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Histology labs eye new kids on the block

William Check, PhD

“To every thing there is a season,” the Book of Ecclesiastes tells us. After a long season of static practice, the field of surgical pathology is entering a season of technological advancement. And it is doing so with a vengeance.

“For the most part, we have been practicing surgical pathology in the same ways for at least 50 years,” says Azorides Morales, MD, professor and chairman of the Department of Pathology at the University of Miami School of Medicine and chief of pathology services at Jackson Memorial Hospital, Miami. “I do not mean to say that there haven’t been advances. There is no question that a lot of instrumentation has been developed. And hardly a day goes by when someone doesn’t develop a new marker or a new technique. But the basic handling of solid tissues hasn’t changed.”

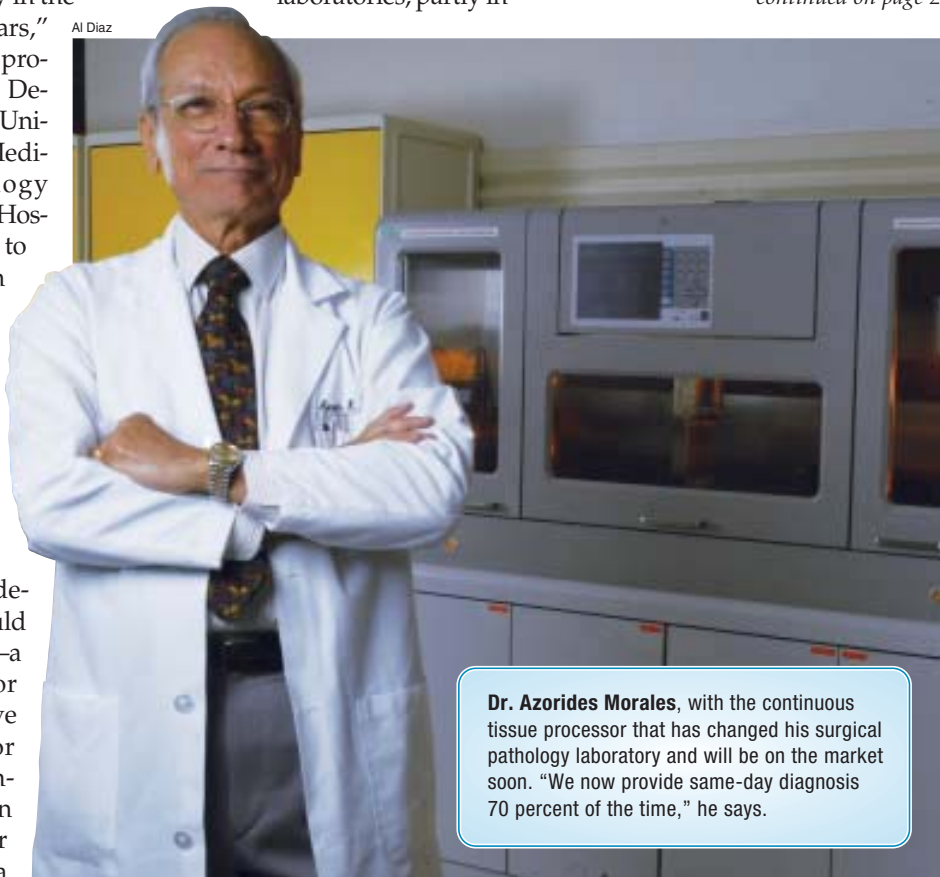
Dr. Morales has helped develop an instrument that could radically alter this situation—a continuous tissue processor based on modified microwave technology. “This processor provides the ability to complete surgical reports within two to three hours after surgery and to do them in a

continuous fashion,” says Dr. Morales. About 70 percent of surgical cases in his section are processed and signed out on the same day the specimen gets to the laboratory. “This is truly unheard of,” Dr. Morales says.

Technological advances downstream of tissue processing are finding their way into more histology laboratories, partly in

response to increasing specimen volume. “The number of specimens we handle goes up by at least 10 percent per year,” says Brendan Boyce, MD, head of surgical pathology at the University of Rochester Medical Center, NY, “because more patients are being screened, such as with colonoscopy.

