Leading Edge Diagnosis and Care for Genetic Diseases

When your child has a genetic condition, it can be a scary road to travel without proper understanding of the risks, medical issues and treatment plans. The University of Michigan Health System provides comprehensive care in the diagnosis, treatment and counseling for numerous genetic conditions, including those associated with birth defects, chromosomal and structural abnormalities, autism, intellectual disability, and inborn errors of metabolism (newborn screen). Clinical care often requires clinical genetic testing.

The Michigan Medical Genetics Laboratories (MMGL) is a comprehensive CLIA-certified clinical genetics testing laboratory that provides state of the art clinical testing and Research & Development for genetic diseases. There are two separate MMGL sections: the Biochemical Genetics Laboratory and the Molecular Genetics Laboratory. This article will focus on the Molecular Genetics Laboratory; an article for the Biochemical Genetics Laboratory will appear in a future issue.

The Molecular Genetics Laboratory develops, validates, and performs various clinical assays, including DNA sequencing, SNP chromosomal microarrays, relative-quantitative PCR, methylation sensitive PCR, multiplex PCR, fragment analysis by capillary electrophoresis, and
Multiplex Ligation-Dependent Probe Amplification (MLPA) to detect the underlying causes for genetic diseases. Identification of the genetic aberrations facilitates clinical diagnosis, management, and accurate genetic counseling.

The MMGL Molecular Genetics Laboratory began offering Chromosomal Microarray analysis in 2007 using an oligonucleotide array (44,000 markers) for patients with developmental delay or intellectual disability, autism spectrum disorders, or multiple congenital anomalies. In 2010, the American College of Medical Genetics and Genomics recommended using microarrays as a first-tier diagnostic test for postnatal testing instead of karyotypes (Manning et al., Genet Med, 12(11):742-745, 2010). In February 2012, the Molecular Genetics Laboratory transitioned to a higher density SNP chromosomal microarray (330,000 markers). Currently, our laboratory is working with a Cytogenetic Consortium on developing a higher density array (~850,000 markers) that should be available for clinical testing in the first quarter of 2013. Our laboratory has performed and analyzed over 3000 array cases.

The MMGL Molecular Genetics Laboratory deployed in 2010 a rapid, accurate and inexpensive high throughput fragment analysis assay for Fragile X syndrome, instead of using southern blots, for the detection of CGG repeat expansion in the Fragile X mental retardation (FMR1) gene associated with Fragile X spectrum disorders. This fragment analysis assay accurately sizes FMR1 alleles, detects Fragile X full mutation expansions, and determines the FMR1 methylation status.

In the second half of 2012, the MMGL Molecular Genetics Laboratory successfully developed an Autism/Intellectual Disability panel that enables detection of the underlying genetic aberration of 30-50% of all autism spectrum disorder cases. This Autism/Intellectual Disability panel consists of three reflexive tiers; including 16 different assays that are performed in an order based on the reported mutation detection rate of each tier (see Test Updates on page 5).

In addition, the MMGL Molecular Genetics Laboratory has developed a variety of molecular genetic tests to meet the needs of the Pediatric Genetics, Otolaryngology, Neurology, Pulmonology and other clinics at the University of Michigan Health System (UMHS). Our laboratory has developed Sanger sequencing assays for OTC, GJB2, NOG, CFTR, PTPN11, SOS1, KRAS, SETBP1, and SLC7A7 genes, as well as deletion/duplication assays by MLPA for SMN1 and SMN2, CFTR, MECP2, and PTEN genes, to name a few.

Cutting edge Research & Development leading to new clinical assays is an ongoing activity at the MMGL Molecular Genetics Laboratory. Recently, our R&D unit has focused on developing efficient, comprehensive diagnostic gene panels using Next-Generation Sequencing technology, which are currently going through clinical validation. To support these efforts, the MMGL Molecular Genetics Laboratory has hired experienced senior faculty, technologists, and bioinformaticians to design and develop a pipeline for accurate and efficient Next-Generation Sequencing, data analysis and interpretation.

