



The One

a publication that highlights new tests and announcements from ARUP

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Technical Bulletins

* Please note that test details contained in this publication may change. For the most up-to-date test information, please see ARUP's technical bulletins at <http://www.aruplab.com/Testing-Information/technicalbulletins.jsp>.

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Upcoming Trade Shows

FEBRUARY 28-MARCH 2

United States and Canadian Academy of Pathology (USCAP); San Antonio, TX

FEBRUARY 28-MARCH 2

Society for Inherited Metabolic Disorders (SIMD); Pacific Grove, CA

MARCH 9-11

American Pathology Foundation (APF); Las Vegas, NV

MARCH 17-19

American College of Medical Genetics (ACMG); Vancouver, Canada

Editorial Board:

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Integrated Marketing Communications

Donna Cooper, MS, MBA
Marketing Analytics

Contributors:

Archana Agarwal, MD

Roy Bastien

Phillip Bernard, MD

Katherine Geiersbach, MD, FCAP

David Grenache, PhD

Kimberly Hart, MS, LCGC

Thomas Haven, PhD

Harry Hill, MD

Kamisha Johnson-Davis, PhD

Patti Krautscheid, MS, LCGC

Danielle LaGrave, MS, LCGC

Lester Layfield, MD

Christine Litwin, MD

Stephanie Marin, PhD

Gwen McMillin, PhD

Stephen Merrigan

Jaqueline Rogerio, MD

Patricia Slev, PhD

Michelle Wallander, PhD

APRIL 13-15

G-2 Molecular Diagnostics Conference; Boston, MA

MAY 3-5

Executive War College: Conference on Laboratory and Pathology Management; New Orleans, LA

MAY 8-10

Clinical Virology Symposium; Daytona Beach, FL

MAY 15-17

VHA Leadership Conference; San Diego, CA

New Business Analytics and Reporting Application (BARA)

BARA has been designed to allow ARUP clients to quickly perform test-utilization report extractions. The application is easily adaptable to each client's specific situation and includes a scheduling feature that allows ARUP clients to specify a date (e.g., monthly or quarterly) for report generation, along with accompanying date-range parameters for utilization data. Notification will be sent via email when the scheduled report is available for viewing.

As of May 2011, utilization reports will no longer be distributed by ARUP Laboratories. We strongly encourage all clients to migrate to this new application.

BARA benefits include:

- Elimination of dependency on ARUP to generate test-utilization reports

- Ability to schedule report generation based on user-specified date range
- Ability to create on-demand reports
- Grouping functionality that will allow users to generate utilization data for multiple locations
- Printable, downloadable formats available in PDF, Microsoft Word, Microsoft Excel, or HTML
- Convenient availability via ARUP Connect™
- Instant notification via email when a new scheduled report is available

ARUP Consult® Now Available for Apple Devices at No Cost or Obligation at the App Store

ARUP Consult® is a physician's guide to laboratory test selection and interpretation. This web- and mobile-based product offers point-of-care access for health care providers and encourages improved patient care and reduced health care costs.

ARUP Consult is available at no cost or obligation at the following:

- www.arupconsult.com
- App Store (for Apple devices)
- m.arupconsult.com (for mobile devices)

Features:

- Content authored by experts at the University of Utah and ARUP Laboratories
- Algorithms to support clinical decision-making
- Clinical background, diagnostic advice, screening and monitoring recommendations, and test-selection guidance
- References hyperlinked to leading journals and guidelines

FREE CME and CEUs | www.arup.utah.edu ARUP Online Scientific Resource for Research and Education

ARUP's Online Scientific Resource for Research and Education currently provides more than 20 CME courses for physicians and offers more than 17 P.A.C.E.® credits to laboratory professionals. All courses are free of charge, with new topics added monthly. Courses are developed by the ARUP Institute for Learning and ARUP's medical directors, who are faculty in the Department of Pathology at the University of Utah School of Medicine, along with other highly qualified expert contributors. Course topics include

current issues and recent developments in the fields of pathology and laboratory medicine.

For more information, email learning@aruplab.com.

The University of Utah School of Medicine designates these educational activities for *AMA PRA Category 1 credit(s)* and affirms that they meet the criteria for Self-Assessment Modules (SAM) credit to apply toward the Maintenance of Certification (MOC) by the American Board of Pathology.

Bordetella pertussis Antibodies by Immunoblot

For the qualitative and semi-quantitative detection of anti-*Bordetella pertussis* antibodies

Test Highlights

- This assay is used to detect IgG, IgA, and IgM antibodies against *Bordetella pertussis*.
- The PT-100 band in the IgG immunoblot can be used to help diagnose acute infection or recent vaccination.

Disease Overview

- Pertussis (whooping cough) is a highly transmittable respiratory infection caused by acute infection of the tracheal epithelial cilia by the slow-growing, gram-negative coccobacillus *Bordetella pertussis*.
- Pertussis symptoms in unvaccinated individuals include fits of coughing, inspiratory whoop, and posttussive vomiting.
- Individuals with partial immunity may have milder cold-like symptoms and chronic cough.

Epidemiology

- In the 1940s, a whole-cell pertussis vaccine was introduced, which has caused the number of pertussis cases to drop dramatically. However, there are still a significant number of cases in the United States, with 25,616 cases reported in 2005.
- The World Health Organization (WHO) estimates that 50 million cases and 300,000 deaths occur every year due to *Bordetella pertussis* infections.

Indications for ordering

- The immunoblot assay should be ordered for adults and children if the following pertussis symptoms exist: a cough lasting more than 14 days, paroxysmal coughing, inspiratory whoop, and posttussive vomiting.
- Infants should be tested if the following pertussis symptoms exist: a cough lasting longer than 14 days, possibly an inspiratory whoop, gagging, gasping, cyanosis, or apnea.
- Additional testing by nasopharyngeal culture is also recommended.

Methodology

- This assay utilizes a *B. pertussis*-specific protein, pertussis toxin (PT), and a *Bordetella*-specific protein, filamentous hemagglutinin (FHA).
- The *B. pertussis* IgG immunoblot differs from the IgA and IgM immunoblot in that it contains two pertussis toxin (PT) bands calibrated with the international WHO standards for PT in

international units (IU/mL). The PT-100 IgG band and the PT IgG correlate with 100 IU/mL and 8 IU/mL, respectively. These two calibrated bands allow the differentiation of high- and low-level specific PT IgG antibody, and are useful in the differentiation of recent infection or vaccination from past infection or vaccination.

Interpretation

- A positive PT-100 IgG band indicates a significantly high level of anti-PT IgG antibodies that correlates with either acute *B. pertussis* infection or recent *B. pertussis* vaccination (<36 months). A positive PT IgG band (without a positive PT-100 band) indicates a lower level of anti-PT antibodies that is consistent with past *B. pertussis* infection or past vaccination.
- *B. pertussis* specific IgA or IgM antibodies are detected if the respective PT band is positive.
- *Bordetella* IgG, IgA, or IgM antibodies are detected if the respective FHA band is positive.
- All other results are considered negative, and no *B. pertussis*-specific IgG, IgA, or IgM antibodies are detectable.

