BC Cancer Agency
CARE & RESEARCH
VANCOUVER

October 17, 2005

Dr. Donald Cook
Clinical Chief
Department of Laboratory Medicine
St. Clare's Mercy Hospital
154 LeMarchant Road
St. John's, NL
A1C 5B8

Dear Dr. Cook:

Re: External Quality Review of the Immunohistochemistry Service

Please find enclosed my report as requested. I would be happy to clarify any issues that may arise once you have had a chance to discuss my report with the Leadership Team of the Laboratory Medicine Program.

In addition, please convey to Dr. Robert Williams, Vice President, Medical Services, that beyond the specifics of my report, there should be recognition of the following issues that have bearing on the sustainability of a quality laboratory program:

- Pathologists' compensation should be competitive with those of other provinces, otherwise your department will face ongoing staff turnover as pathologists move to more rewarding positions elsewhere. Unless this "revolving-door" syndrome is dealt with, it will only lead to deterioration of the quality of staff as you will continue to lose your best people.
- For a high quality cancer program in the province, your department must invest in subspecialization, continuing education, and central pathology review for the entire province, in order to provide the highest quality of service and cancer diagnosis, so that your oncologists can manage their patients optimally. All cancer patients deserve the same standard of care regardless of where they live. Accurate pathology diagnoses, grading and staging are essential for clinical decision making and these activities cannot be compromised.

With the 2 recommendations implemented, you will be able to attract and retain the best pathologists.

I also enclose my expense receipts. I waive my consultation fees as a courtesy to a colleague.

Sincerely,

D. Banerjee, MB, ChB, FRCPC, PhD
Provincial Program Leader – Cancer Pathology
Director, Department of Pathology & Laboratory Medicine

Encl.

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External Quality Review of the Health Care Corporation of St-John's Laboratory Medicine Program's Immunohistochemistry Service

October 17, 2005

Dr. D. Banerjee

Provincial Program Director, Cancer Pathology

& Director of Laboratories

BC CANCER AGENCY

Clinical Professor of Pathology and Laboratory Medicine

University of British Columbia

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May 23/07

Background

In 1997, a Dako Immunostainer was installed for the immunohistochemistry service, replacing the testing of estrogen (ER) and progesterone receptors (PR) by the biochemical method.
This was replaced in 2004 with the Ventana Benchmark system with on-board deparaffinization and antigen retrieval.

The incident problem case:

The case is that of a patient with invasive lobular carcinoma whose tumour was tested in 2002 on the Dako Immunostainer and reported as negative for ER and PR and when retested in 2005 on the Ventana Benchmark, was strongly positive for both receptor proteins.

It should be noted that invasive lobular carcinomas are frequently ER+ (92%),[1] thus the initial negative result should have been questioned.

Four other patients previously tested as negative in 2002 were also retested, and all tested positive with the Ventana System. This led to a review of other (57) cases reported in 2002 as negative which on retesting on the Ventana Benchmark, resulted in a high conversion rate from negative to positive (38/57=67%).

Review of cases

I reviewed a number of cases from the retrospective testing set with Dr. Donald Cook. All of the cases that had converted from negative to positive by switching platforms had one or more of the following characteristics:

1. Poor fixation
2. Negative internal controls (normal ductal epithelium, when present, was completely negative).
3. Absent internal controls (no normal ductal epithelium present to evaluate).

It is apparent that too much reliance is being placed on external positive controls with no attention paid to internal controls.

Literature Review of Dako vs. Ventana Immunostainer Performance

A Medline search revealed no recent published studies comparing the Ventana Benchmark and the DAKO immunostainer. One study was published in 1998, but this was based on earlier versions of the two systems, and may not be relevant for the current discussion.[2]

Literature review about the effects of formalin fixation on ER immunostaining:

Fixation time in formalin does not affect ER results as long as 2 mm thick slices of tissue are placed in fixative within 15 minutes of surgical excision and adequate heat-induced antigen retrieval is performed.[3] However, the effects of under-fixation due to >2 mm thick slices remains a possibility. It should be noted that earlier literature on ER immunohistochemistry using frozen sections or cytology preparations indicates that inadequate fixation leads to loss of ER protein

through diffusion out of the cell nucleus.[4] It is possible that inadequate exposure to formalin would result in diffusion of ER protein out of the cell nuclei during tissue processing. No amount of antigen retrieval would have any effect if the protein has been completely lost during processing. Since the Ventana System did detect ER protein in previously negative cases, one must conclude that even if there was partial loss of ER protein due to poor fixation, the failure of the Dako system was largely due to inadequate antigen retrieval or inadequate antibody and/or detection system optimization, or a combination of these factors. It should be noted that many laboratories, including ours, use the Dako system successfully, having optimized all the steps. It remains possible that even with complete optimization of antigen retrieval and immunostaining protocols, if fixation is not optimized, there will be an irreducible number of false-negative cases. Thus the importance of proper fixation cannot be overemphasized.

Choice of antibody:

Although most laboratories are using mouse monoclonal antibodies against ER protein for ER IHC, the recent availability of rabbit monoclonal antibodies with superior staining performance suggests that the sensitivity of ER IHC could be improved even further, in some cases obviating the need for antigen retrieval. Rabbit anti-estrogen receptor monoclonal antibody (clone SP1) has reactivity even without heat-based antigen retrieval of fixed, paraffin-embedded tissue resulting in intense nuclear immunostaining with very low cytoplasmic staining. SP1 yields the same results as the mouse monoclonal antibody to estrogen receptor (clone 1D5), but the antibody affinity of SP1 is 8 times higher than that of 1D5.[5,6] The SP1 antibody results correlate better with patient response to tamoxifen than the mouse monoclonal antibody results (David Huntsman; personal communication).

