

Lynch Syndrome Screening in Colorectal Carcinomas

Jennifer J. Findeis-Hosey, MD

Lynch syndrome is a genetic syndrome which puts patients at increased risk for the development of numerous cancers, including colorectal and small bowel carcinomas, endometrial carcinoma, skin tumors, brain tumors, and cancers of the renal pelvis and ureter[1]. In 1966 Henry Lynch and colleagues detailed two large Midwestern families with a propensity for carcinomas, especially primary to the uterus and colon[2]. Subsequent genetic studies have detailed that Lynch syndrome is an autosomal dominant disorder which is the result of germline mutations in components of the mismatch repair (MMR) protein complex. Mutations are most frequently encountered in the *MLH1*, *MSH2*, *MSH6*, and *PMS2* genes. These MMR proteins are normally responsible for correcting aberrant or 'mismatched' DNA pairs which can form during the DNA replication process. DNA replication typically involves adenine binding to thymine (A-T) and guanine binding to cytosine (G-C). When there is an atypical binding, such as adenine to guanine, the MMR protein complex identifies and removes the incorrect nucleotide so that the appropriate nucleotide can be inserted in the replicated DNA strand. When there is a mutation in one of the MMR genes resulting in a faulty MMR protein, the mismatched DNA pairs cannot be appropriately corrected, resulting in the propagation of errors in the patient's DNA and an increased risk of developing cancer.

It is estimated that 1-7% of patients with colorectal carcinoma have Lynch syndrome, making it the most common heritable form of colorectal carcinoma[3]. Clinical criteria heavily relying on family cancer history has historically been used to screen patients for Lynch syndrome, most notably in the forms of the Amsterdam and revised Bethesda criteria. Unfortunately, it has been demonstrated that upwards of 40% of patients with Lynch syndrome will be missed by these clinical screening protocols[4]. As such there is the need to explore laboratory-based methods to screen for Lynch syndrome.

There are multiple laboratory-based testing modalities that can be performed to assess for abnormal MMR genes and proteins, including immunohistochemistry (IHC) and microsatellite instability testing. IHC staining can be used as a protein-based screening methodology to assess for presence or absence of MMR proteins within tumor cells. IHC testing typically involves examining the presence or absence of nuclear staining for the MMR proteins MLH1, MSH2, MSH6, and PMS2 in the tumor cells (Fig. 1). In patients without Lynch syndrome the MMR proteins should be normally expressed within nearly all nuclei, so all four IHC stains should demonstrate nuclear positivity (Fig. 1B). In Lynch syndrome one or more of these proteins



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Deadlines for Submission are:

December 1 – Winter

June 1 - Summer

Membership in the New York State Histotechnological Society, includes a subscription to *On Stage*. The annual membership fee is \$25.00. Please direct membership inquiries to:

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Happy New Year!

I am looking forward to all the new things 2016 has in store for all of us! It is my pleasure to welcome the following individuals to the New York State Histotechnological Society (NYSHS) Board of Directors (BOD):

Vice-President: Amy Farnan

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In 2016 there will be several positions open on the NYSHS BOD. Please consider running for election to serve the members of NYSHS.

This year the 41st annual NSH Symposium Convention in DC was another well put together meeting. Kudos to the NSH staff for planning another great meeting. There were over 125 workshops which included a wide variety of topics such as General Lab Practices, Immunohistochemistry, Leadership and Safety, as well as many posters. There were over 90 vendors with their products on display.

I'm very excited for this year's NYSHS annual spring meeting. We are in the process of finalizing plans for the meeting program. The 2016 Annual Spring Symposium will be held at the Holiday Inn Express and Suites in Latham, New York on Friday April 22nd and Saturday April 23rd, 2016. This year's theme is "SPECTRUM OF KNOWLEDGE". Be sure to keep an eye on our website, www.nyhisto.com for any updates as they become available. I hope to see all of you there!