Limitations

- This test has been validated for serum and plasma samples only; no other sample types may be used.
- As with all serological assays for pertussis, interpretation of results must be used in conjunction with the patient's clinical symptoms, medical history, and other clinical and/or laboratory findings to produce an overall clinical diagnosis.

References

1. Centers for Disease Control. Prevention of pertussis, tetanus, and diphtheria among pregnant and postpartum women and their infants. *MMWR* 2008; 57:1-47.
2. Mandell GL, Bennett JE, Dolin R. 2010. *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*, 7th ed. Philadelphia, PA: Churchill Livingstone Elsevier.
3. Melker HE, et al. Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*. *J Clin Micro* 2000;38(2):800-6.

For specific collection, transport, and testing information, refer to [Bordetella pertussis Antibodies, IgA, IgG, & IgM by Immunoblot \(2004328\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

EML4-ALK Translocations by RT-PCR

For prediction of patient response to ALK inhibitors in non-small cell lung carcinoma

Test Highlights

- Detects the two most common *EML4-ALK* translocation variants in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung carcinoma (NSCLC) tissue.
- Aids in the selection of patients who may benefit from treatment with ALK inhibitors.
- Interpretation of *EML4-ALK* RT-PCR is less subjective than *ALK* FISH.

Clinical Background

- Lung cancer is the most common and deadly form of cancer in the United States, with a five-year survival rate of approximately 15 percent.
- A subset of NSCLC patients harbors translocations involving the 5' portion of the *EML4* gene and the 3' portion of the *ALK* gene.
- The incidence of *EML4-ALK* in NSCLC is low (2–7 percent) but can be higher (13 percent) if the patient population displays the following characteristics: adenocarcinoma, non-existent or light history of smoking, younger age, and lack of *EGFR* or *KRAS* mutations.
- The resulting *EML4-ALK* fusion protein displays constitutive *ALK* kinase activity, which can be targeted with *ALK* kinase inhibitors.

Genetics

- Multiple small inversions on chromosome 2p generate in-frame fusions of the *EML4* and *ALK* genes.
- While the breakpoints in *EML4* can vary (fusion at exons 2, 6, 13, 14, 15, 18, and 20), the breakpoint in *ALK* consistently occurs at exon 20 of the kinase domain.
- The majority (~70 percent) of translocations involve *EML4* exon 13 (variant 1) or *EML4* exon 6a/b (variant 3a/b).
- Due to the small inversions, detection of some *EML4-ALK* variants is challenging with commercially available *ALK* break-apart FISH probes.

Indications for Ordering

- *EML4-ALK* RT-PCR should be performed on FFPE adenocarcinomas of the lung, especially tumors lacking *EGFR* and *KRAS* mutations.

Interpretation

- The presence of an *EML4-ALK* translocation predicts a favorable response to *ALK* inhibitor therapy.

Limitations

- This assay will only detect *EML4/ALK* translocations involving *EML4* exons 6 or 13 and *ALK* exon 20.

Methodology

- RNA is extracted from FFPE tissue blocks and reverse transcribed into cDNA. The cDNA is subjected to PCR amplification using oligonucleotide primers specifically designed to detect *EML4-ALK* transcript variant 1 (*EML4* exon 13 fused to *ALK* exon 20) and *EML4-ALK* transcript variant 3a/b (*EML4* exon 6a/b fused to *ALK* exon 20).
- Amplification of the *MRPL19* gene is also performed for each sample to ensure RNA quality.

Related Tests

- *EGFR* Mutation Detection by PCR and Fragment Analysis ([2002440](#))
- *KRAS* Mutation Detection ([0040248](#))

References

1. American Cancer Society. 2010 Cancer facts and figures. 2010; Atlanta, GA: American Cancer Society.
2. Ries L, et al, eds. 2005. Cancer Statistics Review, 1975–2002. Bethesda, MD: National Cancer Institute.
3. Soda M, et al. Identification of the transforming *EML4-ALK* fusion gene in non-small-cell lung cancer. *Nature* 2007;448:561–6.
4. Solomon B, Varella-Garcia M, Camidge DR. *ALK* gene rearrangements: a new therapeutic target in a molecularly-defined subset of non-small cell lung cancer. *J Thor Oncol* 2009;4(12):1450–4.
5. Kwak E, et al. Clinical activity observed in a phase I dose escalation trial of an oral c-Met and *ALK* in-hibitor, PF-02341066. *J Clin Oncol* 2009;27:15s.
6. Kwak EL, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010;363(18):1693–703.
7. Camidge DR, et al. Optimizing the detection of lung cancer patients harboring anaplastic lymphoma kinase (*ALK*) gene rearrangements potentially suitable for *ALK* inhibitor treatment. *Clin Cancer Res* 2010;16(22):5581–90.

For specific collection, transport, and testing information, refer to [EML4/ALK Translocation by RT-PCR \(2004547\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Everolimus (Afinitor®)

For therapeutic drug monitoring (whole blood) in patients with SEGA associated with tuberous sclerosis who require therapeutic intervention but are not candidates for curative surgical resection

Clinical Background

- In March 2009, the FDA approved Afinitor (everolimus) for the treatment of patients with advanced renal-cell carcinoma (RCC) after failure of treatment with sunitinib or sorafenib. In October 2010, the FDA approved Afinitor (everolimus) for the treatment of patients with subependymal giant-cell astrocytoma (SEGA) associated with tuberous sclerosis (TS) who require therapeutic intervention but are not candidates for curative surgical resection.
 - Tuberous sclerosis affects one to two million people worldwide, causing benign tumors to form in many vital organs, including the brain, kidney, heart, eyes, lungs, and skin. SEGAs, benign brain tumors, occur in up to 20 percent of patients with TS and can pose a significant medical risk, including potential for swelling in the brain and hydrocephalus.^{2,3}
 - Loss or inactivation of TSC1 or TSC2, the oncogene suppressor tuberous-sclerosis complexes, leads to activation of downstream signaling. In tuberous sclerosis (TS), a genetic disorder, inactivating mutations in either the TSC1 or the TSC2 gene lead to hamartoma formation throughout the body.¹
- Afinitor (everolimus) is an inhibitor of mTOR, a serine-threonine kinase downstream of the PI3K-AKT pathway. The mTOR pathway is dysregulated in several human cancers. Inhibition of mTOR by everolimus has been shown to reduce cell proliferation, angiogenesis, and glucose uptake in *in vitro* and/or *in vivo* studies.¹
 - Everolimus is a substrate of CYP3A4 and PgP. Following oral administration, Afinitor (everolimus) is the main circulating component in human blood. Six main metabolites of everolimus have been detected in human blood, including three monohydroxylated metabolites, two hydrolytic ring-opened products, and a phosphatidylcholine conjugate of everolimus.¹
 - The dose of Afinitor (everolimus) for the SEGA population is individualized to the patient's body surface area. The dose is titrated to achieve a whole-blood trough concentration of 5–10 ng/mL.¹
- The blood-to-plasma ratio of Afinitor (everolimus), which is concentration-dependent over the range of 5 to 5,000 ng/mL, is 17 to 73 percent. The amount of Afinitor (everolimus) confined to the plasma is approximately 20 percent at blood concentrations observed in cancer patients given 10 mg of Afinitor (everolimus) per day. Plasma protein binding is approximately 74 percent in both healthy subjects and patients with moderate hepatic impairment.¹ Routine Afinitor (everolimus) whole-blood therapeutic drug concentration monitoring is recommended for all SEGA patients.¹

Indications for Ordering

- Afinitor (everolimus) is approved for the treatment of SEGA associated with tuberous sclerosis (TS) in patients who require therapeutic intervention but are not candidates for curative surgical resection.