“We have been automating as
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Dr. Azorides Morales, with the continuous tissue processor that has changed his surgical pathology laboratory and will be on the market soon. “We now provide same-day diagnosis 70 percent of the time,” he says.

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much as we possibly can," Dr. Boyce says. His section uses automated coverslippers, an automated slide labeler, and automated processors for both special stains and immunohistochemistry.

David Hicks, MD, section head of surgical pathology at the Cleveland Clinic Foundation, also finds increasing volume a challenge, particularly in light of staff shortages. "We do approximately 80,000 surgical specimens per year," Dr. Hicks says, "so we have had to be creative about how best to utilize staff, as well as to respond to pressures for timeliness and quality and error reduction.

"One way to do this is to move in the direction of automation in histology," he continues. "In a sense, histology has lagged behind." He has automated most routine histochemistry special stains as well as immunohistochemistry.

Besides increasing efficiency, automation can reduce errors, says Christopher Otis, MD, director of surgical pathology, Baystate Medical Center, Springfield, Mass., and associate professor of pathology at Tufts University School of Medicine, Boston. "One of the growing concerns is correct identification of specimens, avoiding giving a diagnosis to the wrong person," Dr. Otis says. "Hospitals are becoming increasingly concerned about how we track cases as they come through the laboratory, from the time the biopsy is obtained and accessioned to when it is signed out by the pathologist and the report is delivered to the clinician." For Dr. Otis' laboratory, which processes between 800 and 1,200 blocks daily, this can be a challenge.



Dr. Otis

To meet this challenge, he has adopted more advanced applications of information technology, such as bar coding and block labeling.

On the test side, immunohistochemistry is advancing as well. "Immunostains are definitely a growing area," Dr. Hicks says, "particularly tumor markers. The number of antibodies on our menu is growing, and test volume is growing as well."

In the opinion of Allen M. Gown, MD, medical director and chief pathologist at PhenoPath Laboratories, Seattle, "Batteries of immunostains have largely replaced special stains."

"In the past," he says, "we used methods like reticulum stains, because there was not much else we could do to further identify the nature of certain tumors." Special stains are still useful for identifying organisms such as mycobacteria and fungi, Dr. Gown notes.

"We have had a fairly large expansion of reagents useful in diagnostics," adds Paul Swanson, MD, director of anatomic pathology at the University of Washington Medical Center, Seattle. "Immunostains are much more widely used, although it is not necessarily true that diagnostic accuracy or predictive value of the technique has advanced in parallel." (See "Special stains and immunostains," page 7.)

Of the many automated instruments, a continuous tissue processor could have the greatest impact. "It will revolutionize the practice of anatomic pathology," Dr. Morales says. In traditional surgical pathology, everything is handled in batches. "We gross tissues all day long," he says. "Then they are placed in automated processors overnight. In the next day or two they are handled by histotechnologists in batches. In laboratory medicine we don't do that any longer," he notes. "When specimens come to the laboratory,

for the most part we place them in analyzers one at a time. Continuous processing allows us to respond to patients' needs immediately."

In surgical pathology the technology needed to expedite tissue processing has not been available. Solid tissue converted into slides is subject to many agents—heat, vacuum, agitation—and the process takes 10 to 12 hours, so it is done overnight. Tissues are presented to a pathologist at a minimum of 24 hours after material is received.

Microwave technology, introduced into pathology 20 or more years ago, accelerates to a considerable extent the diffusion of solvents into tissues. "Unfortunately," Dr. Morales says, "for the most part the microwaves available for use in histology are modified kitchen microwaves." That situation is changing. Dr. Morales was approached in 1996 by father and son physicians named Essenfeld in Caracas, Venezuela, who were working to optimize microwave exposure for solid tissues. Dr. Morales joined forces with them, and in one year the team had developed a manual microwave method for histology. An automated version soon followed, and Sakura Finetek, Torrance, Calif., then designed and built a new processor that is now in beta testing. In several years of clinical work with the prototype, Dr. Morales says, "we have processed close to 1 million samples and we have not spoiled a single one."

