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Molecular Diagnostics

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## Emerging role of Immunohistochemistry in Molecular Diagnostics and Theranostics

Personalized medicine creates an urgent need for rapid and accurate molecular characterization of cancers in the clinical diagnostic setting. Although many conventional molecular techniques (e.g. PCR-based) are available, such testing approaches prove to be relatively time consuming, technically demanding, and require a well-developed laboratory infrastructure. In addition, accurate results are at the mercy of tissue heterogeneity and sampling error and must overcome suboptimal preservation of RNA and DNA in formalin-fixed paraffin-embedded (FFPE) tissues. For these reasons, routine conventional molecular testing is difficult to deploy, particularly in resource-challenged academic centers.

Rising to this challenge, immunohistochemistry (IHC) can be performed on routinely processed FFPE tissue in a standard surgical pathology laboratory and can play a central role in complementing or reducing the need for commonly used molecular platforms, especially in the first-line screening process for molecular abnormalities. In some instances, the cost of molecular profiling can be reduced several-fold when using IHC in a testing algorithm as compared to direct nucleic acid-based testing.

Validation and feasibility of using IHC as a molecular screening test in various diagnostic scenarios has been and will continue to be an important subject of pathology-based studies. The introduction of HercepTest™ in 1998 as an IHC-based companion diagnostic test represented the first wave of molecular screening algorithms and has undergone further refinement and continued use to the present day.

Introduction of pre- to post-analytic standardization guidelines and quality assurance programs for IHC testing, in conjunction with major improvements in test sensitivity through use of polymer-based IHC detection and an increasing role for digital image analysis will allow IHC to shed its image as a finicky, unreliable and subjective test. In particular, the execution of predictive (so-called Class II) IHC tests must now occur within well-defined technical guidelines and under appropriate supervision and quality assurance.

In a climate of exciting advances and increased accessibility to conventional molecular testing (such as next-generation sequencing), IHC molecular screening has only continued to expand by virtue of significant analytic, practical and cost advantages, in spite of some major disadvantages (summarized in Table 1).

LAB  
CONNECTIONS

**Your feedback, suggestions and new ideas are welcomed. Submit to the Editorial Office:**

Dr. Cheryl Main, Editor, Email: mainc@hhsc.ca; Michelina Bozzo, Editorial Assistant, Email: bozzom@hhsc.ca

	IHC molecular screening	Molecular tests
<b>Advantages</b>		
Cellular and spatial specificity	Yes	No (excluding FISH)
One-day turnaround time	Yes	No
Result from single tissue section of a minimal sample	Yes	Not always
Additional specialized equipment and expertise	No	Yes
Cost per test	Low	High if non-multiplexed
Compatible with archival FFPE tissue	Most of the time	Not always
<b>Disadvantages</b>		
Robust results with improper fixation, alcohol fixatives	No	Generally better
Multiplex testing with comprehensive profiling	No	Yes
Cost per gene tested	Medium to high	Low if multiplexed
Equivocal result obtained	Can be common	Generally rare

**Table 1. Advantages and disadvantages of IHC molecular screening in an academic hospital diagnostic laboratory setting.**

Following HercepTest™, another major example of “molecular IHC” is screening for ALK-positive non-small cell lung cancers (NSCLC) to select patients for TKI therapy. Deployment ALK IHC is an example of an affordable and robust screening test used for a relatively rare disease (less than 5%) occurring within a massive starting number of NSCLC patients (about 5,000 per year in Ontario). HRLMP receives Ministry funding for this test, serves as a reference center for ALK testing, and was one of the first institutions in Canada offering routine ALK testing.

As additional relevant molecular markers emerge, IHC could serve a central role in tumor profiling comparable to the importance of immunophenotyping in the diagnosis of hematolymphoid malignancies. Examples of IHC screening tests, some of which are currently or expected to be offered by HRLMP in the near future, are provided in **Table 2**.

The future of IHC molecular screening is unknown. For the time being, this approach allows pathologists to overcome a few of the barriers encountered with implementing conventional molecular profiling given

limited resources. It would be possible, for example to create antibody “cocktails” that can detect multiple low-occurrence mutant proteins indiscriminately and simultaneously. A smaller pool of positive samples could then be subject to more specific tests without the need for costly in-depth profiling of all incoming samples. It is more likely, however, that with the dropping costs of next-generation sequencing and PCR-based tests and increasing convenience and compatibility with FFPE samples, IHC will serve to complement higher throughput molecular testing panels.

