In this era characterized by increasing emphasis on personalized medicine, the repertoire of molecular diagnostic assays applied to anatomic pathology specimens continues to evolve. Salient examples include molecular analysis for EGFR and KRAS mutations as well as ALK rearrangements in non-small cell lung cancer (NSCLC)\(^1\). Patients with NSCLC harboring EGFR mutations are candidates for targeted therapy with tyrosine kinase inhibitors, specifically gefitinib or erlotinib. Those with NSCLCs harboring ALK rearrangements are candidates for crizotinib therapy. Furthermore, the BRAF inhibitor, vemurafenib, has been approved for use in treating patients with advanced stage melanoma in which a V600E substitution in BRAF is detected\(^2\).

Molecular testing on tumor specimens is especially important in the setting of advanced stage disease where the information obtained has prognostic and therapeutic implications. It is in this setting where minimally invasive small biopsy procedures, including fine needle aspiration (FNA), are becoming the preferred method to obtain tissue for diagnosis. This is facilitated by advances in interventional imaging modalities that allow for improved access to deep-seated lesions for tissue acquisition. In some instances, cytologic samples represent the only available material for molecular testing.

Tissue sampling via FNA represents an essential practice in anatomic pathology as it is cost-effective, associated with minimal complications, and can be performed rapidly and efficiently. FNA allows for sampling of a wide area of the targeted lesion and results in acquisition of tumor cells with lower contamination by stromal tissue\(^3\). Importantly, FNA allows for rapid on-site assessments of adequacy, which represents a valuable opportunity for pathologists to serve as liaisons within a multidisciplinary team composed of anatomic pathologists, molecular pathologists, and oncologists. In this context, the pathologist is in a position to examine the aspirated cellular material microscopically at the time of the procedure while the patient is still accessible. Cytologic direct smears represent a facile preparation method for this activity. Once a particular malignancy is identified, the pathologist...
in collaboration with his or her clinical colleagues is often charged with the task of triaging the FNA material for relevant molecular diagnostic assays. This requires ensuring that sufficient material has been aspirated for both cytomorphologic evaluation and necessary molecular tests.

As the application of molecular diagnostics to cytology specimens continues to grow, pathologists face increasing demands to optimally triage tissue for various molecular assays. The challenge is compounded by the reality that a finite amount of material is obtained during FNA procedures. Cytology specimens can be processed by a variety of preparation methods including the aforementioned direct smears, liquid-based slide preparations (e.g., ThinPrep®), cytospin slides, and cell blocks. This versatility in cytology specimen processing results in exciting opportunities to optimize tissue triaging protocols for ancillary testing.

Cell blocks are traditionally used for molecular and immunocytochemical studies. A cell block is typically prepared by centrifuging a cell suspension (e.g., needle rinses obtained from FNA passes or fluid specimens), congealing the cell pellet in a matrix (e.g., HistoGel® or plasma/thrombin), fixing the congealed pellet in formalin, and paraffin-embedding the material for preparation of hematoxylin and eosin (H&E) stained sections. Formalin-fixed, paraffin-embedded (FFPE) cell block preparations are advantageous in that they are treated similarly to FFPE surgical pathology tissue blocks and multiple thin serial sections can be obtained for a battery of ancillary studies. Nonetheless, the notable disadvantage of cell blocks is that insufficient cell block cellularity can be encountered in a significant proportion of cases. Furthermore, the actual cellularity of the cell block is not known at the time of the FNA procedure as cell block preparation requires processing and the H&E stained cell block section is not available for review until well after the conclusion of the procedure. We investigated the issue of cell block cellularity in our practice by examining cell blocks prepared from 76 consecutive endobronchial ultrasound-guided FNAs of malignant lesions. We found that 28 (37%) were acellular and 15 (20%) exhibited sparse cellularity. These scenarios can lead to repeat FNA procedures in order to retrieve additional material for cell block preparations. Nonetheless, sufficient cellularity of these subsequent cell block preparations is not necessarily guaranteed.