To design a new microwave oven specifically for histology, the team contacted Microwave Materials Technologies, Knoxville, Tenn., which developed a way to provide microwave energy uniformly throughout the retort chamber where solutions are placed. "All samples receive just about the same energy," Dr. Morales says. In addition, they receive very low wattage radiation. "Rather than pulsing mi-

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crowave energy, we have it on continuously at low wattage, 150 watts for a few seconds at the most," he explains. For the rest of the time it is at 50 to 60 watts. "We are handling tissue in a very gentle fashion that prevents overheating that could damage it."

New reagents were introduced to aid this process. "We eliminated or reduced to a significant extent the toxicity of the reagents that we use," Dr. Morales says. Conventional processing typically uses formalin, alcohol, xylene, and paraffin, whereas the new process uses a mixture of acetone, isopropyl alcohol, polyethylene glycol, and mineral oil and paraffin. And the volume of reagents has been reduced by about 80 percent.

To accommodate the automated instrument, other steps have had to be standardized. "Most difficult was to standardize grossing," Dr. Morales says. To standardize the thickness of tissue sections, his team developed simple grossing boards that have slots with preset depths. "If we want 1.5-mm-thick sections, we put the tissue in that slot and slice it," Dr. Morales says.

The processor accepts samples semicontinuously, from one to 40 samples every 15 minutes. Processing takes one hour. A histotechnologist can remove samples every 15 minutes and proceed with embedding, microtomy, staining, and other steps, providing virtually continuous flow.

Dr. Morales says this flow is a "tremendous advantage for the staff, not only pathologists but also histotechnologists." In most histology laboratories, histotechnologists come in at odd hours of the morning, such as 3 AM. With the new processor, everything is done during the day. One group of histotechnologists starts at 7 AM and another at 11 AM.

"The OR for the most part is inactive on weekends," Dr. Morales says, "so histotechnologists only come on the weekend for emergencies." This makes it more attractive for people to work there. Moreover, he says, "we don't run the instrument overnight, so if anything goes wrong, we can handle the problem right away."

With the automated processor, pathologists are working only 1.5 to two hours behind the surgeons. "We now provide same-day diagnosis 70 percent of the time," Dr. Morales says. "Before, less than one percent of our samples were diagnosed and a report provided on the same day." Rapid turnaround lessens patient anxiety and helps the flow of patients through the institution. Moreover, Dr. Morales notes, "We no longer receive calls in the laboratory asking for a diagnosis. I can tell you this is irreversible here."

Dr. Morales says "one of the most fascinating aspects" of the new processing method is that it is "molecular friendly."

"That means that if we handle the tissue appropriately before we place it in the instrument, we can extract RNA and proteins from the paraffin block," he says. "With conventional overnight processors that is basically impossible, because the chemicals used are not molecular friendly."

Sakura Finetek is commercializing the new processor. "It is my understanding that it will be available by the end of 2003," Dr. Morales says. Large laboratories like his, which has 30,000 surgicals per year and processes about 120,000 pieces of tissue, will find the processor desirable, he says. The new instrument, Tissue-Tek Xpress, handles more than 900 samples in an eight-hour shift, doing the work of three conventional Sakura Tissue-Tek VIP processors. Dr. Morales suggests that it may be advantageous for smaller laboratories as well. "Do you want to wait till the next day or next week

to provide diagnosis to your patients?" he asks. "And do you want to archive every single piece of tissue that you handle for possible future molecular assays?"

Sakura has developed an automated embedding device that complements the automated processor. Currently, after tissue is processed, histotechnologists remove it from cassettes and embed it in paraffin. In the new system, tissue will go directly from the processor into the embedding instrument, called Tissue-Tek AutoTEC, without further handling. AutoTEC also uses a special tissue cassette. "What we have been using from the beginning of time can't be sectioned," Dr. Morales says. With the new cassette, tissue is embedded and then sectioned through the cassette. "This will increase efficiency tremendously," he adds.