MMGL’s first Next-Generation Sequencing gene panels target over 300 genes that are associated with over 700 common Mendelian diseases, including neuromuscular, cardiovascular, developmental, and metabolic diseases. These panels should be available for clinical testing in the first quarter of 2013. Such comprehensive panels will significantly expand our clinical testing capabilities for known genetic conditions and will enhance efforts in personalized genomic medicine.

To complement the high quality genetic testing and interpretive reports provided by the MMGL Molecular Genetics Laboratory, the Division of Pediatric Genetics at C.S. Mott’s Children’s Hospital offers supportive services to assist patients and their families. The Pediatric Genetics physicians are trained to help with the interpretation of complex genetic information. Pediatric Genetics clinics are held daily at the University of Michigan Health System, as well as frequent outreach clinics in Traverse City and Marquette, Michigan, where patients and their families can meet with experts who can explain their DNA test results and what they mean for their children, now and in the future.

The MMGL Molecular Genetics Laboratory has exceptional staff trained to analyze and interpret complex data sets, who work under the supervision of the Molecular Genetics Laboratory Director, Marwan Tayeh, Ph.D., FACMG, and the MMGL Medical Director, Jeffrey Innis, M.D., Ph.D., FACMG. Both are available for consultation with physicians and other care providers regarding genetic testing options and
Spotlight on
Marwan Tayeh, Ph.D., FACMG

Dr. Tayeh received a B.S. in Applied Biological Science (1995) and an M.S. in Human Genetics (1999) from Jordan University of Science and Technology, Irbid, Jordan. He received his Ph.D. in Genetics from the University of Iowa (2007), where he studied the molecular pathogenesis of Bardet-Biedl Syndrome (BBS). Dr. Tayeh completed his Clinical Molecular Genetics fellowship training at Emory University (2009) and became a Diplomat of the American Board of Medical Genetics (ABMG; 2011). He worked at PreventionGenetics LLC. (2010-2011) as a Clinical Molecular Geneticist where he developed more than 60 Sanger sequencing tests that focused on Ciliopathies, Marfan-related syndrome, and brain anomalies as well as a comparative genomic hybridization (CGH) gene-centric array that targeted more than 500 genes.

Dr. Tayeh is a recipient of the Richard King Award from the American College of Medical Genetics (ACMG) for best publication of the year (2010) for the manuscript entitled “Targeted comparative genomic hybridization (CGH) array for the detection of single- and multi-exon gene deletions and duplications”, which describes the development, validation and implementation of a high resolution oligonucleotide microarray that has the ability to detect intragenic deletion and duplication mutations within 76 targeted genes. Dr. Tayeh has authored a number of publications focusing on Bardet-Biedl syndrome (BBS) and comparative genomic hybridization array (aCGH).

Dr. Tayeh’s research interests focus on establishing genotype-phenotype correlations among Ciliopathies genes, including Bardet-Biedl syndrome, Joubert syndrome, Meckel-Gruber syndrome and Nephronophthisis; determining the pathophysiology of Ciliopathies; and developing high-throughput technologies and Next-Generation Sequencing to improve clinical molecular testing in diagnostic laboratories.

Dr. Tayeh joined the University of Michigan in October 2011 as an Assistant Professor of Pediatric Genetics and as the Director of the Molecular Genetics Laboratory of the Michigan Medical Genetics Laboratories (MMGL).
Sepsis Survivors Prone to Respiratory Viral Infections

UNIVERSITY OF MICHIGAN HEALTH SYSTEM STUDY SUGGESTS A NEW THREAT FOR THOSE RECOVERING FROM SEVERE SEPSIS

by University of Michigan Health System News

A respiratory virus common among infants and children during cold and flu season may become a threat to the old too, especially those who have survived sepsis.

A University of Michigan Health System study showed that following sepsis, a life-threatening blood infection, survivors may be more prone to respiratory viral infections, such as RSV, which can clog airways, cause wheezing and is a leading cause of bronchiolitis in infants.

The U-M study published in the November issue of Shock suggests a new threat for those recovering from severe sepsis.

“Prior research on survivors of sepsis have focused on secondary bacterial infections, however, recently we have identified that vulnerability of survivors of sepsis is not limited to bacterial infections, but also viral infections of the lung,” says study author Sumanta Mukherjee, Ph.D., an immunology expert in the U-M Department of Pathology.

