Best Practice Recommendations for Standardization of Immunohistochemistry Tests

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# Canadian Association of Pathologists
## 60th Annual Meeting

### PROGRAM OUTLINE

#### FRIDAY JULY 10

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>1900 – 2100</td>
<td>Registration Open</td>
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#### SATURDAY JULY 11

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<th>Time</th>
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<tbody>
<tr>
<td>0730 – 1800</td>
<td>Registration Open</td>
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<tr>
<td>0830 – 1200</td>
<td>Morning Scientific Workshops W101-W105</td>
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<tr>
<td>1330 – 1700</td>
<td>Afternoon Scientific Workshops W201-W205</td>
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<tr>
<td>1800 – 2000</td>
<td>Residents' Symposium and Dinner</td>
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#### SUNDAY JULY 12

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<tbody>
<tr>
<td>0730 – 1800</td>
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<tr>
<td>0830 – 1200</td>
<td>Morning Scientific Workshops W301-W305</td>
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<tr>
<td>1330 – 1700</td>
<td>Afternoon Scientific Workshops W401-W405</td>
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<tr>
<td>1900 – 2100</td>
<td>President's Reception</td>
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#### MONDAY JULY 13

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<tbody>
<tr>
<td>0730 – 1800</td>
<td>Registration Open</td>
</tr>
<tr>
<td>0800 – 1130</td>
<td>S501 Symposium – Hematopathology</td>
</tr>
<tr>
<td>0800 – 1130</td>
<td>S502 Symposium – Cytology</td>
</tr>
<tr>
<td>1130 – 1230</td>
<td>S503 – CAP Junior Scientist Award Lecture</td>
</tr>
<tr>
<td>1230 – 1330</td>
<td>Lunch in Exhibit Hall</td>
</tr>
<tr>
<td>1330 – 1430</td>
<td>S601 – CAP William Boyd Award Lecture</td>
</tr>
<tr>
<td>1430 – 1530</td>
<td>Platform Presentations</td>
</tr>
<tr>
<td>1530 – 1800</td>
<td>Poster Presentations</td>
</tr>
<tr>
<td>1800 – 2000</td>
<td>Exhibitors’ Wine &amp; Cheese</td>
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<tr>
<td>1900 – 2100</td>
<td>Guillermo Quinonez Seminar on the Medical Humanities</td>
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### TUESDAY JULY 14

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<tbody>
<tr>
<td>0730 – 1800</td>
<td>Registration Open</td>
</tr>
<tr>
<td>0800 – 0830</td>
<td>Anatomic Pathology Section Annual General Meeting</td>
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<tr>
<td>0830 – 1200</td>
<td>S701 Symposium – Anatomic Pathology</td>
</tr>
<tr>
<td>1200 – 1330</td>
<td>CAP Annual General Meeting Luncheon</td>
</tr>
<tr>
<td>1330 – 1700</td>
<td>S801 Symposium – Advanced Diagnostics</td>
</tr>
<tr>
<td>1330 – 1700</td>
<td>S802 Symposium – Forensic/Neuropathology</td>
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<tr>
<td>1700 – 1830</td>
<td>S803 Symposium – Quality in Pathology</td>
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<tr>
<td>1900 – 2200</td>
<td>Gala Banquet and Award Presentations at Pier 21</td>
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### WEDNESDAY JULY 15

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<tbody>
<tr>
<td>0730 – 1100</td>
<td>Registration Open</td>
</tr>
<tr>
<td>0830 – 1200</td>
<td>S901 Symposium – Dr. Cam Coady Slide Seminar</td>
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### SCIENTIFIC WORKSHOPS

#### SATURDAY JULY 11

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>0830 – 1200</td>
<td>Morning</td>
</tr>
<tr>
<td>W101:</td>
<td>Interpretation of the Non-neoplastic Mucosal Biopsies of the Gastro-intestinal Tract</td>
</tr>
<tr>
<td>W102:</td>
<td>Case Challenges in Surgical Pathology</td>
</tr>
<tr>
<td>W103:</td>
<td>Hematopathology for the Surgical Pathologist – Potential Diagnostic Pitfalls</td>
</tr>
<tr>
<td>W104:</td>
<td>Laboratory Practice Management Skills 101</td>
</tr>
<tr>
<td>W105:</td>
<td>Benign Pediatric Hematopathology: Pitfalls and Pearls</td>
</tr>
<tr>
<td>1330 – 1700</td>
<td>Afternoon</td>
</tr>
<tr>
<td>W201:</td>
<td>Diagnostic Challenges in Surgical Neuropathology</td>
</tr>
<tr>
<td>W202:</td>
<td>Liquid Based Cytology: An Atlantic Perspective</td>
</tr>
<tr>
<td>W203:</td>
<td>The Role of the Pathologist in Solid Organ Transplantation</td>
</tr>
<tr>
<td>W204:</td>
<td>The Role of Immunohistochemistry in Investigating</td>
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<tr>
<td>W205:</td>
<td>Primary Unknown Malignancy</td>
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#### SUNDAY JULY 12

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<th>Time</th>
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<tbody>
<tr>
<td>0830 – 1200</td>
<td>Morning</td>
</tr>
<tr>
<td>W301:</td>
<td>Recent Advances in the Diagnosis and Classification of Kidney and Urinary Bladder Tumours</td>
</tr>
<tr>
<td>W302:</td>
<td>Glandular Lesions of the Uterine Cervix: Cytologic and Histopathologic Features</td>
</tr>
<tr>
<td>W303:</td>
<td>Pathology of Diffuse Non-Neoplastic Lung Disease</td>
</tr>
<tr>
<td>W304:</td>
<td>Breast Pathology: New Entities and New Insights into Old Entities</td>
</tr>
<tr>
<td>W305:</td>
<td>Recent Developments in the Surgical Pathology of Salivary Glands</td>
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SUNDAY JULY 12

1330 – 1700
Afternoon
W401: Forensic Pathology and Sudden Death – Looking Beyond the Standard Autopsy
W402: Fine Needle Aspiration Technique: “State of the Art” - Hands-on Workshop
W403: Controversies in the Diagnosis and Classification of Ovarian Tumours
W404: Diagnostic Approach to Selected Categories in Soft Tissue Tumours with Emphasis on Current Concepts: Interpretation of Tru-cut Biopsies and Various Immunohistochemical and Molecular Diagnostic Tests
W405: Standardized Handling and Reporting of Cancer Specimens: Colorectal and Endometrial Malignancies

SYMPOSIA AND LECTURES

SATURDAY JULY 11

Evening
S101 Residents’ Symposium

MONDAY JULY 13

Morning
S501 Symposium – Hematopathology
S502 Symposium – Canadian Society of Cytology
S503 Lecture – CAP Junior Scientist Award Lecture
Afternoon
S601 Lecture – CAP William Boyd Award Lecture
Evening
Guillermo Quinonez Seminar on the Medical Humanities

TUESDAY JULY 14

Morning
S701 Symposium – Anatomic Pathology
Afternoon
S801 Symposium – Advanced Diagnostics
S802 Symposium – Forensic/Neuropathology
S803 Symposium – Quality in Pathology

WEDNESDAY JULY 16

Morning
S901 Symposium – Dr. Cam Coady Slide Seminar
# BUSINESS MEETINGS

**Preliminary Business Meetings Schedule**

## SATURDAY JULY 11

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>0800 – 2200</td>
<td>Pathologists Assistants Conference Day and Dinner (offsite)</td>
</tr>
<tr>
<td>0800 – 2200</td>
<td>Pathologists Assistants Conference Day and Dinner (offsite)</td>
</tr>
<tr>
<td>1200 – 1330</td>
<td>Workshop Directors Lunch</td>
</tr>
<tr>
<td>1700 – 1800</td>
<td>Section of Anatomic Pathology Executive Committee</td>
</tr>
<tr>
<td>1700 – 1800</td>
<td>CPD Committee</td>
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<tr>
<td>1900 – 2200</td>
<td>Residents’ Section Symposium and Dinner</td>
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## SUNDAY JULY 12

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<tbody>
<tr>
<td>0800 – 1300</td>
<td>CAP Executive Committee, Working Lunch</td>
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<tr>
<td>0800 – 1000</td>
<td>RCPSC General Pathology Specialty Committee</td>
</tr>
<tr>
<td>0900 – 1100</td>
<td>Canadian Society of Cytology Executive Committee</td>
</tr>
<tr>
<td>1000 – 1200</td>
<td>RCPSC Anatomic Pathology Specialty Committee</td>
</tr>
<tr>
<td>1200 – 1400</td>
<td>RCPSC Anatomic and General Pathology Specialty Committees, Working Lunch</td>
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<tr>
<td>1200 – 1330</td>
<td>Workshop Directors Lunch</td>
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<tr>
<td>1330 – 1700</td>
<td>CAP Council</td>
</tr>
<tr>
<td>1700 – 1800</td>
<td>Section of Forensic Pathology Executive Committee and Annual General Meeting</td>
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<tr>
<td>1730 – 1830</td>
<td>Workload and Workforce Planning Training Session</td>
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## MONDAY JULY 13

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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>0700 – 0900</td>
<td>National Standards Committee</td>
</tr>
<tr>
<td>1030 – 1100</td>
<td>Section of Hematopathology Executive Committee and Annual General Meeting</td>
</tr>
<tr>
<td>1100 – 1130</td>
<td>Canadian Society of Cytology Annual General Meeting</td>
</tr>
<tr>
<td>1800 – 2300</td>
<td>Canadian Chairs of Pathology, Working Dinner</td>
</tr>
<tr>
<td>1900 – 2100</td>
<td>Guillermo Quinonez Seminar on the Medical Humanities</td>
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## TUESDAY JULY 14

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<tbody>
<tr>
<td>0700 – 0800</td>
<td>Journal Editorial Board</td>
</tr>
<tr>
<td>0800 – 0830</td>
<td>Section of Anatomic Pathology Annual General Meeting</td>
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<tr>
<td>1200 – 1330</td>
<td>CAP Annual General Meeting and Luncheon</td>
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## WEDNESDAY JULY 15

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<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>0700 – 0830</td>
<td>Media Training Workshop</td>
</tr>
<tr>
<td>0900 – 1100</td>
<td>Annual Meetings Committee</td>
</tr>
<tr>
<td>1300 – 1500</td>
<td>Public Relations Strategy Session</td>
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This image shows an invasive ductal carcinoma from a 53-year-old woman, 2.3 cm modified Bloom Richardson Grade III/III (no special type). The tumour was sentinel lymph node negative, ER positive (i.e., 95% invasive tumour cell nuclei stain positively using Ventana SP1), PR positive (i.e., 70% invasive tumour cell nuclei stain positively using Novocastra Clone 16), HER-2/neu negative by F.I.S.H.

Immunohistochemistry details: fixation used: 10% neutral buffered formalin; time to fixation: <1 hour; duration of fixation: 6–48 hours; Ventana Ultraview Detection System used; positive and negative external laboratory controls and internal normal control breast tissue stained appropriately. (Courtesy of Dr. Bruce Youngson, Department of Pathology, University Health Network, Toronto, Ontario.)
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Welcome to the second issue of *Canadian Journal of Pathology* and to the 60th Annual Conference of the Canadian Association of Pathologists. We hope that you enjoyed reading the introductory issue and will consider contributing to it. The second issue’s content is more in line with what will be the sort of material that you have been waiting to see, waiting to read in your journal, and the type of content that we envisioned when we began planning *Canadian Journal of Pathology*. Our specialty has been changing and continues to do so at an increasing pace. Whereas the complexity of pathology cases keeps on increasing, the number of pathology staff positions is not keeping pace with our workload. The complexity of reporting, of immunohistochemistry tests for many oncology cases, and of molecular diagnostics, as well as cytogenetics and similar studies keeps on increasing. These results are all correlated and collated and presented as a single diagnosis to our clinical colleagues by us, the pathologists. No wonder, then, that the same diagnostic results that may have taken a few minutes in the past now take considerably more time. Among the most striking examples of these are all neoplasms, and among these more specifically are breast cancers and prostate cancers today, and in the future perhaps all malignant neoplasms. Most of these biopsies presently are much smaller than in the past, and there are more samples of many areas of the organ, entailing many deeper sections, immuno stains, and a far larger number of slides per case than ever before.

The future looks challenging and exciting, with many changes predicted, some of which are here already. To borrow a phrase, “The future is here!”

The examination and study of tumours in other tissues has continued to increase. The evaluation of the sentinel lymph nodes has resulted in another significant load of work. Thyroid tumours that were once largely diagnosed on H & E stains today entail trays and trays of slides full of immunohistochemistry for most thyroid cancers. Other tumours are no exceptions. It is for these reasons that the complexity constantly increases; add to this the need to perform multiple deeper sections on each slide, and one realizes how time consuming each report has become. The increasing complexity of tumours and the ever-smaller endoscopic and needle biopsies of early lesions are pushing the need for subspecialization and subspecialty consultations. These also emphasize the need for continuing professional development or, as some call it, lifelong learning!

The first report of the National Standards Committee/Immunohistochemistry, specifically the standards for class 2 tests (FDA classification), which have such significant prognostic value for our patients with breast cancer, is released in this issue. This committee’s chairs, Dr. Emina Torlakovic and Dr. Blake Gilks, did tremendous work in organizing the first national conference on immunohistochemistry techniques (Vancouver, British Columbia, April 2–3, 2009). The conference, cosponsored by CAP and CPAC (Canadian Partnership against Cancer), had over 100 registrants, and the evaluations were uniformly positive. The plan is to organize more of these conferences, every 2 years. The next one will likely be held in Montreal.

The sudden appearance of the H1N1 virus in human beings in several countries has once again raised the spectre of serious viruses gone berserk, with the possibility of numerous deaths in this pandemic. Fortunately, laboratories today, especially since the SARS outbreak in our country and in others, have had improvements in equipment and in skilled personnel. The Canadian Public Health Institute in Winnipeg, Manitoba, was able to sequence the gene, and hopefully there will soon be a vaccine against this virus; although with this virus’s reputation to mutate and to cross species, causing symptoms in both species, it may be much more difficult to raise the vaccine. The virus continues to cause problems in many countries around the world, though it seems to be less virulent than anticipated, except in those with
other pre-existing illnesses.

As we are getting ready to go to press comes the news of potential breast cancer diagnostic problems in Quebec, perhaps similar to those that occurred in Newfoundland. These are related to estrogen and progesterone receptor testing. This raises the spectre once again of problems in pathology diagnoses, the media attention on laboratories, and the urgent need for uniform standards for laboratory accreditation, external quality assurance programs, appropriate equipment, and good staffing of laboratories across the nation – something that we have been pushing for some time now.

Residency programs in most provinces run well and produce pathologists with good training and good future prospects for themselves, their institutions, and the specialty. The Royal College specialty committees in anatomical pathology and general pathology have begun to take note of the need to respond to the changing trends in pathology training, pathology examinations, and residency requirements. With these changes in place, we should be able to produce the pathologist of the future – one who is well trained in immunohistochemistry and has a good knowledge of molecular diagnostics to be able to build on in the future as new molecular diagnostic tests arrive. We will also produce the pathologist who is well versed in the use of digital technologies and who will be able to work with the new paradigms in pathology diagnostics. The next generation of pathologists will soon, more than likely, become the pioneers of “Star Trek” medicine. They will have to learn and develop greater skills in working with others, especially those in similar diagnostic specialties such as microbiology, infectious diseases, and diagnostic radiology. They will also likely have to remain responsive to changes in the environment. The CanMEDS physician competency framework and the RCPSC Maintenance Certification Programs should make it easier to respond to future changes in our work environments. Will these programs be enough? Or will more be needed? At the least, we need new modalities of CPD, with a greater emphasis on user-driven learning programs.

All the new skills cannot be learned in the present residency program; in fact, residency training will continue to evolve and change with the times. Some of these additional skills, including diagnostic skills in subspecialties, will need the provision of greater funding for fellowships, with available positions in those areas of expertise and the desire among pathology residents and the employing institution to send staff for higher and subspecialized training. One of the options may be to revamp the pathology residency program, to make it competency based rather than numbers or time based. With the ongoing evolution of pathology diagnostics and their increasing complexity, the onus will be on us as individuals to constantly upgrade our skills.