Existing forms of automation can help solve another problem in surgical pathology: human errors that can lead to misdiagnosis. "Surgical pathology is unlike most clinical pathology laboratories, where many things are automated and mistakes can usually be backtracked to a central point where accessioning and bar coding take place," Dr. Otis says. Multiple steps are inherent to histology laboratories, starting in the clinician's office or the clinic where specimens are labeled by hand. After delivery, pathology accessions specimens by hand through a computer system. "Computer systems help in a lot of ways," Dr. Otis says. "They standardize through default specimen-type dictionaries how to handle tissues from the beginning. That eliminates some error."

Next, grossing is done by hand. "You must make sure you pick up and gross the right specimen," Dr. Otis says. It's possible to make embedding mistakes in which specimens are switched. Slide labeling

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creates “a big potential for error.” A technologist can hand-label a slide with a specimen accession number and then put the wrong slices on it. It’s also possible to put the wrong computer-printed labels on slides. “All this falls into the preanalytical area, which is fraught with potential error,” Dr. Otis says.

He describes a hypothetical but plausible error in the preanalytical stage. “It would not be uncommon to get specimens from two consecutive patients on whom the same immunostain was ordered,” he says. Assume that the stain ordered was p16 antigen, which helps identify high-grade squamous intraepithelial lesion. What if the labeled slides got tissues from the wrong patients because they picked up the wrong tissue sections? In essence, they were crisscrossed. “Perhaps that incident would be identified at the multi-headed microscope conference,” Dr. Otis says, “because the morphology did not make sense with the IHC results. Even so, that raises a question—how many times do we fail to recognize that kind of event? What is the denominator? No laboratory has a handle on that. It would be naive to assume that it never happens.”

In the analytical phase, the pathologist might pick up the wrong slide, one that doesn’t correlate with the protocol requisition slip linking the slide to the patient. “This is the critical part of signout,” Dr. Otis notes. “With us, each slide has at least a surgical pathology number, the specimen type, and the patient’s name.”

Postanalytical problems can occur at transcription if the pathologist dictates the wrong number. That would yield the correct diagnosis for the tissue but link it to the wrong patient. Errors might not be picked up at sign-out. “Since many of us are using computerized electronic

signouts, we won’t have the requisition at the time of signing out to verify that what we are seeing correlates to the correct patient,” Dr. Otis says.

“Fortunately, these things happen very infrequently, because we have set up checks and balances throughout the system. But we can do better,” he admits.

To reduce the risk of error further, Dr. Otis’ section has started to use bar coding for accessioning cases. Bar coding gets away from manual input of patient information, after the initial assignment of a bar code to each patient at entry into the hospital or when the specimen arrives in pathology. “Our requisition forms have bar-code labels and we are working on getting a bar code on slides,” Dr. Otis says. If you have a reader next to each microscope, that eliminates some potential for switching slides. Bar coding a slide is even more useful: You slip the slide under the reader and the computer information appears as well.

At that point, Dr. Otis says, “You are in the histology laboratory asking, Is the right slide picking up the right tissue section and getting the right paper label? That gets back to bar-code technology that labels a specimen paraffin block. Under a bar-code reader, the bar code tells what is to be done with that block, such as how many slides to make.” The slide producer puts information on slides with indelible ink and produces the number of slides that paraffin block requires with the patient number and other identifiers on it. “We are now working on bar coding blocks and slides,” Dr. Otis says. “That will eliminate preanalytical errors of patient and case number and block identification.”

Acknowledging the possibility of error and identifying each step where error might occur is critical, Dr. Otis says. “In our laboratory, we handle about 45,000 surgical specimens per year, 800 to 1,200 per day,”

he says. “It is optimistic to think that out of those 1,000 or so blocks, errors will not happen. They will, and in most cases they can be resolved. Any laboratory that handles that amount of material has to have steps in place to minimize misidentification.” The same is true of smaller laboratories.

To complicate matters, histology work is being centralized because it’s hard and possibly not cost-effective to automate small histology laboratories. “We have already integrated two other hospitals’ histology laboratories,” Dr. Otis says, “which adds new potential errors—lost specimens and mislabeled specimens.” Possible safeguards include integrating computer systems and eliminating all steps except picking up specimens and delivering them to the central hospital laboratory. Also, Dr. Otis has set up standardized surgical pathology reporting checklists for the whole health system.

