Quality Review of the Immunohistochemistry Laboratory Health Care Corporation of St. John's

Prepared For:

Leadership Team of the Laboratory Medicine Program
Health Care Corporation of St. John's

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1. Introduction

1.1 Executive Summary

The Immunohistochemistry testing performed at the Health Science Center, Health Care Corporation of St. John's was questioned in May 2005, when a change in a patients ER/PR Receptor status occurred.

The inconsistencies associated with formalin fixation, tissue processing and pretreatment methodology compounded by a rotating staff directly affected the repeatability of the Immunohistochemistry results. Also, the lack of Standard Operating Procedures across the Pathology Laboratory contributed to the problem. The high degree of proficiency required to perform, evaluate and interpret Immunohistochemistry testing must be assessed, as both the Technical and Professional staff are accountable for the final outcome. The aforementioned is critical because the reproducibility of Immunohistochemistry results has a direct impact on the Clinicians' therapeutic decisions especially with respect to ER, PR and HER 2-neu.

1.2 Background

The Surgical specimens under review for ER/PR testing were procured, grossed and processed at either the Health Science Center (HSC) or the St. Clare's Mercy Hospital site of the Health Care Corporation of St. John's. All Immunohistochemistry (IHC) testing is performed at the Health Science Center.

From 1997 until 2004 the semi-automated DakoCytomation Autostainer was used for IHC Staining. A Heat Induced Epitope Retrieval pretreatment using Steam was performed manually outside the Autostainer and concentrated Primary antibodies were diluted at the discretion of the Laboratory.

In 2004 the DakoCytomation Autostainer was replaced with the automated Ventana BenchMark IHC/ISH Staining Module which incorporates onboard Antigen retrieval. Predilute antibodies from Ventana were purchased to replace some of the concentrated DakoCytomation Antibodies. IHC is provided by a rotating Registered Technologist staff.

Prior to January 2003, the Formalin fixative was prepared in-house at the HSC and distributed, however, no documentation concerning the pH of this fixative was found. It has since been replaced with commercially prepared 10% Neutral Buffered Formalin fixative.

1.3 Objective

The objectives of this review were as follows:

1. Review the current practices and procedures within the IHC service.
2. Identify issues of concern which may have contributed to the present situation.
3. Provide recommendations pertaining to issues identified.

1.4 Scope

This review will be focused on the Immunohistochemistry service and the procurement and processing from both sites, as tissue fixation and processing provide the basis for accountable Immunohistochemical staining.

1.5 Methodology

Individuals were interviewed to obtain pertinent retrospective and/or current information – Registered Technologists, Pathology Manager, Laboratory Director, Chief Pathologist and Pathologists. The interviews took place from September 20 – 23, 2005. An initial review of the work processes was done.

The intent of this review is strictly professional, and to share relevant knowledge and expertise. All recommendations from my observations are based on College of American Pathologists and Ontario Laboratory Accreditation standards.

2. Histology Laboratory

2.1 Fixation/Grossing

Presently for large Surgical specimens, fixation, grossing and processing are site dependant. Problems with fixation have been identified with breast tissue samples in particular, and those blocks were subsequently reprocessed. The majority of the Breast cases originate from the St. Clare's site. The lack of consistency with fixation and the issue of reprocessing both have had a direct effect on the staining outcome with IHC.

A courier system is in place which transports biopsy specimens to the HSC for grossing and processing from St. Clare's. All embedding, cutting and staining is performed at the HSC on the biopsy specimens, the slides and blocks are couriered back to St. Clare's upon completion.

Frozen sections are performed at both sites. I did not have a chance to review the Quick Section room at HSC site but did at St. Clare's, where the OR Nurse Manager explained how the specimens are handled. Noted however, refrigeration storage is not available for large or unfixed specimens after hours.

Very informal protocols and documentation exist in either laboratory. Procedure Manuals detailing the Standard Operating Procedures do not exist at either site.