For physicians, the findings highlight the potential for viral infections of the lung to complicate the recovery of patients suffering from severe sepsis.

Sepsis is a condition that’s becoming one to watch among the elderly, as hospitalizations for sepsis doubled in the U.S. from 2000-2008; a majority of them over age 65, according to federal figures.

Patients with sepsis are most often treated in intensive care units for the systemic inflammatory response that can damage organs and rapidly become life-threatening. Those who survive sepsis may develop an altered inflammatory response.

Using animal models, the authors revealed a propensity for survivors of sepsis to produce IL17, a type of immune signal molecule that can restrict immunity to some viral infections.

“Critical care physicians should be aware of RSV and other viral infections of the lung when managing the short-term and long-term care of patients diagnosed with severe sepsis,” says William F. Carson IV, Ph.D., a research investigator at the U-M Medical School.

Learning more about IL17 and offsetting its ability to worsen lung inflammation could lead to better treatments for patients suffering from viral infections such as RSV, authors say.

Additional authors: RM Allen, Nicholas W. Lukacs, Ph.D., and Steve L. Kunkel, Ph.D., of the University of Michigan Health System.


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Photomicrographic detection of respiratory syncytial virus (RSV) using indirect immunofluorescence technique. CDC/Dr. Craig Lyerla.
Test Updates

New Tests

ADAMTS13 ACTIVITY AND INHIBITOR ASSAYS

The MLabs Special Coagulation Laboratory began performing ADAMTS-13 Activity and Inhibitor assays effective November 5, 2012. ADAMTS-13 testing was previously sent to BloodCenter of Wisconsin.

Severe deficiency of ADAMTS-13 (activity <5-10%) may be acquired or congenital, and is a relatively specific finding in patients with a clinical diagnosis of thrombotic thrombocytopenic purpura (TTP). TTP is a rare and potentially fatal thrombotic microangiopathy syndrome, characterized by symptoms such as thrombocytopenia, microangiopathic hemolytic anemia (intravascular hemolysis with presence of schistocytes), neurological symptoms, fever, and renal dysfunction. In this patient population, persistence of severe ADAMTS-13 deficiency during clinical remission is associated with an increased risk for recurrent clinical episodes of TTP. Mild to moderate deficiency of ADAMTS-13 activity has also been observed in multiple medical conditions.

Test includes ADAMTS13 Activity. If activity is <30%, the inhibitor assay will be performed at an additional charge. By ordering this test the clinician acknowledges that additional reflex testing will be performed and billed at a separate additional charge if indicated.

Reference Range: Activity: >67%, Inhibitor: <0.4 Units.

AUTISM / ID PANELS

The MLabs Molecular Genetics Laboratory began offering Autism/Intellectual Disability (ID) Panel testing effective October 29, 2012.

Autism/ID panel analysis can be performed on patients who meet the criteria for Autism Spectrum Disorders (ASD) or Intellectual Disability. ASD is a clinically heterogeneous group of disorders that are characterized by impaired social relationships, impaired language and communication, and repetitive behaviors, or a narrow range of interests that appears in the first 3 years of life. Intellectual Disability (also known as developmental delay or formerly, mental retardation) is characterized by significantly sub-average general intellectual functioning associated with impairments in adaptive behavior and manifested during the developmental period (Miller et al. Am J Hum Genet 86:749-764, 2010; GeneReviews).

The Autism/ID Panel consists of three reflexive tiers that are performed in an order based on the reported mutation detection rate of each tier:

Tier 1 includes: Chromosomal Microarray Analysis (SNPM1S), Fragile X Syndrome Mutation Detection (FRXFAS), and Prader-Willi / Angelman Syndrome by PCR (PWSMPS), which may detect the underlying genetic cause in 17-24% of ASD/ID cases (Miller et al. 2010; Miles et al. Genet Med 13:278–294, 2011; Kelleher et al. Cell 135:401–406, 2008). If no mutations are detected in Tier 1, then Tier 2 will be performed.