- Routine Afinitor (everolimus) whole blood therapeutic drug concentration monitoring is recommended for all patients using a validated assay. Trough concentrations should be assessed approximately two weeks after commencing treatment. Dosing should be titrated to attain trough concentrations of 5 to 10 ng/mL.
- Avoid concomitant use with strong CYP3A4 or PgP inhibitors. If moderate inhibitors of CYP3A4 and/or PgP are required, reduce the Afinitor (everolimus) dose by approximately 50 percent. Subsequent dosing should be based on therapeutic drug monitoring (TDM). If strong inducers of CYP3A4 are required, double the Afinitor (everolimus) dose. Subsequent dosing should be based on TDM.

Interpretation

- In a population pharmacokinetic evaluation in cancer patients, no relationship was apparent between oral clearance and patient age or gender.
- The average AUC of everolimus in eight subjects with moderate hepatic impairment (Child-Pugh class B) was twice that found in eight subjects with normal hepatic function. In addition, clearance of everolimus is estimated to be 20 percent higher in patients of African descent as compared to Caucasian patients.
- In patients with SEGA, higher everolimus trough concentrations appear to be associated with larger reductions in SEGA volume. However, responses have been observed at trough concentrations as low as 3 ng/mL, so additional dose increase may not be necessary once acceptable efficacy has been achieved.

Methodology

- Liquid chromatography coupled with mass spectrometry (LC/MS) is the analytical method of choice for immunosuppressant drug detection.
- Afinitor (everolimus) detection by LC/MS provides excellent sensitivity (2.0 ng/mL) and specificity. Interferences from commonly used drugs and associated metabolites have not been observed.

Additional Ordering Notes

- Since more than 75 percent of Afinitor (everolimus) is bound to erythrocytes, whole blood (EDTA anticoagulation) is required.

References

1. Afinitor (everolimus). Package insert. East Hanover, NJ.; Novartis Pharmaceuticals Corp; 2009.
2. Tuberous Sclerosis Alliance. Subependymal giant cell tumor (SGCT) or subependymal giant cell astrocytoma (SEGA). http://www.ninds.nih.gov/disorders/tuberous_sclerosis/detail_tuberous_sclerosis.htm. (accessed on September 8, 2010).
3. Adriaensen ME, et al. Prevalence of subependymal giant cell tumors in patients with tuberous sclerosis and a review of the literature. *Eur J Neurol* 2009;16:691–6.

For specific collection, transport, and testing information, refer to [Everolimus by Tandem Mass Spectrometry \(0092118\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Hemoglobin Lepore (*HBD-HBB* Fusion) 3 Mutations

Detects hemoglobin Lepore resulting from rearrangements of the delta and beta globin genes

Disease Overview

- Hemoglobin (Hb) is a tetrameric molecule that reversibly binds oxygen in red blood cells. Adult Hb is composed predominantly of two alpha globin chains and two beta globin chains.
- Hb Lepore is a hemoglobin variant resulting from a fusion between the delta globin gene (*HBD*) and the beta globin gene (*HBB*).
- Hb Lepore causes beta thalassemia. Thalassemias result from an imbalance in the quantity of alpha and beta globin chains.
- In its heterozygous form, Hb Lepore causes a beta thalassemia minor phenotype and is associated with mild anemia, hypochromic microcytosis, and moderately increased fetal hemoglobin.
- Homozygosity for Hb Lepore is rare. The associated phenotypes for homozygosity or compound heterozygosity for Hb Lepore/beta thalassemia are variable and often include beta thalassemia intermedia and major.
- The combination of Hb Lepore with structural hemoglobinopathies also leads to variable clinical presentations.
- Co-inheritance of alpha globin mutations or additional genetic modifiers may influence clinical presentation.
- The presence of Hb Lepore is often suggested by hemoglobin electrophoresis or HPLC. Molecular confirmation may be useful for optimal management, genetic counseling, and prenatal diagnosis.

Epidemiology

- There are three major forms of Hb Lepore:
 - Hb Lepore-Washington-Boston is the most common; it is reported in many populations but most common in Italians.
 - Hb Lepore-Baltimore is observed in Yugoslavian, Brazilian, American, Northern Sardinian, Spanish, and Portuguese individuals.
 - Hb Lepore-Hollandia is rare; it is observed in New Guinea and Bangladesh.

Genetics

- Autosomal recessive inheritance.
- Hb Lepore is classified as a β^+ thalassemia mutation, as it results in reduced beta globin chain synthesis.
- Due to the high homology of the DNA sequences of delta globin and beta globin genes, unequal crossovers can occur during recombination. This results in a delta/beta fusion gene that produces the abnormal delta/beta hybrid chain.
- The fusion gene involves the 5' portion of the delta globin gene and the 3' portion of the beta globin gene, and results in a deletion of approximately 7.4 kb. The fusion gene retains the promoter of the delta globin gene, decreasing transcription efficiency and production of the delta/beta hybrid chain.
- The three common Hb Lepore mutations described above are distinguished by their characteristic breakpoints within the delta and beta globin genes.
- Other rare delta/beta globin gene rearrangements have been described.

Indications for Ordering

- Molecular confirmation of a suspected Hb Lepore variant identified by hemoglobin evaluation.
- Carrier screening for individuals with a family history of Hb Lepore.

Interpretation

- For optimal test interpretation, please submit a Patient History for Hemoglobinopathy/Thalassemia Testing Form detailing clinical findings, family history, and ethnicity.
- Negative: None of the three common Hb Lepore mutations were identified. This result does not exclude beta thalassemia, as other beta globin gene mutation(s) are not identified by this assay.
- Heterozygous: One copy of an Hb Lepore mutation was identified. Carriers of Hb Lepore typically present with beta thalassemia minor, while individuals who are compound heterozygous for Hb Lepore and a second beta globin mutation may be variably affected.
- Homozygous or compound heterozygous: Two Hb Lepore mutations were identified, consistent with a diagnosis of beta thalassemia; associated phenotypes for homozygosity or compound heterozygosity are variable and often include beta thalassemia intermedia and major.

Methodology

- Multiplex PCR and gel electrophoresis to detect the three common Hb Lepore mutations: Hb Lepore-Washington-Boston (g.63632_71046del), Hb Lepore-Baltimore (g.63564_70978del), and Hb Lepore-Hollandia (g.63290_70702del).
- Clinical sensitivity and specificity are unknown.
- Analytical sensitivity and specificity for the mutations tested are 99 percent.

