QUALITY MANAGEMENT PROGRAM - LABORATORY SERVICES

CONFIDENTIAL
ON-SITE CONSULTATION REPORT
NEWFOUNDLAND

Name of Laboratory:	Immunopathology Laboratory, Eastern Health Authority, St. John's, Newfoundland
Owner/Administrator:	Eastern Health Authority, Health Sciences Centre
Address:	300 Prince Phillip Dr. St. John's Nfld A1B 3V6
Telephone Number of Laboratory:	709 737 6400
Date and Time of On-site Consultation:	December 7, 2007
Team Leader:	Dr. Gregory Flynn, QMP–LS, Managing Director
Consultants:	Mr. Bryan Hewlett, QMP–LS, EQA Technical Co-Ordinator Ms. Laurie Mason, QPM–LS, Anatomic Pathology Committee Chair and Pathology Manager, University Health Network

1.0 QMP–LS performed an on-site consultation at the request of the Laboratory Manager, Barry Dyer; Laboratory Director, Terry Gulliver; and Dr. Nash Denic, Chief Pathologist of Eastern Health Hospitals.

2.0 **Type of Laboratory**: Immunohistochemistry Laboratory, Department of Pathology. This lab provides diagnostic immunopathology studies for all of St. John's, and is the regional centre for all breast cancer studies in Newfoundland and Labrador.

3.0 **Facilities/Space/Safety**: The Immunohistochemistry laboratory is adjacent to the histology and gross dissection laboratories. It is a self-contained laboratory performing the following functions:

a. Paraffin section microtomy for all immunohistochemistry staining
b. Automated staining area for routine immunohistochemistry staining
c. Frozen sectioning and manual staining for immunofluorescence studies
d. Consumables storage
e. Desk for clerical functions

Space is suitable to the functions performed. No safety concerns were identified during this visit.

4.0 **Laboratory Personnel**: Dr. Ford Elms is the pathologist with oversight responsibility for the immunohistochemistry laboratory. The lab is staffed by two full-time Medical Laboratory Technologists (MLT's) who have worked in this lab for several years. One is planning to retire in spring 2008, and the other within two years. In planning for these retirements, two additional MLT's are in full-time training in immunohistochemistry.

5.0 **Workload/Staff**: the facility reports an average of 1500 immunohistochemical slides per month. This includes surgical pathology, cytopathology and autopsy cases. An average of 75 slides per day is run on the 2 Ventana stainers, each with a capacity of 20 slides per run. This results in an average of 4 runs per day, in total. All slides for immunohistochemical staining are cut by staff in this laboratory, with a turn-around time of 12–24 hours to slide send-out.

Staff also perform the following functions:

- specimen procurement for on-site renal biopsies
- cut frozen sections on renal and muscle biopsies, and perform immunofluorescent studies on these cases
- cut and stain frozen sections for intra-operative diagnosis
- attend on-site fine needle aspiration procedures to receive specimens, a determination of specimen adequacy is not performed (Cytology lab and staff reside at another site)

Staffing levels are sufficient for the paraffin sectioning and immunohistochemical slide volumes performed, however the impact of the ancillary procedures performed/attended has not been quantified or included in this consultation. The turnaround time reported meets standard practice.

6.0 Equipment:

- 2 Ventana Benchmark stainers
- 2 Microm microtomes
- 1 Thermo-Shandon refrigerator
- 1 Cryostat (shared with histology)
- 1 Cytospin

The equipment in the laboratory is appropriately selected and maintained for the testing done. Maintenance procedures are performed on a regular basis by staff, and annually by the vendor where appropriate.

7.0 Technical Methods/Procedures:

Guidelines for formalin fixation of breast specimens is documented in the laboratory manual, however, the times cited do not specifically relate to points of handling in the system, and should be referenced to standards of practice. We recommend that the guidelines be more specific as to minimum time of fixation from procurement to chemical processing procedures, include references for formalin fixation duration (see appendix A), and be consistent with regulatory and best practice requirements.

This laboratory is a reference centre, receiving specimens from all over the province. We recommend the laboratory establish the effects of their transportation and fixation times against known positive material. In doing this the laboratory can respond to variances to ensure consistent testing outcomes. We suggest fixing known positive materials for the varying time periods experienced in their services. These controls can be run alongside similarly handled specimens for better quality control. This is particularly important for quantitative breast marker studies.

The automated immunohistochemistry procedures are performed using defined Ventana protocols. Breast cancer studies for estrogen receptor; progesterone receptor and HER2Neu have been validated by the laboratory through prospective parallel testing procedures, and through a retrospective review of previously stained cases. The staining process includes on-board steps for tissue pretreatment and staining. The current detection system utilizes the I-View kit from Ventana. All primary antibodies are purchased as pre-diluted products from Ventana.