Today, diagnostic radiology appears to play a more important role in diagnostics. These professionals seem to have an ever-increasing ability to electronically enhance what is seen and to offer a higher diagnostic speed, skill, and accuracy. We the pathologists, the makers of the definitive tissue diagnosis, must continue to work and evolve to allow this tissue diagnostic specialty to remain the gold standard for treatment to which all other modalities will be compared. This applies also to maintaining the quality of autopsy as of surgical pathology.

We cannot afford to abandon our position or even delegate our responsibility to our patients by allowing others to do what we do best, still wish to do, and have the greatest degree of expertise in. This does not mean that we must practise hegemony; however, we are the ones best trained and experienced in tissue diagnostics and who can, in fact, proffer the most accurate, appropriate, and timely diagnoses. We must continuously ensure that the results are the most accurate possible, given in the most appropriate time and with the appropriate amount of detail. In the absence of these, we may well run the risk of being sidelined in the future.

The role of laboratories, of pathologists, and of laboratory staff in maintaining standards and quality is unquestionable. Our reputation in this regard has until recently been second to none. However, the critical role of that large part of pathology, the autopsy, has been declining everywhere. This is unfortunate, and we must make efforts to reverse it. Its role in clinical quality, education, and training is unrivalled and must be maintained.

With this issue, I have fulfilled the responsibilities I undertook with regard to the Journal, namely to bring out a good and appropriate journal with a guarantee that the process will continue, and the hope that it will prosper and grow in the next few years. Under Dr. Godfrey Heathcote and his editorial staff, the Journal will, I am sure, flourish. (See “The Incoming Editor-in-Chief,” p. 32.) The new editorial board will be meeting for the first time in Halifax, Nova Scotia, and I am sure they will be able to resolve residual concerns and will, in fact, be the finest talent. The Publications Committee chaired by
Dr. Runjan Chetty will set editorial policy and be charged with maintaining continuity of editorial staff.

I take this opportunity to thank the members of the executive who have worked tirelessly with me over the past three years, those who while located at the college office have given their best and helped us unstintingly. The administrative staff at the college have consistently been working to the best of their capabilities and the best that anyone can demand of them. They are an integral and a very large part of CAP.

Every one of the members of the executive through these roughly three years has given his or her best for you, the members of CAP. I am indebted to each of them for their continued and unstinted support through these tumultuous times. I could not have asked for a better or more helpful team. In particular, I must thank Dr. Donald Cook, who, even though facing difficulties in the laboratories in St. John’s, nevertheless stayed on for more than 2 years past his term and did this graciously and pleasantly.

I take this opportunity also to thank everyone of you, the members of CAP, who have helped in whichever form, whether it be in speaking at short courses or workshops or participating in them as discussants, those who have spoken at the evening sessions, and those who are continuing to do so. All those of you who have attended these sessions have been a tremendous help since we could not have put on these courses and symposia without you.

Lastly, an ancient Chinese proverb that most of us are familiar with says, “May you always live in interesting times.” I can tell you honestly that the past 3 years have been interesting, and more. While I do wish that my successor too lives in interesting times, I do hope for all our sakes that the times will be less tumultuous.

I thank you all for having given me the opportunity to serve you for these past many months and the opportunity to be your president for the past 2 years and 10 months. I will always cherish these years and your support. You gave me and your executive the opportunity to do our best and to do what was necessary as events evolved. We all believe that we lived up to your expectations. I wish you all good health and every success.

I must thank a few others without those help it would not have been possible to spend the many hours I have on this aspect of my work. First among these are my wife and my family for their faith and boundless support. Next, my institution, from the president down to our technical staff and, most notably, my chief, Dr. Sylvia Asa, who often provided valuable criticism and advice. And, finally, my university department chair, Dr. Avrum Gotlieb, whose experience and expertise I was always able to call upon in times of crises.

I would like to welcome you to the 60th Annual Conference of the Canadian Association of Pathologists. We hope that the program that the Local Arrangements Committee, chaired by Dr. Laurette Geldenhuys and her entire hardworking group, as well as the Continuing Professional Development Group and the Annual Scientific Meeting Committee have prepared represents a whole new experience and the beginnings of a new annual conference. This year, we have tried ever harder to make this an annual meeting that you will not forget – a meeting that you will remember for its educational value and for the friendships you had, friendships you cemented, and new friendships you make here in Halifax. I am heartened by the increasing support that the resident members show by attending in good numbers. We are planning to change the format of the annual meeting based on the survey we undertook earlier this year. We thank you for having participated in the survey. Your responses are our guide to the format of future annual conferences.

Dr. Godfrey Heathcote has a team in place that will carry the Journal forward for the next 3 years. They include many from among you and others in this country who are leaders in your fields, all masters of our art and teachers to boot. In this issue, you will find letters from some young residents talking about their hopes, aspirations, and concerns. These views are coming from those who will be most directly affected by current changes and should be of significant concern to every one of us.

This has been an eventful period in my life and an eventful quarter since the publication of the last issue of the Canadian Journal of Pathology! We hope that you enjoyed the first issue, and will enjoy this, and others to come, even more.

Ever has it been that love knows not its own depth until the hour of separation.

– Kahlil Gibran

Jagdish Butany
Best Practice Recommendations for Standardization of Immunohistochemistry Tests

Canadian Association of Pathologists, National Standards Committee/Immunohistochemistry; Emina Emilia Torlakovic, MD, PhD; Robert Riddell, MD, FRCPath, FRCP; Bryan Hewlett, ART (CSMLS), MLT (CMLTO); Diponkar Banerjee, MBChB, FRCP, PhD; Hala El-Zimaity, MD, MS, FRCP; Gregory Flynn, MD, FRCP; Dragana Pilavdzic, MD, FRCP; Peter Dawe, MS; Anthony Magliocco, MD, FRCP; Penny Barnes, MD, FRCP; Richard Berendt, MD, FRCP; Donald Cook, MD, FRCP; Blake Gilks, MD, FRCP; Gaynor Williams, MD, PhD; Bayardo Perez-Ordonez, MD, FRCP; Bret Wehrli, MD, FRCP; Paul E. Swanson, MD; Christopher N. Otis, MD; Søren Nielsen, HT, CT; Mogens Vyberg, MD; Jagdish Butany, MBBS, MS, FRCP

Preface
Immunohistochemistry (IHC) and immunocytochemistry (ICC) assays are highly complex diagnostic analyses used to aid in the accurate identification and biological characterization of tissue types in neoplastic and non-neoplastic diseases. Although currently applied mainly to the diagnosis of neoplasms, some of these tests provide information of important prognostic and predictive value in selected human neoplasms, and as such are often critical for the appropriate and effective treatment of the patients. These assays require specialized training in the selection of the appropriate tissue fixation and processing, preparation of the IHC/ICC slides, selection of controls, pre-treatment, detection systems, reagents, and extensive training in test selection and the interpretation of results. This document addresses several topics relevant to test quality and recommends standards for appropriate IHC/ICC test development, performance, and interpretation in diagnostic pathology and laboratory medicine.

With the understanding that medical knowledge and technology are constantly evolving, these recommendations comprise current principles and best practices for quality assurance in clinical IHC testing that anticipate the need for expansion and improvement of test options. The recommendations seek to assist Canadian pathologists and clinical IHC laboratories in the development and introduction of appropriate quality assurance procedures to do the following:

- Promote development and implementation of standards for quality assurance
- Provide a standardized approach to tissue handling,

Emina Emilia Torlakovic, MD, PhD, is with the University of Saskatchewan, Saskatoon SK; Robert Riddell, MD, FRCPath, FRCP, is with Mount Sinai Hospital, Toronto ON; Bryan Hewlett, ART (CSMLS), MLT (CMLTO), and Gregory Flynn, MD, FRCP, are with QMPLS, Toronto ON; Diponkar Banerjee, MBChB, FRCP, PhD, is with PHSA Laboratories, Vancouver BC; Hala El-Zimaity, MD, MS, FRCP is with McMaster University, Hamilton ON; Dragana Pilavdzic, MD, FRCP, is with Sir Mortimer B. Davis Jewish General Hospital, Montreal QC; Peter Dawe, MS is with the Canadian Cancer Society, St. John’s NL; Anthony Magliocco, MD, FRCP is with the Tom Baker Cancer Centre, Calgary AB; Penny Barnes, MD, FRCP, is with the QEII Health Sciences Centre, Halifax NS; Richard Berendt, MD, FRCP is with the Cross Cancer Institute, Edmonton AB; Donald Cook, MD, FRCP, is with St. Clare’s Mercy Hospital, St. John’s NL; Blake Gilks, MD, FRCP, is with the Vancouver Hospital and Health Science Centre, Vancouver BC; Gaynor Williams, MD, PhD, is with the University of Manitoba, Winnipeg MB; Bayardo Perez-Ordonez, MD, FRCP, and Jagdish Butany, MBBS, MS, FRCP, are with the University Health Network, Toronto ON; Bret Wehrli, MD, FRCP, is with London Health Sciences Centre, London ON; Paul E. Swanson, MD, is with the University of Washington Medical Center, Seattle, WA; Christopher N. Otis, MD, is with Baystate Health and Turfts University School of Medicine, Springfield, MA; Søren Nielsen, HT, CT, and Mogens Vyberg, MD, are with the Institute of Pathology, Aalborg Hospital/Aarhus University Hospital, Aalborg, Denmark.

Correspondence may be directed to Emina Emilia Torlakovic, MD, PhD: emina.torlakovic@usask.ca.

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test performance and test interpretation

- Facilitate introduction of newly developed quality assurance frameworks and maintenance of stringent standards for test performance and interpretation for prognostic and predictive tests, the results of which are used for stratification of patients for appropriate therapies

- Increase public and professional confidence in the quality of immunohistochemical testing

These recommendations for quality control (QC) and quality assurance (QA) in clinical IHC provide the basis for reasoned QC/QA in the clinical IHC laboratory and to ensure both the accuracy of the tests and their interlaboratory reproducibility. This document addresses almost exclusively indirect IHC methods performed on formalin-fixed/paraffin-embedded tissues used by most practicing anatomic pathologists and hematopathologists.

This document includes recommendations on good laboratory practices, designed to ensure that appropriate quality processes are considered; It does not prescribe the use of specific reagents, methodologies, or laboratory equipment.

In summary, this document provides a framework for laboratory measures for clinical IHC, which should be of help to pathologists and medical laboratory technologists, as well as organizations involved either in the development or the implementation of laboratory quality assurance programs for the practice of clinical immunohistochemistry.

The Canadian Association of Pathologists is neither a regulatory nor a licensing body. These standards are proposed and recommended for use as one of the tools to achieve the above objectives in the area of high complexity laboratory testing.

Use of Standard Terminology in Clinical Immunohistochemistry

The use of standard terminology will improve communication between pathologists and laboratory staff and ensure appropriate test classification, which will further determine the level of appropriate quality control (QC) and quality assurance (QA) measures that need to be implemented by clinical IHC laboratories.

Proposed Terminology

Clinical IHC laboratory – Any diagnostic pathology laboratory employing immunohistochemical tests for the purpose of diagnosing and/or characterizing human disease, the results of which will be evaluated by a pathologist and incorporated into the pathology reports.

Immunohistochemical test (IHC tests) – Tests that employ immunoaassays to produce colour change colocalizing with an epitope of interest in tissue sections. IHC also encompasses testing on cell blocks or clot specimens prepared from cytologic and hematologic materials. While the majority of the IHC tests use immunoenzymatic detection methods and in particular horse radish peroxidase (HRP) and the chromogen diaminobenzidine (DAB) to demonstrate a positive reaction, IHC tests also may utilize other methods of detection (e.g., immunofluorescence, alkaline phosphatase, and others).

Immunocytochemical tests (ICC tests) – Tests that employ immunoassays to produce colour change colocalizing with an epitope of interest in cytological smears, cytospin preparations, or monolayer preparations. ICC tests often use alkaline phosphatase-based detection systems (with red chromogens), although peroxidase-based techniques are also commonly employed. Since processing of cytological samples is often substantially different than in IHC tests, ICC tests require different QC/QA measures with an emphasis on the use of appropriate positive and negative controls prepared under the same conditions.

Pre-analytical variables of IHC tests – Any and all steps in tissue processing, including intraoperative tissue handling/treatment (prolonged ischemia, delayed fixation, etc.), type and length of fixation, decalcification, and elements of tissue handling. The pre-analytical component is concluded at microtomy and the placement of the tissue section on pre-treated glass slides.

Analytical variables of IHC tests – The analytical variables phase begins with the handling of the cut slides in a clinical IHC laboratory. It is completed with the cover-slipping of the stained slides.

Post-analytical variables of IHC tests – Interpretation and reporting of the results, which also includes interpretation of positive and negative control results.

Class I IHC/ICC tests – Any IHC or ICC test that is interpreted in the context of histo- or cyto- morphology and clinical data. This class of in vitro medical tests includes the great majority of IHC and ICC tests most of which are used for determination of cell differentiation (e.g., cytokeratins, vimentin, S-100, CD45). Any IHC/ICC test that is reported as a stand-alone result to a clinician for prognostic or predictive purposes is by definition class II (see below).

Class II IHC/ICC tests – Any IHC or ICC test that is not directly confirmed by routine histopathologic or cytologic internal and external control specimens. These tests are
ordinarily reported as independent diagnostic information to the ordering clinician. Claims regarding clinical utility associated with these data must be widely accepted and supported by valid scientific evidence. These test results are often used to determine patient management. Examples of class II tests are those intended for semi-quantitative measurement of an analyte, such as hormone receptors in breast cancer.

**Qualitative IHC tests** – Test results that are interpreted only as “positive” or “negative.” Some quantification may be involved, since a cutoff point/threshold for interpreting the result as positive is often quantitatively defined (i.e., > 10% reactive cells designated as a positive result). The cellular localization of the evaluated epitope/antigen must be taken into account when interpreting these tests. These tests can be optimized without reference control material by using appropriately selected positive and negative controls. However, the calibration of these tests needs to be validated and verified, both in-house and further by participation in external QC/QA programs.

**Quantitative IHC tests** – The test results are interpreted and reported according to an accepted scoring scheme. The tests are usually interpreted in a semi quantitative manner (from 0 to 3+), but may also be a subject to evaluation by image analysis or manual cell counts. It is assumed that the tests are optimized and calibrated in such a manner that the intensity of staining, percent positive cells, and distribution of staining proportionally reflects the levels of target antigen expression. These tests cannot be run without prior calibration against reference control material or a specified tissue equivalent. Optimally, the performance of class II quantitative IHC tests will be informed by national/international consensus guidelines that address issues relating to and performance guidelines for pre-analytical, analytical, and post-analytical variables.

**Prognostic IHC tests** – The results of these tests independently forecast clinical outcome. They may be either qualitative or quantitative. They are considered class II IHC tests.

**Predictive IHC tests** – The results of these tests independently predict response to a particular therapy. They may either be qualitative or quantitative. They are considered class II IHC tests. These tests are currently limited to only those for which targeted therapies are developed (e.g., HER2/neu in breast carcinoma, CD117 in gastrointestinal stromal tumour, CD antibodies in lymphomas/leukemias).

**Controls** – A device, solution, lyophilized preparation, cell line(s), or human tissues intended for use in the quality control process.

**Calibration of IHC tests** – Calibration of the IHC tests is based on results obtained by analysis of appropriately selected positive and negative controls or designated reference controls. Calibration of qualitative tests (class I) is based on the use of semiquantitative controls that include at least one tissue sample with weak expression of the target antigen and one sample with moderate or strong expression of the target antigen. A single tissue fragment may also be suitable if it predictably represents cells with low and high antigen expression. For details on control design see positive controls (below). Calibration of prognostic and/or predictive IHC tests (class II) is based on the use of reference, previously validated control materials (calibrated control samples, which may consist of tumour samples, histoids, matrix models, or cell lines) with predetermined and reproducible levels of target antigen expression. Samples negative for the antigen of interest should be included. Both commercial and homemade calibrated control samples are acceptable if scientifically validated. Optimal calibration may or may not be equivalent to maximal sensitivity of the tests, since some tests may intentionally be calibrated so that they do not detect very low levels of expression of certain antigens/epitopes.