Recommendations:

1. Standard Operating Procedures relating specifically to the Grossing/Fixation Procedures must exist for each tissue type to ensure the reproducibility and reliability of results.

2. Three Pathology Assistant positions to be created to facilitate this, rotating between the 2 sites.
   The PA's would be responsible for Quick Sections.
   Autopsy coverage would be arranged on an on need basis with HSC – Pathology Manager.

3. Refrigerator to be available in the Quick Section rooms.

2.2 Processing

Currently each site maintains a Tissue Processor. All specimens grossed each day are placed in the tissue processor at that particular site with the exception of biopsy specimens. The biopsy specimens are handled at the HSC site.

Procedure Manuals outlining the Standard Operating Procedures for the Tissue Processors were not found. Neither signed documentation for the daily maintenance of the processors nor temperature monitoring of the Paraffin wax was found.

Recommendations:

4. To ensure that the Tissue Processing is reproducible, it should be amalgamated to one site. The St. Clare's Tissue Processor should be relocated to the HSC site.
   A Laboratory Assistant to document and maintain the cleaning schedules at one site for the equipment, eliminating duplication.

5. Signed daily cleaning and maintenance schedules for the Tissue Processors must be maintained and retained. Temperature of the paraffin wax must be recorded daily – this is especially important as some antibodies are heat labile.

6. Standard Operating Procedures to be written for the Tissue Processing including identifying different processing, cleaning and maintenance schedules, directions to load and unload the processors, editing and copying the programs and a STAT case policy. Please note this is not a mutually exclusive list.

7. All blocks to remain at HSC site.

8. Couriering of stained slides to remain status quo.

3. Immunohistochemistry Laboratory

3.1 IHC Staffing

Presently three Registered Technologists rotate through the IHC area with an additional Registered Technologist performing the microtomy. Approximately 80 Immunohistochemical tests are performed daily with the majority done on the automated Ventana Benchmark IHC/ISH Staining Module. The HER 2–Neu and Immunofluorescent staining are performed manually on the workbench.

There is no Medical Section Head for the IHC Laboratory. Each Pathologist requests their Immunoperoxidase stains on a request form. Request forms originating from St. Clare's are faxed to HSC. However, any follow up is communicated with the Technologist in the IHC Laboratory for that particular day, or the Pathology Manager.

Both the Technologists and the Pathologist's I spoke with are frustrated with the present situation as there are no clear lines of communication. In addition, the Technologists are overwhelmed as they do not completely understand the theory of IHC and this testing requires high technical proficiency to troubleshoot the methodology.

Recommendations:

9. Three Technologists to be dedicated to perform IHC staining, optimization and validation. The microtomy function to be evaluated at the Pathology Managers' discretion.

10. One of the three Registered Technologists' is to be given the additional responsibility and title to oversee the IHC Labaoratory. The classification to be determined by the Pathology and Laboratory Manager.

11. A Medical Section Head to be designated and responsible for the IHC Laboratory.

    A backup Designate should be selected for the unavoidable times when the Director is not present. This would assist with Succession planning.

12. Lines of authority, communication and organization to be determined prior to establishing these positions.

The Technologist responsible for the IHC Laboratory will report to both the Pathology Manager and the Medical Section Head depending upon the issue at hand.

The Pathologists need to establish a mechanism as to how their IHC concerns/issues will be addressed. Documentation of all concerns/issues must be maintained with the corrective action.

13. Succession planning to be evaluated at both the Technical and Professional level.

3.2 IHC Documentation

Documentation in general was deficient.
Key areas identified in IHC:

- No Test Procedure manual including Standard Operating Procedures.
- Lacking organized current Manufacturer Specification Data Sheets.
- Minimal record keeping of daily/weekly/monthly equipment maintenance.
- No Pipette and thermometer calibration of accuracy.
- No reagent, antibody or detection system evaluation & no validation documentation.
- No equipment documentation and no calibration records
- Retirement of procedures or antibodies not documented
- Retention of records not done.
- Lacking completed Error log and Corrective action
- No Distilled Water monitoring and documentation.