Limitations

- Only the three common Hb Lepore mutations will be detected. Rare delta/beta rearrangements and other mutations in the alpha, delta, or beta globin genes will not be identified.
- This assay may not be able to distinguish between homozygosity for a common Hb Lepore mutation and compound heterozygosity for a common Hb Lepore mutation and a rare delta/beta globin gene deletion.
- Rare diagnostic errors can occur due to primer-site mutations.

Related Test

- Hemoglobin Evaluation with Reflex to Electrophoresis and/or RBC Solubility ([0050610](#))

References

1. Chaibunruang A, et al. Interactions of hemoglobin Lepore ($\delta\beta$ hybrid hemoglobin) with various hemoglobinopathies: a molecular and hematological characteristics and differential diagnosis. *Blood Cells Mol Dis* 2010;44(3):140–5.
2. McKeown SM, et al. Rare occurrence of Hb Lepore-Baltimore in African Americans: molecular characteristics and variations of Hb Lepores. *Ann Hematol* 2009;88(6):545–48.
3. Goncalves I, et al. Fetal hemoglobin elevation in Hb Lepore heterozygotes and its correlation with β globin cluster linked determinants. *Am J Hematol* 2002;69:95–102.
4. Hartevelde CL, et al. Hb Lepore-Leiden: a new δ/β rearrangement associated with a β -thalassemia minor phenotype. *Hemoglobin* 2008;32(5):446–53.

For specific collection, transport, and testing information, refer to [Hemoglobin Lepore \(*HDB-HBB* Fusion\) 3 Mutations \(2004686\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Interleukin 28 B (*IL28B*)-Associated Variants, 2 SNPs

Detects DNA variants associated with treatment response for chronic genotype 1 hepatitis C virus (HCV) infection

Disease Overview

- Hepatitis C is an infectious disease mediated by the hepatitis C virus (HCV). Transmission may occur from contaminated blood or tissue products, IV drug use, intranasal cocaine use, organ transplant, or an HCV-positive sexual partner. Hepatitis C is the most common cause of progressive liver disease, cirrhosis, and hepatocellular carcinoma (HCC) in North America.
- In the United States, hepatitis C causes an estimated 10,000 deaths annually and is responsible for 60–70 percent of chronic hepatitis and up to 50 percent of cirrhosis and HCC. Hepatitis C is also the primary reason for liver transplant in the United States.
- HCV is a small, enveloped, single-stranded RNA virus. Viral genotyping has identified at least six major HCV genotypes that are further subtyped based on sequence characteristics. Genotype 1 (subtypes 1a and 1b) accounts for 75 percent of hepatitis C infections in the United States.
- Approximately 80 percent of those with hepatitis C develop chronic HCV infection. Chronic HCV infection is characterized by the presence of anti-HCV antibodies with serum amino transferase (ALT) and detectable levels of viral HCV RNA persisting for more than six months.
- In individuals with chronic infection, the risk of developing cirrhosis ranges from 5 to 25 percent over a period of 25 to 30 years. Persons with HCV-related cirrhosis are at risk of liver failure (30 percent over 10 years) as well as HCC (1–3 percent/year).
- Due to the slow evolution of chronic HCV infection, it is difficult to demonstrate treatment response using clinical endpoints. Sustained virological response (SVR), defined as the absence of HCV RNA in serum 24 weeks following completion of therapy, is the most accepted measure of treatment success.
- Current recommended therapy for chronic hepatitis C infection consists of weekly peginterferon (PEG-IFN α) injections and daily oral ribavirin (RBV), together which improve ALT levels and clearance of HCV RNA. The specific therapy regimen, including dosing and duration of therapy, depends on numerous clinical factors and HCV genotype.
- Combination therapy is effective in eliminating HCV RNA in 40–50 percent of individuals with the genotype 1 HCV and in 70–90 percent of those with HCV genotype 2 or 3. Individuals of European ancestry have a substantially higher probability of treatment response than African-Americans.
- Given the high cost of treatment and significant adverse side effects, which result in discontinuation of hepatitis C therapy in 10–15 percent of those treated, pre-therapeutic identification of factors predicting response is helpful in estimating the likelihood of an SVR. However, the lack of favorable factors should not be used to deny therapy, as there is no alternative to interferon therapy in the HCV-infected population.

- Two single nucleotide polymorphisms (SNPs), rs12979860 and rs8099917, located near the *IL28B* gene-encoding interferon- λ -3 (IFN- λ -3), have been independently associated with response to PEG-IFN α /RBV therapy in Caucasians.
- IFN- λ -3 is believed to interact with a cellular trans-membrane receptor to upregulate the JAK-STAT pathway. This results in antiviral activity.

Epidemiology

- Persistent hepatitis C infection affects approximately 180 million individuals worldwide, or approximately 3 percent of the world's population.
- In the United States, approximately 4.1 million individuals, or 1.6 percent of all Americans, have anti-HCV antibodies.
- Estimated allele frequencies for the favorable rs12979860 C allele: East Asian:0.90; Caucasian:0.75; Hispanic:0.70; African-American:0.50.
- Estimated allele frequencies for the favorable rs8099917 T allele: Caucasian:0.75; Asian:0.88; unknown in other ethnicities.

Genetics

- The favorable rs12979860 C allele has been associated with a two to threefold greater rate of SVR following PEG-IFN α /RBV therapy in individuals with HCV genotype 1, while the T allele is a risk factor for non-response.
- The rs12979860 C/C genotype has also been associated with a threefold increase in natural clearance of HCV compared to the C/T and T/T genotypes combined.
- The favorable rs8099917 T allele is associated with a higher rate of SVR after PEG-IFN α /RBV therapy compared to the G allele, which is a risk factor for non-response.
- The rs8099917 T/T genotype is also associated with increased natural clearance of HCV compared to the G/T and G/G genotypes.

Indication for Ordering

- Pre-therapeutic prediction of response to PEG-IFN α /RBV therapy for individuals with chronic genotype 1 HCV genotype infection.

Interpretation

- Genotype should be interpreted with clinical information. Lack of favorable genetic factors should not be used to deny therapy.
- rs12979860 genotype
 - C/C: Two copies of the favorable C allele are associated with an increased likelihood of sustained virological response (SVR) following HCV PEG-IFN α /RBV therapy and increased spontaneous clearance of HCV.
 - C/T: One copy of the favorable C allele is a risk factor for non-response to HCV PEG-IFN α /RBV therapy.

Interleukin 28 B (*IL28B*)-Associated Variants, 2 SNPs, continued

- T/T: Lack of the favorable C allele is a risk factor for non-response to HCV PEG-IFN α /RBV therapy.
- rs8099917 genotype
 - T/T: Two copies of the favorable T allele are associated with an increased likelihood of sustained virological response (SVR) following HCV PEG-IFN α /RBV therapy.
 - T/G: One copy of the favorable T allele is associated with a mild increase in the likelihood of sustained virological response (SVR) following HCV PEG-IFN α /RBV therapy.
 - G/G: Lack of the favorable T allele is a risk factor for non-response to HCV PEG-IFN α /RBV therapy.

