Medicine is currently witnessing a near unprecedented expansion of molecular advancements leading to new diagnostic, prognostic and therapeutic innovations in the field of oncology. What just a few years ago seemed out of reach has rapidly become a reality. The utilization of knowledge of a specific driving molecular aberration in individual patients for diagnostic or prognostic classification, therapeutic choice, or enrollment in a clinical trial is no longer just around the corner but is actually here – truly, the future is now. And all of this has been possible as a result of a multi-disciplinary team effort being currently made by various institutions and hospitals across the globe, all working with one goal in mind: to surmount the morbidity and mortality unleashed by cancer.

Notable progress at the molecular discovery and launch end also places a pressing demand upon the delivery modules for the integration of relevant and impactful discoveries into existing models of clinical care. To facilitate clinical translation of emerging molecular advances Jeffrey L. Myers, M.D. and Jay L. Hess, M.D., Ph.D., of the Department of Pathology at the University of Michigan Health System (UMHS) launched a “service line” concept in which content subspecialists (i.e., diagnostic pathologists, molecular biologists, cytogeneticists, medical oncologists, etc.) work closely together to advance molecular diagnostics in areas of clinical need. In this pilot model, dedicated molecular diagnostic service line laboratories serve as launch platforms for amalgamation of novel, clinically significant molecular oncology results into assays for diagnostic, prognostic, and/or therapeutic use. A diverse array of specialists and personnel within the Department of Pathology at UMHS, including the Clinical Cytogenetics Laboratory, Michigan Center for Translational Pathology (MCTP), and the Molecular Diagnostics Laboratory, has contributed to making this concept a practical reality.

The field of genitourinary oncology has undergone tremendous transformation, with several landmark discoveries made in the last decade or so. Our understanding of pathogenesis of prostate cancer...
has been revolutionized, first by the discovery of ETS family gene fusions in clinically localized and metastatic prostate cancer\textsuperscript{2,3}, and subsequently by the identification of RAF family gene fusions in advanced metastatic prostate cancer\textsuperscript{4}. What was once considered to be a complete enumeration of renal tumors comprising only four diagnostic subentities has now evolved into a complex and clinically useful World Health Organization (WHO)-driven classification system of renal cancers, including several new and intriguing subtypes such as translocation-associated renal cell carcinoma (t-RCC) and the increasingly recognized inherited renal neoplasms. In addition, actionable genomic alterations have been identified in a significant subset of high-grade bladder cancers\textsuperscript{5} and we are beginning to unravel the complex etiology, pathogenesis, and clinical development of testicular cancers\textsuperscript{6}.

The promise and realization of personalized medicine has been one of the most important advancements in the genomic era. For oncology, the potential for personalized medicine is based on the premise that integrated, real-time sequencing of an individual tumor can identify its unique constellation of driver mutations and subsequently reveal targetable biologic networks. This concept, encompassing non-genitourinary malignancies as well, has been established at UMHS through the MI-ONCOSEQ initiative and is currently being introduced in varying forms by other institutions around the world\textsuperscript{7}. Service line laboratories, once implemented, would be able to provide an easy and effective confirmation and clinical implementation route for aberrations discovered through a variety of sequencing strategies\textsuperscript{1}.

**CLINICAL DESIGN AND IMPLEMENTATION**

The genitourinary service line laboratory at UMHS currently offers fluorescent in situ hybridization (FISH) assays for TFE3 and TFEB gene aberrations and is actively working on new assays for inclusion in its clinical menu. Renal cell cancer is less common than prostate cancer but twice as lethal, with advanced metastatic renal tumors demonstrating a very dismal prognosis. The tremendous evolution in classification of renal tumors has been associated with the development of diverse therapeutic choices, wherein specific subtypes of renal tumors might dictate availability and response to specific treatments. For example, vascular endothelial

