A Process Review of the James Paton Memorial Regional Health Pathology Laboratory under the Central Regional Integrated Health Authority

on

October 3, 2008

by

Bryan R. Hewlett

James Paton Memorial Regional Health, Gander, NFL

A review of James Paton Memorial Regional Health pathology laboratory was conducted on October 3, 2008. The review was a process of observation, questioning of pathologists and technologists and examination of the resulting product at each of the different work areas in the histology laboratory. Particular attention was directed to the pre-analytic processes used to prepare a tissue specimen for sectioning prior to staining and examination.

The Histology laboratory policy and procedure manual was briefly reviewed. Based on the contents of the existing manual, a number of questions were developed to ask staff.

I found the pathologists and technical staff to be very enthusiastic and forthright in their answers and dedicated to the tasks they were performing.

Fixation

Observation of the specimen receiving area, specimen triage and the grossing of the specimens, along with a review of documentation leads me to believe that the issue of fixation has been partly addressed in principle. Specimens from the OR, an outlying small hospital and satellite clinics are delivered on a regular daily schedule. Breast samples are delivered immediately on procurement and lab staff is notified.

Breast requisitions are date and time stamped as to procurement and time received by the lab. Other specimens are not handled in this manner. It would be my recommendation to apply the same date and time stamping procedures to all specimens.

Specimens procured after hours are placed in fixative and stored at room temperature until the next day. This is suboptimal, if the specimens are small (1.0cm in largest dimension) they are best placed in formalin and fixed at room temperature, if the specimens are larger then the fresh tissue should be refrigerated and the pathologist on call should be notified to deal with them as soon as possible.

The fixation time of most specimens is now 24 hours or longer with the exception of a few 'Rush' specimens. The Pathologists are preparing larger specimens in such a manner as to optimize fixation prior to further grossing.

The procurement time, date and time received in the lab, time into fixative for fresh breast specimens, date and time of grossing are all recorded. This information provides documentation that is available to users of the tissue block for all future studies. This should be extended to include all specimens.

In addition, the date and time of tissue processing is subsequently documented and may ultimately used to determine total fixation time. This is in compliance with the existing Canadian Consensus Guidelines for HER2/*neu* testing and the soon to be published guidelines by the *Ad Hoc* committee on ER testing.

The 10% buffered formalin currently in use is a purchased product.

The purchased fixative formulation is not the conventional phosphate buffered formalin used and the pH is not stated on the product label. The constituents of the solution listed on the MSDS sheet supplied by the manufacturer are not the same as those listed in a conventional Sörenson's phosphate formalin buffer and nowhere is the final pH indicated. The pH is checked on opening the bulk container and recorded.

The Eastern Health group and potentially a Provincial purchase of large volumes of commercial formalin should allow for the specification of the formulation and pH by the purchaser. Requesting a conventional phosphate buffer with a pH of 7.2-7.4 would provide a product that is compliant with all current immunohistochemical antibodies and guidelines.

All grossing is performed by the pathologists, assisted by a technologist. CAP checklists are used for various specimen types for inclusion in a synoptic reporting format. Special instructions for the technologists are directly communicated and documented on process worksheets. In addition, diagrams are used as necessary. The technologist reports only occasional problems with tissue thickness. These occasional problems are immediately brought to the pathologist's attention.

Processing

There is some evidence that an occasional intermittent problem exists with the processing of the tissue blocks. A random review of large blocks in the storage files found only occasional blocks that showed evidence of inadequate processing. The tissue in these blocks was retracted from the surface and dry. This is indicative of tissue that was insufficiently cleared and/or infiltrated with paraffin. I did not find blocks containing smaller pieces of tissue that showed similar inadequate processing. This indicates that sporadically large tissue blocks were likely too thick to be adequately processed by the current routine processing schedule. It should be born in mind that such occasional events occur in most histology laboratories due to momentary lapses in judgment of tissue block thickness during grossing. Observation of the Pathologists during grossing of large specimens, showed the appropriate selection of tissue thickness being placed in cassettes.

Another artefact observed rarely in a few blocks was a very evident interface line separating the tissue and the surrounding supporting paraffin. This can occur if the tissue is allowed to cool or is drained of molten paraffin before being placed in the mould during the embedding process.

A review of the schedule used on the processor showed that there is an inadequate number (only 2) of final alcohols in the dehydration sequence. The sequence is 60%, 80%, 90%, 95%, and 100%. There are also only 2 xylene changes. It is recommended that the sequence be changed to match the suggested sequence for the St. John's laboratory (see appendix #1).

I observed that the racks loaded in the processors had the organized cassettes appropriately packed. The technologist reduces the number of cassettes in each compartment of the rack from 10 to 8. By providing more space between individual cassettes, this helps to ensure superior reagent flow around the tissue. The staff is still able to maintain the order of cassettes in the rack, an excellent QC practice.

Documentation of the processor's scheduled maintenance and reagent changes was available and indicated that this had occurred within the specified time limits. It is my opinion that the frequency should be increased slightly, since I detected the presence of xylene in the third and fourth waxes. Under normal usage these two waxes should be free of xylene contamination. This and the lack of a third 100% alcohol in the sequence could be causes of the occasional inadequate processing observed.

The embedding center in use had an empty heated holding well for the specimens. I recommend that the embedding center holding well be filled with liquid paraffin to hold all specimens during embedding. This will prevent the blocks cooling and liquid paraffin draining from the tissues. Maintenance of the liquid paraffin phase at the outside tissue surface during embedding will also prevent separation interfaces forming between tissue and the supporting paraffin.