In addition to the variable cellularity exhibited in cell blocks, it should be noted that needle rinses collected from FNA procedures are pooled specimens stemming from multiple needle passes. Consequently, it is conceivable that the tumor cells obtained from an on-target needle pass could be diluted by background benign cellular elements (e.g., lymphocytes) obtained from off-target needle passes. This has significant implications for molecular testing as the analytic sensitivity of molecular diagnostic tests is dependent on a percent tumor cellularity threshold. If an excess
Spotlight on
Michael H. Roh, M.D., Ph.D.

Michael Roh joined the faculty of the Department of Pathology in July 2009, but his Michigan roots run much deeper. Mike matriculated in the Medical Scientist Training Program at the University of Michigan in 1998 after graduating from Johns Hopkins University. He completed his Ph.D. thesis in 2003 under the mentorship of Ben Margolis, M.D., and was awarded his joint M.D. and Ph.D. degrees in 2005 after completing medical school and serving for one year as Postdoctoral Research Assistant in the Margolis Laboratory. He spent the next four years at Brigham and Women's Hospital in Boston completing residency training in anatomic pathology and fellowship training with Christopher Crum, M.D., in Women's and Perinatal Pathology and with Edmund Cibas, M.D., in Cytopathology. In 2009, we celebrated his return to Michigan as Assistant Professor of Pathology with clinical responsibilities in cytopathology and gynecological pathology.

In this issue of MLabs Spectrum, Dr. Roh summarizes his state-of-the-art approach to diagnostic cytopathology that he helped to develop here at Michigan in collaboration with colleagues in anatomic pathology as well as our Molecular Diagnostics Laboratory. Since his arrival Michael has built a record of success across all of our missions, always with an eye toward putting patients and service first. He has published over 20 papers in the peer reviewed literature in just the last four years, building on a solid foundation of previously published work reflecting his interests in the molecular pathogenesis of Müllerian neoplasms and application of novel diagnostic techniques to cytology specimens. The 2011 publication entitled *The application of molecular diagnostic studies interrogating EGFR and KRAS mutations to stained cytologic smears of pulmonary adenocarcinoma* (Am J Clin Pathol 136: 564-71), on which Dr. Roh was a co-first author, was designated a 2011 Top 10 Game Changer in Pathology by Medscape (ranked #5). Eight abstracts comprising work done with Michigan colleagues were presented at national and international meetings in 2011 and 2012 and further showcase Mike’s collaborative successes in advancing our understanding of how to more effectively apply cutting edge diagnostic strategies to cytology samples.

Dr. Roh’s passions extend beyond the cytopathology and research laboratories to education. His commitment to teaching others dates to his earliest days as a student. During the first two years of medical school he served as an instructor for the MCAT Preparation Course offered by Kaplan, Inc. right here in Ann Arbor. He is currently Co-Director of the M1 Pathology Curriculum at the University of Michigan Medical School, and since January 2012 is Program Director for our Cytopathology Fellowship. Michael has also been a popular speaker at our annual New Frontiers in Pathology seminars, and will be joining us as faculty for our next New Frontiers meeting scheduled for August 3-5, 2012 at The Homestead Resort in Glen Arbor, Michigan (learn more at [http://www.pathology.med.umich.edu/newfrontiers/](http://www.pathology.med.umich.edu/newfrontiers/)). Come join us and learn first hand about some of his approaches to complex diagnostic problems using modern diagnostic tools.

Effective July 1, 2012, Dr. Roh was appointed Director of Cytopathology and Medical Director of our Cytology Laboratory. He served in these capacities on an interim basis for the previous six months. During that time he has walked the talk when it comes to the Michigan Difference, modeling what it means to reap the rewards realized from working with others to continuously improve patient care and education, while expanding the boundaries of knowledge in an environment that celebrates innovation.
lesions were also tested for tissue blocks prepared from the excised metastatic cases with surgical followup, sections of the FFPE mutation (V600K) was detected in 4 cases. For the was seen in 16 cases and the second most common mutation (V600E) at expected frequencies; specifically, 20 (41%) of the 49 cases. The most common mutation (V600E) available in 34 cases.