Tier 2 includes: MECP2 and PTEN full gene sequencing and deletion-duplication analyses. Tier 2 may detect the underlying genetic cause in 5-20% cases of ASD/ID (Miles et al. 2011; Butler et al. J Med Genet 42:318–321, 2005; Varga et al. Genet Med 11:111–117, 2009). If no mutations are detected in Tier 2, then Tier 3 will be performed.


The Autism/ID Panel may be ordered as a single reflexive test; each Tier and each assay in the panel may also be ordered individually.

CDKL5 GENE SEQUENCING

The MLabs Molecular Genetics Laboratory began offering full gene sequencing of CDKL5 (OMIM:300203) effective October 30, 2012.

The CDKL5 gene is located on chromosome X at Xp22.13 (GRCh37/hg19). Mutations in CDKL5 gene are associated with Infantile Epileptic Encephalopathy 2 (EIEE2, OMIM:300672; also known as Atypical Rett Syndrome) and Angelman-Like Syndrome (OMIM:105830). The CDKL5
gene encodes a Serine/Threonine protein kinase suggested to be involved in MECP2 protein function, which indicates that CDKL5 and MECP2 may belong to the same molecular pathway (Mari et al. Hum Molec Genet 14:1935-1946, 2005; Lin et al. Hum Molec Genet 14:3775-3786, 2005.). See NCBI Gene for additional details.

**CFTR GENE PANEL**

The MLabs Molecular Genetics Laboratory began offering CFTR gene panel testing effective December 19, 2012. The CFTR gene panel analysis can be performed on patients who have disease characteristics consistent with autosomal recessive cystic fibrosis (CF, OMIM:219700) or congenital absence of the vas deferens (CAVD, OMIM:277180). The CFTR Panel is used to detect the presence of CFTR mutations in patients with CFTR-related disorders affecting the respiratory tract, pancreas, sweat glands, intestine, male genital tract, and liver, and for the confirmation of a diagnosis of Cystic Fibrosis in patients who had no mutations detected in a targeted mutation analysis for the 23 CF mutations recommended by ACMG/ACOG but have a clinical presentation consistent with cystic fibrosis. CFTR Panel testing consists of CFTR full gene sequencing (Tier 1) and if only one or no CFTR mutation is detected, then CFTR gene deletion and duplication analysis (Tier 2) is performed.

**HERPES SIMPLEX DNA, TYPES 1 & 2, QUALITATIVE**

The MLabs Microbiology Laboratory began offering Herpes simplex virus type 1 and type 2 testing for swab specimens collected in M4-RT transport from anogenital, skin, and oral mucosa lesions effective November 14, 2012. HSV Culture for these specimen types has been discontinued for patients >=3 days old; HSV Culture will continue to be performed for specimens from infants <3 days old, eye, and respiratory specimens.

**MECD GENE DELETION AND DUPLICATION ANALYSIS**

The MLabs Molecular Genetics Laboratory began offering MECP2 gene (OMIM:300005) deletion and duplication analysis effective October 24, 2012. MECP2 gene deletion and duplication analysis can be performed on patients with negative MECP2 sequence analysis or relatives of a patient with a known MECP2 deletion/duplication mutation. Approximately 30% of patients with Rett syndrome (OMIM:312750) and 7% of patients with atypical Rett syndrome have a deletion mutation within or encompassing the MECP2 gene (Laccone et al., Hum Mutat. 2004; 23:234–44; Archer et al., J Med Genet. 2006; 43:451–6).

MECP2 duplication mutations have been reported in approximately 2.5% of males with severe intellectual disability (Van Esch et al., Am J Hum Genet. 2005; 77:442–53; Lugtenberg et al., J Med Genet. 2006; 43:362–70), approximately 1% in X-linked intellectual disability cases, and rarely in females with severe encephalopathy (Lugtenberg et al., Eur J Hum Genet. 2009; 17:444–53, GeneReviews). MECP2 duplication syndrome is a severe neurodevelopmental disorder in males characterized by infantile hypotonia, severe mental retardation, poor speech development, progressive spasticity, recurrent respiratory infections (in ~75% of affected individuals) and seizures (in ~50%). There is no typical duplication size, other than typically the entire MECP2 gene is involved and will usually be picked up on a chromosomal microarray as well as by MECP2 del/dup analysis. Duplications ranging from 0.3 to 4 Mb are seen. These male patients have infections like individuals with common variable immunodeficiency but typically have normal immunoglobulins. The immune problem may be due to duplication of the adjacent IRAK1 gene. These patients may present for immunodeficiency workup, hypotonia, spasticity or seizures.