The 2016 Region I meeting will be held in Mystic, Connecticut on April 15th and 16th. We will post information on our website as it becomes available at: www.nyhisto.com

Best,
Sarah Mack

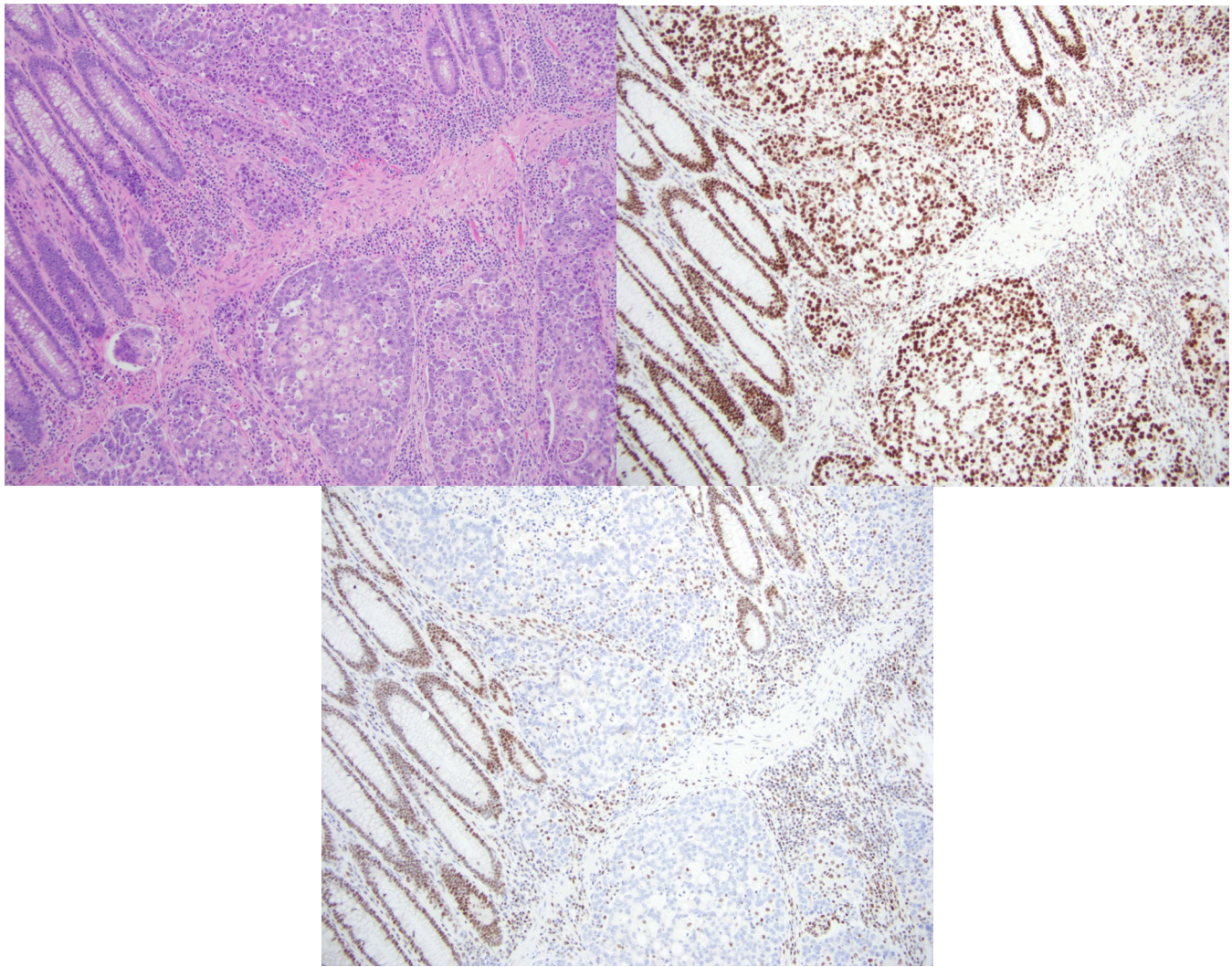


Figure 1. Immunohistochemistry utilizing antibodies against mismatch repair proteins to screen for Lynch syndrome. (A) Colorectal adenocarcinoma nestled between normal colonic epithelium. (B) Retained MSH2 nuclear staining is seen in both tumor and normal cells. (C) Loss of MLH1 staining in tumor cells while normal epithelial cells still retain staining; concerning for Lynch syndrome. Internal an on-slide positive controls and internal negative controls are evaluated. Convincing nuclear staining in $>1\%$ of tumor cells is considered positive

will demonstrate loss of staining (Fig. 1C). Given that this is a protein-based assay, IHC testing functions as a screening modality since protein expression may be abnormal due to non-germline mutations or hypermethylation.