EGFR and stained cytologic direct smears could be utilized for EGFR and KRAS mutational analysis. We examined 21 cases diagnosed as adenocarcinoma and 10 cases diagnosed as non-small cell carcinoma, not otherwise specified (NSC, NOS)⁵. Tumor cells were retrieved from the smears via manual microdissection (Figure 1) using the Pinpoint Slide DNA Isolation System™ (Zymo Research, Orange, CA). After successful DNA isolation and PCR amplification in every case, we detected EGFR and KRAS mutations at expected frequencies. Specifically, we observed EGFR and KRAS mutations in 3 (14%) and 8 (38%) of the 21 adenocarcinomas, respectively. Furthermore KRAS mutations were detected in 3 (30%) of the 10 NSC, NOS cases. Cell blocks were co-tested in parallel. In one case, a false negative result was observed in the cell block preparation due to sparse overall cellularity and less than 10% tumor cellularity. There were no cases in which a mutation was detected in the cell block material but not in the direct smear derived cellular material. These results indicate that direct smears represent a reliable source of cellular material for molecular diagnostic assays.

We next extended our analysis by applying BRAF mutation testing to archived direct smears prepared from metastatic melanoma FNAs that were decoverslipped prior to tumor cell microdissection. Again, in collaboration with the Molecular Diagnostics Laboratory, we examined a total of 49 FNAs of metastatic melanoma for which surgical followup was available in 34 cases. BRAF mutations were observed at expected frequencies; specifically, 20 (41%) of 49 cases. The most common mutation (V600E) was seen in 16 cases and the second most common mutation (V600K) was detected in 4 cases. For the cases with surgical followup, sections of the FFPE tissue blocks prepared from the excised metastatic lesions were also tested for BRAF mutations. A 100% concordance rate between the BRAF mutation test results from the FNAs and subsequent excisions was observed. This not only confirms the idea that FNA material is representative of the targeted lesion for molecular diagnostic purposes but also reaffirms the notion that direct smears represent a robust source of cellular material for molecular testing.

The results of these studies provide opportunities to optimize cytologic specimen triaging for molecular diagnostics. In our practice, when appropriate, we prepare multiple—typically between 3 and 6—direct smears for a given FNA needle pass by distributing the cellular material over multiple slides. One smear is air-dried for rapid Diff-Quik staining, the second smear is alcohol-fixed for subsequent Papanicolaou staining in the cytopreparatory laboratory, and the remaining unstained, air-dried smears can be utilized for ancillary studies. By visualizing representative tumor cells on the Diff-Quik stained smear at the time of the procedure, the pathologist can infer that tumor cells are present on the remaining smears prepared from the same needle pass. A more direct visualization approach, via light microscopic examination of an unstained smear with the condenser flipped, can also be employed to confirm the presence of tumor cells on the unstained smear (Figure 2). Unstained smears can be immediately submitted for immunocytochemistry and the immunostained slides can be obtained within hours. Of note, we have previously reported this approach to the subclassification of pulmonary NSCLC and the diagnosis of melanoma, Merkel cell carcinoma, and metastatic malignancies in effusions.

If molecular testing is necessary, one of the extra unstained, air-dried smears can be Diff-Quik stained, left uncoverslipped, and sent directly to the molecular diagnostics laboratory for tumor cell microdissection and subsequent DNA isolation for molecular analysis. If extra uncoverslipped Diff-Quik stained smears are not available, previously coverslipped smears can be decoverslipped in xylene and triaged for molecular testing. This approach is flexible and forgiving. In our practice, we prefer to utilize Diff-Quik stained smears over Papanicolaou-stained smears for molecular testing based on recent findings by Killian and colleagues. They observed increased DNA fidelity in the former as DNA degradation, as a function of time, was observed in the latter.

Overall, the advantages of utilizing direct smears for molecular testing include: ease and cost-effectiveness of smear preparation; the ability to directly visualize...
and select the cellular material to be utilized for molecular testing; and elimination of the sole reliance on cell blocks for molecular testing. By ensuring that adequate material has been obtained for both cytomorphologic evaluation and necessary ancillary tests at the time of the FNA procedure, this approach could significantly reduce the frequency of repeat procedures and minimize delays in patient management for cases in which insufficient cell block cellularity is encountered.

REFERENCES


Test Updates

New Tests

**NLGN3 AND MBD5 GENE SEQUENCING**

The MLabs Molecular Genetics Laboratory began sequencing the MBD5 (OMIM:611472) and NLGN3 (OMIM:300336) genes effective May 7, 2012.