Calibrated controls have been used for breast marker studies, and are reviewed by the pathologist with oversight for Immunopathology, Dr. Ford Elms. These controls should be used to assess the effects of any changes to the system, including new reagent lots, new controls, and antibody titration studies.

The technical methods employed for breast marker studies have undergone rigourous review. The specificity and sensitivity of these methods is considered by the laboratory to be consistent with that of Mt. Sinai Hospital in Toronto, their consulting laboratory in both the retrospective review and parallel prospective studies. We have no recommendations for change to this staining system. A suggested source of control tissue for low expression estrogen receptor positivity is myoendometrium.

We recommend that all system tests, calibrations and validations be done in a controlled step-wise manner, and that these steps and their outcomes be recorded. A process map, procedures and standard forms outlining this would be helpful to staff. Each validation protocol should be reviewed, and the subsequent steps approved by assigned authorizers. Findings and changes should be communicated to laboratory staff to ensure effective implementation.

Please see section 9.0 for Quality Control recommendations.

8.0 **Documentation/Record Keeping**: Slides run on the automated Ventana system are labeled with patient identification in both written and barcoded formats. The system tracks and retains a log of all steps performed to each slide in a run, maintenance performed, reagent inventory, and reagents used for each run. Print outs are available, some of which are printed out and retained in hard copy by staff. System back-ups are performed every 6 months.

Manual logs of reagent inventory are also kept for historical tracking. Manuals containing evidence of equipment maintenance are also kept.

We recommend that Ventana data back ups be performed on a daily or weekly basis to protect the integrity of the Ventana system information. Please see section 9.0 for Quality Control recommendations.

9.0 **Quality Assurance**

9.1 **Internal Quality Control**: Known positive control tissue sections are mounted on each patient slide and reviewed prior to release. Negative controls are prepared by using normal isotype serum on duplicate patient sections instead of primary antibody. Although the slides are reviewed, the interpretation of positive and negative controls is not recorded in the laboratory, or by the pathologist, and reviewed for quality assurance purposes. We recommend the laboratory staff document the control results as a cumulative record, prior to release, and that regular reviews be performed by a senior technologist trained for quality assurance purposes.

Positive control slides are not retained in the laboratory for quality tracking or teaching purposes. We recommend that a control library be created and referenced against documented results. This could also be a valuable teaching tool for newer MLT's, residents and pathologists. We also recommend that as new control blocks are obtained, they be tested and referenced to the current control material prior to being put in use. Each newly validated control should then be added to the library.

Controls for cytology specimens are not handled in a similar manner to the specimens tested by immunohistochemistry. We recommend that controls for cytology specimens be prepared, fixed and handled in a manner consistent to that of test specimens. The effects of fixation and handling should be considered.

9.2 **External Quality Assurance**: the laboratory participates in EQA programs provided by the College of American Pathologists (CAP), the United Kingdom National Quality Assurance System (UK-NEQAS), and the Quality Management Program for Laboratory Services in Ontario (QMP–LS). These are all voluntary programs.

Results for QMP–LS surveys are known to our team. In the May 2007 QMP–LS survey for estrogen receptor, CD3, CD20 and vimentin staining very good results were attained. Four surveys have been completed for NEQAS, and annual surveys for CAP. Staff indicate all results meet or exceed expectation.

Results of these surveys are not routinely reviewed with staff. We recommend that this be done to ensure staff may appropriately respond to issues if they arise, and as a valuable teaching tool for staff in training. We also recommend that digital pictures be taken of slides prior to submission so that results may be compared with images when reviewing.

10. **Manuals**: Manuals are available outlining analytical procedures, equipment maintenance and reagent inventory. Many analytical procedures have recently been written using new formatted templates, in accordance with the program in development for the entire laboratory system. The procedures are given unique numbers and titles, however the system does not prevent the inadvertent use of an un-approved procedure, nor the unauthorized duplication of a document.

    The document control system is undergoing change, and an electronic system to manage this is being considered. However several documents in the procedural manual show no evidence of review by the issuing authority, and do not have an issue date or version date on them. There are elements of an analytical procedure that could be included to assist staff in their work, including; quality control materials to be used, expected quality control outcomes, safety, related procedures and current references. We recommend these be considered, as appropriate, in document templates.

    Where applicable, procedures should reference current product inserts. These product sheets should be checked and reviewed for changes, then filed for reference. We suggest they be marked with the date put into, and taken out of service.

    Outdated documents should be removed from use and archived in a separate area.

11. **Education**: Records of staff continuing education sessions are maintained in the laboratory. Records of training are in development with the 2 new MLT's. A documented competency program is not in place, however elements of a program are practically conducted. We recommend that a formal program should be established and records kept.