Methodology

- PCR and fluorescent probe hybridization to detect SNPs rs12979860 C>T and rs809917 G>T.
- Clinical sensitivity and specificity are unknown.
- Analytical sensitivity and specificity for the SNPs detected are 99 percent.

Limitations

- Only the specific SNPs targeted will be detected.
- The tested SNPs are associated with treatment response in Caucasians with chronic genotype 1 HCV infection; the association of these SNPs with treatment in patients of other ethnicities or for other HCV genotypes is unknown.
- Mutations in other genes and non-genetic factors that may affect response to hepatitis C therapy are not detected.
- Rare diagnostic errors can occur due to primer-site mutations.

Related Tests

- Hepatitis C Virus RNA Quantitative, Real-Time PCR with Reflex to Genotype ([2002685](#))
- Hepatitis C Virus RNA Quantitative, Real-Time PCR ([0098268](#))
- Hepatitis C Virus Genotyping by PCR & Sequencing ([0055593](#))

References

1. Ge D, et al. Genetic variation in *IL28B* predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461(7262):399–401.
2. Ghany MG, et al. American Association for the Study of Liver Diseases: practice guidelines; diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009;49(4):1335–74.
3. Thomas DL, et al. Genetic variation in *IL28B* and spontaneous clearance of hepatitis C virus. *Nature* 2009; 461(7265):798–801.
4. Suppiah V, et al. *IL28B* is associated with response to chronic hepatitis C interferon- α and ribavirin therapy. *Nat Genet* 2009; 41(10):1100–4.
5. Tanaka Y, et al. Genome-wide association of *IL28B* with response to pegylated interferon- α and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009;41(10):1105–9.
6. Rauch A, et al. Genetic variation in *IL28B* is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterol* 2010;138(4):1338–45.

For specific collection, transport, and testing information, refer to [Interleukin 28 B \(*IL28B*\)-Associated Variants, 2 SNPs \(2004680\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

LMNA-Related Disorders (LMNA) Deletion/Duplication

To confirm a clinical diagnosis of muscular dystrophy-related laminopathy

Disease Overview

- Mutations in the *LMNA* gene cause a broad range of clinical diseases collectively termed laminopathies.
- *LMNA*-related disorders include Emery-Dreifuss muscular dystrophy type 2 (EDMD2) and Limb Girdle muscular dystrophy 1B (LGMD1B).
- Clinical findings are highly variable.

DISEASE	CLINICAL FEATURES
EDMD2	Joint contractures, progressive muscle weakness and wasting, cardiac disease with conduction defects and arrhythmias; age of onset is variable.
LGMD1B	Progressive proximal lower limb weakness and atrioventricular cardiac conduction complications.
DCM	Progressive ventricular dilation and impaired systolic function leading to congestive heart failure.

Prevalence

- Unknown for laminopathies caused by deletions/duplications.

Genetics

- Lamin A/C codes for isoforms A and C of the protein lamin, a structural component of the nuclear membrane.
- Type A lamins are encoded by the *LMNA* gene, which is composed of 12 exons and is located at 1q21.2-q21.3.
- Alternative splicing of the *LMNA* gene results in the production of multiple proteins, including Lamin A and Lamin C, which have been shown to provide mechanical support to the nucleus and anchor heterochromatin to the inner nuclear membrane.
- Mutations occur throughout the gene and are predominantly missense.

Indication for Ordering

- To confirm a clinical diagnosis of non-X-linked EDMD2, LGMD1B, or inherited DCM.

Contraindication for Ordering

- Order *LMNA*-Related Disorders (*LMNA*) Sequencing (ARUP test #2004543) to confirm a diagnosis of Hutchinson-Gilford progeria, Charcot-Marie-Tooth 2B1, familial partial lipodystrophy (Dunnigan type), mandibulo-acral dysplasia, atypical Werner syndrome, or restrictive dermopathy.

Interpretation

- Positive: Detection of a single pathogenic *LMNA* mutation is consistent with a diagnosis of an autosomal dominant laminopathy.
- Negative: Lack of detection of an *LMNA* mutation decreases, but does not exclude, the possibility of a laminopathy.

Methodology

- Multiplex ligation-dependent probe amplification (MLPA) of the *LMNA* gene.
- Analytic sensitivity and specificity of MLPA are 90 and 98 percent, respectively.
- Clinical sensitivity is dependant upon the specific *LMNA*-related disorder.

Limitations

- Breakpoints of large deletions/duplications detected in *LMNA* will not be determined

Related Test

- *LMNA*-Related Disorders (*LMNA*) Sequencing (2004543)

References

1. Worman HJ and Bonne G. Laminopathies: a wide spectrum of human diseases. *Exp Cell Res* 2007;313(10):2121–33.
2. Genschel J and Schmidt HH-J. Mutations in the *LMNA* gene encoding lamin A/C. *Human Mutat* 2000;16:451–9.
3. Online Mendelian Inheritance in Man. www.ncbi.nlm.nih.gov (accessed on November 1, 2010).
4. Online GeneTests. www.genetests.org (accessed on November 1, 2010).

For specific collection, transport, and testing information, refer to [LMNA-Related Disorders \(LMNA\) Deletions/Duplications \(2004543\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

LMNA-Related Disorders (LMNA) Sequencing

To confirm a clinical diagnosis of a laminopathy

Disease Overview

- Mutations in the *LMNA* gene cause a broad range of clinical diseases collectively termed laminopathies.
- *LMNA*-related disorders include:
 - Hutchinson-Gilford progeria syndrome (HGPS)
 - Emery-Dreifuss muscular dystrophy type 2 (EDMD2)
 - Limb Girdle muscular dystrophy 1B (LGMD1B)
 - Charcot-Marie-Tooth 2B1 (CMT2B1)
 - Familial partial lipodystrophy (FLPD) Dunnigan type
 - Dilated cardiomyopathy (DCM)
 - Mandibulo-acral dysplasia (MAD)
 - Atypical Werner syndrome (WS)
 - Restrictive dermopathy (RD)
- Clinical findings are highly variable.

DISEASE	CLINICAL FEATURES	INHERITANCE
HGPS	Accelerated aging, profound failure to thrive, characteristic facies, alopecia, joint degeneration, growth retardation; average life span is 13 years.	All affected individuals carry a de novo, dominant p.Gly608Gly mutation in <i>LMNA</i> exon 11.
EDMD2	Joint contractures, progressive muscle weakness and wasting, cardiac disease with conduction defects and arrhythmias; age of onset is variable.	Typically autosomal dominant; autosomal recessive cases are very rare.
LGMD1B	Progressive proximal lower limb weakness and atrioventricular cardiac conduction complications.	Autosomal dominant.
CMT2B1	Symmetrical distal muscle weakness and atrophy, depressed or absent tendon reflexes; approximate age of onset is 14 years.	Autosomal recessive; very rare.
FLPD	Post-pubescent progressive loss of subcutaneous fat from the extremities and excess fat accumulation on the face and neck.	Autosomal dominant.
DCM	Progressive ventricular dilation and impaired systolic function leading to congestive heart failure.	Autosomal dominant.
MAD	Post-natal growth retardation, craniofacial and skeletal anomalies, mottled cutaneous pigmentation; symptoms are evident at approximately 4 years of age.	Autosomal dominant.
Atypical WS	Progeroid syndrome with features of partial alopecia, premature aging, short stature, hypogonadism, osteoporosis, premature atherosclerosis, weak voice, cataracts; approximate age of onset is 13 years.	Autosomal dominant.
RD	Skin tightness causes fetal akinesia or hypokinesia deformation sequence; lethal.	All reported cases are de novo, caused by an autosomal dominant mutation.