The MBD5 gene is located on chromosome 2 at 2q23.1. This gene encodes a member of the methyl-CpG-binding domain (MBD) family. The MBD domain is involved in recruiting a specific methyl-CpG-binding protein at the site of DNA methylation. In addition, this protein contains a PWWP domain (Pro-Trp-Trp-Pro motif), which is involved in cell division, growth and differentiation. Mutations in MBD5 cause mental retardation autosomal dominant type 1 (OMIM:156200). Haploinsufficiency of MBD5 is associated with a syndrome involving microcephaly, intellectual disabilities, severe speech impairment, and seizures. [RefSeq, Mar 2010] See NCBI Gene www.ncbi.nlm.nih.gov/gene/55777 for additional details.

The NLGN3 gene is located on chromosome X at Xq13.1. This gene encodes a member of a family of neuronal cell surface proteins. Members of this family may act as splice site-specific ligands for beta-neurexins and may be involved in the formation and remodeling of central nervous system synapses. Mutations in this gene are reported to be associated with Asperger syndrome susceptibility, X-linked 1 (OMIM:300494) or Autism susceptibility, X-linked 1 (OMIM:300425). [RefSeq, Oct 2009] See NCBI Gene www.ncbi.nlm.nih.gov/gene/54413 for additional details.

**UBE3A GENE SEQUENCING**

The Molecular Genetics Laboratory began sequencing the UBE3A gene (OMIM:601623) effective April 16, 2012. The UBE3A gene is located in chromosome 15 at 15q11.2. This gene encodes an E3 ubiquitin-protein ligase, part of the ubiquitin protein degradation system. This imprinted gene is maternally expressed in brain and biallelically expressed in other tissues. Maternally inherited deletion of this gene causes Angelman Syndrome, characterized by severe motor and intellectual retardation, gait ataxia and/or tremulousness of the limbs, hypotonia, epilepsy, absence of speech
or severe speech impairment, characteristic facies, and a unique behavior with an inappropriate happy demeanor that includes frequent laughing, smiling, and excitability. Microcephaly and seizures are also common. Developmental delays are first noted at around age six months; however, the unique clinical features of AS do not become manifest until after age one year, and it can take several years before the correct clinical diagnosis is obvious.

The diagnosis of AS rests on a combination of clinical features and molecular genetic testing and/or cytogenetic analysis. Analysis of parent-specific DNA methylation imprints in the 15q11.2-q13 chromosome region detects approximately 78% of individuals with AS, including those with a deletion, uniparental disomy (UPD), or an imprinting defect (ID). Less than 1% of individuals with AS have a cytogenetically visible chromosome rearrangement (translocation or inversion). UBE3A sequence analysis detects mutations in approximately 11% of individuals with AS. Methylation analysis and UBE3A sequence analysis identifies genetic alterations in approximately 90% of individuals with AS. The remaining 10% of individuals with classic phenotypic features of AS have the disorder as a result of an as-yet unidentified genetic mechanism.

Test Methodology, Reference Range, and Specimen Handling Changes

**D-DIMER**

Effective May 16, 2012, the MLabs Coagulation Laboratory will implement a change in the testing of D-Dimer.

The new D-Dimer assay (Siemens Innovance®) has a FDA-cleared clinical indication for use in excluding both deep vein thrombosis (DVT) and pulmonary embolism (PE) in conjunction with a non-high pretest probability assessment (PTP) model.

Elevated D-dimer levels are observed in all diseases and conditions with increased coagulation activation, e.g., thromboembolic disease, DIC, acute aortic dissection, myocardial infarction, malignant diseases, obstetrical complications, third trimester of pregnancy, surgery or trauma. However, symptoms being present since a certain period of time, e.g. longer than a week, may produce normal D-Dimer values.

Reference Range: <0.59 mg/L FEU. The cut-off value for exclusion of PE/DVT is a D-Dimer result of ≤0.50 mg/L FEU.

**DRUG SCREEN BY IMMUNOASSAY, URINE**

Effective April 2, 2012, the interpretation associated with MLabs Drug Screen by Immunoassay, Urine, has been updated to alert clinicians to possible Amphetamine interference. The following information has been added: Ranitidine and metabolites of Trazodone and Labetalol may cause false positive results for Amphetamine.