**Antibody optimization of IHC tests** – Antibody optimization is just one part of an analytical component, in which it will be demonstrated that a certain clone or a specific lot of the primary antibody accurately and reproducibly detects its target epitope. This is not equal to “validation” or “verification” of a clinical IHC tests (see below).

**Primary antibody selection** – Clinical IHC tests are often named according to the antigens/epitopes they detect. This should not be equated with primary antibodies that are available for detection of the antigen/epitope of choice. Antibody reactivities may have been proposed by the commercial provider or based on initial published literature regarding the characteristics of a particular anti-
body, but the tissue distribution of antibody reactivity with a given clone may expand as greater experience is gained and either a wider than previously recognized distribution of the target epitope is defined or cross-reactivities with non-target antigens is encountered. Since many clinical laboratories usually do not have the means to extensively evaluate a particular primary antibody beyond internal validation and optimization as defined elsewhere in this document, a selection of a given primary antibody should be based on mature published literature or the results of external QA/QC programs.

**Validation and verification of clinical IHC tests** — A “valid” assay performs as designed to detect the specified antigen. A “verified” assay detects the antigen as designed in a specified tissue/specimen type. The process of validation or verification of class I and class II IHC test is very different. Validation and verification of class I tests is usually performed by using in-house samples of positive and negative controls. Detailed knowledge of antigen/epitope distribution and levels of expression in different tissues is required for appropriate selection of controls. In contrast, an ideal validation of class II IHC test should be based on reference material from completed prospective randomized studies, though it may also be achieved if the test and reagent set produce results on local samples that are substantially equivalent to the originally validated IHC protocol. Concordance of ≥95% for both positive and negative results with reference laboratory results or other reference method (FISH for HER2/neu) is recommended. The number of test samples that is required for test validation is determined by power analyses based on the proposed concordance rate and known characteristics of both the calculation to be used and the expected “pass rate.” The same or different cut-off point may be used for “pass” or “fail” by external quality assurance (EQA) programs that provide proficiency testing for class II tests. Concordance at this level usually parallels a kappa-value of ≥0.80 or “perfect or near perfect” agreement with a reference laboratory or method. This is a desirable target for tests, as the results are intended to be used to determine best therapies for cancer patients. Some EQA programs may provide test samples that also quantitatively and qualitatively support IHC test validation and verification. Participation in such programs provides appropriate support for initial and continuous re-validation of these tests.

**Principles/Best Practices for Quality Assurance of Clinical IHC Testing**

**Scope: Internal**

QC/QA standards are based on performance of daily positive and negative controls. Therefore, the scope of this section is focused on the selection and evaluation of positive and negative controls for clinical IHC tests. Such controls are calibrated according to recommended standards. Most of the published literature refers to class II tests, for which specific guidelines have been published or are in preparation. However, for all other class II tests and the great majority of class I tests, there are no published guidelines. A useful source to consult is the continuously updated published literature at PubMed Search (see http://www.ncbi.nlm.nih.gov/sites/entrez). Among few others, the NordiQC organization also posts some useful specific recommendations for control tissues, antibody selection and optimal methodology (see http://www.nordiqc.org/Techniques/Recommended_control_tissue.htm).

Standardization in clinical immunohistochemistry relies on consensus regarding an “optimal result” as well as optimal/standardized selection of positive controls. Therefore, appropriate selection of the positive controls is critical for the introduction and validation of IHC tests in the clinical IHC laboratory and monitoring of daily IHC runs.

**Positive Controls**

Positive controls consist of tissue samples that contain an antigen of interest that can be detected by using primary monoclonal or polyclonal antibodies designed to bind to the selected epitope(s) in either fresh, frozen, or formalin-fixed/paraffin-embedded (FF/PE) samples. Positive controls are valid only if they are fixed and prepared in the same manner as the tissue samples that are tested in the assay.

**Selection of Materials/Tissues for Positive Controls**

1. Positive controls for fresh air-dried (or briefly fixed in ethanol or methanol or cytological spray fixative) cytological preparations must be only fresh air-dried (or fixed in the same fixative) cytological preparations derived from previously characterized patients’ samples (i.e., pleural fluid with metastatic melanoma) or previously characterized cell lines.

2. Positive controls for frozen tissue samples must be previously characterized frozen patients’ samples or frozen cell blocks from previously characterized cell lines.

3. Positive controls for formalin-fixed/paraffin-embedded (FF/PE) tissues must be previously characterized patients’ samples or FF/PE cell blocks from previously characterized cell lines. The former of these two is preferred.

4. The use of normal tissues with predictable antigen
expression, rather than tumour samples with variable expression of antigen, is highly recommended in the selection of positive controls, although neoplastic tissues may be considerable value, indeed even required, in selected settings (ALK – anaplastic lymphoma kinase, for example).

5. Inclusion of non-expressor cells/tissues with expected negative results in a given positive control is highly recommended. The inclusion of such tissues provides a means for detecting unintended antibody cross-reactivity to cells or cellular components and represents a “specific negative control.”

6. Positive controls for acetic zinc formalin (AZF)-fixed/paraffin-embedded decalcified specimens are tissues fixed in AZF, embedded in paraffin and decalcified by using the same decalcifying procedure as for patients’ samples. Similarly, any other type of tissue processing creates a need for positive controls processed exactly the same way. It is not only inappropriate to use positive controls which are processed differently than tested samples, but it may be diagnostically misleading.²

Positive Controls Design

Qualitative IHC Tests Need Semiquantitative Positive Controls

Semiquantitative positive controls are created by inclusion of tissues that optimally show predictable high, intermediate, and low levels of expression of the tested epitope. Samples with no reactivity for the given epitope should also be included. Such controls should ideally contain tissue with low levels of expression and either intermediate or high level if both of the latter are not available. Small tissue arrays with several tissue cores with various expression levels of the epitopes are recommended as best practice. Alternatively, a single tissue fragment is sufficient if it reproducibly contains such representative areas. Examples: (a) benign tonsillar tissue contains mantle zones with low level of CD23 expression and germinal centers with follicular dendritic cells with high level of CD23 expression; (b) appendix contains mucosa with crypts that demonstrate graded expression of Bcl-2; and (c) liver tissue exhibits low levels of CK8 (or low molecular weight cytokeratin) expression in the hepatocytes and high levels in the bile ducts.

Quantitative IHC Tests (HER2, ER, PR) Need True Quantitative Positive Controls That Are Calibrated According to Reference Material/Standard

So called “reference material” can be designed by using appropriately validated IHC kits or cell lines. It can also be created by using appropriately validated tumour samples. See also “Validation and Verification of clinical IHC tests” (Recommended Terminology).

Types of Positive Controls

External Positive Controls

External positive controls are previously characterized positive tissue samples or cell lines that are tested in parallel with patients’ samples. For IHC tests in general, one such external positive control is sufficient per run. However, for clinical IHC testing, it is recommended that the appropriate positive control be placed on a slide together with the patient material. This external control sample needs to be indelibly identified as such.

Automated IHC platforms/instruments can in some instances have pipetting failure with random skipping of a specimen. In some cases, where an internal control is not present or is not informative, such instrument failure cannot be detected by any other means, other than having the external positive control placed on the same slide. It is

If positive controls are not decalcified as the tested sample, they are much more likely to produce good positive signals with methods optimized on non-decalcified samples. False negative results are not uncommon with such practice. It continues to be a challenge for reference laboratories to perform IHC tests on tissue samples from different laboratories, for which the reference laboratory may not have appropriate positive controls. This is particularly true for bone marrow specimens, for which tissue processing protocols vary widely.

²It is wrongly assumed that such internal controls are always better than external controls and that when internal control is present, no external control is needed. In the first place, not every tested antigen has a normal internal counterpart that can be used for this purpose. Internal controls are critical in evaluation of tissue preservation; however, this is useful only when an internal control is expected to be positive, but the test produces negative result. In such cases, if the lesion/tumour is also negative, a false negative result cannot be ruled out. Internal controls rarely, if ever, provide semiquantitative information regarding different target antigen levels of expression, which are essential for properly calibrated IHC testing. A notable exception is the presence (or intentional inclusion) of normal breast tissue with tumour tissue to represent a reference point for interpretation of breast cancer markers results. For some particular applications (e.g., CK5/6 staining of basal cells in prostate biopsies) the internal positive and negative control are sufficient and, in fact, superior to external controls, as they also allow assessment of factors related to tissue fixation and processing.
not known how often this machine failure occurs (no published data are available); however, it can be stated that “unpublished experience of reference laboratories” strongly favours this approach to positive controls. This approach is also extremely useful in the clinical setting when an “unexpected negative” result is encountered by the pathologists. The presence of an external positive control on the same slide as patient’s sample will greatly decrease number of repeat tests and will demonstrate that analytical component of the IHC testing was valid.

Internal Positive Controls

Internal positive controls are tissues in the patient’s sample that contain the target antigen within normal tissue elements, in addition to the tissue elements to be evaluated. Internal positive controls are very useful if the tissue studied is expected to show at least some degree of expression of targeted epitope. This rule can be applied only if the test tissue is expected to demonstrate target antigen expression. Even if this is the case, the pathologists should consider the variation between normal and tumour expression of most antigens. Many diagnostic criteria refer to IHC tests results based on detection of low levels of antigen(s), which are used to establish proper diagnosis (e.g., CD15 in Hodgkin lymphoma, cytokeratin in small cell carcinoma), which may not be demonstrated if appropriate positive controls with exact/similar low levels are not used. Such controls are not useful if the tissue used for the study shows only aberrant tissue (“tumour only”).

Negative Controls

Specific Negative Controls/Negative Tissue Control

Specific negative controls or negative tissue controls are those tissues that are known not to contain the antigen of interest. This type of negative control enables detection of unintended antibody cross-reactivity to cells or cellular components and they may be a portion of a patient sample (“internal negative control”), which parallels the concept of “internal positive control.” Specific negative controls are used to document no reaction in cells/tissues that are known to have no expression of the tested epitope(s). If non-specific negative controls are negative and specific negative control positive, the false positive result is due to variables associated with the primary antibody. Occasionally polyclonal antibodies may be contaminated with other antibodies due to impure antigen used to immunize the host animal. This problem may be detected by use of specific negative controls. See also “Non-Specific Negative Controls.”

Nonspecific Negative Controls/Negative Reagent Controls

Negative reagent controls are used to confirm the specificity of the test and to assess the degree of nonspecific background staining present by omitting the primary antibody. Commonly, the primary antibody is replaced by one of the following: (1) antibody diluent, (2) same species nonimmune immunoglobulin of the same dilution and immunoglobulin concentration, (3) an irrelevant antibody, or (4) buffer. Non-specific negative controls can detect unintended background staining. The main cause of non-specific background staining is non-immunological binding of the specific immune sera by hydrophobic, ionic, and electrostatic forces to certain sites within tissue sections. This form of background staining is usually uniform. Prolonged fixation in formalin or other aldehyde-based fixatives should be avoided as it may produce non-specific background. This background staining from overfixation can be remedied by postfixation with Bouin’s, Zenker’s, or B5 fixatives, but this is not useful in daily practice. Endogenous peroxidase activity is found in many tissues and can be detected by reacting fixed tissue sections with DAB substrate, which is routinely eliminated by pretreatment of the tissue section with hydrogen peroxide prior to incubation of the primary antibody.

Non-specific negative controls are sections prepared from the same block of patient material that is used for clinical testing. The purpose of negative reagent controls is to detect either the lack of specificity of the test or nonspecific background staining, and for this reason has been referred to as the “methodology control.” Negative results in negative controls represent medical evidence that staining for a particular epitope in the test tissue is not a false positive result due to variables other than the primary antibody. Therefore, negative controls are critical for daily QC/QA documentation in clinical immunohistochemistry. The following principles should be adhered to:

1. Negative tissue controls need to be processed in the same manner as the slides for specific IHC tests, including various epitope retrieval procedures.

2. The number of negative reagent controls is determined by the number of different pretreatment procedures: one negative reagent control should be prepared for each methodologic variation employed in a given clinical case. For example, if three different epitope retrieval procedures are used (for example – HIER in citrate buffer, HIER in EDTA, and protease digestion), three negative controls (one processed HIER in citrate buffer, one with HIER in EDTA, and one with protease digestion) must be prepared.
3. Negative controls are typically run by omission of the specific primary Ab in the protocol and replacement by an appropriate (presumably) non-reactive moiety. For monoclonal primary antibodies, the optimal choice is an antibody of the same isotype, present in the same Ig concentration as the test primary antibody, using the same diluent, but non-reactive with human epitopes. Specially prepared commercially available negative controls may be used. For polyclonal antibodies, negative reagent controls should be a dilution of Ig fractions of whole serum of normal/non-immune serum of the same animal source. Mouse ascites fluid can also be used as a negative control. Finally, the primary antibody may be replaced with cell culture medium (McCoy’s tissue culture medium is commonly used).\(^4\) Importantly, none of the non-specific negative controls are able to detected an undesirable/unexpected cross-reactivity of the primary Ab with some epitopes. In addition, when two or more antibodies are applied to serial sections, which is often the case in clinical laboratory, negative stain areas of one slide represent the negative control for other antibodies. This combined approach is recommended.

4. Negative results with negative controls do not ensure the specificity of the IHC tests in all cases. Therefore, when unexpected reactivity of the primary Ab is encountered, false-positive results need to be considered.\(^5\)

**EXCEPTIONS**

**Class I tests.** When a panel of antibodies is used for tissue analysis, the results of other tests in the panel may provide sufficient negative control information so that no additional negative controls are needed. This approach involves proactive interpretation of “negative control” results by the pathologist who orders the panel. When ordered, panels may not be sufficient or appropriate to serve as negative controls and, therefore, additional negative controls could be ordered by the pathologists.

**Class II tests.** If published guidelines address the type of negative controls to be used for the particular class II tests, the guidelines should be followed. If negative controls are not described by the published guidelines, principles suggested in items 1 to 4 above should be followed.

### Documentation of Positive and Negative Controls Results

**Note:** In establishing retention requirements, care should be taken to comply with provincial and federal regulations as these may exceed the following CAP NSC/IHC recommendations.

The retention of laboratory documentation should be maintained in such manner that it demonstrates that the test(s) were performed correctly on the correct patient, that the reagents and equipment used to perform the test(s) were operating correctly and that pathologists were given correct information, which would allow correct interpretation of the IHC results. This information is largely included in the laboratory QA/QC records. The recommendation on retention of laboratory documentation on positive and negative controls in IHC laboratories is addressed here only from the aspect of internal audit/review and its use for troubleshooting and does not address requirements for accreditation or legal purposes, which may vary in different jurisdictions/provinces.

1. Every clinical IHC laboratory needs to keep daily records of all positive and negative controls and their results. Review and sign off of the positive and negative control results by pathologists in charge is recommended.
2. If positive or negative controls indicate a failed run, documentation of corrective action is required.
3. Electronic documents with secure back-up are recommended.
4. A two-year period is generally recommended for the retention of all laboratory records including the records of positive and negative control performance. However, shorter periods for retention are also

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\(^4\) For clinical IHC laboratories, where a large number of different monoclonal antibodies are in use, the most practical solution is to use cell culture medium instead of primary antibody for all negative controls.

\(^5\) Internal (or specific) negative control is essential for recognizing unexpected crossreactivity of the primary Ab, which can produce false positive results. Evaluation of cells that are expected to produce negative results in the tissue section should always be performed. Such expected negative results are more consistent in benign tissues and often unknown or unpredictable in tumours. External controls, if included in the same slide, may also provide useful information if they contain expected negative tissues.

\(^6\) Practical approach to recording and retention of control results is to document specific suboptimal and inadequate results. Good results can be reported in aggregate fashion.
acceptable as follows:

a. Class I IHC test records may be kept for only 6 months. Last 10 results should be readily available for review.

b. Class II IHC test records should be available for at least 12 months. Last 10 results should be readily available for review.

c. If the laboratory regularly participates in EQA programs that support test validation, the records should be kept for the period of the last two challenges for both class I and class II tests.