In Dr. Otis’ view, automation in histology has lagged behind because of marketplace issues. “Histology laboratories are a relatively small marketplace,” he says, “not like automated clinical chemistry analyzers. Companies have to see whether it’s worth it to invest R&D for hardware design compared to what they will get back on their investment.” The computerized nature of most histology laboratories will start to make that return attractive, he predicts. There are now several choices in bar coders and block labelers that are integrated and interfaced with computer programs that label the number of sections needed. “In the next few years this will all come into place,” Dr. Otis says.

One of the biggest reductions in human labor has come from the adoption of automated stainers. “We are certainly aware of the shortage of histotechnologists,” Dr. Gown, of PhenoPath, says. “Au-

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tomation is one way that laboratories can address that." Automation has made immunostains more accessible in general, he notes. "The capability of doing special stains by instrument has been a big advance."

Dr. Hicks' laboratory at the Cleveland Clinic uses the Ventana Nexxus for special stains. It basically automates all steps, including reagents, temperatures, and times. "We have some stains that are not yet on the machine," Dr. Hicks says, "and we have been working with Ventana to adapt some of our most frequently ordered special stains to automation." This would be a huge help, he says, and would reduce staff exposure to potentially toxic reagents. Some of the special stains currently automated in Dr. Hicks' lab are Congo red, acid-fast bacterial dye, Gomori silver stain for fungal organisms, trichrome for collagen, and Steiner stain for spirochetes. "The stain set we would really love to see on the machine is the Movat stain for elastic fibers, which is helpful for evaluating blood vessels," he says.

In the immunohistochemistry laboratory, Dr. Hicks has Ventana Benchmark stainers, which accept freshly cut slides and have the online capability to bake and deparaffinize and do antigen retrieval as well. "Basically, all of our immunostains are on the instrument, other than immunofluorescence," Dr. Hicks says. "It has reduced our turnaround time for most immunostains from 24 hours to around two to six hours." Immunostains ordered by noon are reported out the same day. "The clinicians love it," he says. "Automated staining really has been a plus for the laboratory, as the number of antibodies we do has risen. And new antibodies are easier to work up and incorporate into the instrument."

Automated staining instruments have two further advantages, in Dr.

Hicks' experience. First is improved consistency and quality, since the machine controls all parts of the staining process. For immunostains particularly, this has been a boon, with about a 70 percent reduction in repeat stains. Repeats are ordered if controls are not staining appropriately, the pathologist feels that the stain is too weak or too strong, or a specimen came off the slide (which can happen during manual antigen retrieval but is less likely with an instrument). The laboratory absorbs the cost of repeat stains, so reducing the number saves time and money.

Second are worker issues, from increased productivity to greater histotechnologist job satisfaction. "We can handle higher volumes of work with the same number of technologists," Dr. Hicks says, "because they are not changing reagents, running timers, and moving slides." And technologists now have time to look at the stains they run and evaluate them, so they don't have to wait until a pathologist sees a slide to recognize problems. "Our technologists enjoy troubleshooting and often call problems to my attention," Dr. Hicks says. "The more you work with them, the better they get, which reinforces my experience that they want to know and understand how the results of their work are used and they appreciate when someone takes time to explain it to them."

(Because Dr. Hicks finds that "more-informed technicians do a better job and are more interested," he has started a twice-monthly lecture series for histology technologists in which staff pathologists, house staff, or trainees talk on technical topics related to the laboratory. "The laboratory has responded tremendously to that," he says, "and staff has also. We have had no problem getting staff to give these talks." Dr. Hicks himself frequently gives workshops and lectures at National Society of Histotechnology meet-

ings, mostly about immunohistochemistry.)

Dr. Hicks is now working on a further step in automating the histology laboratory. This includes testing a new automated routine H&E stainer and interfacing the LIS with laboratory instruments to allow transfer of stains ordered to the instruments' computers. In this way, a bar code can be generated for the stainers, eliminating the need for redundant data entry by the technical staff and potentially reducing errors. Dr. Hicks calls this project "a major task." The new routine stainer will automate baking, deparaffinization, staining, and even coverslipping, he says. "The hope is that this approach will free up valuable technician time and make our staff more productive."