IHC test	Basis of IHC test	Application
MMR (mismatch repair)	Detects expressed normal DNA repair proteins	Screen for inherited cancer syndrome
PTEN	Detects normal PTEN tumor suppressor protein	Prognostic value in prostate cancer
ROS-1	Detects over-expressed gene fusion product	Seen in <2% of NSCLC, predictive of TKI response
BRAF V600E	Detects specific mutant protein	Predictive maker for Melanoma, used for colon cancer screen with MMR IHC
p16	Detects over-expressed protein	Surrogate marker for HPV infection
Combined p53, p21, PLK1	Identifies true p53 mutated tumors	Prognostic value in breast cancer
TLE1	Overexpressed in synovial sarcomas	Alternative diagnostic screen from SYT-SSX gene fusion tests
INI1	Detects normal INI1 tumor suppressor protein	Diagnostic value in rhabdoid tumors of CNS, kidney, soft tissue

**Table 2: Examples of IHC molecular screening tests in non-hematolymphoid malignancies.**

### References:

1. Canadian Association of Pathologists National Standards Committee / Immunohistochemistry Best Practice Recommendations for Standardization of Immunohistochemistry Tests. *Am J Clin Pathol.* 2010;133:354-365.
2. Ultra-deep sequencing confirms immunohistochemistry as a highly sensitive and specific method for detecting BRAF V600E mutations in colorectal carcinoma. *Virchows Arch.* 2013 Nov;463(5):623-31.
3. Comparison of Reverse Transcription-Polymerase Chain Reaction, Immunohistochemistry, and Fluorescence In Situ Hybridization Methodologies for Detection of Echinoderm Microtubule-Associated Proteinlike 4–Anaplastic Lymphoma Kinase Fusion–Positive Non–Small Cell Lung Carcinoma: Implications for Optimal Clinical Testing. *Archives of Pathology & Laboratory Medicine* Jul 2012, Vol. 136, No. 7 (July 2012) pp. 796-803.

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## Education News

Congratulations to **Dr. Jeremy Daniels** who received a Quality Assurance Award for his project entitled "Benford's Law for Forensic Pathology Quality Assurance". Dr. Daniels is currently a resident in the Anatomical Pathology resident training program.

For information and the latest news on our residency training programs follow the link:

<http://fhs.mcmaster.ca/pathres/news/index.html>

Information on the postdoctoral fellowship:

<http://fhs.mcmaster.ca/pathology/education/postdoctoralfellowshiptraining.html>

## News from the HRLMP

### Mitigating patient risk: expecting the unexpected



**October 18, 2014**

Mohawk College, 135 Fennell Ave. West

#### Objective

Attendees will explore how laboratories prevent errors in order to reduce the risk of harm to patients.

#### Who Should Attend?

Medical laboratory technologists, laboratory physicians, residents, students, other healthcare professionals.

#### Continuing Education Credits

Application for this event to be a group learning activity in progress.

#### Register early – space is limited.

[www.iqmh.org](http://www.iqmh.org)

## 7<sup>th</sup> Annual HRLMP Rapid Fire Showcase

A Potpourri of Innovative Science

Saturday November 22, 2014

8:15 – 12:15

Miller Auditorium, SJHH

## News from Genetics

The Cytogenetic Laboratory is excited to introduce a molecular based assay (QF-PCR) for the assessment of aneuploidy involving chromosomes 13, 18, 21, X and Y in perinatal tissues (ie. placenta, cord tissue or fetal tissue). This assay has been used within the Molecular Cytogenetic Laboratory for several years for assessment of aneuploidy in prenatal (amniotic fluid, chorionic villi) and neonatal (peripheral blood) specimens, but had not been routinely available for other perinatal tissues. Validation of this assay for use with placental, cord and fetal tissues expands the currently available armamentarium within the HRLMP for the investigation of genetic causes contributing to fetal death or congenital anomalies in recent pregnancies.