Microsatellite instability (MSI) testing is a polymerase chain reaction (PCR)-based assay that looks for variability in a pre-defined panel of microsatellite DNA regions. These microsatellites are repetitive DNA sequences usually formed from repeats of DNA sequences between one and six nucleotides long, which exist most often in regions of non-coding DNA. Normally, the MMR protein complex functions to keep these microsatellite regions at a relatively consistent length for a given person. When there is a mutation in one of the MMR genes and the MMR protein complex does not function appropriately, the size of these microsatellite regions can become very variable within a given person and this variability can be detected through PCR testing. When at least 30% of the examined loci within a MSI panel demonstrate



instability in the resulting PCR product, it is termed microsatellite instability or MSI-high (MSI-H). Similarly to the IHC-based assay, MSI testing should be regarded as a screening modality. Although it utilizes DNA, MSI testing involves the examination of tumor DNA, which may or may not be reflective of a germline mutation since tumors often harbor mutations that are not present in the germline.

Irrespective of the screening methodology, universal screening of colon cancers for Lynch syndrome is supported throughout the literature[5]. If a patient is identified as possibly having Lynch syndrome, they should be directed to a genetic counselor for in-depth assessment of family history and for germline mutation analysis. This mutational analysis will assess if there truly is a mutation in one of the MMR proteins. If a patient is found to have Lynch syndrome they should undergo life-long screening given their increased risk of developing a wide range of cancers. This screening involves endometrial biopsies to assess for uterine cancer in women, as well as colonoscopies and urine cytology in both men and women to assess for colon cancer and cancers of the urinary tract, respectively.

At the University of Rochester Medical Center we perform MMR IHC testing on all colorectal carcinoma specimens. We have historically performed this testing on all colorectal carcinoma resection specimens which are the result of the surgical resection of a large segment of bowel involved by the tumor mass. While this provides a reliable method for Lynch syndrome screening, we have found that patients are unlikely to follow through with visits to genetic counselors for definitive testing, presumably secondary to the myriad of physical and psychosocial factors that come with the diagnosis and treatment of a new cancer. Recently we have begun MMR IHC testing on all colorectal carcinoma biopsy specimens in the attempt to bring Lynch syndrome screening to the forefront.

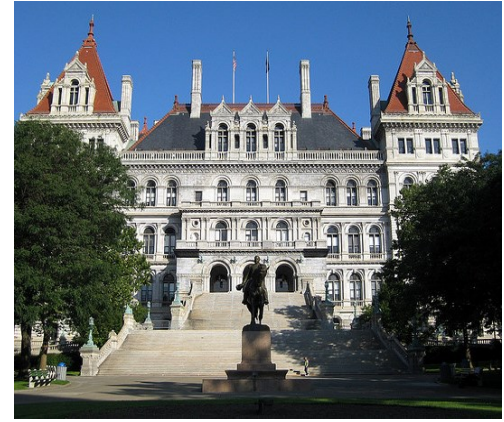
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SAVE THE DATE!!!!

New York State Histotechnological Society's
Annual Spring Meeting
"SPECTRUM OF KNOWLEDGE"
Friday April 22nd and Saturday April 23rd, 2016
Holiday Inn Express and Suites,
Latham, New York



Check our website for updates: www.nyhisto.com

Histology Tips & Tricks

Angela Fogg

Histology tips and tricks is probably one of the most interesting topics of our profession. We have all gone to conferences and talked with our peers about practices and procedures. At our NYS meeting in Canandaigua, we had a lecture given by Angie Cahill about mitigating contamination in the H&E process. It was refreshing to hear how many different ways we can avoid and learn how to correct cross contamination of our specimens. I am sure that many of our readers have come up with some of their own excellent ideas how to accomplish some of Histology's little or big tasks and hopefully in a less complicated manner.