Interlaboratory variability

A number of publications indicate poor concordance between laboratories for ER assays, especially for the weakly positive cases, and this is attributed to variation in antigen retrieval protocols.[7,8]

Conclusions about the reasons for test failure

1. Is the Dako system faulty? This is unlikely as there are many laboratories using the Dako system successfully. The reason for test failure was most likely due to a lack of test optimisation, including antigen retrieval method and antibody/detection system titration, as positive controls showed weak staining in general, and internal controls failed in all of the false negative cases.
2. Is the Ventana System too sensitive? There is no evidence that the Ventana system creates false positive results. However, the system still requires optimisation to avoid non-specific cytoplasmic staining.
3. Is there a problem with tissue fixation? There appears to be inadequate attention paid by the grossing pathologists to the thickness of tissue slices, quality and adequacy of fixation and there is no standardised fixation protocol that everyone adheres to.
4. Inadequate or no attention is being paid by the reporting pathologists to the status of internal controls, with inappropriately exclusive reliance on external positive controls. Negative test results in the absence of positive internal controls should have triggered

corrective procedures (optimisation of method, choice of a better fixed block, choice of a block with benign ductal epithelium included, etc.) and should not have been released without troubleshooting, and in the event that poor fixation resulted in internal control failure in all available blocks, this should have been noted in the reports as an uninterpretable cases due to failure or absence of internal controls.

6. Inappropriate choice of blocks with no representative normal ductal epithelium
7. Better education required for technologists, pathologists and clinicians about the pitfalls of IHC, the importance of quality control, and interpretation of IHC results.

Other System Flaws Observed:

1. Lack of dedicated immunohistochemistry technologists. A rotation system is used. This prevents the technologists from gaining in-depth expertise in troubleshooting.
2. Lack of an officially designated pathologist as director of immunohistochemistry service - technologists thus get conflicting feedback from a large number of pathologists. There is no accountability for the quality of the service.
3. Lack of standard operating procedures for grossing, fixation, tissue processing, block selection, positive control block selection, method optimisation through systematic titration, incubation time and antigen retrieval time for each analyte.
4. Lack of subspecialization amongst pathologists, leading to the lack of in-depth knowledge about IHC technical and interpretation details and pitfalls.
5. Disconnect between Laboratory Program Director, Division Manager, Clinical Site Chief, and Laboratory Director in decision making. The organizational charts indicate a complete separation of reporting structures into technical and clinical streams with no matrixed cross-reporting between technical and medical leadership. This leads to frustration and resentment on both sides, lack of communication, lack of accountability, and lack of buy-in. The Division Manager and Program Director appear enthusiastic and keen on modernizing the laboratory, but their efforts have not been appreciated by the pathologists and workflow changes have not been mapped out and implemented (e.g. Sakura Express implementation has failed due to lack of planning of workflow changes). Superior outcomes could be achieved by ensuring better linkages between technical, managerial and medical leadership.
6. Attendance by both medical and technical staff at various conferences with a focus on new technology should be encouraged. Consensus-driven innovation should be the goal.
7. The department needs dedicated Pathologist Assistants to ensure gross room consistency in tissue handling, trimming, and fixation.

Recommendations:

1. Pathologists should subspecialize if possible, covering 2 or more sites each, with one designated leader for each major tumour site.
2. One pathologist should be appointed Section Medical Director for the Immunohistochemistry Service, with a 3 Year term and defined performance expectations. All feedback from individual pathologists should be channeled through the tumour site pathologist leaders to the Section Medical Director, who would instruct technologists about method optimization, choice of antibodies, introduction of new antibodies, etc.

3. Consideration should be given to switching to the rabbit monoclonal antibody SP1 for ER IHC.

4. An appropriate number of technologists must be dedicated to the IHC service and be accountable to the Section Medical Director for all technical quality issues, test menu, choice of reagents and equipment purchase decisions must be based on appropriate discussion and consensus. Technologists should be capable of quality assurance of each staining run and not release slides if internal and external controls have failed. QA/QC failures noted by the reporting pathologists should be documented and reviewed periodically by the Section Medical Director, with corrective measures implemented as soon as possible.

5. Tumour site pathologist leaders must regularly attend appropriate educational and scientific conferences in order to stay current with standards of practice.

6. Pathologist assistants should be hired and trained in order to standardize and optimize gross room procedures and free up pathologists to complete microscopy and sign-out in a timely manner.

7. In order to allow implementation of the Sakura continuous flow tissue processing system, pathologists and technical staff should jointly redesign workflow practices.

8. The Ventana platform is performing adequately, and with improvement in and standardization of fixation protocols, there is no reason that the service could not be resumed without further delay.

9. The laboratory should subscribe to external quality assurance programs such as CAP (http://www.cap.org/apps/docs/proficiency_testing/histoqip_details.htm) or NEQAS (http://www.ukneqasicc.ucl.ac.uk/index.shtml) and should continue to monitor performance by interlaboratory comparisons with appropriate large volume teaching hospital laboratories in Canada of the US.

10. Consideration should be given to organisational chart redesign in order to provide better joint technical and medical accountability, planning, and communication.

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[1] J Clin Oncol. 2005 Jan 1;23(1):41-8.

[2] Clinica Chimica Acta 278 (1998) 185–192

[3] J Clin Pathol 1995;48:429-432

[4] J Clin Pathol. 1982 Apr;35(4):401-6.

[5] Appl Immunohistochem Mol Morphol. 2005 Mar;13(1):91-5.

[6] Am J Clin Pathol. 2005 Aug;124(2):295-302.

[7] Am J Clin Pathol. 2002 Nov;118(5):675-82.

[8] Am J Clin Pathol 2001;115:44–58.