**IMPORTANT INFORMATION:**

1. Due to structural differences FENTANYL is not detected by the Opiate assay. Please contact MLabs for requests to analyze for this drug.
2. Rifampin can cause false positive urine opiate screen.
3. The presence of hydrocodone in a urine sample can cause a false positive urine oxycodone screen.
4. Sympathomimetic amines may show a positive result for amphetamine class. Ranitidine and metabolites of Trazodone and Labetalol may cause false positive results for Amphetamine.
5. Lorazepam is not detected by the Benzodiazepine immunoassay screen.
6. If the drug screen failed to report a specific drug of interest, please contact MLabs for additional help.
7. Positive results by immunoassay should be considered presumptive. Confirmation by an alternate method may be requested by contacting MLabs.

Threshold cut off levels: Amphetamines 1000 ng/mL; Barbiturates 200 ng/mL; Benzodiazepines 100 ng/mL; Cannabinoids 50 ng/mL; Cocaine metabolites 300 ng/mL; Opiates 300 ng/mL; Oxycodone 100 ng/mL.

**HEPATITIS B AND C VIRUSES BY PCR**

Effective June 4, 2012, the QHCV and QHBV assays will be performed using the Abbott Realtime HBV Test and the Abbott Realtime HCV Test, performed using the Abbott M2000 system. Sample collection and processing for these tests will remain the same. Serum, the preferred specimen type, or plasma must
be removed from the cells within 6 hours of collection. Serum or plasma should be sent to the laboratory as soon as possible, but may be stored refrigerated for 3 days or frozen for longer storage.

The dynamic range for the **Hepatitis B Virus DNA** (HBV) Test is 10 IU/mL (1.0 log10) HBV DNA/mL to 1,000,000,000 IU/mL (9.0 log10) HBV DNA/mL. Quantification values for HBV average 0.5 log10 IU/mL lower than values determined by the previous assay.

**Reference Range:** Not Detected (<10 IU/mL HBV DNA)

If the result is greater than 9.0 log10 IU/mL, a dilution of the sample will be run for an exact quantification. There is an additional charge for this quantification (QHBVEP). Please contact the MLabs Client Service Center if actual viral load is needed.

The dynamic range for the **Hepatitis C Virus DNA** (HCV) Test is 12 IU/mL (1.08 log10) HCV RNA/mL to 100,000,000 IU/mL (8.0 log10) HCV RNA/mL. Quantification values for HCV average 0.3 log10 IU/mL lower than values determined by the previous assay.

**Reference Range:** Not Detected (<12 IU/mL HCV RNA)

If the result is greater than 8.0 log10 IU/mL, a dilution of the sample will be run to determine an exact quantification. There is an additional charge for this quantification (QHCVVEP). Please contact the MLabs Client Service Center if actual viral load is needed.

**LAMOTRIGINE**

After a review of newer medical literature and the therapeutic ranges currently in use by other laboratories, the MLabs Drug Analysis & Toxicology Laboratory made a change to the reference range for the Lamotrigine assay effective June 5, 2012.

Longer-term experience with the anticonvulsant drug lamotrigine, plus newer studies of serum concentrations versus therapeutic efficacy, have shown that a higher therapeutic range appears appropriate for this drug. Although there remains a wide range in effective therapeutic thresholds and therapeutic concentrations in blood, a therapeutic range of 3.0 – 14.0 µg/mL has been recommended.

There will not be an actual reported reference (normal) value. The range and additional assay information will be reported as interpretive data as follows:

**Reference Range:** The proposed therapeutic range for control of seizures is 3.0 – 14.0 µg/mL. Drug pharmacokinetics varies widely, being especially affected by decreased renal function and co-medications.

**OLIGOCLONAL BANDS, CSF**

Effective April 2, 2012, the MLabs Immunopathology Laboratory has discontinued Oligoclonal Band (CSF) testing due to the low volume of requests and availability of isoelectric focusing methodology at Mayo Medical Laboratories (MML). Requests for this test will be sent to MML.

**Collection Instructions:** Collect both blood and CSF (0.5 mL); specimens must be collected within 1 week of each other. Collect blood in a red top tube and aliquot 0.5 mL (minimum 0.4 mL) of serum into a plastic vial. Refrigerate both serum and CSF specimens.