5. It is recommended that pathologists include the results of positive and negative controls for all class II IHC tests in the pathology reports. This is not currently recommended for class I tests, though it is entirely relevant to include this information with all test results.

6. Reporting of the results of the class I IHC controls is optional in pathology reports; however, appropriate documentation needs to be performed by the IHC clinical laboratory as above.

New Test Validation
The introduction to clinical use of any new test must start with test verification and validation (see Proposed Terminology for definitions). However, all previously validated IHC assays must be completely revalidated if significant changes are made to the assay procedure. Significant changes include: change of primary antibody clone or provider, new detection system, new buffer type or any other component of antigen retrieval step, new machine for immunohistochemistry, or switch from pre-diluted to in-house diluted (or the other way around) primary antibody. For new primary antibody lots, a smaller concordance study for lot variation alone is recommended to include a 10 case run in parallel with the original lot.

Class II Immunohistochemistry Test Principles/Best Practices

Scope
There is only one IHC test so far for which testing guidelines have been issued, namely HER2. In Canada, eleven expert pathologists issued a document entitled Canadian Consensus for HER2 Testing Guidelines in Breast Cancer, which basically reflects ASCO/CAP Guidelines; however, Canadian modifications are introduced based on the experience of the 11 consensus group participants. It details pre-analytical, analytical, and post-analytical steps for HER2 IHC testing. The impact of the pre-analytical component was the most challenging to define, since various epitopes may have widely different responses to formalin-fixation and tissue-embedding or other steps in tissue processing. The following recommendations for class II tests include most recent published articles and appear to be safe for all class II tests (prognostic and predictive IHC markers) including HER2, estrogen receptor (ER) and progesterone receptor (PR), and CD117 IHC tests:

Pre-analytical Component/Tissue Processing
Since ER/PR and HER2 tests are generally performed on the same specimens, at a minimum, compliance with HER2 guidelines may be sufficient for most analytic variables. However, more recently published requirements for ER/PR testing may be more informative with respect to appropriate minimum fixation times. The following recommendations are based on published peer-reviewed recommendations for tissue fixation and processing of samples for breast carcinoma markers. At this time, there is no evidence to suggest that the proposed recommendations would not be safely applied to all other class II tests and to all class I tests. The following recommendations incorporate accumulated scientific knowledge on formalin-fixation effects on epitope preservation.

Tissue Handling and Fixation
Tissue processing, in particular the type of the fixative used and the fixation time prior to loading the tissue onto the tissue processor needs to be recorded and included in pathology report. Use of decalcified tissues is not recommended unless permitted by the specific class II test guidelines. If decalcified tissues are used for testing, following times need to be recorded: Fixation time prior to decalcification; type of decalcifying reagent; and time of decalcification.

Fixation of tissues should be performed in 10% neutral-pH (pH 7.2–7.6 aqueous), phosphate-buffered formalin for a minimum of 8 hours (24–72 hours optimal). A fixation time of 8 to 72 hours is generally recommended. Therefore, tissues should be fixed in formalin for at least 8 hours before being loaded onto the tissue processor. Non–formalin-based fixatives and or other fixation methodologies should not be used for class II IHC tests, because of the lack of published evidence on the performance characteristics of other tissue processing methods. However, if any scientifically supported tissue processing method is validated in future, such a method may be used for clinical IHC testing. It is of high importance to remember that any such pre-analytical methods must also be validated for all other IHC tests (both class I and class II) that may be applied to tissues thus prepared. The time from surgical excision of the specimen to place-
ment in fixative should be optimized. Epitope degradation due to delayed fixation is a potentially serious problem that generally cannot be modified/corrected by epitope retrieval techniques (these mainly adjust for fixative-induced modifications of the epitope, but not for the effects of delayed fixation, which usually leads to irreversible tissue deterioration). Both, prolonged prefixation ischemic time and fixation of less than 8 hours may irreversibly modify our ability to detect specific epitopes. Samples should be sliced immediately at 5–10 mm intervals after appropriate gross inspection and margin designation, and then placed in sufficient volume (20:1) of 10% aqueous neutral buffered formalin.

A longer fixation time of up to 10 days is acceptable and is not an exclusion criterion for most IHC testing, as long as the specimen has been adequately sectioned to allow adequate fixation as described above. Class II IHC tests may require validation (consult published guidelines for each test separately) of protocols for fixation times exceeding 72 hours.

Note the following:

- Under-fixation is more deleterious than over-fixation.
- Fixation requirements are particularly specified for ER/PR/HER2 IHC and FISH testing on core biopsies and surgical excisions. Consensus guidelines for other class II tests are not available yet and thus are not specified in this document. They will be incorporated into this document as they become available.
- Long term archival storage of tissue blocks (for even 20 or more years) does not preclude HER2 testing as long as the archived material has not been subject to significant temperature fluctuations over time.

**Analytical Component**

Assay validation and verification, antigen retrieval, selection of positive controls, and use of laboratory methods as per reference 10.

**Assay Validation**

As many as 50 – 100 samples may be required when validating a new antibody for a class II test. An assay accuracy of a 95% concordance rate is recommended for test validation for both the positive and negative categories. Ensure adequate validation, preferably by using 50% cases that are unequivocally positive and 50% cases that are the mixture of weakly positive and unequivocally negative. Much smaller number of samples may be sufficient for some class II test (e.g., CD117). Validation documentation must be kept as long as reported results of these tests are kept. Any significant modifications to the procedure require additional validation to ensure accurate performance (see re-validation for definitions).

**Type of Antigen Retrieval**

Stringent compliance with validated Standard Operating Procedures developed in Assay Validation must be adhered to. QC documentation must be in place indefinitely or as long as pathology reports that include the IHC test results are mandated to be retained.

**Use of Standardized Control Materials**

The controls should include positive and negative cases as well as a low protein expressor cases. The control tissue should be fixed and processed in the same manner as the patient samples. The number of samples is determined based on the design of the validation sample as well as on the power analysis based on the selected level of performance and known characteristics of the test sample. It is recommended that the design of the in-house validation sample be supported by the recommendations from the statistician versed in such studies. The material is validated in a prospective clinical trial results or by using the results of procedures that are validated based on such prospective clinical trial.

**Use of Automated Laboratory Methods**

The use of correctly operated automated staining protocols and equipment are acceptable and desired for clinical testing; however, validated methods must be used. Records of recommended maintenance and service records must be retained indefinitely or as long as the pathology reports that include results of the IHC tests are retained.

**Post-analytical Component/Interpretation of Results**

Every test may ultimately have its own guidelines for performance and interpretation, although guidelines for class II tests will likely be more rigidly defined than those for class I tests. HER2/neu is detailed in the *Canadian Consensus for HER2/neu Testing Guidelines in Breast Cancer* and the ASCO/CAP document. Similar guidelines for the interpretation of the ER and PR tests are being or have been developed. Image analysis may also be suitable for the interpretation of the results as it was reported to be as good as expert pathologist’s scoring and some systems are already approved for such use by the USA Food and Drug Administration (FDA). It is recommended that image analysis scoring results also be validated by either participation in the extra laboratory QC/QA program or by the reference laboratory. The post-analytical components of the class I test are generally test specific. However, it could be said that for general purposes a cut off value of 10%
positive cells is used to designate a test as positive or negative. Only exceptionally, the test may be positive in smaller numbers of cells if the pattern of staining is sufficiently restricted to certain lesions and documented by the published literature. This is well illustrated using an example of a CMV immunoreactivity in morphologically altered cells where even one positive cell is sufficient for interpretation as a “positive test result”. On the other hand, some class I markers are considered as “positive” only if strongly expressed by a majority of cells as this is the case in interpretation of some tests, most notably TdT and CD99. Therefore, approved training in pathology is required for clinical application and interpretation of IHC tests’ results.

Proficiency Testing: Monitoring the Quality of Laboratory Performance

Proficiency Testing and Certification of Class II IHC Tests versus Laboratory Accreditation

Laboratory accreditation is currently under the jurisdiction of provincial regulatory bodies in several provinces in Canada. However, class II test certification is distinct from the current processes for laboratory accreditation. The IHC test certification is defined as successful participation in external quality assurance program for IHC testing at the pass rate of minimum of 90% with both positive and negative results of the reference value used by the EQA program. This definition assumes the requirement of sufficient number of test samples for meaningful statistical analysis based on power analysis. It appears that at least 40 samples are required to achieve the desirable pass rates (90% to 95%), or to approach good correlation with “near perfect” agreement based on kappa-values of 0.80. This pass rate may or may not be required by the provincial or other accrediting bodies. Canadian provinces or territories that do not have accrediting bodies may use the above recommendation for safe clinical practice. Therefore, participation in EQA programs that provide such test samples would support “certification” of clinical IHC test. Laboratory accreditation further mandates/enforces compliance to designated provincial standards, which may be, higher or lower than the above.

The above recommended requirements for class II IHC test certification may be regularly updated to follow published literature and developing national and international guidelines.

The CAP National Standards Committee/Immunohistochemistry suggests that implementation of the national and international published guidelines for class II tests and their continuous validation could be facilitated by development of the following:

1. Establishment of a Canadian National Checklist for clinical IHC Laboratory certification. This would ensure that all Canadian clinical IHC laboratories fulfill minimum, standard, requirements and elements of implementation for class I and class II tests, which are very different and need to be adequate for the type of tests that are performed by clinical laboratories. Class I IHC Test Checklist and class II IHC Test Checklist would form the basis for a step-wise approach to appropriate daily QA/QC measures, as well as for selection of appropriate EQA programs that would support test validation and verification.

2. Certification for each prognostic and predictive test (class II IHC tests) separately is proposed. Historical evidence in EQA has indicated that good or even optimal performance in one test does not guarantee equally good performance in another (similar or otherwise) IHC test. Therefore, the NSC/IHC recommends certification of each class II test separately.

3. The certification of class II tests includes demonstration of at least 90% concordance for both positive and negative results at least twice annually; the participation interval is aligned with ASCO/CAP recommendations for IHC breast cancer markers. Large testing centers do not automatically qualify as reference laboratories. Any IHC laboratory that provides evidence of ≥95% concordance or kappa-value of >0.80 with both positive and negative reference values can be considered a “reference laboratory” as this cut off point correlates with “near perfect agreement with reference value, which is not the result of chance agreement” (see above regarding kappa-values in EQA). Also, see above for definitions of IHC test validation.

4. If a laboratory did not pass with a recommended score of at least 90% concordance, it is recommended that the unsuccessful laboratory send all samples to be tested, to another certified laboratory or designated reference laboratory until corrective action has been taken and repeat tests passed with an acceptable success rate. Participation in any EQA program that provides such samples for test validation is accept-

Certification for performing class I IHC tests, would continue to be conducted according to current practices (in agglomerate) with recommendation to use Canadian IHC QA Checklist for class I IHC tests.
considered as good practice:

2. Clinical IHC laboratories should be directed by a fully trained and certified designated pathologist with experience in immunohistochemistry, who is the director of IHC laboratory and is responsible for complying with internationally and/or nationally/provincially designated standards for class II tests.

3. The director of the IHC laboratory and the laboratory manager and ultimately the director/head of the department of pathology in that institution are responsible for the results of the IHC tests. (It is reiterated that the director of the IHC laboratory must have final authority over the IHC laboratory and that the head of the Department of Pathology/Laboratories must have overall responsibility for the work/results of that department.)

4. The medical director (pathologist) must have the final authority for selecting appropriate tests, antibody clones, detection systems, or any other significant component of clinical IHC testing.

5. IHC laboratory director (pathologist) is expected to recommend the implementation of appropriate pre-analytical, analytical, and post-analytical components of the IHC testing. Such recommendations should be recorded. (The IHC director cannot be held responsible for laboratory errors if the above recommendations are not implemented by the laboratory and hospital management.)

6. Any clinical IHC laboratory should have at least one fully trained and qualified technologist for the type of the tests that are performed by the clinical laboratory. Support technicians/technologists without special training in IHC techniques may support IHC laboratory operations (e.g., cutting tissue sections).

7. The training and continuing professional development of the technologists for the IHC laboratories has to be documented by the department management.

8. Rotation of technologists designated to the clinical IHC laboratory to oversee and perform clinical IHC tests in addition to other histology duties is appropriate if the highly qualified personnel are not replaced by the less experienced technicians/technologist without special training in immunohistochemistry techniques.

9. There are no recommendations for support technologists/technologist at this time; they may rotate between IHC duties to other laboratory activities.

**Education and Training Standards for Laboratory Personnel**

**Scope**

Minimum standards for clinical IHC staff including laboratory directors are not currently set. The NSC/IHC at this time is recommending that the following would be considered as good practice:

1. Clinical IHC laboratories should be adequately staffed to meet the volume and complexity of the laboratory testing.

2. Clinical IHC laboratories should be directed by a fully trained and certified designated pathologist with experience in immunohistochemistry, who is the director of IHC laboratory and is responsible for complying with internationally and/or nationally/provincially designated standards for class II tests.

3. The director of the IHC laboratory and the laboratory manager and ultimately the director/head of the department of pathology in that institution are responsible for the results of the IHC tests. (It is reiterated that the director of the IHC laboratory must have final authority over the IHC laboratory and that the head of the Department of Pathology/Laboratories must have overall responsibility for the work/results of that department.)

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**Bibliography**


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2-YEAR RESIDENCY TRAINING PROGRAM IN TRANSFUSION MEDICINE

Applications are invited for the two (2) year Residency Training Program in Transfusion Medicine (sub-speciality with examination), to prepare the candidate for consultancy role in Transfusion Medicine. This is a Joint Program with the University of Ottawa, Queen’s University and Canadian Blood Services. At the end of the two (2) year program, there is certification by examination by the Royal College of Physicians and Surgeons of Canada. Salary is commensurate with PGY year plus research and travel monies provided. Training includes nine (9) months at a blood centre, nine (9) months at a hospital (Transfusion Medicine service), four (4) months Elective, and two (2) months selective.

Eligible applicants will be nearing completion of training in, or have a Royal College of Physician and Surgeons of Canada Certification in one of the following:

- Internal Medicine + Hematology
- Pediatrics + Pediatric Hematology Oncology
- Hematological Pathology
- Anesthesiology
- General Pathology

**Deadline Date for Applications:** July 31, 2009

**Date of Commencement of Program:** September 1, 2009

Applications will be considered until the position is filled.

In accordance with Canadian immigration requirements, this advertisement is directed primarily, but not solely, to Canadian citizens or permanent residents of Canada. The Ottawa Hospital and University of Ottawa are committed to equity in the workplace: women, aboriginal peoples, members of visible minorities and persons with disabilities are encouraged to apply.

Please submit curriculum vitae and the names of three referees to:

**Dr. Antonio Giulivi, Division Head**

Hematopathology and Transfusion Medicine
The Ottawa Hospital, General Campus
501 Smyth Road, EORLA Lab
3rd Floor, Rm. #3887, Box #115
Ottawa, ON K1H 8L6
Annual Meeting Survey

Martin Bullock, MD, FRCPC

In January 2009, the Canadian Association of Pathologists (CAP) conducted a web-based survey of the general membership to assess the member’s opinions about the annual general meeting and other activities of the association. By conducting the survey, the CAP executive committee hoped to gain more information about what the members wanted the meeting to provide from educational, social, and scientific perspectives, where and when meetings should be held, and how members felt the meeting could be improved. The survey consisted of 17 questions. A maximum of 167 members (about 18% of the membership) responded to any of the questions, but most questions were answered by 130–160 members. Most of the questions permitted free text comments to be added.

General Findings
Of the 167 respondents, the majority (55%) attend the meeting less frequently than every other year or not at all. Twenty-six percent stated they attend every year, 19% every other year. The most frequent reasons for infrequent attendance were: the desire/need to attend other meetings (43%) and scheduling conflicts (39%). About one quarter of respondents chose cost of attendance and location as reasons for not attending. About 20% stated that the meeting did not fit their educational needs. There were many specific comments that the timing of the meeting (early summer) was not convenient, mainly because of conflicts with vacation. There were several comments that the meeting should aim to provide higher quality speakers, topics of more practical value to practicing pathologists, and integrate more lab management and QA topics. Educational value for dollar spent was a concern, and comparisons were made with other meetings (especially USCAP) and courses.