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**Figure 1.** A and B, H&E images of Xp11 t-RCC demonstrates papillary architecture with cells exhibiting voluminous clear and eosinophilic cytoplasm (A - 200x, B - 400x). C, TFE3 FISH employing TFE3 break-apart FISH probe strategy demonstrates TFE3 translocation - co-localized green and red signal indicate wild-type TFE3 allele, TFE3 translocation is indicated by a single green signal and single red signal. D and E, H&E images of t(6;11) t-RCC with nests and sheets of cells separated by thin vascular septae; biphasic morphology with clusters of smaller cells dispersed between larger cells (D - 200x, E - 400x). F, TFEB FISH employing TFEB break-apart FISH probe strategy demonstrates TFEB translocation - co-localized green and red signal indicate wild-type TFEB allele, TFEB translocation is indicated by a single green signal and single red signal.
growth factor (VEGF) inhibitors are either currently approved or in clinical trials for the treatment of clear cell renal cell carcinoma. Recent breakthroughs have also been reported in the treatment of other renal tumors, including t-RCC, a unique subtype of renal cell carcinoma that commonly occurs in children but is increasingly being identified in adults as well. These cancers generally present at advanced stage in children, and are frequently associated with an aggressive clinical course and poor survival in adults. In a recent study, targeted therapy for patients with metastatic t-RCC achieved prolonged progression free survival similar to patients with clear cell renal cell carcinoma. These examples underscore the clinical value for the accurate sub-classification of renal tumors, including eligibility for and response to specific treatments.

Two main classes of t-RCC are currently recognized: translocations involving the TFE3 locus on chromosome Xp11 and a t(6;11) translocation generating the TFEB gene fusion. Xp11 RCCs often morphologically demonstrate high nuclear grade, prominent papillary and/or alveolar pattern and clear to granular eosinophilic cytoplasm (Figure 1). The t(6;11) carcinomas may exhibit nests, sheets and tubules of cells with clear to eosinophilic cytoplasm separated by thin vascular septae. These tumors may also present with a biphasic morphology with a minor subpopulation of smaller cells with high nuclear/cytoplasmic ratio with or without the presence of associated basement membrane material. While morphologic features often correlate with translocation category and subtype, significant morphologic overlap exists among different translocation groups and non-translocation associated tumors. Our morphologic recognition of these renal tumors is undoubtedly improving and newer patterns have recently been associated with t-RCC. Still, these renal tumors can be diagnostically challenging without the aid of appropriate ancillary studies. Whereas immunohistochemistry (IHC) for TFE3 or TFEB proteins (or their common transcriptional target cathepsin K) is specific for t-RCC, there is poor reproducibility of TFE3 or TFEB IHC, and the sensitivity is not high enough to exclude the possibility of false-negative results. FISH, however, has been robustly validated for the accurate diagnosis of these renal tumor subtypes and exhibits a high degree of sensitivity and specificity. Importantly, because it shares molecular rearrangement of the
TFE3 gene locus, the UMHS TFE3 FISH assay can also be used to confirm the diagnosis of alveolar soft part sarcoma, if indicated.

FISH is performed on unstained formalin fixed paraffin embedded (FFPE) tissue sections of cases under investigation using a dual color break-apart probe set specific to the TFE3 gene locus at Xp11.2. Similar methodology is applied to detect the break-apart probe patterns specific to the TFE3 or TFEB gene breakpoint region. Splitting of the probes is observed when a rearrangement involving these genes is present (Figure 1). Probe signals are evaluated in interphase nuclei and cases are interpreted according to normal cutoff values determined by the laboratory. Control specimens are run concurrently to ensure quality control.

