A Process Review of the Charles S. Curtis Memorial Hospital Pathology Laboratory under the Labrador-Grenfell Regional Integrated Health Authority

on

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by

Bryan R. Hewlett

Charles S Curtis Memorial Hospital, St Anthony, NFL

A review of Curtis Memorial Hospital pathology laboratory was conducted on October 6 pm and 7 am, 2008. The review was a process of observation, questioning of technologists (unfortunately the pathologist was away) 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 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 only been partly addressed in principle. A new fixation policy has been written, but has not yet been implemented since certain unique and difficult physical circumstances apply. Specimens from the hospital OR are received on a regular basis throughout the day. Requisitions are date and time stamped as to procurement and time received by the lab. Breast samples are delivered immediately on procurement and lab staff is notified. Either the pathologist, or in his absence a trained technologist, will slice the tissue at appropriate intervals, insert formalin soaked paper towels and immerse the specimen in an appropriate volume of fixative for a minimum of 24 hours.

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.

Specimens from Labrador city and Goose bay are placed in fixative and delivered on an irregular daily schedule depending on availability of transport. This is appropriate for small (<1.0cm thick) specimens but completely unacceptable for larger specimens including breast. The larger specimens need very large containers of fixative and weigh a considerable amount when appropriately packaged for air shipment. Arrangements will have to be made to train appropriate personnel at these locations to slice the larger tissues appropriately, fix them for the correct period of time and then replace the fixative with a smaller amount for shipping. This will invariable prolong fixation past the recommended 48 hours in the fixation policy adopted from St John's. The policy will need to be re-written to accommodate these lengthened fixation times.

The fixation time of most specimens is now 24 hours or longer with the exception of a few 'Rush' specimens. The Pathologist and technologists 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. (Note: prolonged fixation times exceeding 72 hours will not meet the *Ad Hoc* committee on ER testing guidelines but will meet the Canadian Consensus Guidelines for HER2/*neu* 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 using inappropriate dipstick technology and recorded. There is no working pH meter in the laboratory.

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 of large and complex specimens is performed by the pathologist assisted by a technologist. When the pathologist is away, the large specimens are shipped out of province. The technologist grosses small simple specimens. 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 some problems with tissue thickness. These problems are immediately brought to the pathologist's attention.

Processing

There is evidence that an ongoing problem exists with the processing of the tissue blocks. A random review of large blocks in the storage files prior to June 2008, found numerous blocks that showed evidence of inadequate processing. The tissue in these blocks was retracted from the surface and dry. In some cases the retraction was severe. This is

indicative of tissue that was insufficiently dehydrated, cleared and/or infiltrated with paraffin. I did find a few blocks containing smaller pieces of tissue that showed similar inadequate processing. This indicates that larger tissue blocks were likely too thick to be adequately processed by the routine processing schedule. Examination of tissue blocks prepared since June, 2008, when the new tissue processor was installed, also found a number of large tissue blocks showing evidence of inadequate processing. Observation of the technologist during grossing of small 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 of graded alcohols in the dehydration sequence. The sequence is 70%, 80%, then a jump to 100%. There are also only 2 changes of the xylene substitute Safeclear II. This new processor also only allows three paraffin wax changes. It is recommended that the sequence be changed to match the suggested sequence for the St. John's laboratory (see appendix #1).

It was noted that a xylene substitute (Safeclear II) was being used for the clearing process. Although in itself use of such substitutes may be a more ecologically friendly approach and is to be applauded, such a change in process reagents may have an impact on subsequent IHC. The soon to be published *ad hoc* committee recommendations on ER testing state, that use of such substitutes needs to be cross-validated against material (100 cases) processed in the conventional way. The reference lab performing IHC needs to be informed of this and close cooperation would determine suitability of this process change.

I observed that the racks loaded in the processors had the organized cassettes appropriately packed and that this processor rack was fitted with coil wires to separate cassettes. By providing more space between individual cassettes, this ensures 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 not available as a hard copy. Instead the instrument log records this information electronically. Examination of the instrument log 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 safeclear in the third wax. Under normal usage the second two waxes should be free of clearant contamination. The instrument has facilities for connection of an electronic data storage device to back up stored data and hence a printout may be obtained of the process log. I recommend that these facilities be used.

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.

The laboratory has some new instrumentation with more apparently on the way, although how they will accommodate it in such already skimpy quarters is difficult to understand. The cramped, disorganised, non-ergonomic and frankly unsafe existing working environment of this histology lab needs to be rectified urgently.

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 technologist check's the control slides for H&E and special stains, documentation is kept in Meditech. This is an onerous task, given that the only microscope in the lab has a broken lamp housing, making it impossible to adjust for correct illumination. It also has insufficient objectives of the wrong magnification and is essentially unusable. The technologists are responsible for checking the quality of cutting and staining, together with troubleshooting and documenting any required remedial action. It is hard to perform that task with inadequate equipment. A new instrument is necessary.

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.

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.

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. The entire laboratory requires an updated quality assurance system, hospital administration needs to address this and provide resources appropriate to the importance of a quality regional laboratory service.

Staffing

Histology is a laboratory discipline that requires very specific skills that need time and practice to develop and hone. Only one technologist spends the majority of their time in histology. The other technologists rotate through all departments of the lab. The current staffing levels appear to be barely adequate for the workload. The technologists feel out of the loop and that they don't matter. The role of the technologists 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 some 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 on-site is just one application of this. The more difficult task relating to fixation at the distant sites still has to be addressed. This quality activity needs to be expanded to all areas of the histology lab and to the lab in general.
The 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, does not yet place 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, validation of the use of a xylene substitute and consultation with the IHC lab regarding other aspects of tissue preparation, the effects of poor tissue preparation on IHC testing will be minimized.

Appendix 1

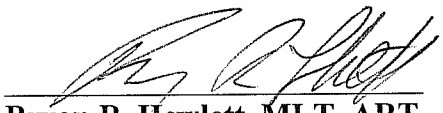
Routine Overnight Process Schedule

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