Of factors that respondents considered important when deciding whether or not to attend the meeting, more than 75% considered cost of travel to be very or moderately important. Greater than 50% considered price of lodging and local attractions as very/moderately important. Climate of the location and availability of restaurants were considered to be very/moderately important by fewer than 50%.

Fifty-seven percent of respondents felt that there is value in holding the meeting in conjunction with other societies’ annual meetings, such as the CSCC or CAMB. However, few respondents (16%) named another specific society with which they would like to meet. Among others, the responses included the CANP, American pathology organizations (USCAP, CAP, and ASCP), and provincial associations (e.g., OAP).

Social Activities
When asked which of the main social activities during the meeting they considered valuable, the responses were as follows: exhibitor’s wine and cheese (87%), president’s reception (74%), gala banquet (71%), and local tours (57%). Only 37% felt that they would utilize a spousal program if offered.

Poster and Paper Sessions
The members were asked to rank their satisfaction with the poster sessions from a choice of five categories ranging from “very unsatisfied” to “very satisfied.” They were asked about the overall quality of the posters, the poster viewing time, and the interaction with authors. The range of responses was similar for each category. While only a low percentage (less than 10%) was unsatisfied with any of the three elements, even fewer were “very satisfied,” with the majority being either “neutral” or “satisfied” (about evenly split). Several respondents commented that there was little interest in posters and that they were in inconvenient/poor or remote locations.

There was a greater degree of satisfaction with the oral paper sessions. Respondents were asked to rank their satisfaction with oral paper sessions with respect to the 15 minute format, the discussion during the sessions, and the open discussion to complement the session. Very few respondents were unsatisfied. About 60% of respondents were “satisfied” or “very satisfied” with the 15 minute format and discussion during the session. Half the respondents were “neutral” with respect to an open discussion, with most of the remainder expressing some degree of satisfaction.

Workshops and Symposia
In general, there was a high degree of satisfaction with the symposia. Members were asked to rank their satisfaction with the symposia topics, format, and opportunity for discussion. The percentage of respondents who
were “satisfied” or “very satisfied” with those three components ranged from 65–69%. Seven percent of respondents were “very unsatisfied” or “unsatisfied” with the symposia topics. The specific comments with respect to symposia were variable and sometimes conflicting. There were a few comments that the symposia were too “AP-oriented,” that the interest to individuals varied considerably from year to year and that the sessions should be more practical.

Eighty-two percent of respondents felt that the workshops are beneficial. When asked when the workshops should be held, about half felt they should be during the meeting, with 40% favouring before and 10% after.

Other
Members were asked to comment on how CAP membership could be increased. As expected, there was a wide variety of responses, but a strong theme was that the CAP should become more relevant by increasing its focus on issues of concern to all Canadian pathologists, including standards of practice, lab accreditation, workload, and compensation. There were multiple suggestions to increase cooperation with provincial pathology associations and organizations in Europe, Britain and the USA. Several respondents felt that the CAP should offer more CME (online and otherwise) and that it should encourage the interest of general pathologists by offering more clinical pathology topics. While there were a few comments that membership fees should be decreased, there were more comments about value for dollar spent, rather than lower fees.

Conclusion
The survey provided important information that should prove helpful in planning future meetings. Attendance is limited by competition with other meetings that members may feel better fulfill their educational needs. The early summer timing is inconvenient for many members. Cost of travel and lodging is a concern to many, and therefore concentration on the most readily accessible Canadian cities should be a consideration. The respondents were generally satisfied with symposia and workshops, although many felt that the workshops should occur during the main meeting rather than on the weekend before. The paper and poster sessions received variable ratings. Consideration should be given to ways of increasing interest in the posters. The expense of organizing local tours and spousal events is probably not worthwhile. The survey was limited by the fact that of the respondents, only 45% attend the meeting at least every other year, so comments may not reflect the current reality. Many members felt strongly that the CAP should become more involved in providing practical guidance to Canadian pathologists, for example through practical CME and by increasing emphasis on standards of practice.
Laboratory Medicine Residency Training 2007–2008

Aaron Pollett, MD, FRCPC

The Professional Affairs Committee of the Canadian Association of Pathologists performs a periodic survey of all Canadian laboratories to find the number and specialty of physicians in active practice. As part of this survey, residency program directors are also asked for details of physicians in training. The CAP manpower and resident database is voluntarily maintained and requires accurate responses to the surveys and request for information.

In the past 5 years the number of residency positions available through the Canadian Residency Matching Services (CaRMS) has increased from 37 in 2004 to 65 in 2009 (see Table 1 for a breakdown by discipline for position in 2009). At the same time, the number of laboratory medicine positions available for international medical graduates (IMGs) has increased from seven in 2004 to 15 in 2009. Not all available positions for laboratory medicine trainees are filled through the CaRMS match. In the past 5 years the percentage of positions filled from the primary match ranged from 59% in 2004 to 84% in 2007 (69% of the positions were filled in 2008 after the primary match). Table 2 shows the number of available and filled positions for the various areas of laboratory medicine since 2002.

Table 1. Number of Laboratory Medicine Specialty Positions (Allocated Quota) in the 2009 CaRMS

<table>
<thead>
<tr>
<th>Discipline</th>
<th>Canadian Graduates</th>
<th>IMG Positions</th>
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CaRMS = Canadian Residency Matching Services; IMG = international medical graduate.

Table 2. Adjusted Allocated Positions (quota)/Accepted Positions for Canadian Graduates in Primary CaRMS Match, 2002–2008, for Laboratory Medicine Specialties

<table>
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CaRMS = Canadian Residency Matching Services.

Aaron Pollett, MD, FRCPC, is a member of the Professional Affairs Committee, Mount Sinai Hospital, Toronto, Ontario.
If we assume that all positions filled through the primary CaRMS match stay within the program, and that all accepted IMGs are in the program for 5 years, the number of laboratory medicine trainees estimated in 2008 was 222. This is close to the 240 residents currently listed in the CAP manpower database. There has been an approximate year-over-year growth of 29% in 2007 and 12% in 2008 and a 70% increase in the number of laboratory medicine residents compared to 5 years ago (140 pathology residents were in the CAP database in 2003).

Table 3 shows the breakdown of current pathology residents by discipline and training level (PGY year). Of the 240 residents in the database, 87 (36%) are indicated as having entered through the IMG program. Approximately 63 residents are expected to be graduating from residency positions in July 2008.

There is rising concern that there will not be sufficient positions for the laboratory medicine residents when they complete their training. While the number of available positions with the residency programs has increased by 70% in the past 5 years, the number of full-time practicing laboratory medicine professionals has increased by approximately 5%. The majority of this increase in the workforce is the result of filling vacant positions. There have been very few new positions created for laboratory medicine professionals in the past 5 years. The majority of full-time practicing pathologists in Canada are now over 50 years of age, with an average age of 53.5. This has lead to the concern that there will be a shortage of pathologists within the next 5–10 years as the number of full-time laboratory medicine practitioners retire. This retirement replacement need is very difficult to estimate. There is no mandatory age of retirement and approximately 12% of the current full-time pathology manpower is over the age of 60.

The incidence of cancer has increased by approximately 4% per year for the past 5 years. This increase is largely due to the growing and aging population within Canada. Screening programs and cancer wait-time improvement has become a focus of the provincial cancer care organizations. The rising incidence of cancer and other cancer care initiatives have increased the workload within pathology laboratories with essentially no increase in the pathology workforce. Alberta has been successful in the creation of several positions in the past few years, but this has been in response to a growing population and a system strained by large cutbacks which occurred in the 1990s. In Ontario, there have been less than five new positions in the past 5 years, despite large institutions demonstrating an increase in workload by 20–25%. Our training programs have been very successful in growing the laboratory medicine residency programs, and we are in a position to increase the pathology workforce in Canada with highly trained laboratory medicine professionals. There is a need to increase the number of laboratory medicine positions across the country to handle the increasing workload and ensure that all Canadians have access to high-quality laboratory medicine.

<table>
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</table>
The origins of surgical pathology as a specialty were thought to be born in the immediate aftermath of the Renaissance. At the same time that chiaroscuro emerged with its interpretations of light and dark embodied in the art of tenebrism, so too the appreciation of the subtleties and nuances of microscopy were stirring and taking a meaningful shape in pathology. Through the post-Renaissance period, surgical pathology grew and was adorned by beautifully crafted descriptions of diseases and tumours. The art of surgical pathology expanded and refinements of description devoted to morphology were the initial landmark changes. From its romantic Renaissance origins, surgical pathology in the mid-20th century had evolved into a pivotal specialty on which treatment options were based. The surgical pathologist was a fully fledged member of the management team, and intra-operative consultations became the daily norm, especially in North America. Surgical pathology remained somewhat static into the latter parts of the 20th century with electron microscopy and then immunohistochemistry taking centre stage as the new development that would impact diagnostic practice. The past 15–20 years have seen pathology undergo its own form of the industrial revolution, with the emergence of molecular biology and pathology as an irresistible force that has changed the way we think about and practice pathology. Luddite thinking has been shed slowly and painfully at first, but the inexorable march, hearteningly, is being embraced more readily. With this have come more challenges and difficulties for the surgical pathologist. The milieu and landscape in which we now practice has changed, and with this change the surgical pathologist and indeed pathologists of all persuasion, are confronted by a series of new questions and applications.

The Challenges

As if technological advances were not enough, surgical pathology has found itself in the midst of a maelstrom of public mistrust, especially here in Canada. Public perception of medicine in general has changed and with an increasingly sophisticated and informed general public the ambiance is now shrouded by litigation and second guessing. Somewhere along the line, we as a profession have become inured to the opinion of the public, about how they are treated, are they getting the best treatment, and what is in their best interests. While this has not been a wilful neglect of the very people we care for, it has been an imperceptible reality.

As a profession we need to stand back, be introspective, and institute the necessary checks and balances that ensure optimal diagnostic accuracy and hence the best patient care. While it is true that errors have been made and these are regrettable, it is appropriate to keep in mind that errors are unavoidable. However, as a specialty we have to ensure that the best possible steps are in place to minimize errors and harm to patients. We must be mindful of public perception at all times and responsible, rational behaviour is mandatory at all times from all parties concerned. Whistle blowing is an acceptable form of drawing attention to a problem if done properly and not sensationalized. It is an unfortunate dictum of life that we dwell on the negatives, forgetting the positives that far outweigh the former. While we must strive for perfection, it must be borne in mind that surgical pathology is a subjective field with personal bias like the interpretation of the shades of light and dark of chiaroscuro. Problem and/or difficult areas abound in surgical pathology, and herein lies the challenge: to minimize the difficult areas and replace subjectivity with objectivity.
Maintenance of Standards and Safe Practice

The licensing and examining bodies have a set of standards that have to be met before anyone is allowed to practice pathology. These standards should constantly be modified and adapted so that the modern surgical pathologist is in tune with the demands of modern day medicine. Regular maintenance of knowledge is required and maintenance of certification is to be supplemented by re-certification or re-validation, a more rigorous process that will ensure fitness to practice. In the interest of good practice and transparency it is a development that must be welcomed by all medical practitioners. The vexing issue currently is the mechanism and implementation of this goal. It is incumbent upon us to ensure that a fair, just, and efficacious method is found as it is in the best interests of everyone.

In the meantime, several other strategies are in place to ensure quality. Quality assurance is at the forefront of all laboratories, both at internal and external levels. Steps are in place to monitor all aspects of quality from slide generation, to the final end product which is the surgical pathology report. Internal consultation between colleagues on all types of cases is routine and is encouraged. In an attempt to ensure accuracy and as part of internal consultation, several centers also ensure that two pathologists view and concur on every cancer diagnosis. This has obvious benefits but may sometimes be impractical. A potential negative impact is to take away an individual pathologist’s ability to diagnose independently, something that the entire training mechanism is geared towards. Perhaps a variation on this would be that a predetermined percentage of cancer cases have two pathologists examine them. In addition, one must also rely on pathologists having the necessary insight to realize when they are confronted by a difficult case. Allied to this is random audit within a department. A pre-determined percentage of cases (usually about 10%) are randomly vetted by a quality assurance team within a department. Discrepancies are discussed and those with clinical significance result in an amended report and the responsible clinician being contacted. Clinopathological meetings (rounds) or multi-disciplinary management meetings are an extremely important facet of quality assurance. The role of the clinicians and ancillary diagnostic modalities in this setting provides essential feedback to the pathologist.

Everything that can be done to ensure accuracy and timely output should be done within a laboratory. Turnaround times are monitored scrupulously to ensure that effective treatment can be instituted and difficult/problem cases referred to appropriate centres. Other innovations that are now integrated into routine practice in some institutions are standardized synoptic reporting which ensures that key information is transmitted to treating physicians in a uniform manner. Laboratory information systems should be fully computerized allowing for swift transfer of results and reports, and information to be placed in cancer repositories for planning and epidemiological studies. External quality assurance is also fairly routine and many laboratories undergo strenuous external review of all facets of their operation before achieving accreditation. It will be obligatory to participate in some form of external quality assurance across the board in due course. The College of American Pathologists and the Royal College of Pathologists in the United Kingdom have established guidelines regarding requirements for accreditation, best practice and multidisciplinary team meetings. In addition, the Association of Directors of Anatomic and Surgical Pathology have also published recently on quality assurance and improvement in surgical pathology.

Sub-specialization

The expansion of knowledge has meant that the need for sub-specialization is greater than ever before. Not only are new therapeutic molecules that impact on patient management discovered regularly, but smaller lesions are being identified by sophisticated radiological techniques which means surgical pathologists have to be acutely aware of early and/or extremely subtle lesions that may have clinical consequence. This brought a new kind of pressure for the general surgical pathologist: how does one maintain sufficient expertise in all fields? There is compelling evidence to support sub-specialist expertise so that every patient has access to the best opinions. The balkanization of surgical pathology is inevitable. However, it must be borne in mind that only a small, yet significant, proportion of cases need expert opinion: remember, common things are common. Under no circumstances should a so-called “triage pathologist” emerge out of fear or a headlong dash for expert opinions. Above all, the individual surgical pathologist needs to have insight: when someone is out of their depth and when to solicit another opinion cannot be mandated. However, advances in telepathology have meant that time and distance are no longer rate limiting factors to obtaining a second opinion. Regionalization of specialized cancer
services has also meant that quality is ensured but distinct referral pathways will also aid in the acquisition of second opinions. It is idealistic to think that all surgical pathologists will be au fait with all specialties and the nuances within each. Training should be geared to preparing pathologists with an overall understanding of what is needed for a modern surgical pathologist. The specialty is well catered for in terms of meetings, conferences, and updates that provide an excellent opportunity to upgrade knowledge. Electronic means of acquiring knowledge is at hand with numerous educational resources from dedicated websites to journal being available at the click of an icon.

We have to ensure that colleagues are not isolated because of geographical factors in a large country such as Canada. It is not unrealistic to suggest that remote solo practices are not to be encouraged. If unavoidable, then measures need to be in place to allow ready access to colleagues, and telepathology is the ideal mechanism to make this possible.

The Future

The future of surgical pathology is bright, enticing, and full of promise. It attracts the brightest minds as it still has the mystique that only a blend of art and science can conjure. The ingredients have been made more intoxicating as we stand squarely at the interface of applied and basic molecular biology (“translational pathology”). The importance of morphology should not be underscored in the frantic pursuit of a molecular solution. Instead, it is becoming abundantly clear that the two are inextricably linked with both having an impact on each other. Both should be harnessed in a meaningful manner that will allow for the progress that we all demand. There is much to be proud of in terms of what has already been achieved in pathology. As a specialty we do have some daunting challenges that are not insurmountable but which can be addressed with the openness, honesty, and élan that befits the jewel in the crown of modern medicine.