ADDITIONAL ASSAYS UNDER DEVELOPMENT

The expansion of knowledge regarding the molecular pathogenesis of tumors has created a number of exciting prospects with the potential to improve and alter oncologic clinical practice. For genitourinary cancers, these discoveries are already translating into improved diagnostic, therapeutic and prognostic tools having a significant impact on clinical decision making. Such assays currently under development at UMHS include: enumeration probes to confirm trisomy 7 and 17 in patients with papillary renal cell carcinoma; break-apart FISH probes to detect ERG, ETV1, BRAF and RAF1 gene fusions in prostate cancer; and locus control probes to detect deletion of PTEN in prostate cancer. Immunohistochemistry and/or RNA in situ hybridization for targetable biomarkers may provide new therapeutic options for patients, especially in the field of prostate cancer. In the future, these services will also be offered by the genitourinary service line at UMHS.

THE FUTURE

New FISH assays have been fully validated and are now available for use on FFPE tissues of a subset of renal and soft tissue tumors not only for UMHS patients but also those seen by our consultation service. This collective initiative speaks not only to the potential value of a service line model in selected subspecialties but perhaps more importantly to the results realized from working together to achieve a better and more effective cancer diagnosis and cure. New assays are being currently added to the molecular arsenal available from the Department of Pathology at UMHS, which will undoubtedly result in improved patient management as the availability of clinically translatable discoveries increases.

REFERENCES

Test Updates

New Tests

**BILIARY TRACT MALIGNANCY BY FISH**

The MLabs Molecular Diagnostics Laboratory began performing Biliary Tract Malignancy by FISH effective January 20, 2014.

This test detects aneuploidy of chromosomes 3, 7, and 17 via fluorescence in situ hybridization (FISH) in bile duct brushing and aspirate specimens. Detection of chromosomal gains is associated with malignancy. As an adjunct to conventional cytopathology this test may be useful in the assessment of pancreatobiliary strictures for malignancy.


Collection Instructions: Obtain bile duct brushing or aspirate. Place brush directly into a ThinPrep (Non-Gyn) or conical tube containing CytoLyt® solution, swirl the brush vigorously in the solution, then snip into the container, or place aspirate specimen into CytoLyt® container. Refrigerate. Please note that specimens submitted in ThinPrep (Gyn) vials containing PreservCyt® are not acceptable.

**BRCA TESTING**

The MLabs Michigan Medical Genetics Laboratories (MMGL) Molecular Genetics Laboratory now offers a full complement of BRCA testing:

**BRCA Gene Sequencing**: BRCA1 and/or BRCA2 gene sequencing can be performed on a patient who has a family history or is suspected of having breast, ovarian, prostate, or pancreatic cancer or for determining a genetic mutation for a patient with a clinical diagnosis of one of these cancers. Individuals with BRCA2 mutations may also be at an increased risk for melanoma. Germline mutations in the BRCA2 gene are associated with an increased risk for these cancers.

**BRCA Deletion/Duplication Analysis**: This test is used to detect the presence of BRCA1 (OMIM:113705) and/or BRCA2 (OMIM: 600185) deletions and duplications. BRCA gene deletion and duplication analysis can be performed on patients with no BRCA mutations detected by sequence analysis but have a clinical presentation consistent with BRCA-related disorders or on relatives of a patient with a known BRCA deletion/duplication mutation.

**BRCA Targeted Sequencing, Familial**: BRCA1 and/or BRCA2 targeted sequencing can be performed on a patient with a known familial mutation.

**BRCA Ashkenazi Jewish Founder Mutations**: Three Ashkenazi Jewish founder mutations can be detected by this targeted sequencing assay: BRCA1 gene c.68_69delAG (BIC: 185delAG) and c.5266dupC (BIC: 5382insC) and one in BRCA2 c.5946delT (BIC: 6174delT).

Please visit our website at www.mlabs.umich.edu for full test descriptions as well as specimen collection and handling requirements. For assistance with sending a specimen contact MLabs at 800-862-7284.