Recommendations:

14. A Procedural Manual outlining the Standard Operating Procedures is to be created outlining all methods and antibodies currently in use.
The manual should be written in compliance with the Clinical and Laboratory Standard Institute (CLSI) using GP2-A4 guidelines for Clinical Laboratory Technical Procedure Manuals.
The Procedure Manual must be available for reference at the workbench. It is to be approved by the Medical Director and reviewed annually by the Medical Section Head.

It would be prudent to send the Technologist responsible for compiling and maintaining the Procedural Manual to a computer course for Word and Excel programs.

Note:
The Ventana BenchMark Operator's Manual was available at the work bench. It is an acceptable component of the overall IHC departmental procedures but does not replace a Procedure Manual.

15. Antibody Data Specification Sheets to be available for all antibodies currently in use.

The Data sheets should be kept in alphabetical order and be available at the workbench for both Technical and Professional reference.
It would be prudent to maintain the specifics of the Lot Number in use, such as the Clone, Expiry Date, Storage conditions and Specific Staining Instructions with the Data sheet for easy reference.

16. Routine Equipment Maintenance must be performed and documented.

Internal Check:
The Ventana BenchMark Staining Module has a daily/monthly/quarterly Maintenance program.
The Registered Technologists currently perform the daily maintenance but do not always document it, this must change.

The quarterly maintenance program includes a computer portion which the Registered Technologist is unable to access. This documentation should then be provided from the Information Technology department and included with the Equipment Maintenance records.

The microscope must be set up for Kohler Illumination daily to ensure optimal resolution and contrast.

Laboratory personnel are responsible for the reliability and proper function of the instruments they work on. Hence, service and repair records or copies must be available to the technical staff operating the equipment. As well, an alternate protocol must exist in case of failure of the automated equipment.

External Check:
Annual Preventative Maintenance records must be maintained.
The Microtome, weigh balance, Microscope and any other piece of equipment not mentioned are to be annually inspected.

17. Guarantee Pipette and temperature accuracy and calibration.

The accuracy of the volume the pipette delivers must be guarded to ensure the reproducibility of the antibody dilutions.

A standard thermometric device or reference thermometer (NIST-certified or guaranteed by manufacturer to meet NIST Standards) is to be available to check thermometers used on all temperature controlled instruments.

Digital Temperature readings do not suffice and thermometers readings are to be recorded.

18. Documented evaluation must be performed to ensure the sensitivity and specificity of the test results. Each component used in IHC staining must be optimized and validated individually to ensure the outcome and to assist in troubleshooting. Presently this is not being done.

For all Detection System components (matched lot reagents must be tested in unison), Parallel testing of old vs. new lot suffices.

Reagents with a pre-defined pH should be monitored. The pH must be taken and recorded when a new lot is received.

Ensure all storage and handling conditions are met.

Records of reagent check records must be kept.

An inventory log would assist in record keeping.

Primary Antibodies
Pre-diluted and Concentrated
Any change in lot number or concentration, the specificity of the antibody must be verified prior to use.
The staining results should be compared to the previous lot using the appropriate controls.

The validation documentation must be approved and signed off by the Medical Section Head prior to use in Routine Service.
Stained Slides used for validation to be kept for reference.

19. Equipment selection criteria and calibration to be documented.

No documentation for the selection criteria used for the Ventana Benchmark IHC/ISH Staining Module.

For any new equipment or instruments purchased, Protocol and Records must be maintained of the Calibration, Performance and Functional checks and verification performed.

Evidence of training and competency of Technical staff on Equipment must be available.

20. Retired Procedures and antibodies information must be retained for 2 years and readily available.
It is recommended that these records are retained for longer for Reference.

21. All issues, concerns and corrective actions must be documented. This should be done by both the Technical and Professional Staff.

22. Distilled water is available on tap in the laboratory. Documentation of the suitable quality of this water for the testing being performed was not found nor was it known.