DakoCytomation, too, is marketing automated histology instruments; it makes a histochemistry stainer and an immunostainer. "Dako's instruments are quite good also," Dr. Hicks says. "For us, the main issues were the individual needs of our laboratory and a history of collaboration with Ventana. I know a number of excellent laboratories that are equipped with Dako instruments and are happy with them."

"It is not the specific instrument that is important," he emphasizes, "but the concept of automation that adds efficiency and productivity in histology. The goal here is not to reduce [the number of] people, but to make them more productive."

Dr. Swanson's laboratory at the University of Washington Medical Center uses DakoCytomation instruments. "We just brought in the Artisan system for histochemistry," he says. Like Dr. Otis, he finds that an automated instrument increases reproducibility and allows more efficient use of technologists.

However, he is not automating immunohistochemistry. "Basically,

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unless there is a clear quality or manpower issue in the laboratory, immunostaining performed manually can still provide a superior product," he explains. "I have worked in three different large academic centers in my career, and I have been blessed to work with laboratorians with technical expertise in IHC who provide day in and day out reproducible quality results." Eventually, he thinks that an instrument may be more cost-effective in terms of manpower. Still, he says, "if there are no quality issues on the table, I don't see the need for automation [of IHC]." In smaller laboratories, in settings where there are worker shortages, or when it is difficult to devote one or more technologists to IHC, automation may be a more urgent consideration, in Dr. Swanson's view. "We may yet find that automation suits our needs, but at the moment that is not the case."

Beyond current automation devices, Dr. Boyce, of the University

of Rochester Medical Center, sees yet another need. "What I would like to see developed is a robotic device for handling small specimens like biopsies," he says. "We can get from one to 15 bottles from individual patients, each of which has a number of pieces of tissue in it. Someone has to take off the tissues, dictate what was received, how many pieces of tissue, describe them, then pass them into a teabag and into a cassette." This process is labor-intensive. A robot that would suck out the contents of each bottle, scan the number of pieces of material, and count and size them would be "a tremendous advance," Dr. Boyce says. There would have to be a mechanism to make sure all contents of the container have been removed, that nothing has stuck to the bottle. The robot could feed directly into an automated processor of the type Dr. Morales developed.

In general, Dr. Boyce says: "A great deal of what we do, I believe, could be done by robots with technical oversight. We shouldn't have

people doing tedious tasks, standing for hours on end taking lids off containers and describing what they see. We have at least one person doing this full-time essentially all day."

Dr. Boyce's section grosses about 40,000 specimens per year, and large reference laboratories can do twice that many. "I would have thought some of these large places would have developed something like this," he says. "I suspect it hasn't happened because no one has really pushed forward to make it happen. It may also be that the cost of machines versus the number of specimens has been prohibitive up to the last few years. But as more people get more biopsies taken and we deal with more specimens, the time is coming when most laboratories could benefit from robots."

When that happens, histology will truly be enjoying the season of automation. □

William Check is a medical writer in Wilmette, Ill.

Special stains and immunostains

To demonstrate the value of immunostains, Allen M. Gown, MD, medical director and chief pathologist at PhenoPath Laboratories, Seattle, goes back to his days as a resident in the 1970s.

"When we worked up a case and H&E didn't give the final answer, we first elected special stains," he says. That included things like reticulum stain. Now when a diagnostic dilemma arises, in most laboratories the first step is to think of immunostains. "Antibodies to type IV collagen can be used routinely to address many of the same issues that were addressed by reticulum stains," Dr. Gown says. Looking for organisms might be one place where traditional stains prevail. "Even then sometimes immunostains do a good or even better job," he says, citing Steiner stain for *Helicobacter pylori*.

Dr. Gown prefers to distinguish immunostains from special stains. "Maybe because of the way immunostains were introduced into the laboratory, in some people's minds they are a kind of special stain," he says. In his view, special stains are empirical—they just happen to work—whereas immunostains are defined by an antibody of known specificity.