Over the years I have had the unique experience of having Histology students come to the lab to do the externship to prepare for the ASCP examination. After which, I also had the opportunity to hire some of those students to work at the facility. Whether they were previous students or new hires, I have always been compelled to share things I was taught, by someone more experienced than I was or things that I learned from trial and error and found they worked. Sometimes it's the little things that can save the day or help even if it becomes a stepping stone to a solution. I also don't think that everything we hear works 100% of the time. I have learned to accept this ever since I read an article Lee Luna wrote, "What works today in Histology may not work tomorrow." I am not sure if that is a direct quote but I have lived by it for years. Through the years I have passed these on to others and received messages from folks thanking me for sharing.

So, one more time to share a few with you.

NOTEBOOKS

The first thing I tell every student or new employee who comes to do their clinical or when a new hire starts is, that no matter if you a newly certified Histotech or if you are a seasoned vet it is important to bring a notebook with you and take notes. You cannot remember everything you hear and you will remember better if you write it down. I still have my notebook 45 years later and still refer to some of the information, the stuff that never out dates of course!!

LEARNING YOUR NEW JOB

In this day and age our society is constantly on the move so remember when you decide to take a new job in a new place that everyone does things, at least a little bit, differently than the last. All your education is never wasted but the "Order of the Day" changes from place to place. Put your, we used to do it this way, aside until you learn their way.

TECHNICAL TIPS!

Some you already know, some I learned from others and some by trial and error. And no doubt a lot can be found in any one of our great Histology textbooks.

BLOCK WON'T RIBBON

Your block won't ribbon, smooth the edges of the paraffin that come in contact with the blade. I use my thumbnail, it's quick, not scientific but it works most of the time. Sometimes even a little piece of dirt or crystal in the paraffin can be the problem.



BLOCK DIDN'T PROCESS WELL

Got a block that didn't process well, need a section today, put it in the cryostat for about 10 minutes. When you take it out you have to work quickly, first cool your water bath down so the section doesn't melt away. When you put the block in the microtome don't touch the tissue surface. Your thumb will warm the surface. When you take it out of the cryostat, don't try to cut too fast, this will cause heat friction and the process may not work as well. It will only work for the first 2 to 6 sections off the block depending on the size of the tissue but it just may be the difference between a diagnosis today or reprocessing the block for tomorrow.

DRY TISSUE

Have really dry tissue? We all have come across that from time to time. There are many ways to correct this problem. This list is just a few.

- Soak in cold water
- Soak in warm water
- Soak in soapy water
- Soak in ammonia water
- Soak in cold ammonia water
- Soak in decal solution

Use a gauze covered ice cube to soak while the block is still on the microtome.

All of these have merits. Over soaking is always a concern but, so far the only one I have found that has **not** caused the tissue to stain incorrectly is the ice cold ammonia water for about 10 to 15 minutes. It is necessary to dry the block off before you cut it or the sections will "dance" on your water bath due to surface tension.

COVERGLASS REMOVAL

Need to remove an old coverglass. The conventional way, was or is to put the slide into Xylene and let it soak...for 3-4 days sometimes. It is important here to note, you **cannot** do this with the coverslipping film it is plastic and will melt. Put the slide in direct contact with a hot surface, 75-100 degrees, a bacti-incinerator works well if available. Warming the back of the slide leave it in contact for 30 seconds to a minute, the mounting media will become soft enough so that the coverglass can be very carefully moved off of the slide. The coverglass must be removed before the slide goes into Xylene, otherwise the cold Xylene will harden the mounting media and you have to start again.

WRINKLES IN THE TISSUE ON THE WATERBATH

I heard this next one from someone tried it and it works MOST OF THE TIME.

If a tissue section has minor wrinkles and you really really need that section, put a drop of 95% alcohol on the slide before you pick up the section it will create a small wave/ripple affect and smooth out the small wrinkles.

NOTE: It does not work with folded sections.

REPEATING SPECIAL STAINS

Last item I would like to share. Special stains-If your special stain does not work, you can re-stain it using the same slide **ONLY** if the special stain does not involve oxidation or reduction steps.

Just hoping you may have found something you didn't know and there is something you can use.

Remember, "The best fun is work; if it isn't fun it's work."





Call for NYSHS Nominations Awards for 2016

To be eligible, please send an application to the Awards Chairperson specifying the award you are applying for and include the following items: A completed application with name and address of your current employer or school, your current mailing and email address and a brief paragraph showing evidence of your commitment to the profession. One letter of recommendation from a supervisor, pathologist, histotechnologist or professor. *Please specify which awards you are applying for in your application.*

You must be a member in good standing (the current year). We prefer email submission of applications and letters of recommendation. Awards applications are available on the [NYSHS website](#) Please download the application below and send completed application and supporting documentation to the awards chairperson:

Applications are due March 31st, 2016.