Tissue culture and classical cytogenetic approaches continues to be the standard methodology for chromosome analysis of perinatal tissues within the HRLMP Cytogenetic Laboratory; however, this technique relies on sufficient growth of tissues in culture in order to isolate dividing cells for chromosome analysis. Factors such as poor growth of abnormal tissues or receipt of non-viable or necrotic tissue (ie. due to intrauterine fetal demise) can negatively impact culture success thus leading to cytogenetic test failure. Validation of QF-PCR for analysis of these tissues is predicted to enhance the currently available services by enabling assessment of the most common chromosomal causes of pregnancy loss or congenital anomalies even in the absence of viable cultures. These include testing for trisomy 21, trisomy 13, trisomy 18, Turner syndrome and triploidy. Limitations of this assay include inability to detect aneuploidies involving chromosomes other than 13, 18, 21, X or Y, chromosome rearrangements or mosaicism. As such, classical cytogenetic analysis of these tissues remains the most sensitive assay available within this laboratory for the investigation of molecular cytogenetic abnormalities in these tissues. However, DNA extracted

from these tissue for QF-PCR may also be used for additional genetic testing if clinically warranted, thus paving the way for future test development for the clinical investigation of these cases.

If there are any questions regarding the genetic laboratory services available through the HRLMP for the investigation of perinatal tissues, please contact Dr. Elizabeth McCreedy ([mccready@HHSC.CA](mailto:mccready@HHSC.CA), tel: 905-521-2100 ext 73706).

### News from Hematology

On July 25<sup>th</sup>, the Hamilton Health Sciences Stem Cell and Bone Marrow Transplant Program was informed that its Foundation for the Accreditation of Cellular Therapy (FACT) accreditation had been renewed. FACT is a non-profit corporation co-founded by the International Society for Cellular Therapy (ISCT) and the American Society of Blood and Marrow Transplantation (ASBMT) for the purposes of voluntary inspection and accreditation in the field of cellular therapy and is the only international standard used in Europe, Canada, Australia, New Zealand and the USA. FACT accreditation involves evaluation of written documents, as well as on-site inspection of clinical, marrow and apheresis collection, and stem cell processing facilities, to evaluate all quality aspects of cellular therapy treatments, including clinical care, donor management, cell collection, cell processing, cell storage and banking, cell transportation, cell administration, cell selection, and cell release. Successful accreditation is the result of the emphasis that Hamilton's clinical and laboratory programs place on collaborative high quality and evidence-based patient care and laboratory practice - congratulations to all involved!

### News from Pathology

Congratulations to **Dr. Vina Alexopoulou** who received the award for Leadership in Education at the 65<sup>th</sup> Canadian Association of Pathologist Conference (CAP-ACP) meeting this year. She is an exceptional leader and teacher who has been an integral part of the Laboratory Medicine Resident Training programs at McMaster. Well done Vina!

We are very happy to announce that **Dr. Miranda Schell** is joining the HRLMP Anatomical Pathology Division as an Anatomical Pathologist starting on Monday, July 14, 2014. She is a recent graduate of our AP training program.

Dr. Schell will be based at the MUMC site and will ultimately provide GI consultation services in addition to her AP responsibilities.

Please join us in welcoming Dr. Miranda Schell!

### News from Microbiology

In light of the current outbreak of Ebola virus disease (EVD) in Western Africa, the Public Health Agency of Canada is advising Canadian healthcare professionals to be on the lookout for illnesses compatible with EVD in recent travellers, including healthcare workers from Guinea, Liberia and Sierra Leone.

Although there have not been any cases reported in Canada, we need to remain vigilant for persons with EVD symptoms and who have returned from affected areas within 21 days of symptom onset. **All patient assessments should include travel history.**

Signs and symptoms of EVD include sudden onset of fever along with one or more of the following:

- headache
- sore throat
- joint and muscle aches
- weakness
- diarrhea
- vomiting
- stomach pain
- lack of appetite
- jaundice
- rash

Transmission can occur through direct contact with the body fluids of affected cases. Strict infection control practices should be implemented for any suspected or probable cases of EVD.

This includes:

- Patient placement in Airborne and Droplet and Contact Precautions
- It is essential to **notify Infection Prevention and Control and the Microbiologist on call** when there is a potential case of viral hemorrhagic disease.
- **DO NOT** collect any specimens without speaking to the microbiologist on call.
- All probable cases of EVD must be immediately **reported to the Public Health Agency of Canada through its 24-hour emergency line: 1-866-262-8433.**