**CALR MUTATION**

The MLabs Molecular Diagnostics Laboratory began offering CALR Mutation analysis effective March 25, 2014. CALR gene mutations occur at a frequency of 20-30% in primary myelofibrosis (PMF) and essential thrombocythemia (ET). Testing for CALR mutations can aid in the diagnosis of these myeloproliferative neoplasms. CALR mutations are typically exclusive of the JAK2 V617F mutation and are common (~50-75%) in PMF and ET cases that test negative for JAK2 V617F. The utilization of JAK2 V617F, CALR, and also MPL mutation testing provides detection of a clonal marker in the majority (>85%) of patients with PMF and ET. This CALR mutation test qualitatively detects all CALR exon 9 insertion and deletion mutations to a sensitivity of 2-5% mutant allele.

**DIGEORGE PANEL**

Effective February 6, 2014, the MLabs Molecular Genetics Laboratory began performing a DiGeorge Panel. This panel is used to confirm a clinical or a suspected diagnosis of DiGeorge syndrome in a patient. Tier 1 of this panel includes rqPCR using multiple TaqMan probes within the most common 22q11.2 deletion. This panel test will automatically reflex to Tier 2 (Chromosomal Microarray, CMA) (CPT 81229) when rqPCR detects a normal genomic copy number or if only one the TaqMan probes detects an abnormal copy number. This panel test will automatically reflex to Tier 2 (Chromosomal Microarray, CMA) (CPT 81229) when rqPCR detects a normal genomic copy number or if only one the TaqMan probes detects an abnormal copy number. This panel test will automatically reflex to Tier 2 (Chromosomal Microarray, CMA) (CPT 81229) when rqPCR detects a normal genomic copy number or if only one the TaqMan probes detects an abnormal copy number, then a reflexive CMA will not be performed.
**MYD88 (L265P) MUTATION**

The MLabs Molecular Diagnostics Laboratory began offering MYD88 L265P Mutation Analysis effective March 11, 2014.

The L265P mutation in the MYD88 gene is detected in approximately 90% of lymphoplasmacytic lymphoma/Waldenstrom Macroglobulinemia (WM), 30% of activated B-cell type diffuse large B-cell lymphoma (ABC-DLBCL), and half of IgM monoclonal gammapathy of undetermined significance (IgM-MGUS) cases. MYD88 L265P testing can aid in the diagnosis of these neoplasms. The presence of MYD88 L265P is also associated with higher risk of disease progression in IgM-MGUS. This test qualitatively detects the MYD88 L265P mutation in peripheral blood, bone marrow, and formalin fixed paraffin-embedded tissue to a sensitivity of 2% mutant allele.

**TFE3 AND TFEB REARRANGEMENTS BY FISH**

The MLabs Cytogenetics Laboratory began offering TFE3 and TFEB Rearrangements by FISH assays effective February 28, 2014. The primary value of these assays is in accurate diagnosis of a subset of uncommon renal tumors referred to as translocation-associated renal cell carcinomas (tRCC). Translocation-associated renal cell carcinomas tend to occur in children or young adults and are associated with a poor prognosis (especially in adults). Two main classes of tRCC are currently identified: translocations involving the TFE3 locus on chromosome Xp11 and a t(6;11) translocation generating the TFEB gene fusion. These renal tumors can be morphologically challenging without the aid of ancillary studies. Whereas IHC for TFE3 or TFEB or their common transcriptional target, cathepsin K, is specific for tRCC there is poor reproducibility of TFE3 or TFEB IHC and the sensitivity is still not high enough to exclude the possibility of false-negative results. FISH, however, has been clearly documented for the diagnosis of these renal tumors and exhibits a high degree of sensitivity and specificity. FISH for TFE3 and TFEB gene fusions is now offered as one of the clinical assays at UMHS. This TFE3 FISH assay can also be used to confirm the diagnosis of alveolar soft part sarcoma if needed or indicated.

Collection Instructions: Submit a formalin-fixed, paraffin block of the tumor tissue (with matched benign renal tissue if available in case of renal cell carcinoma); store at room temperature.