**PTEN GENE DELETION AND DUPLICATION ANALYSIS**

The Molecular Genetics Laboratory of The Michigan Medical Genetics Laboratories began PTEN gene deletion and duplication analysis effective October 22, 2012. PTEN gene deletion and duplication analysis can be performed on a patient whose PTEN sequence analysis was negative and for carrier testing in families with a known PTEN deletion/duplication. Approximately 10% of individuals with Bannayan-Riley-Ruvalcaba Syndrome (BRRS), who do not have a mutation detected in the PTEN coding sequence, have intragenic deletions within or encompassing the entire PTEN gene (Zhou et al., Am J Hum Genet. 2003b;73:404–11). Also, PTEN intragenic deletions have been reported in Cowden Syndrome (CS) patients (Zbuk et al. Nat Rev Cancer.2007; 7:35–45; GeneReviews).
Test Methodology, Reference Range, and Specimen Handling Changes

**AMINOLEVULINIC ACID, URINE**

Effective August 30, 2012, the specimen type for the Aminolevulinic Acid, Urine assay has changed from a 24 hour to a random urine collection.

Reference Range: &lt; 1 yr: &lt; or = 10 nmol/mL; 1-17 yrs: &lt; or = 20 nmol/mL; &gt; or = 18 yrs: &lt; or = 15 nmol/mL.

**BUTALBITAL, SERUM**

Effective October 25, 2012 the following reference range was implemented for the Butalbital, Serum assay:

Reference Range: &lt; 10 mcg/mL.

**BETA GALACTOSIDASE, LEUKOCYTES**

Please note the following change to the reference range for the Beta Galactose, Leukocytes, assay effective November 15, 2012:

Reference Range: &ge; 1.56 nmol/min/mg

**CARBOHYDRATE DEFICIENT TRANSFERRIN FOR CDG**

Additional analyte have been added to Carbohydrate Deficient Transferrin for Disorders of Glycosylation testing effective January 3, 2013. Please note the following updated Reference Ranges:

<table>
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<th>Ratio</th>
<th>Normal</th>
<th>Indeterminate</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferrin Mono-oligo/Di-oligo</td>
<td>≤ 0.06</td>
<td>0.07 - 0.09</td>
<td>≥ 0.10</td>
</tr>
<tr>
<td>Transferrin A-oligo/Di-oligo</td>
<td>≤ 0.011</td>
<td>0.012 - 0.021</td>
<td>≥ 0.022</td>
</tr>
<tr>
<td>Transferrin Tri-sialo/Di-oligo</td>
<td>≤ 0.05</td>
<td>0.06 - 0.12</td>
<td>≥ 0.13</td>
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<tr>
<td>Apo CIII-1/Apo CIII-2</td>
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<td>2.92 - 3.68</td>
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<tr>
<td>Apo CIII-0/Apo CIII-2</td>
<td>≤ 0.48</td>
<td>0.49 - 0.68</td>
<td>≥ 0.69</td>
</tr>
</tbody>
</table>

**INFLEXIMAB AND INFLEXIMAB ANTIBODIES**

Prometheus Laboratories introduced the Anser™ IFX test beginning July 30, 2012. The Anser™ IFX test is a next generation quantitative monitoring assay that allows healthcare providers to simultaneously measure and monitor serum infliximab (IFX) and antibodies to infliximab (ATI) levels at any time during therapy. Incorporating drug monitoring may clarify what factors are contributing to a patient’s loss of response and help guide treatment decisions by providing information to help determine an appropriate course of action. This test has replaced the serum Infliximab/HACA assay.

Please be advised that the CPT code for this assay, 84999 (unlisted chemistry procedure), is not covered by many insurance carriers. It is recommended that prior authorization is obtained before ordering this test.

For specimens referred from a physician office, MLabs will request that Prometheus bill the 3rd party insurance carrier. The patient will be responsible for any copay or deductible balance billed by Prometheus. For additional information regarding test coverage and cost, contact the Prometheus Billing Department at 888-892-8391.