Because they are based on antibodies, immunostains have proved more of a challenge to automate than special stains. Immunostains require special considerations in tissue preservation and preparation to optimize their use. "They are less forgiving than special stains," Dr. Gown says. He cites antigen retrieval as something generally required for immunostains but not for special stains.

Forefront immunostains include antibodies to relatively esoteric tumor-specific markers, such as the FLI-1 gene product found in Ewing's sarcoma and the WT-1 gene product that characterizes desmoplastic small cell tumor. Both of these gene products represent C'



Dr. Gown

terminal elements of chimeric fusion proteins created by tumor-specific chromosomal translocations.

For such tumor-type specific markers, says Paul Swanson, MD, director of anatomic pathology at the University of Washington Medical Center, Seattle, "when the translocation-defined portion of the protein is expressed in the appropriate histologic context, it can be a reliable marker for that disease." In these cases, immunohistochemistry detects what otherwise would be identified by cytogenetic analysis or rtPCR for the chimeric fusion transcript. "We cannot claim either sensitivity or specificity benefits with immunostains relative to cytogenetics," Dr. Swanson says. "But sometimes because of tissue handling, conventional cytogenetics or molecular techniques cannot be performed. In particular, rtPCR is extremely dependent on fresh or properly prepared tissue," Dr. Swanson says. If tissue is formalin-fixed and routinely processed, it can be hard to retrieve intact mRNA.

In these situations, IHC offers an alternative. Immunostains that recognize lineage-restricted markers composed of a family of transcription regulators have also appeared on the forefront of diagnostic pathology. Most widely used among these is thyroid transcription factor 1, or TTF-1, says Dr. Swanson. "It is associated with elements of foregut development in fetal tissues," he says. It is also expressed in differentiated thyroidal and pulmonary epithelium, and as such is a very sensitive and reasonably specific marker for thyroid and pulmonary glandular and endocrine neoplasms. It also has limited value in extrapulmonary neuroendocrine carcinoma.

A related marker, CDX-2, is preferentially expressed in midgut and hindgut epithelium. "It is a reasonably specific and sensitive marker for gastrointestinal carcinomas, especially carcinoma of the colon and rectum," Dr. Swanson says.

TTF-1 and CDX-2 are particularly relevant to the diagnosis of metastatic carcinoma, especially in those cases

where primary site of involvement is uncertain or unknown (occult clinical disease). "A lot of IHC today revolves around that broader question," Dr. Swanson says.

Other recent markers purport to distinguish between noninvasive proliferative lesions and overtly invasive malignant disease. High-molecular-weight keratins have been joined by the p53 analogue p63 as markers of basal cells in prostate. P63 also complements antibodies to smooth muscle determinants as a marker of myoepithelial cells in breast and other organs. Immunoreactivity for these markers in the appropriate context is evidence of an intact or attenuated basal cell or myoepithelial layer (non-neoplastic or in situ disease), whereas the absence of these markers is presumptive evidence of extension of carcinoma beyond normal structures.

In contrast, α -methyl acyl coA racemase (p504s) is selectively expressed in malignant epithelium of prostate cancer, ostensibly providing a positive marker for carcinoma instead of the traditional inference of malignancy from the loss of normal elements. Dr. Swanson calls it "somewhat controversial."

Still other markers of increasing relevance to diagnostic pathology are those, such as Her2/*neu*, topoisomerase-2 α , and thymidylate synthetase, that predict response to specific classes of chemotherapeutic agents, a role until recently played only by estrogen and progesterone receptor proteins.

"For me, the important question posed by these markers is not whether a selective panel of chemotherapeutic agents or other treatment options is available to the patient," Dr. Swanson says, "but whether such treatment is of proven or expected clinical value." In a similar vein, one might question if there is a defined benefit to the physician and the patient in making an immunohistochemical determination of the origin of metastatic disease.

"In most cases there is," Dr. Swanson says, "but not always. What we detect by immunohistochemistry may help dictate management, but will it reliably predict outcome?"