    We suggest that interpretive microscope sessions between the pathologist and MLT's be a component of training. This is particularly important for the new staff in developing skills for interpretation of staining outcomes and troubleshooting methodology.

    Education programs for pathologists and MLT's may be difficult to obtain given the more remote location of Eastern Health, however we suggest that there are technical experts who could be approached to do sessions through various formats, including teleconference and telepathology.

12. **Textbooks, Journals and QMP–LS Literature**: Textbooks are available to staff. In addition, we suggest that the laboratory review materials available through the Clinical and Laboratory Standards Institute (CLSI) for guidance on laboratory testing standards. Because the laboratory participates in the EQA program through QMP–LS, they will also have access to on-line laboratory accreditation standards mandatory in Ontario laboratories. We recommend they access and review these as they develop their quality management system. Should a decision be made to pursue accreditation through this program these documents would be invaluable in their preparations.

13. **Other Observations**: The reporting of receptor status is performed by two pathologists who are at a separate hospital site (St. Clare's). It is not ideal to have reporting remote from the IHC laboratory, but it is a good practice to consolidate the interpretation. The pathologists have adequate

equipment to perform the task and participate in an exchange of samples with a reference laboratory for quality control. One pathologist keeps these QC records manually in a binder. It is recommended that there be sufficient support staff so that these records are maintained and held in a central office location.

14. **Summary of Consultants' Findings**: The QMP–LS team of consultants wish to thank the staff and leadership of the Eastern Health Authority Health Sciences Laboratory for the opportunity to provide an On-site Consultation of the Immunohistochemistry Laboratory. The staff were very welcoming and helpful. They openly discussed their current status and asked that we help them find ways to continue the process of improvement. The laboratory is functioning at a comparable level to similar labs in Ontario. The lab has a demonstrated commitment to external quality assurance and to service excellence.

    We appreciate the effects that physical isolation has on the maintenance of excellence. Opportunities for continuing professional and technical development through a variety of means could be considered. The laboratory could consider developing a relationship with other laboratories that have expertise in telepathology and conferencing by telephone or Internet. It may also be more economical to bring technical experts to the laboratory to share expertise with all staff as opposed to sending a few people from time to time.

    In conclusion the IHC laboratory is producing good results which would be interpretable anywhere. There are improvements that could be made to the selection, use, and recording of control material, but these are incremental in nature. The Administration should be confident that at this time, the IHC laboratory is operating a high quality controlled ER/PR program. Maintaining that quality requires professional and technical oversight on an ongoing basis, particularly as the IHC laboratory will experience significant turnover, and there is nothing that will change the geography of Newfoundland and Labrador. Communication and cooperation with the referring hospitals will also be required to promote standardization and ensure high quality results. Participation in External quality assurance programs is an essential part of monitoring such a sophisticated testing system that is sensitive to many potential variables.

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Appendix A: Penetration and Fixation Rates of formaldehyde

Formaldehyde is one of the most rapidly penetrating fixatives used. Unfortunately, it is one of the slowest to fix the tissue. This paradox was finally explained by Burnett[1] in 1982.

An excellent description of the properties of formaldehyde may be found in John Kiernan's book.[2]

The penetration rate of formaldehyde in mm/hr is a variable thing. It depends on how the data is obtained. It may also vary slightly depending on tissue type. The penetration rate of formaldehyde fixatives has been extensively studied, often with conflicting results. The penetration of non-coagulating fixatives is difficult to measure.

The original experiments of Medawar[3] utilized plasma clots with an indicator to mark depth of penetration. Medawar showed that fixatives obey the diffusion laws, that is, the depth penetrated was proportional to the square root of time. Medawar determined a coefficient of diffusibility for each fixative, the Medawar constant *K*.

Using the equation $d = K\sqrt{t}$, where *d* is distance penetrated in mm, *t* is time in hours and *K* the Medawar constant for the fixative in question, it is possible to determine the penetration rate.

Medawar determined *K* = 5.5 for formaldehyde. Using this value NBF would penetrate 27.5 mm in 25 hours. Plasma clots are easier to penetrate than solid tissues, so the rate is probably less.

Baker[4] chose a gelatin/albumen gel to more closely mimic solid tissue and determined

K = 3.6 for formaldehyde, or 18 mm in 25 hours. Baker also pointed out that the actual penetration into tissue would probably be less, possibly due to the resistance of lipid containing cell membranes. He quotes the data of Tellyesnicsky[5] who, mainly using liver tissue samples, indicated a more conservative *K* = 0.78 for formaldehyde. That would translate to 3.9 mm in 25 hours.