Incidence

- The birth incidence of HGPS is approximately one in eight million.
- DCM occurs in approximately one in 2,500 births and is familial in 30–60 percent of cases, of which approximately 8 percent are caused by *LMNA* gene mutations.
- The incidence of other *LMNA*-associated disorders is unknown.

Genetics

- Lamin A/C codes for isoforms A and C of the protein lamin, a structural component of the nuclear membrane.
- Type A lamins are encoded by the *LMNA* gene, which is composed of 12 exons and is located at 1q21.2-q21.3.
- Alternative splicing of the *LMNA* gene results in the production of multiple proteins, including lamin A and lamin C, which have been shown to provide mechanical support to the nucleus and anchor heterochromatin to the inner nuclear membrane.
- Mutations occur throughout the gene and are predominantly missense.
- The G608G mutation in exon 11 of the *LMNA* gene is present in all individuals with HGPS.

Indication for Ordering

- To confirm a clinical diagnosis of HGPS, non-X-linked EDMD2, LGMD1B, CMT2B1, FLPD, inherited DCM, MAD, atypical Werner syndrome, or RD.

Contraindication for Ordering

- To determine carrier or affected status in relatives of an individual with a previously identified *LMNA* mutation, order Familial Mutation, Targeted Sequencing (ARUP test #2001961).

Interpretation

- Positive:
 - Detection of a single pathogenic *LMNA* mutation is consistent with diagnosis of an autosomal dominant laminopathy or may indicate carrier status for an autosomal recessive *LMNA*-related disorder.
 - Detection of two pathogenic *LMNA* mutations is consistent with diagnosis of an autosomal recessive laminopathy.
- Negative: Lack of detection of an *LMNA* mutation decreases, but does not exclude, the possibility of a laminopathy.
- Gene variants of uncertain significance may be detected by *LMNA* sequencing.

Methodology

- Bidirectional sequencing of the entire *LMNA* coding region and intron-exon boundaries.
- Analytical sensitivity and specificity are 99 percent.
- Clinical sensitivity is dependant upon the specific *LMNA*-related disorder.

Limitations

- Some regulatory region mutations and all deep intronic mutations of *LMNA* will not be detected.
- Rare diagnostic errors may occur due to primer-site mutations.

LMNA-Related Disorders (LMNA) Sequencing, continued

Related Tests

- LMNA-Related Disorders (LMNA) Deletions/Duplications ([2004539](#))
- Familial Mutation, Targeted Sequencing ([2001961](#))

References

1. Eriksson M, et al. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 2003;423:293–8.
2. Worman HJ, Bonne G. Laminopathies: a wide spectrum of human diseases. *Exp Cell Res* 2007;313(10):2121–33.
3. Genschel J and Schmidt HH-J. Mutations in the LMNA gene encoding lamin A/C. *Human Mutat* 2000;16:451–9.
4. Online Mendelian Inheritance in Man. www.ncbi.nlm.nih.gov (accessed on November 1, 2010).
5. Online GeneTests. www.genetests.org (accessed on November 1, 2010).

For specific collection, transport, and testing information, refer to [LMNA-Related Disorders \(LMNA\) Sequencing \(2004543\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

PAM50 Breast Cancer Intrinsic Classifier

For determining breast cancer subtype and other tumor characteristics that are useful for patient management

Clinical Background

- Breast cancer is the most commonly diagnosed carcinoma among American women and the second-leading cause of cancer-related death.¹
- Approximately 190,000 women in the United States are diagnosed with breast cancer each year, and more than 40,000 die from the disease.
- Early diagnosis and tailored therapies can significantly affect overall survival.
- Immunohistochemistry (IHC) staining for ER, PR, and HER2/*neu* (HER2) is the standard methodology for making a molecular diagnosis and determining treatment.
- Gene-expression profiling of breast cancer can identify different biologic subtypes (i.e., Luminal A, Luminal B, HER2-enriched, and Basal-like) that correspond to differences in patient outcome.^{2,3}
- Efficacy of adjuvant and neo-adjuvant drug regimens has been shown to vary among subtypes.^{2,3}

Indications for Ordering

- The PAM50 Breast Cancer Intrinsic Classifier test is recommended for all patients diagnosed with invasive breast cancer, regardless of stage or ER status.

Pathophysiology

- The distribution of breast cancer subtypes varies in the population:
 - Luminal A: 35–40 percent
 - Luminal B: 25–30 percent
 - HER2-enriched 10–20 percent
 - Basal-like: 10–20 percent
- Luminal A tumors usually have intermediate to high expression of *ESR1* and estrogen-regulated genes and rarely have high *ERBB2* expression.
- Luminal B tumors usually have intermediate to high expression of *ESR1* and estrogen-regulated genes and often have higher proliferation than Luminal A tumors.
- HER2-enriched tumors usually have intermediate to high expression of *ERBB2* and intermediate to low expression of *ESR1* and estrogen-regulated genes. Approximately one-third of tumors subtyped as HER2-enriched are not HER2+ by immunohistochemistry (2+ or 3+ HER2 score) or fluorescence in-situ hybridization (DNA amplified for *ERBB2*).

- Basal-like tumors usually have low expression of *ESR1*, *PGR*, *ERBB2*, and estrogen-regulated genes, but have high proliferation.

Methodology

- RNA is extracted from formalin-fixed, paraffin-embedded tissue and converted to cDNA using both random and gene-specific primers.
- RT-qPCR is then performed on 50 classifier genes and five control genes simultaneously in a plate pre-manufactured at ARUP to determine RNA expression levels.
- Subtype predictions are done using a previously reported centroid-based algorithm.^{2,3}

Interpretation

- The subtype assignment corresponds to the overall tumor biology as characterized by the expression of 50 classifier genes and normalized to five control genes.^{2,3}
- Quantitative gene expression scores and subtype classification should be interpreted together.
- Gene expression scores provide an objective and quantitative method for measuring standard biomarkers used in breast cancer (e.g., *ESR1*/ER, *PGR*/PR, *ERBB2*/HER2).

Limitations

- This test should not be used as the sole means of diagnosis for patient management.
- The test is intended only for invasive breast cancer.
- The specimen must contain at least 75 percent breast cancer.

References

1. Centers for Disease Control and Prevention, National Program of Cancer Registries. <http://apps.nccd.cdc.gov/uscs/> (accessed on November 5, 2010).
2. Parker JS, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol* 2009;27(8):1160–7.
3. Nielsen TO, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor positive breast cancer. *Clin Cancer Res* 2010;16(21):5222–32.