Dominic Europa Award



2016 Corporate Educational Scholarships



Gulf Coast Instrument Company Scholarship:

Is sponsoring two \$250 scholarships. The award is presented to a histology student or a histotech.

(Continued on page 10)



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Mercedes Medical Scholarship: The award is presented to a histology student or a histotech who wishes to attend a professional meeting. The **\$250** award is sponsored by Mercedes Medical.



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Tissue Processing Protocols

To ensure the quality of microscopic slides for patient diagnosis, many variables & parameters must be explored

By M. Lamar Jones, HT(ASCP)

Processing of tissue specimens for patient diagnosis has been somewhat overlooked in many ways. What actually goes on inside the tissue processor? What does each of the processing reagents do to the tissue? Which instrument platform is recommended? What processing cycle should be used? What tissue processing workflow is needed and how should it be implemented?

Principle

Tissue processing is the next step after fixation. The tissue sections will go through the process of dehydration, clearing and infiltration, exposing tissues to various chemicals for certain processes. During tissue processing, one step will build upon another. Although many references include the embedding process in tissue processing, embedding is really a separate process after paraffin infiltration.

Dehydration

After proper fixation, the tissue sections begin the process of dehydration, or the gradual removal of water. At the molecular level, water is present in free and bound tissues. The free water is removed; dehydration is accomplished by using graded alcohols.

During tissue processing, many cellular entities or structures are also removed such as fats, lipids (by the higher grade alcohols) and proteins (by the lower alcohols). Particular dehydrating reagents can be used to help with tissue shrinkage. Most dehydration processes usually begin in 70% alcohol, then at least two stations of 95% and at least two stations of 100% alcohol. Thorough and complete fixation must occur before the tissues are submitted to processing such as dehydration. If tissues are poorly fixed, the alcohol will continue the fixation process only to create possible artifacts and alter morphology.

As well, the time that tissues should remain in the dehydrating reagents should be tested and validated. Biopsies should be validated on a short processing cycle, whereas the larger tissues should be on at least a 10-12 hour processing cycle or a rapid processing cycle. Thin tissue sections (1-2 mm in thickness) usually require about 30 minutes per station on a conventional processor. Thicker tissue sections (3-4 mm in thickness) usually require about 60 minutes or more per station on a conventional processor.

For most histology laboratories, either ethyl (ethanol), isopropyl (isopropanol) or a "reagent grade" alcohol is preferred. Ethyl alcohol can cause hardening and brittle effects on collagen, connective tissues, colloid tissues and even bloody tissues. Isopropyl alcohol is a great substitute for ethyl alcohol in tissue processing and actually hardens tissue less than ethyl alcohol. Isopropyl can be used as a clearant and tissues can go directly to paraffin.

Acetone is a rapid dehydrate and can cause some shrinkage if the time is not controlled. Tissues that contain a lot of fats and lipids can be dehydrated with acetone. Acetone can also be used as a clearant.

Clearing

Clearing is the removal of the alcohol after dehydration and serves as a transition step between dehydration and paraffin infiltration. The term clearing is derived from the fact that some solvents have a high index of refraction and actually render tissues "clear" or transparent, which is the clearing endpoint. Xylene is the most commonly used hydrocarbon for the clearing station in conventional tissue processing. Some xylene grades contain benzene; xylene for routine histology should be benzene and sulphur free. The time that tissue remains in xylene should be controlled to -eliminate over-



hardening of the tissues.

Xylene substitutes work well, although they do not tolerate water. As a rule, the xylene substitutes must be used in a series of three stations on the tissue processor. Xylene substitutes can be slow but offer good results as a clearant

Infiltration

Infiltration, sometimes referred to as "interpenetration," is the final stage of tissue processing prior to the embedding step. Infiltration is the submerging of tissue into a medium that will internally support tissue spaces and cell walls for internal support during microtomy. For most routine histology tissue processing, a fine grade of paraffin is used. Paraffin is a petroleum-based substance that is crystalline in structure. The melting points of paraffin can range from 38-68° C depending on its use. Additives such as rubber, bayberry, beeswax, stearic acid, resins (piccolyte 115), and plastic polymers (dimethyl sulphoxide - DMSO) assist with the sectioning process in microtomy. For histology purposes, the paraffin is usually melted 2-3°C above the melting point.