**Test Methodology:** Isoelectric Focusing (IEF) with IgG Immunoblot Detection

**Reference Range:** CSF Olig Bands Interpretation: <4 bands. A finding of 4 or more cerebrospinal fluid (CSF)-specific bands (i.e., bands that are present in CSF but are absent in serum) is consistent with multiple sclerosis. The presence of oligoclonal band is unrelated to disease activity.

**VITAMIN B1, WHOLE BLOOD**

Effective May 3, 2012, Mayo Medical Laboratories New England Thiamin (Vitamin B1), whole blood assay replaced the Mayo Medical Laboratories Rochester assay. Note that there was a change in specimen requirements from a green top tube (sodium heparin) to a lavender top tube (EDTA); heparinized whole blood will not be accepted.

**Collection Instructions:** Collect specimen in a lavender top tube (EDTA) following an overnight (12 hour) fast; place on ice immediately after collection. Freeze whole blood specimen (5 mL) within 4 hours of collection. Do not freeze glass vacutainer tubes; collect in plastic vacutainer tube or transfer blood to a plastic tube prior to freezing. Protect specimen from light.

**Reference Range:** 70 – 180 nmol/L
MLabs News

U-M DEPARTMENT OF PATHOLOGY NEWS

MLabs welcomes Nathanael Bailey, M.D., Assistant Professor of Pathology who recently joined the Clinical Pathology Division in the Hematopathology section. Following residency training, Dr. Bailey completed a fellowship in Hematopathology at West Virginia University (2009-2010) and a fellowship in Molecular Genetic Pathology at the University of Michigan (2010-2011).

Stand Up To Cancer and the Prostate Cancer Foundation announced a new “dream team” co-directed by Arul M. Chinnaiyan, M.D., Ph.D. This translational cancer research research grant will provide $10 million in funding over 3 years to support a seven-center project, including both clinical and research endeavors addressing therapeutic interventions for advanced prostate cancer.

Congratulations are to be extended to David O. Ferguson, M.D., Ph.D., Associate Professor who was elected to the American Society for Clinical Investigation at the annual ASCP/AAP meeting in Chicago, April 27-29, 2012.

Megan S. Lim, M.D., Ph.D., Professor and Director of Hematopathology, has been asked to serve as a member of the Education Committee for the United States & Canadian Academy of Pathology (USCAP) for the period 2012 - 2015.

Congratulations are to be extended for awards received from the American Society of Clinical Laboratory Science (ASCLS)-Michigan. Kristina Martin, Clinical Pathology Operations Coordinator, received the 2012 ASCLS-Michigan Pam Agren Inspirational and Key to the Future awards. Her contributions as co-chair for the 2012 ASCLS-Michigan Annual Meeting, and Membership Committee involvement were recognized. Kristina was also elected, along with Melanie Herbert, Laboratory Supervisor, Chemical Pathology, as ASCLS-Michigan District Representatives for the 2012 - 2013 term. Andrea Hickey, Clinical Laboratory Scientist II, Blood Bank and Transfusion Service, received the Mentor of the Year award. She was recognized for mentoring society members as the 2011 President and as annual meeting chair.

On March 15, 2012, Jeffrey L. Myers, M.D., was inducted as one of thirty-one inaugural members into the University of Michigan Medical School League of Clinical Excellence, recognizing individual clinicians who have distinguished themselves in providing the finest care to the tens of thousands of patients and their families who come to us annually seeking hope and healing.

Lauren Smith, M.D., was an invited lecturer at the 32nd Annual Graduate Student Symposium in Pharmacological Sciences and Biorelated Chemistry held in April. Her talk was entitled “Putting the ‘me’ in medicine: The ethics of personalized health care.”

Congratulations to Carol Young, Supervisor, Clinical Microbiology/Virology Laboratories for receiving the 2012 Significant Contributor to Clinical Microbiology Award by the South Central Association for Clinical Microbiology (SCACM) at the annual SCACM Spring Meeting in Indianapolis, IN, March 21-24. Carol was recognized for her years of dedicated service to the field of Clinical Microbiology not only through her technical and research activities, but also through her inspiration of co-workers and students to work in and contribute to Clinical Microbiology. Carol has served in almost every board position for SCACM (including President) and through this organization has been a tireless promoter of continuing education opportunities for bench-level technologists.