For specific collection, transport, and testing information, refer to [PAM50 Breast Cancer Intrinsic Classifier \(2004700\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

ST2, Soluble

For use as an aid in the risk stratification of patients with heart failure or acute coronary syndromes

Test Highlights

- Soluble ST2 complements the prognostic value of NT-proBNP.
- Soluble ST2 is useful in risk stratification and assessment in patients with known cardiovascular disease.

Pathophysiology

- ST2 is a member of the IL-1 receptor family that is produced by cardiac fibroblasts and cardiomyocytes under conditions of mechanical stress. The ST2 ligand, IL-33, is also secreted by stretched cardiac fibroblasts and has antihypertrophic effects on myocardium.
- Soluble ST2 (sST2) is believed to function as a decoy receptor by binding IL-33 and abrogating the otherwise cardioprotective effect of IL-33 signaling through the cell membrane-bound form of ST2.¹

Clinical Background

- In multiple peer-reviewed publications, sST2 has been shown to be an aid in the risk stratification of patients with heart failure (HF) or acute coronary syndrome (ACS).²⁻⁴
- sST2 assesses a biochemical pathway different than the natriuretic peptides BNP and NT-proBNP. sST2 has been shown to complement the prognostic value of NT-proBNP.³ Patients with elevated concentrations of both biomarkers are at greater risk of mortality than those with an elevation of only one sST2 or NT-proBNP.
- Unlike the natriuretic peptides, sST2 is primarily useful in risk stratification and assessment in patients with known cardiovascular disease and has not been shown to add to the diagnosis of heart failure or other cardiovascular diseases.^{3,5}

Interpretation

- Serum sST2 concentrations greater than 35 ng/mL correspond to increased 30-day and one-year mortality in patients with heart failure³ and/or acute coronary syndromes.⁴ This risk has been shown to increase continuously with rising concentrations of sST2.⁴⁻⁵
- A decrease in sST2 concentrations is associated with a decreased risk of mortality at one year or increased risk in patients who have had heart transplant, myocardial infarction followed by percutaneous coronary intervention, or coronary artery bypass graft.⁶⁻⁸
- Reference intervals for sST2 were determined to be 10.4–52.1 ng/mL for males and 8.4–33.6 for females, respectively.⁹
- The biological variability or reference change value for healthy individuals was 30 percent.¹⁰

Limitations

- This test uses a kit designated by the manufacturer as “for research use, not for clinical use.” The performance characteristics of this test were validated by ARUP Laboratories. The U.S. Food and Drug Administration (FDA) has not approved or cleared this test. The results are not intended to be used as the sole means of clinical diagnosis or patient-management decisions. ARUP is authorized under Clinical Laboratory Improvement Amendments (CLIA) and by all states to perform high-complexity testing.

- Interference from anti-reagent antibodies in the patient sample has not been demonstrated, but the possibility of interference cannot be ruled out.

Methodology and Analytical Performance

- sST2 is measured using a commercially available, highly sensitive enzyme-linked immunosorbent assay.

Related tests

- B-Type Natriuretic Peptide ([0030191](#))
- proBrain Natriuretic Peptide, NT ([0050083](#))

References

1. Seki K, et al. Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. *Circ Heart Fail* 2009;2:684–91.
2. Daniels LB, et al. Association of ST2 levels with cardiac structure and function and mortality in outpatients. *Am Heart J* 2010;160:721–8.
3. Januzzi JL, Jr, et al. Measurement of the interleukin family member ST2 in patients with acute dyspnea: results from the PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study. *J Am Coll Cardiol* 2007;50:607–13.
4. Eggers KM, et al. ST2 and mortality in non-ST-segment elevation acute coronary syndrome. *Am Heart J* 2010;159:788–94.
5. Sabatine MS, et al. Complementary roles for biomarkers of biomechanical strain ST2 and N-terminal prohormone B-type natriuretic peptide in patients with ST-elevation myocardial infarction. *Circulation* 2008;117:1936–44. (Data analysis was completed with earlier version of ST2 assay, so reference values will differ.)
6. Grabowski M, et al. Abstract 3481: Prognostic utility of serial measurements of a novel biomarker ST2 in STEMI patients treated with primary PCI. *Circulation* 2009;120:S808. (Data analysis was completed with earlier version of ST2 assay, so reference values will differ.)
7. Grant MC, et al. Abstract 4778: Postoperative ST2 blood concentrations predict one year mortality in coronary artery bypass patients. *Circulation* 2008;118:S 942. (Data analysis was completed with earlier version of ST2 assay, so reference values will differ.)
8. Pascual-Figal DA, et al. Characteristics of the interleukin family biomarker ST2 after heart transplantation. ESC Congress 2009. (Data analysis completed with earlier version of ST2 assay so reference values will differ.)
9. Lu J, Snider JV, Grenache DG. Establishment of reference intervals for soluble ST2 from a United States population. *Clin Chim Acta* 2010;411:1825–6.
10. Dieplinger B, et al. Analytical and clinical evaluation of a novel high-sensitivity assay for measurement of soluble ST2 in human plasma—the Presage ST2 assay. *Clin Chim Acta* 2009;409(1–2):33–40.

For specific collection, transport, and testing information, refer to [ST2, Soluble \(2002270\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Targeted Drug Screen Based on LC-MS/TOF

For drug screening performed by liquid chromatography coupled with time-of-flight mass spectrometry (LC-MS/TOF)

Test highlights

- Qualitative identification of 53 drugs or drug metabolites.
- Specific identification does not require confirmatory testing unless quantitative results are needed, a second methodology is required, or if results are inconsistent with expectations.
- Quantitative confirmatory testing is ordered separately, if needed.

Clinical Background and indications for Ordering

- Identification of drugs in serum or plasma is clinically useful to verify compliance with prescribed therapy and to identify inappropriate drug use. Serum or plasma is the best specimen for drug testing when the objective of drug testing is to:
 - Verify compliance with prescribed therapy (e.g., to support pain-management clinics), particularly for patients who cannot provide urine.
 - Correlate clinical signs and symptoms with exposure to a particular drug.
 - Verify drug absorption.
 - Evaluate individual pharmacokinetics.

Interpretation

- Positive drug screen:
 - The patient recently took or was otherwise exposed to a drug or a precursor drug (e.g., a prodrug) to the compound identified; the compound(s) reported could be drug metabolite(s).
 - The patient was exposed to more than one drug; the pattern of drugs and drug metabolites is important for predicting the original drug or drugs that a patient may have taken or otherwise been exposed to.
- Negative drug screen:
 - The drug or drugs of interest are not detected by the drug test ordered.
 - The patient did not take the drug or drugs expected.
 - The drug or drugs expected were not absorbed at the time of specimen collection or were eliminated prior to the time of specimen collection.

Limitations

- Identification of drugs or drug metabolites is dependent on time of specimen collection relative to time of drug administration, drug dose, drug formulation, genetic factors, clinical factors, co-administered drugs, and analytical factors.
- The targeted drug screen does not report drug concentrations; confirmatory testing must be ordered separately if drug concentrations are required.
- Timing of drug administration relative to specimen collection or amount of drug administered cannot be determined from a single blood specimen.