Parameters

- *Agitation:* During the tissue processing cycle, there must be some type of fluid agitation to move reagents through and around the tissue sections. Methods range from the up and down motion, magnetic stirrers in the processing retort, to fluid exchange. In recent years, the pressure and vacuum technology provided unique agitation of reagents for improved tissue processing. Ideally, tissue cassettes should be arranged fairly loose in the processing rack to allow thorough reagent flow in, around and through the tissue cassette.
- *Heat:* The application of heat during the dehydration and clearing steps of tissue processing will considerably reduce processing times. Care must be taken to maintain the heat temperatures no higher than 37°C, as high temperatures may affect immunohistochemistry. The temperature for the paraffin infiltration step should be no more than about 2-3° C above the melting point of the paraffin. If the paraffin temperature exceeds the recommended temperature settings, shrinkage, hardness and brittleness will also occur in the tissue sections. Paraffin temperatures of the tissue processor paraffin tanks must be taken and recorded at least daily.
- *Vacuum and Pressure:* Vacuum used during the dehydration and clearing stages of tissue processing is an advantage especially to porous tissues. Pressure can also add some advantage to the tissues in these stages. Together, vacuum and pressure can pull reagents through and around the tissues to insure more rapid and complete tissue processing.

Tissue Processor Platforms

Two tissue processing platforms are used: conventional and rapid (includes microwave processing). Conventional tissue processors are classified as the usual overnight processors, allowing 8-10 tissue processing programs, 12 or more reagent stations plus paraffin stations, choice of heat, vacuum and/or pressure and other options. Usually these were the carousel rotating processors later advancing to the enclosed processors. The fixation step can be delayed from a few hours to a few days.

Newer processors have two retorts available to operate two processing cycles at the same time for better utilization of the instrument, thus providing a Lean and Six Sigma workflow process.

Rapid (microwave) tissue processors have been available to assist with better turnaround time (TAT); many have been incorporated in the Lean and Six Sigma workflow processes. These require the change in daily application of tissue processing and many of these processors implement the "continuous flow" concept to handle the tissue specimens in a one-piece flow methodology. In most cases the tissues can be processed and slides prepared in the same day.



A few of these rapid processors incorporate at least two processing cycles to accommodate either biopsies or thin sections up to fatty or larger tissues. A reduction of reagents can be used and graded alcohols and xylene are eliminated. These instruments are truly tissue processors -- no online fixation or delay times. Special rapid processing reagents are used, even those to allow the fixation and processing of tissues for molecular assays. Controlled microwave technology is used in many of these rapid processors.

Quality Control

The tissue processing cycles and reagents require specific quality control (QC) measures. The proper changing or rotation of the processing reagents should be on a set schedule in most cases. Documentation of these changes or rotations must be recorded.

As well, a hydrometer must be used to check the strength of the diluted alcohols and recorded. Care must be taken to ensure that the correct reagent is poured into the correct reagent container to avoid processing errors. Temperatures of the paraffins should be checked and recorded daily. Preventative maintenance schedules should be established at least once or twice annually. The quality and even grade of the processing reagents should be adhered to for the proper processing of the tissues.

Validation

New tissue processor instruments are often installed, a processing program loaded and tissue specimens processed. However, there must be a validation process before a tissue processor is put into use to identify and record, for example, the tissue processor platform, what tissue types will be processed, which processing protocol or cycle will be implemented, technical training for each operator, the workflow process, reagents, processing cycles, etc.

One method to validate the tissue types is to use a multi-tissue block and variety of tissues in one block, all the same size. This is not perfect but can save time. If two processing cycles are available, both will need to be validated. The thickness also needs to be validated. Standardization will play a role in the validation process. Upon completion, the slides need to be reviewed by both technical staff and pathologists, then documented.

M. Lamar Jones is with Carolinas College of Health Sciences, Charlotte, NC; and Davidson County Community College, Lexington, NC This article has been reprinted with permission from ADVANCE Newsmagazines

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