References


The Incoming Editor-in-Chief

Dr. Godfrey Heathcote, incoming editor-in-chief, is head of the Department of Pathology at Dalhousie University (2004–) and chief of pathology and laboratory medicine for the Capital District Health Authority in Halifax, Nova Scotia.

Dr. Heathcote has a wealth of academic and administrative experience as well as many publications to his credit related to ophthalmic (and head and neck) pathology, his area of special interest. He is chair of the Canadian Ophthalmic Pathology Society and section editor of general and ophthalmic pathology for the Canadian Journal of Ophthalmology.

Please join me in welcoming Dr. Heathcote. We look forward to his stewardship of the journal.

Jagdish Butany
Go to where the puck is going, not where it has been.

– Walter Gretzky

With these famous words, Walter Gretzky helped to shape the remarkable “anticipatory” skills used so successfully by his son, Wayne, one of the greatest hockey players of all time.

In the various disciplines of pathology and laboratory medicine, we are living in an era of rapid advances in knowledge and unprecedented opportunities for transforming health care. As a former residency program director and department head at the University of British Columbia (UBC) and now department chair at the University of Toronto, I have been asked to comment on the state of post-graduate training in pathology and laboratory medicine disciplines, focusing on some current trends that might shape our future as a sustainable and relevant part of the health care system. Unlike Wayne Gretzky, I have not developed any special aptitude for anticipating what lies ahead (despite having a crystal ball on my office desk); however, I have had the privilege of working in two outstanding institutions with many highly talented and accomplished colleagues, and have learned from those for whom innovation, creativity, renewal, and sustainability are hard-wired. Moreover, there are a number of trends affecting areas outside of pathology and laboratory medicine, and there may be useful analogies that could be applicable to our profession. In this presentation, I will briefly review the role of the university in post-graduate training in the disciplines of pathology and laboratory medicine, identify some current developments for which there is rationale for devoting thought and attention, and speculate on what the future might look like if we answer the call to action for ongoing development and improvement to what we do and who we are.

Role of the University

A major conceptual framework that emerged some years ago was for the accreditation of residency training programs of the Royal College of Physicians and Surgeons of Canada to run through universities, as opposed to individual hospitals, institutes, or agencies. I am not aware of the original basis for creating this particular framework, nonetheless it has had profound implications in shaping the relationship between clinical practice (a responsibility of bodies affiliated with the university) and the academic mission of a university (creating and conveying knowledge through research and education). An easy way to
think about this is that the university is not in the business of patient care, and the roles and responsibilities of each of the parties are articulated in affiliation agreements signed between the university and its partners. While clear for Royal College residency training programs, the role of the university has not entirely been defined for the various post-PhD diploma programs in clinical laboratory science, some of which are run through the university and some of which are not. In my opinion, this is an area that requires revisiting and discussion with government, for harmonization and clarification of expectations and performance. In addition, despite universities being charged with accreditation of residency training programs, there have been episodes of misunderstanding on the part of universities in recognizing post-graduate teaching by faculty, for example in the context of academic promotion. In this area, recent trends are encouraging, as universities seem to be becoming better “educated” about post-graduate teaching, and academic departments are doing a better job of “packaging” these activities in a manner understandable by the greater university.

Some Current Trends Facing Our Profession

Personalized Medicine and Translational Research

Through the late part of the 20th century, the prospective, double-blind randomized clinical trial was considered to be the “gold standard” for evaluating the effectiveness of new therapies in comparison to usual treatment. This model provided a robust scientific and statistical foundation to the evaluation of innovative therapies and represented a marked advance over previous activities such as anecdotal case reports or uncontrolled case series. By contrast, current indications are that we are reaching (or perhaps have already reached) a plateau for the utility of the randomized clinical trial, manifested by lower expectations by which the “success” of new pharmaceuticals is considered more in terms of lack of inferiority, rather than superiority, when compared to standard treatment. Pharmaceutical companies are experiencing difficulties in developing and bringing to market so-called “blockbuster” compounds that served the industry and its shareholders so well for many years, further indicating that new approaches are needed. In 2003, a worldwide vice president at GlaxoSmithKline stated, “The vast majority of drugs – more than 90% – only work in 30 or 50% of the people.” This underscores the need for better predicting who is likely to respond to a given therapy, as well as who is likely to experience side effects. With the completion of sequencing of the human genome, we are now in the “post-genomic era” in which gene-based and other “-omics” (i.e., proteomics, metabolomics, etc.) approaches are being aggressively developed and pursued to fulfill the promise of personalized medicine: “giving the right person, the right treatment, at the right time, in the right amount.” In addition to predicting effects of therapy, the discovery, and validation of biomarkers of disease have profound implications for risk stratification (“pre-diagnosis”) and more refined diagnosis and prognosis of disease (“theranostics”). Our profession is particularly well positioned to accelerate “translational research,” defined here as the process by which discoveries are validated, qualified, and implemented into practice for improved health outcomes. In addition to the intellectual property and commercialization aspects that tend to dominate discussions surrounding translational research, we are poised to contribute to the growing “evidence base” of newly developed and validated biomarkers, to larger purposes such as cost-benefit analyses and informing health care policy and legislation. This represents a marked advance over the previous tendency where new discoveries were promoted loudly, with lesser regard to potential downstream implications. For example, if one considers the implementation of molecular laboratory testing for the SARS-associated coronavirus, the assay itself is no doubt robust and satisfactorily validated, yet going forward, how many clinical specimens would have to be tested, at what cost, before there is a single true positive result? Our profession has the requisite expertise in crucial areas of pre-test and post-test likelihoods, and insight into pre-analytical, analytical and post-analytical considerations regarding laboratory data. This gives us legitimacy and relevance for which our post-graduate trainees can become expert, and continue to build and refine.

Biobanking

Exquisitely phenotyped and annotated biospecimens are the fuel of transitional research. Pathology and laboratory medicine is the obvious steward of human biospecimens, and initiatives such as the BC BioLibrary provide an opportunity for our profession to exhibit innovation and leadership in the design, standards, and public engagement related to biobanking. We are living in a time of increasing accountability to our funders and to the public, and it behooves us to be visible front and centre with respect to biobanking: the risk is that we will become marginalized or forfeit our legitimate and highly value-
added role if we choose not to lead. Post-graduate trainees need to appreciate the gravity of these considerations and themselves become pro-active and assume leadership as we progress toward personalized medicine.

**Disruptive Technologies**

Pathology and laboratory medicine has a rich tradition of applying new technologies to examine diseases in unprecedented ways. In anatomical pathology, the hematoxylin and eosin stain done on sections prepared from formalin-fixed, paraffin-embedded tissues has changed little since the 19th century; this technique remains the cornerstone for rapid and inexpensive screening and diagnosis of disease. Over the years, advances in special stains, histochemistry, electron microscopy, immunohistochemistry, and confocal microscopy have been effectively implemented to refine and improve the capabilities of conventional light microscopy. In recent years, nucleic-acid based technologies such as in situ hybridization and the polymerase chain reaction have transitioned from the research to the clinical laboratory setting. For example, in the diagnostic virology laboratory, viral culture is becoming a technique of the past. Technological advances have also provided increasing automation and range of applications that often blur the boundaries between traditional laboratory medicine disciplines. A mass spectrometer for proteomics can be used for such diverse purposes as cancer diagnosis, microbiology, metabolic disease and organ transplantation. In addition, newer imaging modalities such as micro CT, in which the resolution rivals that of light microscopy, are beginning to blur the boundaries between radiology and pathology/laboratory medicine. What such technological convergence means for the training of the next generation of pathologists, laboratory physicians, and scientists is that acquisition of sound fundamentals in the underlying assumptions and factors (pre-analytical, analytical, and post-analytical) that go into the production of a “result” are paramount. Having such a knowledge base is important to distinguish the domain expertise from that of our other colleagues or the lay public, who may also have access to data and information, but not the additional background and perspective that goes into meaningful interpretation. Our training programs have to meet the challenge of ensuring that graduates understand these considerations and can continue to do so with the advent of future disruptive technologies. This approach is analogous to the major shift in emphasis that occurred in legal education toward the end of the 19th century, when law school curricula changed from having students memorize every statute on the books to learning how to “think like lawyers think” (today this is known as problem-based learning). It took many decades for a similar change in emphasis to occur in undergraduate medical education, which involved a transformation from rote memorization of medical facts, to studying pathogenesis as a means for rationally approaching diagnosis and therapy, to having the ability to access and interpret a myriad of data and information (see below). For post-graduate education, emphasizing solid fundamentals and being able to understand new advances are crucial to ensuring graduates will be well prepared for long-term competence and relevance.

**Electronic Networking**

It is self-evident that post-graduate training has to be relevant to “real-world” practice. Within Canada, there is currently a wide diversity of practice models, and what is relevant to one geographic region (and its associated culture and other characteristics of disease prevalence, incidence, referral patterns and the like) may have little or no relevance elsewhere. While the setting of any individual practice may have unique considerations, any practice model must enable acceptable performance that is accountable to the standards of the profession. With technological advances in videoconferencing and telepathology, the world is becoming increasingly linked and networked, and this has potential for more effectively transmitting new knowledge for continuous improvement. Examples showing the capability of high-speed, broadband connectivity include the UBC medical undergraduate curriculum, which, in the face of expansion to distributed sites on Vancouver Island and in Northern British Columbia, necessitated implementation of innovative technologies so that students at each site would have a comparable educational experience, in fulfillment of accreditation standards. The electronic videoconferencing and distance education approaches in the UBC medical undergraduate curriculum have performed very well in terms of overall functionality and reliability. With the upcoming expansion to the Okanagan, the look and feel of the technology will remain consistent. In Toronto, at the University Health Network (UHN), approximately 17 hospitals in Ontario have high-speed, broadband telepathology connection to the UHN department. There is considerable interest in evaluating the impact of this telepathology system on indices of patient care, with exploration of education and research potential still in the early days.
Consilience: A Path to Wisdom?
The systems theorist and professor of organizational change, Russell Ackoff, has posited that the human mind can be classified into five categories: data (raw material, symbols); information (processed data that answers “who,” “what,” “where,” “when”-type questions); knowledge (application of data and information to answer “how” questions); understanding (appreciation of “why”), and wisdom (evaluated understanding). Of these categories, wisdom is the pathway toward creating the future, and given what is at stake for our profession, the question is, how do we get there? In the academy, the time-honoured way to attain wisdom has been through increased specialization (i.e., being “the big fish in a small intellectual pond”), but there are problems with this approach. Ponds tend to shrink and dry up due to irrelevance (“know more and more about less and less”), fragmentation increases and overall this compromises the ability to tackle the really big, important issues. As a response to this trend, funding agencies have encouraged increased networking and cross-talk between disciplines, with formation of multi-disciplinary, inter-disciplinary, and trans-disciplinary teams. Consilience, from the Latin “jumping together,” is an approach for the unity and integration of knowledge through synthesis and is a direct contradiction to the paradigm of increased specialization.

With technological advances and increased automation, data are being obtained faster than they can be interpreted. Systems biology focuses on understanding the relationships between the components of a biological system, requiring a new skill set that goes above and beyond acquiring data and trying to make sense of information. Pathology informatics offers a compelling approach to this situation, for which training will result in the next generation of our laboratory physicians and scientists becoming more adept at the type of abstract reasoning needed to derive meaning from the mounds of data being generated by ever-increasing sophisticated technologies.

Concluding Remarks
In summary, I have attempted to anticipate “where the puck is going” based on several trends that promise to profoundly impact the future of pathology and laboratory medicine and which have implications for both the structure and content of post-graduate training. Our profession has an admirable track record of being able to adapt to change: the challenge is to deal with a world in which knowledge is rising exponentially. I propose that consilience provides a possible means for us to achieve wisdom and be vibrant leaders who shape the future in a relevant and compelling manner.

Acknowledgements
Special thanks for Dr. Malcolm Silver, who encouraged my career development at crucial stages; Dr. James Cullen, for his inspirational example; Dr. James Hogg, an outstanding role model and mentor for embracing innovation and change; and Dr. David Hardwick, for helping me learn to see the world with curiosity and discipline.

References
Research in Pathology

Avrum I. Gotlieb, BSc, FRCPC, MD

R esearch in pathology has been and continues to be a cornerstone of pathology. Over 150 years ago, Rudolf Virchow carried out observational research on the clinical tissues he and his colleagues obtained from patients with disease. He applied histology to pathology and came to the conclusion that the cell regulates the physiology and pathology of the human being.1 With simple tools and very keen observational skills, he was able to begin the modern era of research into the pathogenesis of disease. Over the years, research has always been a component of the practice of pathology and pathologists developed and embraced state-of-the-art technologies to further their studies into pathogenesis. As medicine advanced, organized pathology developed research, education and clinical platforms which favoured the strong presence of scholarship and knowledge creation. These platforms became embedded in the emerging practice of anatomic pathology as it became an essential patient care specialty in medicine.

Drivers of Pathology Research

Today, research in pathology takes on many forms extending from basic to translational to clinical investigations. The common thread in all these is that the inspiration and ideas to drive the research originates from the cells, tissues, and body fluids pathologists examine in their clinical practice.2 This careful in-depth study of clinical material to describe and characterize the pathology is the first step along the research path. This has been the mainstay of research in pathology and has been invaluable to medical progress in general. Today pathologists investigate tissue, cells, and molecules to provide an integrated understanding of the pathobiology of disease. The goal of the pathologist then is to ask innovative questions and to pursue answers through research. This is done through independent research programs organized and led by individual pathologists as principle investigators (PIs) and/or by collaborative group or team initiatives in which pathologists are the active members. Pathologists with high quality subspeciality training make excellent leaders of research programs. The research programs themselves must be well funded to carry out the expensive state-of-the-art research. However observational studies and clinical-pathologic studies can be done with very little funding and have proven valuable. The aim of research in pathology is to transform pathology practice from purely diagnostic to predictive and curative.

Subspecialization Enhances Research

A focus on subspecialty pathology practice allows pathologists to create research platforms around their clinical practice. As consultants, pathologists need to have in-depth, state-of-the-art knowledge about health and disease. In the modern medicine practiced in Canada today, it is impossible to keep up with all areas. To continue to be truly effective, subspecialization has become a necessary way to strengthen the quality of practice. The formation of site groups and subspecialty groups not only provides excellent ways to deliver consultations to clinical colleagues, but also educational and research platforms. Subspecialization provides high volume case material, time for in-depth study and excellent opportunities to carry out meaningful studies on mechanisms of disease.

Translational Research and Personalized Medicine

To be at the forefront of translational research pathologists need to be well trained in this area of research. Since pathologists sit at the crossroads of basic science and clinical medicine, they will need to be agents of change. They handle human biological materials—tissues, cells, serums, other fluids—and as custodians of this material, pathologists need to ensure that this valuable research resource is utilized in an efficient and effective way to promote high quality health care. This requires training programs in translational approaches at the graduate and fellowship...
levels as well as investments in institutional infrastructure to support this research and develop new technologies that will lead to new basic discoveries that can be moved to clinical practice.

As we discover more about the abnormal biology of disease, we are beginning to understand the heterogeneity of disease in individual patients in order to develop customized treatment. This will require sound research at all levels of investigations. Laboratory physicians have the opportunity to be leaders in the use of both cell and molecular biology and imaging to investigate disease. There have been successes although progress is slow for new discoveries to move into mainstream clinical practice. Pathologists are at the forefront of personalized medicine and are making the investment in time, energy, and money to pursue this goal with vision and vigour. To accomplish this, laboratory physicians will require highly specialized knowledge in the cell and molecular biology of human disease and be willing to engage in interdisciplinary research. This requires adjustments to the curricula of specialty and subspecialty training programs. To achieve robust programs in research in pathology, excellent well-funded research fellowships need to be made available in Canada to train pathology and laboratory medicine subspecialists at our Canadian training centres. Any time we send our trainees out of the country to train, we run a serious risk of losing them to other jurisdictions. We have the capacity to train fellows in Canada and this should be exploited by provincial and federal health care providers. These training platforms should enable residents and fellows the opportunities to become well trained and make new discoveries in molecular laboratory medicine even at early stages in their careers.