At the microtome workstation it was noted that following section pick up on slides, the mounted cut sections were dried prior to staining in a domestic microwave oven. This includes sections that are cut to be sent out for IHC. This is of concern for IHC, since antigen degradation occurs more rapidly during transport of sections following high temperature exposure. The reference IHC lab should be informed of this practice. Their recommendations regarding section handling should be followed. It would be preferable to simply air dry IHC sections and allow the reference lab to dry them just prior to staining. Even more preferable would be to ship the blocks to the IHC lab for cutting.

The use of domestic appliances in laboratories is to be discouraged. These appliances are uneven and uncontrolled in their heating pattern. Only laboratory grade instruments should be considered.

Manuals and Documentation

The Histology laboratory policy and procedure manual was briefly reviewed. Although not in CLSI format, the older type procedure manual covered most basic technical procedures. A complete rewrite of this manual to current accreditation standards would take considerable time, effort and require substantial additional resources. In addition, an

overall laboratory quality system would need to be in place. This would be required if accreditation were to be pursued.

There is evidence of the use of this manual. The staff is aware of the manual and has also demonstrated their knowledge through application in several situations during my visit to the site.

There is evidence of some QC documentation throughout the work processes. A more complete documentation would be valuable, for example a microtomy worksheet with corrective actions taken.

The pathologist's check the control slides for H&E and special stains, but no record or corrective action documentation is kept. One of the most important QC checks in histology occurs at the H&E staining bench. The slides and blocks should be brought together for comparison after staining, to ensure that a complete section of the correct tissue is on the slide. This QC check is neither being performed nor documented at this time. The technologists do check the section for completeness during microtomy and that is appropriate. However, it is the check after staining that is more important.

There needs to be sign off sheets at all of the work stations assigning ownership and responsibility for the task. Where possible the specific case numbers should be listed. Corrective action record sheets should be also in use at the same workstations. The technologists are responsible for checking the quality of cutting and staining, together with troubleshooting and documenting any required remedial action.

An overall QA use of QC information is not evident. Valuable information needs to be collected and used to take corrective actions throughout the process in order to reduce the occurrence. The QA processing of the QC information, the trouble shooting and the ultimate corrective action should be assigned to a senior technologist position in the lab.

Staffing

Histology is a laboratory discipline that requires very specific skills that need time and practice to develop and hone. The current staffing levels appear to be barely adequate for the workload. It is encouraging that two additional staff members are undergoing training at this time. The role of the technologist's should be expanded to encompass all QC and QA activities. Continuing professional development for the pathologists and technologists requires more resources allocated and participation encouraged. It is also not too early to be developing a succession plan.

Summary

There has been a real effort in the front end of the histology laboratory to determine the best patterns of practice and implementation of these practices. Fixation time and the

related tissue sample thickness is just one application of this. This quality activity needs to be expanded to all areas of the histology lab.

Pathologists and technologists all expressed enthusiasm and genuine eagerness to learn about the rationale behind the latest practices and techniques of tissue handling that are so necessary to accommodate ancillary testing methodologies such as IHC.

Compliance

I believe that the laboratory's efforts to date, in regard to the handling of fresh breast specimens, fixation policies/procedures and grossing practices, places them in compliance with the important pre-analytic portions of the Canadian Consensus guidelines for HER2/*neu* testing, the ASCO/CAP guidelines for HER2 testing and the soon to be published *ad hoc* committee ER testing guidelines. With further modifications to the tissue processing and embedding protocols correcting any potential remaining processing insufficiencies, and consultation with the IHC lab regarding section preparation, the effects of poor tissue preparation on IHC testing will be minimized.

Appendix 1

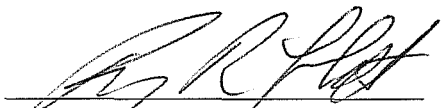
Routine Overnight Process Schedule

station	solution	concentration	Time in Minutes	Temperature °C	p/v	Mix	
1	formalin	10% NBF	60	37	off	fast	
2	alcohol	70%	45	37	on	fast	
3	alcohol	80%	45	37	on	fast	
4	alcohol	95%	45	37	on	fast	
5	alcohol	100%	45	37	on	fast	
6	alcohol	100%	45	37	on	fast	
7	alcohol	100%	60	37	on	fast	
8	xylene		45	37	on	fast	
9	xylene		45	37	on	fast	
10	xylene		60	37	on	fast	
11	paraffin		45	60	on	fast	
12	paraffin		45	60	on	fast	
13	paraffin		45	60	on	fast	
14	paraffin		45	60	on	fast	

Biopsy Program

station	solution	concentration	Time in Minutes	Temperature °C	p/v	Mix	
1	formalin	10% NBF	15	37	off	fast	
2	alcohol	70%	20	37	on	fast	
3	alcohol	80%	20	37	on	fast	
4	alcohol	95%	20	37	on	fast	
5	alcohol	100%	20	37	on	fast	
6	alcohol	100%	20	37	on	fast	
7	alcohol	100%	20	37	on	fast	
8	xylene		20	37	on	fast	
9	xylene		20	37	on	fast	
10	xylene		20	37	on	fast	
11	paraffin		30	60	on	fast	
12	paraffin		20	60	on	fast	
13	paraffin		20	60	on	fast	
14	paraffin		20	60	on	fast	

I believe my observations and the information presented in this report to be accurate and unbiased.



Bryan R. Hewlett, MLT, ART