Type II water is used for microbiology media preparations, histology stains and dyes. reagents to be sterilized and reagents with preservatives. Testing and documentation is to include the tolerance levels of silicates, pH and organic contaminants.

3.3 Immunofluorescent Staining

Currently specimens sent for immunofluorescent staining are not retained after sectioning. The patient samples are left in the cryostat which renders them unusable the following day and they are discarded.

No controls are used and the immunofluorescent staining is performed in a lit environment.

The Fluorescent antibodies are stored correctly.

Recommendations:

23. All tissue samples must be retained for 2 weeks after the final report is issued, the specimen integrity must be maintained. The frozen patient tissue samples are to be labeled and stored correctly to avoid dessication in a frost-free freezer. Specimens will maintain their antigenicity longer when stored at -80°C.

24. Interpretation of every staining procedure must be substantiated with the use of controls, both positive and negative. Controls must be handled exactly as the patient test samples. It is also ideal that the control sections used have a composition similar to the patient samples being tested. Positive controls assure that the specimen staining was correctly performed while negative controls assess non-specific staining.

The use of frozen sections from previously confirmed positive and negative cases offers the easiest and most practical way to obtain controls for Immunofluorescence studies.

25. To obtain the brightest staining with the lowest background, Immunofluorescent staining should be performed protected from light.

3.4 Her 2- Neu Testing

Currently the HER 2-neu testing which provides predictive/prognostic information is performed manually using a kit. The manual pretreatment method for these markers is the same as was used in the past for the ER/PR testing. The issue of reproducibility and consistency is of concern.

Recommendations:

26. This methodology must be performed in the strictest of conditions. Automation of the procedure is suggested.

The variation in pretreatment between testing batches must be guarded to ensure that all test results are reproducible. Strict adherence to time and temperature must be maintained.

3.5 Controls

Currently positive tissue controls are placed on every patient tested slide. No negative controls are used.

No daily assessment and documentation of the controls is performed in the IHC Laboratory. Without assessing the controls, no troubleshooting of the procedure is occurring.

Presently, the onus is on the individual Pathologist to assess the control tissue with the patient sample. No documentation from the Pathologists was found.

Recommendations:

27. Negative reagent controls to be run on every block of patient tissue being tested.

28. Negative tissue control slides to be run for each antibody. A multi-tissue or "Sausage" block will serve this purpose.

29. IHC Registered Technologists to be trained to interpret and make an assessment of the quality and specificity of staining for every positive and negative control daily tested.

    Signed documentation of this assesment must be maintained.

    Also, a review of tissue recognition would be an asset.

4. Surgical Reports

Presently each site has their unique report Headings on the Surgical Pathology reports. Also, a standard reporting format was not identified.

Recommendations:

30. Standard Report Headings for both sites of the Health Care Corporation of St. John's.

31. Standard Reporting for all predictive/prognostic information. Investigate Synoptic Reporting capability with Meditech, Medical Information Technology, Inc.

32. All IHC tests providing predictive/prognostic information must include information in the patient report on specimen processing, antibody clone and the scoring method used.

5. Quality Assurance

Currently neither External Quality Assurance nor Inter-Laboratory comparison (excluding Mount Sinai Hospital Retrospective analysis) is performed.
No documentation was seen concerning Internal Quality Assurance for both the Technical and Professional components.

You need to ensure the quality of your Laboratory's results to determine the accuracy and reliability of the procedure. There must also be a mechanism to evaluate the interobserver variability amongst all the Pathologists interpretation of IHC staining.

Recommendations:

33. Membership in the College of American Pathologists.
Peer assessment/educational program.
Peer performance programs.

34. Inter/Intra Laboratory Comparison between St. Clare's and HSC Pathologists
Inter observer interpretation and quantification
(ER, PR and Her 2-neu)
Case/Control Review
Histology/Cytology Correlation

35. Proficiency Testing – Inter-Laboratory testing, develop a mechanism to compare results with other sites.

36. IHC user group
The ability for the IHC Registered Technologist to discuss issues and advances with their peers.

It would also be advantageous for the Technologists to attend any Medical Rounds relevant to the work they are involved with to understand the larger scope of practice.