Methodology

- Liquid chromatography and time-of-flight mass spectrometry (LC-MS/TOF) is a highly sensitive and specific technology applied recently to qualitative drug screening.
 - Compounds are identified by comparing chromatographic retention time, mass, isotope spacing, and isotope abundance of the suspected positive to expected values.
 - LC-MS/TOF utilizes chromatographic separation combined with high-resolution accurate mass MS/TOF for qualitative compound identification.
 - Mass resolution on the order of 10,000 and accuracy in the milli-Dalton (mDA) range, or ± 0.001 amu, can be achieved.
 - LC-MS/TOF provides drug-screening results that are sufficiently specific, and reflex confirmatory testing is not usually required; LC-MS/TOF testing is therefore "targeted."
 - LC-MS/TOF is more sensitive than many immunoassays, identifying drugs and drug metabolites at lower concentrations for some drug classes.
- The two choices for serum or plasma drug screens at ARUP are compared below:

Traditional Screen (ARUP test #0092420) (immunoassay)		Targeted Screen (ARUP test #2003254) (LC-MS/TOF)	
DRUGS OR DRUG CLASSES DETECTED	CUTOFF (LIMIT OF DETECTION)	DRUG CLASSES, SPECIFIC DRUGS, AND DRUG METABOLITES DETECTED	CUTOFF (LIMIT OF DETECTION)
OPIOIDS			
Opiates	30 ng/mL	Buprenorphine (buprenorphine glucuronide, norbuprenorphine, norbuprenorphine glucuronide); dodeine, dihydrocodeine, fentanyl, norfentanyl, 6-acetylmorphine (heroin metabolite), hydrocodone, hydromorphone, meperidine (normeperidine), methadone (EDDP), morphine, oxycodone, oxymorphone, propoxyphene (norpropoxyphene), tapentadol, tramadol (N-desmethyltramadol, O-desmethyltramadol)	1-25 ng/mL
Oxycodone	30 ng/mL		
Methadone	40 ng/mL		
Propoxyphene	75 ng/mL		
STIMULANTS			
Cocaine	30 ng/mL	Cocaine (benzoylecgonine, cocaethylene, m-hydroxybenzoylecgonine), amphetamine, methamphetamine, MDMA (Ecstasy), MDEA (Eve), MDA	20 ng/mL
Amphetamines	30 ng/mL		
SEDATIVE-HYPNOTICS			
Benzodiazepines	75 ng/mL	Alprazolam (alpha-hydroxyalprazolam), clonazepam (7-aminoclonazepam), diazepam, flurazepam metabolites (desalkylflurazepam, 2-hydroxyethylflurazepam), lorazepam, midazolam, nordiazepam, oxazepam, temazepam, triazolam (alpha-hydroxytriazolam)	25 ng/mL
Barbiturates	75 ng/mL	Amobarbital, butalbital, pentobarbital, phenobarbital, secobarbital	500 ng/mL
Marijuana	30 ng/mL	Marijuana (11-nor-9-carboxy-THC)	60 ng/mL

Targeted Drug Screen Based on LC-MS/TOF, continued

Related tests

- Traditional immunoassay-based drug screen with reflexed confirmation testing (0092420).
- Individual tests designed to support therapeutic drug monitoring applications are available through www.aruplab.com.
- Several urine-based drug-monitoring screens, confirmatory tests, and panels are available through www.aruplab.com.
- For more information about clinical applications of drug testing and specifications of drug-confirmation tests offered through ARUP Laboratories, see <http://www.arupconsult.com/Topics/DrugTesting.html> and <http://www.aruplab.com/Lab-Tests/resources/da-plasma-urine.pdf>

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1. Lynch KL, et al. Performance evaluation of three liquid chromatography mass spectrometry methods for broad spectrum drug screening. *Clin Chim Acta* 2010;411(19-20):1474-81.
2. Gingras M, Laberge MH, Lefebvre M. Evaluation of the usefulness of an oxycodone immunoassay in combination with a traditional opiate immunoassay for the screening of opiates in urine. *J Anal Toxicol* 2010;34(2):78-83.
3. Nielsen MK, et al. Simultaneous screening and quantification of 52 common pharmaceuticals and drugs of abuse in hair using UPLC-TOF-MS. *Forensic Sci Int* 2010;196(1-3):85-92.

For specific collection, transport, and testing information, refer to [Targeted Drug Screen Based on LC-MS/TOF \(2003254\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Voltage-Gated Calcium Channel Autoantibody Testing

For confirmation and monitoring of autoantibodies in patients with Lambert-Eaton myasthenic syndrome (LEMS)

Clinical Background

- Lambert-Eaton myasthenic syndrome is an autoimmune presynaptic disorder characterized by proximal muscle weakness and mild autonomic symptoms (diminished salivary secretion, diminished sweating, and impotence).
- Symptoms result from impaired calcium-dependent release of acetylcholine, which causes insufficient minimum end-plate potential change required for muscle contraction.
- Autoantibodies against P/Q-type voltage-gated calcium channel (VGCC) have been identified in 85 to 90 percent of patients with idiopathic LEMS and in 95 to 100 percent of patients with paraneoplastic LEMS.
- A relatively high association has been reported for P/Q-type VGCC antibodies and cancer, particularly small-cell lung carcinoma. Antigenic mimicry of VGCC antigens by cancer cells has been proposed to account for this association; however, the etiology of idiopathic LEMS remains unclear.
- Autoantibodies that recognize the P/Q-type voltage-gated calcium channel on the presynaptic neuromuscular junction membrane have been identified in the majority of patients with Lambert-Eaton myasthenic syndrome.
- Antibodies to the P/Q-type VGCC have been detected in patients with other paraneoplastic neurologic disorders, in patients with cancer without neurologic symptoms, and 25 percent of patients with amyotrophic lateral sclerosis; thus, these antibodies cannot be considered diagnostic for LEMS.
- Antibodies to N-type voltage-gated calcium channels have also been identified in approximately 75 percent of patients with paraneoplastic LEMS.

Epidemiology

- Incidence and prevalence information for LEMS are limited; the annual incidence is estimated to be approximately 0.5 per million, with a prevalence of around four per thousand in the United States.

Indications for Ordering

- Confirmation of diagnosis and monitoring of patients with Lambert-Eaton myasthenic syndrome.
- Included in the differential diagnosis of other neuromuscular disorders (e.g., myasthenia gravis, neuromyotonia), paraneoplastic syndromes, neurological disorders, and autoimmune neuropathological syndromes.

Interpretation

- Results < 23,000 fmol/L are considered negative.
- Results > 23,000 fmol/L are considered positive.
- The presence of VGCC antibodies should be used in conjunction with clinical findings and should not be used as the sole criteria for diagnosis.

Limitations

- Antibodies detected are isotype-restricted to IgG immunoglobulin.
- Antibodies detected are restricted to the P/Q-type VGCC.

References

1. Lennon VA, et al. