HAMILTON REGIONAL LABORATORY MEDICINE PROGRAM

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IN THIS ISSUE

WHAT'S NEW

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Update on Genetics Services in Hamilton

FISH Based Testing (Fluorescence in Situ Hybridization)

While Chromosome microarrays have replaced many of the FISH tests we used to provide for testing in pediatric developmental anomalies and syndromes, FISH-based testing for oncology has markedly expanded. Over the last year we have modified the breadth of FISH-based testing for a variety of cancer diagnostic indications, as follows:

Myeloma: testing covers FGFR3-IGH rearrangement (4;14 translocation), gain of chromosome 12, deletion of 13q, and deletion of TP53 (17p13.1)
CLL: testing covers ATM (11q), chromosome 12, deletion of 13q, and TP53
Mantle cell lymphoma: CCND1-IGH (11;14)
Marginal zone lymphoma: API2-MALT1 (11;18)
MDS: EGR1 to be performed routinely but by request only (deletion of 5q); CSF1R, deletion of 7q, and deletion of 20q also available but not routine
AML: expanded use of FISH for uncommon fusions (e.g. ETV6; PDGFRa, PDGFRb), and increased use of FISH to detect residual disease or document complex karyotypes; note that ***FLT3-NPM1*** testing is now available (provided as a reflex test by Molecular Hematology service)
ALL: expanded use of FISH for quantitation of residual disease

FISH on pathology specimens is a new focus. We are moving to validate a number of FISH-based tests on formalin-fixed, paraffin embedded materials (FFPE). This will be of considerable help in the assessment of lymphomas and selected solid tumours. For example, we currently offer FISH based testing for Burkitt's rearrangements on blood, marrow, and cytology specimens; we are validating a similar protocol for FFPE specimens to permit direct testing of lymph nodes. For IGH-BCL2 (follicular and double-hit lymphomas), we will transfer our current PCR protocol for FFPE specimens from PCR to a more accurate FISH protocol; another transfer from PCR to FISH is planned with our 1p/19q testing for oligodendrogliomas. Validation will be based upon preparation of single cell (disaggregated) cell suspensions and/or targeted staining of selected areas of tumour sections.

LAB CONNECTIONS

We expect that FISH on FFPE specimens will allow more definitive testing for specific rearrangements in lymphomas; clinicians and pathologists will be able to rule in or rule out specific rearrangements on fresh or fixed nodal tissue (or marrow, if necessary) as a complimentary, reflex test. In turn, we will aim to reduce the routine use of karyotyping marrow specimens for lymphomas. This work is proceeding with the assistance of Anatomic Pathology and the helpful leadership of Dr. J. C. Cutz. We are starting with CMYC and IGH-BCL2 as the points of greatest need in FFPE-based testing. As the lymphoma panel is developed, we will be looking at PTEN and a variety of other probe sets for solid tumours.

Future Developments in Hematology-Oncology

For Myelodysplastic Syndromes (MDS), myeloma and Chronic Lymphocytic Leukemia (CLL), there is a significant demand for testing. Since each FISH probe adds over $100 to the cost of analysis, meeting the clinical demand within budget constraints is a challenge. In these disorders, the patterns of anomalies suggest that microarrays should become a single-step and more cost-efficient analysis. We have been testing the feasibility of this approach with our current microarray platform, but while current microarrays are excellent for detecting gains and losses of chromosome segments, they do not allow recognition of the fusion rearrangements like the 4;14 translocation. New arrays specifically developed for cancer testing are on the horizon.

For myeloma specimens, we will also be testing a new device that permits CD138 sorting and enrichment as part of the FISH protocol, in the hope that this will allow more sensitive detection of abnormalities in specimens with low proportions of myeloma cells. If this technology works, it can be extended to other specific entities by modification of the antibody-based sorting.

In Acute Myeloid Leukemia (AML), the number of specific rearrangements that need to be evaluated continues to expand. CEPBA and CKIT appear to be the next priorities for adding to our test panel, but evidence of clinical utility is coming for IDH1, IDH2, and others. We will be working with the Molecular Hematology lab to coordinate updates to our test menus.

EGFR Testing in Lung Cancer Now Available

In December, Hamilton was approved and funded to perform EGFR testing for Non Small Cell Lung cancer (NSCLC). This test detects the presence of mutations in the EGFR (Epidermal Growth Factor Receptor) gene in tumour tissue that will predict for responsiveness to thymidine kinase inhibitors such as gefitinib. The process for requesting EGFR testing is similar to the KRAS test for colorectal cancer, and is available for patients who are being considered for gefitinib therapy as first line treatment for advanced NSCLC. Testing is based upon FFPE material (biopsies or cell blocks prepared from cytology specimens). Requisitions are available from the web site for our affiliated partner at the Chedoke site (Bay Area Genetic Laboratory: *www.bagl.ca*), and analysis of specimens is performed in collaboration with Anatomic Pathology (Dr. J. C. Cutz). It will also be possible to submit test requests through the IRESSA website once it has been updated by the drug manufacturer (in progress). Our test protocol for EGFR covers point mutations in exons 18, 20, and 21 as well as deletions of exon 19 and insertions in exon 20. The current reporting time is less than 10 days and results are faxed directly to the referring clinician. The cost of testing is supported by AstraZeneca, under a 3 year agreement with the MOHLTC.

KRAS and EGFR are examples of “companion tests” which will become increasingly common and important in oncology. Availability of these tests is required as part of the approval process for new drugs that have been custom designed to counteract the effect of causal genetic mechanisms. The action of the drugs can be so specific that treatment efficacy may depend upon the presence or absence of a single base pair in the tumour genome. Ultimately, our challenge will be to detect the most significant treatment targets out of thousands of genetic mutations in any given tumour – a task that will depend upon the clinical validation of rapid sequencing or array platforms that include sophisticated software packages for analysis and interpretation of clinical significance.

Dr. Ron Carter
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POLG-Related Disorders

The mitochondria of human cells maintain their own copies of a small 16.6 kb circular double-stranded genome which is replicated by DNA polymerase γ (POLG). POLG is encoded by a nuclear gene (*POLG*) on chromosome 15q24, containing 23 exons and spanning 18.5 kb. *POLG* mutations are associated mitochondrial DNA (mtDNA) depletion and/or deletions, and more than 160 different pathogenic mutations have been reported. The *POLG*-related disorders are clinically heterogeneous and include Alpers-Huttenlocher syndrome (AHS), childhood myocerebrohepatopathy spectrum (MCHS), myoclonic epilepsy myopathy sensory ataxia (MEMSA), ataxia neuropathy spectrum (ANS), and both autosomal recessive and autosomal dominant progressive external ophthalmoplegia (arPEO and adPEO).[1] Mutations in *POLG* are the most commonly found nuclear gene mutation in children with mitochondrial disease.

Establishing a diagnosis for *POLG*-related disorders relies on clinical findings and the demonstration of two pathogenic mutations for most disorders except adPEO (one pathogenic mutation). We recently began offering *POLG* mutation testing based on sequence analysis of the coding exons and intron/exon boundaries. This approach has a detection frequency of >95%. A recent survey of ~2,700 patients with clinical presentations suggestive of POLG deficiency identified at least one pathogenic mutation in ~5.0% of patients, and two pathogenic mutations in 3.4% of patients.[2]

Our experience is limited to only 21 cases, and we have yet to identity patients with pathogenic *POLG* mutations; however, several patients will be screened in the near future with a higher pre-test probability of POLG-related disease.

1 Cohen BH, Chinnery PF, Copeland WC. POLG-related disorders. GeneReviews [PMID 20301791].

2 Tang S, Wang J, Lee N-C, Milone M, Halberg MC, Schmitt ES, Craigen WJ, Zhang W, Wong L-JC. Mitochondrial DNA polymerase γ mutations: an ever expanding molecular and clinical spectrum. *J Med Genet* 2011; 48:669-681 [PMID 21880868]

Dr. John Waye
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Quality Snapshot:

Seeking ISO 15189 Accreditation

During our upcoming Ontario Laboratory Accreditation Assessment visit, the HRLMP will also be seeking ISO 15189 accreditation.

ISO 15189 is becoming an international standard of excellence for laboratory medicine. Achieving ISO 15189 accreditation demonstrates our commitment to quality and excellence, while ensuring we have a quality management system designed to identify and correct latent errors and improve patient safety.

Did you know?

External quality assessment is a requirement for Point of Care testing too! Devices used for Point of Care Testing must be compared to the laboratory and other point of care testing devices. This can be achieved by evaluating QMP-LS samples on all devices or testing split samples. Standards of practice should be the same, within and outside of the laboratory…..That's Quality!

Education News

Congratulations to General Pathology resident **Hetal Talati**. At the January 12, 2012 SJH Staff meeting, Hetal was awarded with the Best Resident Award for 2011.

CaRMs interviews for Anatomical Pathology, General Pathology, Medical Biochemistry, and Medical Microbiology will start this month. All programs are working hard to ensure that we continue to train high quality residents.

Congratulations to **Ashwyn Rajagopalan** (AP PGY5 Resident) and **Etienne Mahe** (AP PGY4 Resident) who have presentations scheduled at the United States and Canadian Academy of Pathology Conference to be held in Vancouver, British Columbia March 17-24th 2012. This is the largest and most prestigious international pathology conference.

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

Laboratory Updates:

Consolidation News

The Clinical Chemistry and Immunology Laboratory consolidation Phase 2 construction is nearing completion. MUMC Immunology transferred to the General site in Oct 2011. St. Joseph's Toxicology transferred to the General site in Jan 2012. We begin work on the creation of an integrated work force with the completion of the construction phase coming to a close. Cross training of staff is ongoing, and workflow efficiencies will soon follow.

The updated Chemistry facilities

Microbiology has consolidated all of Bacteriology at the Hamilton General Hospital site. A lot of hard work and planning went into bringing this project to realization. Virology and Molecular Microbiology continue to be located at the St. Joseph Charlton site.

The updated Microbiology facilities

News from Chemistry

The HLA laboratory will have a change in leadership with **Dr Azim Gangji** assuming the lead in an acting capacity. The laboratory will also be moving later this year into vacated space within the current core laboratory space. This model is a more efficient and strategic use of resources and will provide us with better patient care /safety.

Dr. Vijay Grey has announced that she will be retiring at the end of March 2012. She has been a valued member of the HRLMP and will be greatly missed!

News from Pathology

Dr Vicky Chen with Sister Joan O'Sullivan

Dr Vicky Chen, staff pathologist HRLMP, has received the Sister Joan O'Sullivan Award. This is the highest award given by the St. Joseph's Hospital Medical Staff Association, for exemplary clinical and educational dedication. Dr. Chen is finishing her term as DEC for the Department of Pathology and Molecular Medicine at the end of March and will be retiring June 29, 2012.

We welcome **Dr. Harkiran Kaur**, who has joined the anatomical pathology team for a long-term locum at our MUMC site. Dr. Kaur completed fellowships in breast and gynecological pathology at Mount Sinai and Sunnybrook Hospitals, respectively. She completed her residency at the University of Western Ontario.

We are saying farewell to **Dr. Dean Daya** and **Dr. Franco DeNardi**, both staff pathologists at Juravinski site, who will be leaving the HRLMP after a long period of dedicated service.

News from Microbiology

Rapid HIV-1 Antibody testing is available 24 hours/day 7 days/wk for women **in labour without any prior prenatal care**. The test is run through the virology laboratory during regular working hours and is performed on-site in transfusion medicine when virology is closed. If rapid testing is needed, when a woman is in labour, a call must be made to the test location to ensure that the rapid test is done. Please ensure that you order stat HIV tests correctly so that you will get the urgent results you need as rapidly as possible.

Bacteriology will be offering service 24 hours per day, 7 days a week starting **February 27, 2012!**

News from Transfusion Medicine

We are pleased to announce that Transfusion Medicine has successfully completed the implementation of Immucor analyzers for patient blood groups, antibody screens and antibody panel investigations in all Transfusion Medicine laboratories across the HRLMP. Each site has an instrument that is equipped to perform site-specific testing. This will result in higher throughput, enhanced utilization of staffing resources, and opportunities for future consolidation. It also has the benefit of result verification through remote capabilities.