If the patient is a hospital inpatient or outpatient on the date of service, or client billing is specified with the test order, MLabs will bill the client for this assay.

**NICKEL, SERUM**

Effective December 7, 2012, there was a change to the reference range for the Nickel, Serum assay:

Reference Range: &lt; 2 ng/mL

**Discontinued Tests**

**HEPATITIS B VIRUS DNA BY PCR, QUALITATIVE**

The Hepatitis B Virus DNA by PCR, Qualitative assay performed by Focus Diagnostics has been discontinued effective November 5, 2012. The recommended alternative test for monitoring Hepatitis B viral load is the Hepatitis B Virus DNA by PCR, Quantitative assay.

**PLASMINOGEN ACTIVATOR INHIBITOR-1 ACTIVITY**

Effective November 8, 2012, the Plasminogen Activator Inhibitor 1 Activity assay referred to Labcorp Burlington has been discontinued. All samples held at Labcorp Burlington have been cancelled. No alternative testing is available due to unavailability of reagent; the sole manufacturer of PAI-1 activity kits has taken the kit off the market and recalled all previously distributed kits.
MLabs News

MLABS PARTICIPATING FOR PRIORITY HEALTH MEDICARE

Effective September 1, 2012, the University of Michigan Health System is an innetwork, participating provider with Priority Health Medicare Advantage. The Michigan based Priority Health offers three types of Medicare Advantage plans:

- Priority Medicare (HMO/POS)
- Priority Medicare Value (HMO/POS)
- Priority Medicare Select (PPO)

No referrals needed to send clinical laboratory or anatomic pathology testing for Priority Health Medicare Advantage patients to MLabs. Note that although referrals are not necessary, prior authorization may be required for certain services (e.g., genetic testing).

NEW MLABS WEBSITE

MLabs would like to introduce our new Website, a tool for you to easily view the services we offer as a national provider of specialized laboratory testing and consultations. Please take a few minutes to explore our pages at http://mlabs.umich.edu. You can also find us on Facebook at facebook.com/MLabsUoM or follow us on Twitter at twitter.com/MLabsUM.

U-M DEPARTMENT OF PATHOLOGY NEWS

Congratulations to Suzanne Butch, Administrative Manager Blood Bank and Transfusion Service, and W. John Judd, F.I.B.M.S., Emeritus Professor of Pathology. Suzanne and John were awarded the Dale S. Smith Memorial Award at the recent 2012 AABB Annual Meeting in Boston, MA. The Dale Smith Award is awarded by the National Blood Foundation and recognizes groundbreaking work performed in the application of technology to the practice of transfusion medicine. Suzanne and John were recognized for their “pioneering achievement and championing of the electronic computer crossmatch as a means to expand blood banking while ensuring patient care”. The University of Michigan was the first hospital to implement and validate the electronic crossmatch in the United States. At present, nearly 20% of US hospitals have now adopted the electronic crossmatch.

Congratulations to Celina Kleer, M.D., who was selected as the 2013 recipient of the Ramzi Cotran Young Investigator Award from the United States and Canadian Academy of Pathology (USCAP). Dr. Kleer joins two others in the Department, Dr. Arul Chinnaiyan and Dr. Kojo Elenitoba-Johnson, who have received this award intended “to recognize a body of investigative work which has contributed significantly to the diagnosis and understanding of human disease.”

Gabriel Nunez, M.D., Paul H. DeKruif Professor, has been awarded a Grand Challenges Explorations Grant from the Bill and Melinda Gates Foundation. Dr. Nunez will pursue an innovative global health and development research project designed to find new treatments for bacterial infections of the gut that sicken millions of people each year. Dr. Nunez’s work on bacteria that invade the gut focuses on competition between the naturally occurring, or commensal, bacteria that live in the intestinal tract, and invading pathogens.

Congratulations to Jean-François Rual, Ph.D., Assistant Professor of Pathology, who has been awarded a prestigious ASH Scholar Award from the American Society of Hematology effective July 1, 2013. Dr. Rual’s research will focus on the analysis of protein-protein interaction networks in leukemias associated with HOXA9.