Maintaining a Research Agenda

To maintain Research in Pathology programs, stable funding sources are required that keep up with inflation. Funding is needed in five main categories: (1) operating grants, (2) equipment, (3) training grants, (4) personnel (principal investigator/faculty) awards, and (5) overhead. The Canadian Foundation for Innovation has been effective in providing state-of-the-art, expensive equipment for big science. The Canada Research Chair program has provided much needed salary support for Canada’s best biomedical scientists. Canadian Institutes of Health Research provide overhead for operating research grants but at an average of 20% which is not nearly enough to fund the ongoing costs of maintaining the infrastructure of research facilities. We need to provide the intellectual and the financial infrastructure to support an important group of biomedical investigators, physician-scientists and physician-investigators. Pathologists are in a position to explain to the lay community, to politicians and to community leaders that there is much value in biomedical research. Pathologists need to look for partners in government, academia, industry, and patient advocacy groups to move the research agenda forward. Pathologists should accept the advocacy challenge and act to make the case of biomedical research.

Research in pathology is broadening its domain. The gap between Academic Health Sciences Centers (AHSC) and the community setting is narrowing and research in pathology is extending beyond AHSC. Community hospitals are now providing services that were once the exclusive domain of fully affiliated teaching hospitals. With these changes, one can envision subspecialty research teams working side-by-side with community laboratory physicians outside the domain of the university setting to investigate pathobiology of disease.

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Anatomical Pathology Training in Canada: Perspectives from Junior Residents

Zhongchuan Will Chen, MDCM, Eric K. Morgen, MD

As residents in the earliest stage of training in anatomical pathology (AP), our perspectives on residency training are based on our limited personal experience and that of our junior colleagues. Nevertheless, we will attempt to voice junior resident concerns and identify areas where pathology residency programs may need improvement. We will comment on the structure and logistics of pathology residency programs and advocate for increased training in a few areas that we feel are important.

The Royal College has recently discussed changing the structure of pathology training, and while the current 5-year AP training framework generally works well, there is contention among junior residents regarding the rotating clinical internship year (PGY-1). Some feel it is minimally relevant to pathology training and should be eliminated, bringing us into line with American programs, while others argue that it provides invaluable exposure to clinical specialties that have frequent contact with pathologists. Most agree that if the clinical year exists, it should consist of more pathology-relevant rotations such as medical and surgical oncology and less of areas like general internal medicine, obstetrics, or general pediatrics. Another concern is the frequent absence of pathology exposure in PGY-1, such that a resident can begin PGY-2 unclear on such fundamental topics as autopsy procedure, grossing, and the logistics of a histology lab. Without early exposure to pathology, it may also be difficult for residents to decide whether they have chosen the right specialty. Our discipline does not need unhappy residents, nor do we wish to lose those truly suited to pathology who just need more on-service time to realize it. The above situations would both be improved by 2–3 months of mandatory introductory pathology rotations giving a broad-based exposure to autopsy, grossing, microscopy, and the functioning of a histology laboratory.

Many junior residents are worried about manpower and resource allocation in pathology, especially the large number of pathology trainees in Canada. Many believe the supply of pathologists now greatly exceeds demand, an imbalance exacerbated by the current recession. Although rising cancer rates in an aging and growing population should translate to an increase in pathology jobs, there seem to be absolutely no policy initiatives outside of Newfoundland to create such positions. In this context, it is no wonder that sigh-filled anecdotes from graduating residents about their fruitless job searches have already began to fray the nerves of junior residents. In a paradoxical trend, pathology programs across Canada continue to accept a large number of residents, with Toronto's program alone admitting around 13 annually in recent years. Many current residents would prefer to see the number of residency positions significantly decreased until market conditions improve and the demand for pathologists increases.

Moving to the subject of important topics in residency training, quality assurance/improvement (QA/QI), and laboratory management seem a logical place to start. QA/QI is crucial to pathology, with recent media coverage of so-called "scandals" only reinforcing this point. Surprisingly, QA/QI training is either minimal or absent in many residency programs. The recent addition of a quality assurance elective at a Toronto teaching hospital is encouraging, but it would be beneficial to our specialty if
all residency programs incorporated a well-structured, mandatory, 1- to 2-month or longitudinal rotation dealing with QA/QI topics. A closely related area is laboratory management, which is clearly relevant to practicing pathologists, but in which residents also receive little formal training. Fortunately, a QA rotation could easily include a laboratory management component, such as observation/participation in the duties of department leadership in assuring quality, managing resources, maintaining accreditation, etc. Since most residents will need to fulfill one or more leadership roles at some point in their careers, such exposure would prove invaluable.

In recent years, technological expansion in pathology has begun to accelerate, and since today's residents embody our specialty’s future, it is crucial that growth areas are not neglected in Canadian training. One area showing tremendous promise is molecular diagnostics. With origins in the 1940s, the field is by no means new, but recent years have seen a proliferation of exciting clinical applications. This has led to expectations of better diagnostic tests and disease-tailored therapies, extending to calls for “personalized medicine” from all fronts. The progress of molecular diagnostics and its role in pathology practice have gathered unstoppable momentum and, encouragingly, some residency programs have responded, with at least four Canadian universities routinely training residents in molecular pathology. Nonetheless, with 14 pathology programs in Canada, there is ample room for increased focus in this area.

Two other fields that promise to shape the future of pathology lie at the intersection of pathology with computers. The first is digital pathology, the application to pathology of digital imaging techniques such as whole-slide scanning, telepathology (already successfully implemented in Toronto) and multispectral imaging with the potential to bring greater efficiencies, instant glassless consults, improved QA/QC, and the ability to extract more relevant information from the same slide. As more slides become digitized, there will be an increasing role for computer-assisted diagnosis, with software programs accomplishing tedious tasks prone to human error, allowing pathologists to focus on higher-level information. The second field is pathology informatics, whose importance has been recognized for decades and which uses electronic information systems to facilitate various laboratory operations, from accessioning to reporting to retrieval of information for research and teaching. Internationally, there are a growing number of dedicated divisions and training programs in the area. This is in contrast to the Canadian situation, where residents have no options for in-country training and only minimal research opportunities in pathology informatics or digital pathology.

In an era of accelerating change in medical knowledge and technologies, it is not sufficient for universities to maintain training programs at a level that previously provided an exemplary education. To sustain the vitality of our specialty and build it a better future, we must accomplish a “technological revolution” similar to what has occurred in radiology. We must confidently embrace new technologies and use them to provide the best clinically relevant, patient-centred diagnostic information in a collaborative and highly integrated environment with radiologists and clinicians. This vision of true 21st century laboratory medicine can only be realized if we have the foresight to train our residents today in the technologies and methods that will be the mainstay of tomorrow.

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Expectations of a Senior Anatomical Pathology Resident

Marie S. Abi Daoud, MD, MHSc

Often, when talking with one of my staff pathologists, the conversation sometimes veers into, “When I was a resident ... we would come in at 6 am, gross 1,000 specimens, then do 12 autopsy cases, and wouldn’t leave the hospital until midnight.” OK, I’m being facetious; but often as a resident today, I’m always made to feel like when the staff pathologists were residents, things were harder and busier. Well, I’m writing this essay to illustrate that residency today is just as challenging as it was before – just different.

The expectations of a senior resident are high and varied. Let’s first start with acquiring the expected knowledge base to pass the Royal College exam, become a “competent pathologist,” and gain those beautiful five letters after our name. Learning all of anatomical pathology is no small task and it seems like on a daily basis a new diagnostic entity is being discovered, renamed, or reclassified. It’s no longer basal cell carcinoma with squamous differentiation, but rather metatypical BCC. Also, for such a small organ, the thyroid certainly has many types, sub-types, and variants of carcinoma! Then, add into the mix, molecular pathology. Seriously, how many chromosome deletions, translocations, and inversions can one person remember? When we are practising, are we not allowed to Google?

Next on our “to do” list is applying for those all-encompassing, coveted fellowship positions. To be a competitive applicant and score that staff position at the academic centre of our choice, we need to do a fellowship. Sure, we can work in a community centre, with general sign-out, but is that the way pathology is going? Let’s not forget that pathology has been in the media quite a few times. Things have gone awry in Newfoundland, New Brunswick, Ontario, and British Columbia. Which province is next? It seems to me that pathology is moving towards sub-speciality sign-out, with centralization of services. So, now that we as residents are absolutely scared of signing out on our own, of course we want to do a fellowship. In fact, we need to do a fellowship.

Another year of supervision is good for me!

Of course, to get that fellowship position, to get that staff position, we need to do research. When, pray tell shall we fit in our numerous research projects, posters, case reports, and abstract submissions? More and more, time management is becoming a necessary skill. Residents have to find the time to do research while grossing, signing out, and writing up autopsy reports. No worries, we’ll do it!

Next, we move onto the expectation of teaching. I personally love teaching. It means helping others, which is a big reason I chose medicine to begin with. There is a lot of responsibility with teaching. As my program director told me, it can be quite stressful, depending on the level of education of your audience. So, when we have high school students come through the department, we can pretty much tell them anything! However, when it comes to our peers, we better be prepared for tough questions, even more so for grand rounds and the questions from staff. So, preparation for grand rounds, gross rounds, autopsy rounds, journal club, and so on takes time. We do need those reference letters, for those fellowship positions, for that job!

So, last of the expectations are the administrative duties that come along with being a senior/chief resident: call schedule and vacations, stipends, teaching, and preparing junior residents. In short, we need to make sure all is good in the world of pathology residents. Sometimes that is a tall order, but we do it and we learn from it skills that we will take with us when we do become staff, we hope!

All in all, the expectations of a senior resident in anatomical pathology are realistic and “doable.” However, when I become a staff person, I hope that I will remember that being a resident is not easy and every generation deals with its own challenges. Ours are not harder than those past – just different.

Marie S. Abi Daoud, MD, MHSc, is with the Department of Pathology and Molecular Medicine at Queen’s University, Kingston, Ontario. She can be reached at dabi@dal.ca.
Permanent position available for a successful candidate to join a congenial group of laboratory physicians, consisting of five anatomical and general pathologists, a medical biochemist, a haematopathologist and a microbiologist. We have a full spectrum of anatomic pathology, cytology and haematopathology in addition to a very active service in chemistry and microbiology in a dynamic laboratory accredited by the College of American Pathologists since 1987. The laboratory will move to a new facility in Summer of 2009, located near the surgical suites and intensive care units, and with much new equipment. There is a potential for a departmental directorship on rotational basis for an individual with administrative experience or interest. Haematopathology or cytopathology experience would be an asset.

The Moncton Hospital is part of a large network of hospitals, health centres, services and programs under the direction of Regional Health Authority B which provides health services to the southern, central, and eastern regions of New Brunswick. The 365-bed critical care and major referral hospital serves communities throughout New Brunswick, Prince Edward Island and northern Nova Scotia. It offers services in Trauma, Neurosurgery, Burns, Oncology, Infectious Diseases, Neonatal Care, Provincial Child and Adolescent Psychiatry and Vascular Surgery plus extensive services in elevated secondary and primary care. The Moncton Hospital is one of the largest single employers within metro Moncton, employing 3,000 staff and physicians. The Department of Pathology and Laboratory Medicine requires one of the following qualifications: a) certification or Fellowship in Pathology of the Royal College of Physicians and Surgeons of Canada, or eligibility to write the examination for Fellowship in Pathology; b) certification by the American Board of Pathology; c) certification by le Collège des médecins du Québec; d) membership of the Royal College of Pathologists of England and Ireland. The salary range is determined by the New Brunswick Medical Pay Plan, depending on qualification and experience.

Applicants are invited to forward their resume to

Dr. David Kogon

Chief of Staff/Medical Director,
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We are fortunate to be residents in a pathology training program in Canada. Residency programs undergo stringent review on a regular basis to assure that all programs meet accreditation requirements. A pathologist graduating today must not only be a great morphologist but must also possess skills in immunohistochemistry interpretation, molecular and cytogenetic testing, and quality systems in the practice of pathology.

In recent years, we have seen pathology merge closely with the fields of molecular biology and cytogenetics. In some cases, diagnosis relies, to some extent, on immunohistochemistry and molecular and cytogenetic findings, which have important implications for prognosis and treatment. Residency training in some centres has responded to this evolution by increasing our exposure to these fields. Furthermore, opportunities for involvement in research in fields including molecular and cytogenetics, are readily available and encouraged. Similarly, recent news headlines have brought the importance of quality systems in the practice of pathology into focus and training programs are beginning to respond. Quality assurance and quality control has recently become a “hot topic” and has been incorporated into the curriculum through lectures and elective rotations. In these and other areas CAP workshops provide valuable learning tools, but could be expanded to include more of the new technologies as well.

Despite the challenges that the field has faced in the past couple years, we believe that the state of pathology training in Canada is strong. Residency training is evolving to respond to the changing role of the pathologist and we believe that most residents generally feel adequately prepared for practice. As we approach the end of training we look forward to applying our knowledge.

Unfortunately, as we observe the cohort ahead, it is becoming increasingly apparent that the number of job opportunities does not seem to be keeping pace with the perceived demand, number of graduates and residents in training. How can this be when only a few years ago we were told about looming retirements and a dire shortage of pathologists? Our training program responded logically to this and greatly increased the number of residents in training for the last two years. But now we are told that the situation may become somewhat analogous to the 1990s when many qualified pathologists could not find work and resorted to moving to the United States, pursued further training, or changed careers. This is a difficult pill to swallow for many of us with 15 or more years already invested in training and several hundred thousand dollars of debt. Why does the supply and demand for pathologists go through so many peaks and troughs? Who is doing human resource planning? As a profession should we not show accountability to taxpayers to ensure the money they invested in our education does not sit as an idle resource at the end of our training? Clearly, it seems that there is not sufficient dialogue between the government, universities, and our governing bodies. As we have learned recently in pathology, miscommunication is the source of many errors!

We are assured that the government plans to fund some pathologist positions but this may still fall well below the number of graduates per year from pathology at the University of Toronto alone! Furthermore, fewer pathologists are retiring and entry requirements have recently been eased for American-trained physicians. As we look at the number of junior residents in our program, we cannot help but wonder if some of us will be without jobs, at the end of five years of rigorous training!

This is a very unfortunate situation. Individuals, universities, and government have invested so much. New pathology graduates in Canada are highly trained, ready, and eager to enter the work force. We hope that the recent history of the 1990s does not soon repeat itself and we encourage open communication and human resource planning to address pathology needs in Canada.
Colorectal cancer (CRC) is the third most common cause of cancer death in the United States with 148,810 new cases and 49,960 deaths in 2008. Worldwide, CRC is the third most common cancer death, after lung and breast. CRC with distant metastases is challenging to treat and the 5-year survival rate is less than 10%. The existing, non-targeted chemotherapies are only partially effective. These include the combination of oxaliplatin (a platinum containing agent) or irinotecan (a topoisomerase I inhibitor) with an antimetabolite, 5-fluorouracil (5-FU) and leucovorin, also known as folinic acid (FA). Since the introduction of these non-targeted therapies, the mortality rate of CRC has decreased by 20–25% and the median survival has increased from 6 months with no treatment, to 14–16 months. Many patients, however, do not respond, or become resistant to, combination chemotherapies administered without additional treatment.

Recently, the development of targeted therapy has provided more options for CRC treatment. These include bevacizumab, a monoclonal antibody against the vascular endothelial growth factor (VEGF), as well as cetuximab and panitumumab, monoclonal antibodies against the epidermal growth factor receptor (EGFR). These drugs are currently used to treat patients with advanced CRC.
either in combination with chemotherapy or as single agent for patients resistant to chemotherapy. CRC survival has increased by more than 20 months following the introduction of targeted therapies.\textsuperscript{7}

A subset of patients responds to the addition of anti-EGFR monoclonal antibodies to standard chemotherapy. The molecular mechanisms involved in resistance to these drugs are still incompletely known. In order to reduce the costs ($30,000 to $160,000 per year, per patient) and spare patients from unnecessary toxicities (acne-like rash and hypomagnesemia) of anti-EGFR treatments it is essential to discover molecular markers that will help identify patients that can benefit from these drugs.\textsuperscript{8}

This article reviews the structure and function of the KRAS gene, how the maintenance of its normal (wild type) structure in CRC tumours is a reliable molecular marker for identifying patients sensitive to anti-EGFR monoclonal antibody therapy, in contrast to patients with tumour harbouring mutated KRAS. It also discusses the current methods to test for the KRAS genotype in a clinico-pathological setting.