6. Miscellaneous

6.1 Competency Testing

Presently no documentation exits relating to the training of staff to perform their assigned duties.
Competency Assessment for all staff is not performed in the Pathology Division, Laboratory Program. The Pathology Manager has begun to investigate this process.

Recommendation:

37. Staff must be provided with training and given a documented performance appraisal prior to working without supervision.

38. Staff to be assessed for competence to perform tasks following training. Identify requirements of a task, perform gap analysis and develop action plans.

6.2 Reference Tools

Presently the IHC Laboratory does not have the Internet available at the workbench.
No textbooks were found in the IHC Laboratory

Recommendation:

39. Internet availability as a reference tool at the workbench. Purchase Immunohistochemistry textbooks for the Laboratory.

6.3 Continuing Education/Conferences

The National Society of Histotechnology annual convention is an excellent opportunity for Histotechnologists to attend lectures and workshops devoted to Immunohistochemistry.

Recommendation:

40. Funding available for a IHC Technologist to attend the NSH convention.

6.4 Equipment Placement

The Automated Special Stainer is in close proximity to the Ventana BenchMark. Want to ensure that the equipment is not disturbed, it should be unobstructed.

Recommendation:

41. Relocate the Automated Special Staining equipment.

6.5 Safety

The Fume hood located in the IHC Laboratory had Acid bottles (Acetic, Formic and HCl) out on the enclosed work area.

Recommendation:

42. Acid Storage to be in a rigid leak tight safety cabinet which is able to contain an accidental spill.

6.6 Histocollimator

This product assists the Microtomist with aligning and orientating the paraffin blocks for recutting sections.

This device is available through ESBE Scientific, although I do not know if it will fit onto the Leica Microtome.

7. Resources

- Clinical and Laboratory Standards Institute
  GP2-A4
  Clinical Laboratory Technical Procedure Manual; Approved Guideline
  HS1-A2
  A Quality Management System Model for Health Care;
  Approved Guideline—Second Edition
  www.clsi.org

- College of American Pathologists
  www.cap.org

- The National Society for Histotechnology
  The Journal of Histotechnology
  www.nsh.org

- Applied Immunohistochemistry and Molecular Morphology
  Journal AIMM
  www.appliedimmunohist.com

- ImmunoQuery
  www.ImmunoQuery.com

Text Books/Handbooks

- Diagnostic Immunohistochemistry:
  David J., M.D. Dabbs

- Immunohistopathology: A Practical Approach to Diagnosis, 2nd Edition:
  Jules M. Elias

- Introduction to Immunocytochemistry
  J.M. Polak and S. Van Noorden

- Immunochemical Staining Methods Handbook, 3rd Edition:
  DakoCytomation

8. Conclusion

Rotating Pathology Assistants providing the grossing of large specimens would standardize the procedures between the two sites. Standardizing the fixation time of every specimen will have a direct correlation on the quality of IHC staining

The consolidation of Tissue Processing at the HSC site should be implemented. The consolidation would ensure that all specimens are processed in the same manner and that the Tissue Processors are maintained in the same way. Standardizing the processing will positively impact the IHC staining.

Procedure Manuals must be written; in particular for the Manual in the IHC Laboratory, the Standard Operating Procedures must address all methods and antibodies in use. Documentation must be done throughout the Laboratory.

Three non-rotating Registered Technologists to be employed in IHC, with one appointed a level of responsibility. These Technologists will be responsible for all aspects of IHC from validating all elements of every staining procedure to assessing controls.

A Director of the IHC should be appointed. A designate should be selected for when the Director is not available. The Director will assist in the validation process and will act as the conduit between the Technical and Professional staff.

It is essential that the Laboratory becomes involved in a peer assessment program.

Standardization of all processes will result in increased reliability and reproducibility of the IHC results.

The Pathology Laboratory of the Health Care Corporation is more than capable of attaining this degree of competency.