From $d = K\sqrt{t}$, it follows that fixatives penetrate more quickly into small samples of tissue compared to large ones. The initial rate of penetration into tissue is extremely rapid.

The first layer of cells (20 µm) takes a second or so (70 mm/hr). Using Baker's *K* =3.6, the following examples will illustrate further;

1 hour = 3.6 mm,
4 hours = 7.2 mm, averaged to (1.8mm/hr),
9 hours = 10.8 mm (1.2mm/hr),
16 hours = 14.4 mm (0.9mm/hr),
25 hours = 18 mm (0.72mm/hr),
100 hours = 36 mm (0.36mm/hr).

So much for the penetration rate, the real issue is the fixation rate, i.e. penetration rate plus binding time.

Fox et al.[6] used ^{14}C labeled formaldehyde to study the covalent binding time for rat kidney tissues. At a temperature of 25°C, the amount of formaldehyde bound to tissue increased with time until equilibrium was achieved at 24 hours. At 37°C the reaction was faster and equilibrium was reached at 18 hours. A later study by Helander[7] also used ^{14}C labeled formaldehyde to study binding time for the fixation of rabbit liver. At 25°C. equilibrium was achieved at 25 hours.

The correlation of results between these two studies is impressive. Particularly in view of the fact that Fox used **16μm** thick sections of fresh rat kidney, whereas Helander used **4 mm** cubes of fresh rabbit liver. The virtually identical equilibrium times achieved by each study indicate that penetration time is not a factor in the kinetics of the reaction. Despite the fact that thin slices of tissue will be penetrated faster than thicker cubes, it would seem that the binding time is the limiting factor for tissue stabilization. In a further study also by Helander,[8] using rat brain and kidney, equilibrium was not achieved until 50 hours. However, the tissue in Helander's latest study was twice the thickness (8mm) of the original study, a factor to be taken into account when comparing the data.

Failure to recognize the importance of formaldehyde binding time is the leading cause of the tremendous intra and inter- laboratory variability in immunohistochemical (IHC) performance. A clinical laboratory's so called 'routine formaldehyde fixation', actually consists of allowing the tissue to fix for variable periods of time, dictated by the start time of the tissue processor!

Formaldehyde fixes not by coagulation, but by addition, reacting with basic amino acids (primarily lysine and arginine) to form several adducts. These reactions are readily reversible by water and alcohol. These adducts have free hydroxymethyl groups which are capable of further reaction to form stable methylene bridges between proteins (see Kiernan[2] for more information). This type of cross-linking is responsible for the stabilization of proteins that we term fixation.

For both 1 mm thick core biopsies and 4mm thick tissue slices, the minimum stabilization time is 24-25 hours at ambient temperatures. The minimum stabilization time does not, unfortunately, denote complete fixation time. The initial cross-links are still relatively weak and easily reversible; stronger cross-linking continues to occur over time. Complete fixation is thought to take at least 7 days. Even after this time cross-links continue to form slowly.

Werner,[9] quoting the two papers above, considers cross-linking complete in 24–48 hours, but also expresses concern about the 'over-fixation' due to excessive cross-linking, which may occur if fixation is allowed to exceed 24–48 hours. I agree with Werner, in that cross-linking may mask some epitopes, but in my experience this does not occur with the vast majority of antibodies in use until 5–7 days of fixation. Even then, providing the IHC has been optimized, with the majority of antibodies, fixation up to 4 weeks is acceptable. The major strength of formaldehyde, as a fixative for IHC, lies in the fact that the cross-linking is 90% reversible. This reversibility allows the successful use of 'Antigen retrieval' techniques. Far more serious is the problem of short <24 hour fixation.

In aqueous solution, formaldehyde rapidly becomes hydrated to form methylene hydrate (methylene glycol).[1] The equilibrium of the reaction lies so far in favor of the hydrated form, that little(less than 0.1%) true formaldehyde is present.[6] The reactivity of aqueous solutions of formaldehyde is known to physicals chemists as an example of a "clock" reaction. The conversion of methylene glycol to formaldehyde by removal of the little formaldehyde present can be used as a "real-time" clock, measured in hours.[1]

Formaldehyde fixation begins at the periphery of the tissue. The initial layers of cells bind all of the available formaldehyde (<0.1%) and start the 'clock'. Methylene glycol continues to rapidly penetrate the tissue and, over hours, more formaldehyde is generated from methylene glycol. If this process is interrupted before completion, the formation of addition compounds will be incomplete, easily reversed and full stabilization by cross-linking will not occur. Depending upon the time of interruption, the periphery may show adequate cross-linking, whereas the remainder of the tissue is fixed by coagulant alcohol during processing. This may have disastrous effects upon IHC staining. This will occur whether the tissue is a small biopsy or a 4 mm slice.

References

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