 1-day delay caused by overnight tissue processing is almost universal among laboratories. An interinstitutional comparison of surgical biopsy diagnosis TAT of 157 hospitals in the US, Canada, and Australia conducted by the College of American Pathologists (CAP) in 1998 showed that in 90% of participating hospitals, the pathologists signed off 50% of the biopsies between the 2nd and 3rd postcollection days, and 90% of surgeons received only 50% of the final hardcopy reports by the 4th day.[15] As shown in Figure 1, the TAT in our own institution before introduction of CRTP did not differ from those reported by the 157 hospitals that responded to the CAP survey—we provided the pathology report on the same surgical day in only 27 of 23,000 surgical samples received in the laboratory. This is in sharp contrast to the present practice using CRTP, which allows same-day completion of pathology reports in > 80% of biopsies. In addition, the CRTP system improves the workflow of histology laboratories by eliminating batching of tissue samples and reduces volume and toxicity of reagents used in the laboratory.

Although implementation of CRTP requires additional capital investment, overall expenditure for the system is considerably less than that for clinical laboratory or radiology instrumentation. The CRTP system did not result in a reduction in the number of technical staff. It did lead to evenly distributed workload during the day and established more family-friendly working hours for laboratory personnel.[16] In addition, it is difficult to put a dollar value on the expedited surgical pathology report that is an immediate and readily identifiable advantage of the CRTP. Patient and surgeon satisfaction are greatly enhanced by rapid availability of a final diagnosis based on permanent sections, and planning for additional therapy, if necessary, can often be undertaken while the patient is still in the hospital. If appropriate fixatives (eg, UMFIX) are used, the system also allows for preservation of biomolecules in archival paraffin-embedded tissue, obviating the substantial and often insurmountable difficulty of obtaining freshly frozen samples.[5,17] This renders patients' archived tissues as a viable reservoir for performance of current and future molecular tests that, until now, could only be carried out by the immediate freezing of fresh tissue.

In summary, introduction of a microwave-based processing system, combined with a new fixation reagent, converted our surgical pathology practice from one with lengthy delays and molecularly suboptimal histopathology to a robust, rapid-response, molecular-friendly platform.

Author Contributions

Study conception and design: Morales, Nadji, Livingstone
Acquisition of data: Morales, Nadji
Analysis and interpretation of data: Morales, Nadji, Livingstone
Drafting of manuscript: Morales, Nadji, Livingstone
Critical revision: Morales, Nadji, Livingstone

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