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**Test Methodology, Reference Range, and Specimen Handling Changes**

**DRUG SCREEN, SERUM**

Effective January 14, 2014, the interpretive comment reported with Drug Screen, Serum assay results has been revised to read as follows:

“This gas chromatography screen of blood is a panel of tests designed to detect alcohol, benzodiazepines, barbiturates and a variety of other medications. This assay does not routinely identify opiates, marijuana or cocaine. A urine is the preferred specimen for drugs of abuse testing. This test was developed and its performance characteristics validated by the laboratory. It has not been cleared nor approved by the U.S. Food and Drug Administration.”

**GLUCOSE AND A1C**

To meet the reporting recommendations of the American Diabetes Association, the following changes became effective January 21, 2014:

1. The fasting glucose reference range for 12-150 years of age has changed from 73 – 110 mg/dL to 73 – 100 mg/dL.
2. The A1c reference range for all ages has changed from 3.8 – 6.4% to 4.2 – 5.8%. Additionally, the following comment will be appended to A1c results > 6.5%:

   “Results >=6.5% are consistent with the diagnosis of diabetes. Target goal for a diabetic patient or therapy is <7.0%.”

Note that Glucose is a component of the following panels: Basic Metabolic Panel (BASIC), Comprehensive Metabolic Panel (COMP), Pediatric Obesity Panel (POP) and Renal Function Panel (RENAL).

**HEPATITIS C VIRUS GENOTYPING**

Effective February 10, 2014, the MLabs Microbiology Laboratory began performing the Hepatitis C Virus Genotyping assay using the Abbott RealTime HCV Genotype II assay. The assay is a reverse transcription polymerase chain (RT-PCR) assay for determining the genotype(s) of hepatitis C virus (HCV) in plasma and
serum from HCV-infected individuals. There were no changes to specimen collection and handling requirements, reference range, or CPT code.

**HUMAN PAPILLOMAVIRUS DNA PROFILE**

Effective March 17, 2014, the MLabs Microbiology Laboratory has changed methods for performing HPV testing from hybrid capture 2 to polymerase chain reaction. Test results will continue to be reported for HPV High Risk Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and for one additional type, 66, using the Roche cobas® HPV Test. Equivalent to the hybrid capture 2 method, this assay is FDA approved for testing for HPV High Risk Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 using cervical specimens collected in PreservCyt® liquid.

Report changes will include statement of the new methodology listing the High Risk Types, and simplification of the report to state HPV High Risk types were or were not detected.

Collection Instructions: Collect cervical specimen and place into ThinPrep/PreservCyt® vial. Store and transport at room temperature. Specimens collected in Digene transport are no longer accepted by MLabs and will be sent out to Specialty Laboratories.

Samples submitted for both Pap and HPV testing will be forwarded from Cytology to Microbiology immediately after the cytology slide is prepared to allow for concurrent cytologic and HPV evaluation and improved turnaround time. HPV testing will be performed within 24-36 hours of receipt in Cytology, Monday through Thursday. Samples received on Friday will be tested the following Monday. For samples that will receive HPV testing based on cytologic findings, samples will be tested the next business day following transfer from Cytology.

**PORPHYRIN TESTING**

Effective January 7, 2014, the MLabs Special Chemistry Laboratory discontinued performance of quantitative porphyrin testing due to the low volume of requests. Requests for this test will be forwarded to Mayo Medical Laboratories.

Effective January 21, 2014, the Porphyrins, Qualitative Screen Urine which included Coproporphyrins, Uroporphyrins, and Porphobilinogen, has been replaced by a Porphobilinogen, Qualitative, Urine assay.

**Discontinued Tests**

**ADENOVIRUS AND RESPIRATORY CULTURES**

Effective January 27, 2014, Adenovirus Culture and Comprehensive Respiratory Virus Culture are no longer offered by the MLabs Microbiology Laboratory. The recommended alternative test is the Respiratory Pathogen Panel by PCR, which provides more sensitive molecular testing and has a shorter turnaround time. MLabs will continue to offer the Viral Culture, Comprehensive Non-Respiratory.