**EGFR Activation and RAS Signalling**

EGFR is a 170 kD member of the HER tyrosine kinase growth factor receptor family, and is also known as HER1 or Erb-B1.\textsuperscript{9–11} It is a transmembrane glycoprotein consisting of an extracellular, ligand binding domain, a transmembrane region and an intracellular, ATP binding, tyrosine kinase domain. Ten different ligands can selectively bind to the extracellular domain of EGFR. These include the epidermal growth factor (EGF), transforming growth factor receptor alpha (TGF alpha), amphiregulin, betacellulin, heparin binding EGF and epiregulin.

The EGFR gene is not commonly amplified or mutated in CRC.\textsuperscript{12–14} It is, however, over-expressed in 65–70% of colorectal tumours.\textsuperscript{15–17} Higher EGFR expression correlates with tumour progression and hepatic metastasis.\textsuperscript{18}

The binding of EGF ligand to EGF receptor activates a complex intracellular signalling cascade. During the signalling cascade subsequent to ligand binding, EGFR forms a homodimer with another EGFR, or a heterodimer with another member of the EGFR family. Dimerization activates several tyrosine kinase based motifs that initiate transphosphorylation of receptor pairs. Autophosphorylation promotes the binding of adaptor proteins (e.g., Grb2) that recruit SOS1 a guanine nucleotide exchange factor. SOS1 facilitates the conversion of RAS protein from its inactive guanosine diphosphate (GDP) binding state to its active, guanosine triphosphate (GTP) state\textsuperscript{19} (Figure 1).

In its GTP bound state, RAS activates several downstream effector pathways including RAF/MEK/ERK and PI3K (phosphoinositide 3 kinase)/Akt (Figure 2).\textsuperscript{20} These downstream effector pathways act together to induce the expression of cyclin D1 which regulates the progression of cells through the G1 to the S phase of the cell cycle, promoting cellular proliferation, cell survival and apoptosis inhibition.\textsuperscript{21,22}

RAS activation is terminated by GTP hydrolysis via intrinsic RAS-GTPase activity, which is dramatically accelerated by GTPase Activating Proteins (GAP).\textsuperscript{23} Once signal transduction is complete, EGFR receptors are internalized and down-regulated, or regenerated on the cell surface.\textsuperscript{24}

**KRAS Genes and Their Mutation in Colorectal Cancer**

The human RAS proto-oncogenes coding for RAS protein consists of three principal members: KRAS, HRAS, and NRAS. The transforming activities of RAS oncogenes were first identified in the Harvey (HRAS) and Kirsten...
KRAS mutations occur in 30–45% of colorectal carcinomas.\(^\text{26,27}\) These mutations develop early in the progression from adenoma to colorectal carcinoma\(^\text{28,29}\) and are detectable in colon cancer precursor lesion known as the aberrant crypt foci.\(^\text{30,31}\)

KRAS mutations are localized to exons 1 and 2 in four codons (12, 13, 59, and 61). In colon cancer, 98% of mutations occur in codon 12 and 13, both coding for glycine amino acid in the wild-type gene.\(^\text{32,33}\) Codon 12 is more commonly mutated than codon 13 (77% versus 21% respectively). Codon 61 is mutated in 2% of the cases but the effect of this mutation on therapeutic responses is currently unknown.\(^\text{33}\) Occasional case reports of CRC have described mutations in other codons, such as codons 117 and 146, but these are rare and their significance is unknown.\(^\text{23,34}\) Andreyev et al report that mutations in codons 12 and 13 have a negative effect on survival and increase the risk of cancer recurrence.\(^\text{32,35}\)

Mutations target the guanosine triphosphate (GTP) binding domain of KRAS when located in codons 12 and 13, and the GTPase domain when in codon 61. Mutations inhibit the effects of GAPs, giving rise to a permanently activated mutant RAS-GTP complex that no longer depends on EGFR regulation.\(^\text{33}\) This results in abnormal differentiation and unregulated colon cell growth as well as enhanced tumour angiogenesis.\(^\text{36,37}\)

**KRAS Mutation Detection**

Immunohistochemistry is inadequate to detect KRAS mutations because presently available antibodies cannot reliably distinguish wild-type versus mutant KRAS proteins. Molecular biology techniques are currently used to demonstrate KRAS mutations. The use of highly sensitive methods, some of them requiring microdissection, are necessary because non-neoplastic cells are frequently present in tumour specimens. KRAS mutations were previously detected by direct sequencing; however this method has a sensitivity of 25% which is insufficient. Polymerase chain reaction (PCR)-restriction fragment length polymorphisms, PCR-single strand conformation polymorphism with denaturing gradient gel electrophoresis (SSCP/DGGE), and mutant-allele-specific amplification were the other methods being used to detect KRAS mutations. More recently, he amplification refractory mutation system (ARMS) variant of real-time PCR has proven to be a sensitive method for KRAS mutation detection in fixed tissue specimens containing as low as 1% of tumour cells.\(^\text{38,39}\)

A highly sensitive KRAS mutation assay has been recently described, adapting the smart amplification process version 2 (SMAP-2) with the addition of a peptide nucleic acid clamp with exact homology to the wild-type allele.\(^\text{40}\) Mutant DNA could be identified even when it was present in 0.1% of the DNA in a specimen. With this technique, a single assay in a single tube can be used to identify all possible mutations in codon 12 of KRAS. Whatever technique is being used, it requires adequate validation by DNA sequencing.

**Subtypes of Colon Cancer Associated with KRAS Mutations**

CRC occurs as both sporadic and hereditary conditions. Hereditary CRC, which includes hereditary non polyposis colorectal cancer (HNPCC) and the rarer familial adenomatous polyposis (FAP), accounts for approximately 5–10% of all cases.\(^\text{41}\) HNPCC is an autosomal dominant condition caused by germline mutations in mismatch repair (MMR) genes such as hMLH1, hMSH2, hMSH6 that result in deficient DNA repair and the development of tumours with high levels of microsatellite instability in short tandem repeat DNA sequences. FAP-associated CRC, also an autosomal dominant hereditary cancer, accounts for about 1% of all CRCs and is caused by a
germline mutation in the adenomatous polyposis (APC) gene. The specific causes of sporadic CRC are currently unknown, but are probably multifactorial and have been related to environmental factors, including diet and smoking, the effect of which on the KRAS gene are incompletely understood.

KRAS mutations have been found in both sporadic and hereditary CRCs, but there is conflicting evidence on their frequency. Several studies report a similar frequency of KRAS mutations in sporadic tumours and in patients with FAP and HNPCC, however, found a lower frequency of KRAS mutations in HNPCC than in sporadic cancer. Oliveira et al. published the most detailed analysis of KRAS mutation frequency in different subtypes of colon cancer. They report that KRAS mutations are more frequent in HNPCC than in sporadic cancers with microsatellite instability (40% and 22% respectively). However, HNPCC and sporadic CRC cases with stable microsatellite DNA do not have a significantly different incidence of KRAS mutation. In patients with HNPCC, those with germline mutations in hMSH2 and hMSH6 mismatch repair genes, have a higher frequency of KRAS mutations compared to those with hMLH1 mutations. The lowest frequency of KRAS mutations is found in sporadic cancers with microsatellite instability that also have hMLH1 promoter hypermethylation.

The presence of KRAS mutations in primary CRC tumours versus in metastatic sites is an area that requires further investigations. Most studies screening for KRAS mutations evaluate the presence of this molecular marker in the primary colorectal tumour, while the response of anti-EGFR antibodies is assessed in the setting of metastatic disease. The absence of KRAS mutations in the primary tumour does not necessarily mean that KRAS mutation is absent in metastases. The study by Di Fiore et al. reports an identical KRAS mutational status in primary and metastatic tumours; however, their sample size is too small to show any significant results. Further research is required, but it might become important to screen metastases directly for KRAS mutations.

**Targeted Therapies of CRC with Anti-EGFR Antibodies**

Cetuximab is a chimeric, mouse-human immunoglobulin (Ig) G1 against EGFR. The US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) approved its use in 2004. Panitumumab is a fully humanized IgG2 antibody against EGFR. It was approved by the FDA in 2006 and by the EMEA in 2007. Cetuximab and panitumumab bind to the extracellular domain of EGFR with high specificity and affinity, and competitively inhibit the natural ligand from binding to EGFR. The receptor becomes unable to dimerize and the intrinsic tyrosine kinase is not activated, leading to EGFR internalisation and degradation. Binding of anti-EGFR monoclonal antibodies to EGFR prevents the activation of EGFR dependent RAS/RAF/MEK and PI3K downstream signalling pathways. Cellular proliferation and angiogenesis are inhibited and apoptosis is stimulated.

Cetuximab is indicated for use, in combination with irinotecan or on its own, for the treatment of EGFR-expressing stage IV colorectal carcinoma in patients who are refractory to treatment with irinotecan alone or in combination with other chemotherapy drugs. The indication for panitumumab is comparable to cetuximab, but patients must also carry a wild-type KRAS gene.

Cetuximab is given intravenously at weekly intervals with doses of 250 mg/m² until disease progression or toxicity development. Panitumumab is administered intravenously at a 6 mg/kg dose once every 2 weeks until disease progression or toxicity development.

Severe hypersensitive reactions are infrequent, occurring in 3% of patients. The most common side effect is an acne-like rash, experienced by 88–90% of patients. Pruritus, dry skin, skin fissures, and desquamation are also possible but uncommon. Conjunctivitis and hypomagnesemia have been reported in a small number of patients. Infusion related side effects also include nausea, vomiting, headache, dizziness and chills. Response to anti-EGFR treatment is documented by medical imaging, including chest radiography, magnetic resonance imaging, and computed tomography.

Nimotuzumab is another humanized anti-EGFR monoclonal antibody used to treat various carcinomas. Its use in the treatment of CRC with different KRAS genotypes has not been reported in a large series of patients.

**Evidence for the Association of the Therapeutic Response to Cetuximab and Panitumumab with KRAS Status of the Tumour**

Patient response to cetuximab and panitumumab is measured by assessing complete response defined as the complete disappearance of all measurable lesions, without the appearance of any new lesions, and partial response defined as reduction in bi-dimensionally measurable lesions by at least 50% of the sum of the products of their largest perpendicular diameters and an absence of pro-
pression in other lesions, without the appearance of any new lesions.51

As is customary in adjuvant treatment studies, the overall response to anti-EGFR drugs is measured by overall survival (OS), and progression-free survival (PFS). The latter is calculated from the date of randomization to the first observation of disease progression, or to death from any cause within 60 days after randomization or the most recent tumour assessment.

The overall response rate to cetuximab and panitumumab in patients expressing EGFR, but whose tumours have not been pre-screened for KRAS mutation, remains poor. Twenty three percent of patients respond (by tumour shrinkage) when cetuximab is combined with irinotecan and only 11% respond when cetuximab is administered on its own. The OS and PFS are longer in patients receiving both cetuximab and Irinotecan than cetuximab on its own (OS: 8.6 months versus 6.9 months; PFS: 4.1 months versus 1.5 months respectively).51

Using panitumumab, 10% of patients had an objective response. The mean PFS is longer for patients receiving panitumumab than patients receiving basic standard of care (13.8 weeks versus 8.5 weeks respectively) but the mean PFS is similar (8 weeks for panitumumab and 7.3 weeks for basic standard of care). Seventy-six percent of patients in BSC group crossed over to receive panitumumab after disease progression. There are no observed differences between the two groups for OS.52 Patients that do not respond to cetuximab and panitumumab remain resistant to these drugs.

The discovery that the OS in tumours with wild-type KRAS is longer than in patients having KRAS mutations was a major advance in the documentation of the therapeutic benefits of cetuximab.7,8 Disease progression following cetuximab therapy is faster in metastatic CRC patients bearing KRAS mutations than in patients with wild-type KRAS.46,56 The presence of wild-type KRAS correlates with greater disease control.57 After cetuximab treatment, wild-type KRAS expressing tumours demonstrate a greater (> 9.66%) reduction in tumour size than in tumours expressing mutant KRAS.58

Overall, approximately 50% of patients with wild-type KRAS tumours receiving cetuximab respond to it, but the mechanism(s) by which the remaining 50% resist this treatment is unknown.59 One possibility is that unlike the tumour cells in the primary tumour, those present in metastatic sites have mutated KRAS and have clonally expanded. Another mechanism would be that metastatic tumour cells might have evolved a transduction step that blocks signalling from EGFR.

Patients with wild-type KRAS tumours who receive panitumumab monotherapy have higher OS and PFS than those with mutant KRAS tumours. Tumour reduction following panitumumab administration is observed in the wild-type KRAS group but not in the KRAS mutant group.59

Summary and Future Directions

Following the introduction of anti-EGFR therapy the prognostic of patients with metastatic CRC has improved. However, treatment of a patient with anti-EGFR therapy is expensive and side effects, although minor, can be unpleasant. The treatment is successful in some patients but others remain resistant. In order to spare patients from high costs and unnecessary toxicity of these drugs, it was necessary to identify good markers that could separate responders from non-responders. The mutational status of KRAS gene is the best possible marker available today for this purpose. KRAS mutation is present in CRC tumours of most non-responding patients. The majority of patients with a wild-type copy of KRAS have a better response to anti-EGFR treatment, as shown by longer overall survival and progression free survival. KRAS mutations occur frequently in CRC with the highest frequency occurring in the hereditary cancer HNPCC with microsatellite instabilities. The most common KRAS mutations are localized on codons 12 and 13, but mutations in other codons, 59, 61, 117 and 146 are also reported. Mutation on codon 12, changing glycine to valine is associated with the worst outcome for CRC.

The necessity to test colorectal tumours for EGFR expression by immunohistochemistry is controversial. In American and European countries both cetuximab and panitumumab are approved for use in metastatic CRC patients who express EGFR protein in their tumour as demonstrated by immunohistochemistry (EGFR positive). There is evidence, however, that a subset of patients who do not express EGFR (EGFR negative) can also respond to anti-EGFR treatments. Patients with EGFR negative metastatic CRC have a 20–25% response rate to cetuximab plus irinotecan therapy,8,60 which is similar to the 23% response rate to cetuximab given on its own in EGFR positive tumours.51 Based on these results, the immunohistochemical detection of EGFR expression may no longer be suitable for selecting patients for anti-EGFR treatments, as it may exclude patients likely to benefit from such therapies. More research in this area is required.
but it appears that identifying the KRAS gene status is a superior screening marker.

The second challenge concerns the validity of KRAS status as an exclusive marker or the need to search for new markers predictive of responses to anti-EGFR targeted therapies. Some researchers propose that KRAS status serves as the only basis for selecting metastatic CRC patients as candidates for treatment with anti-EGFR drugs. There are, however, reports that may contradict this proposal. Two studies show that KRAS mutants with advanced CRC experienced a significant response to anti-EGFR therapy and had improved OS and long-term disease control. This suggests that the inhibitory signal exerted on EGFR can be transduced by another pathway, independent of KRAS in some tumour cells. Among the most likely signalling candidates, there might be a need to evaluate the role of RAF, MEK, and ERK in relation to response or resistance to anti-EGFR drugs.

Increased EGFR gene copy number has also been proposed to be a candidate as a predictive marker. Different groups have reported varying percentages of CRC patients with increased EGFR gene copy number, some reporting its rarity (10–15%) while others have reported a much higher percentage (31%). A number of research studies conclude that there is a positive association between increased EGFR copy number in patients with metastatic CRC and improved response to anti-EGFR monoclonal antibodies. However, patients without EGFR copy number amplification can still respond to cetuximab. This marker therefore plays only a minor role compared to KRAS when measuring response to anti-EGFR therapy.

References
Molecular Pathology of Colorectal Cancer

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