**C3D CIRCULATING IMMUNE COMPLEXES**

The C3d Circulating Immune Complexes assay performed by Quest Diagnostics was discontinued effective March 10, 2014. The recommended alternative test is the Raji Cell Immune Complex assay referred to ARUP Laboratories.

**ENA 11 PANEL**

Effective February 4, 2014, the double stranded DNA (ds-DNA) test performed as part of the Extractable Nuclear Antibody Panel (ENA11) has been discontinued. Due to this change, a new ENA 10 Antibody Panel (ENA10) has replaced ENA11. All other ENA testing remains the same.

MLabs recommends the DNA Antibody, Double-Stranded or the DNA Antibody, Double-Stranded, Crithidia Substrate assay to detect and quantify ds-DNA.

**INHIBIN, TOTAL**

The Total Inhibin assay performed by Women and Infants has been discontinued effective March 24, 2014. The recommended alternative test is the Inhibin B assay performed by Mayo Medical Laboratories.

**TEICHOIC ACID ANTIBODY**

Effective April 17, 2014, the Teichoic Acid Antibody assay referred to Focus Diagnostics, Inc., has been discontinued. With modern methods for detection of disseminated or localized *Staphylococcus aureus* infection, testing for teichoic acid antibodies does not provide clinical value.

It is recommended that routine blood cultures be performed for evaluation for *S. aureus* bacteremia, including endocarditis. Routine aerobic bacterial cultures can be performed on tissue or aspirates if the infection is localized but bacteremia is not present.
Arul Chinnaiyan, M.D., has been elected to the American Academy of Arts and Sciences 2014 Class of Members. This elite organization is one of the nation’s most prestigious honorary societies and includes more than 250 Nobel laureates and more than 60 Pulitzer Prize winners. Dr. Chinnaiyan was elected due to his work in the medical sciences, clinical medicine and public health. His research focuses on functional genomics, proteomic, and bioinformatic approaches to study cancer.

Congratulations to Kojo Elenitoba-Johnson, M.D., for his election into the American Association of University Pathologists (PLUTO SOCIETY). The PLUTO Society is an honor society recognizing investigative pathologists with significant contributions to the understanding of the pathologic basis of disease.

Lauren Smith, M.D., has been named Chair of the Michigan State Medical Society (MSMS) Bioethics Committee. The mission of the Michigan State Medical Society is to promote a health care environment that supports physicians in caring for and enhancing the health of Michigan citizens through science, quality, and ethics in the practice of medicine.

GENETIC TESTING RESOURCE AND QUALITY CONSORTIUM (GTRQC)

The Genetic Testing Resource and Quality Consortium (GTRQC) is a quality collaborative in development with Blue Cross Blue Shield of Michigan and coordinated through the University of Michigan Medical School, Department of Pathology.

The purpose of the GTRQC is to address the exponential growth in genetic testing and to improve the quality of care for patients needing molecular diagnostic testing by:

1. Determining which tests are clinically actionable and should be reimbursed.
2. Improving the quality of molecular diagnostic testing by reducing unnecessary testing and utilizing best practices when performing needed tests.
3. Educating providers and equipping them with resources and expert analysis of outcomes research to test, advise and treat their patients.

The GTRQC has been holding informational forums across Michigan to answer questions and to gather input as we establish this consortium. GTRQC is currently seeking input from pathologists, clinicians, medical geneticists, genetic counselors and pharmacists involved in performing, ordering or advising molecular diagnostic testing for their patients as we develop our collaborative quality initiative to address the exponential growth of genetic testing and to improve the quality of care for patients needing molecular diagnostic testing.

Additional information is available at www.gtrqc.org.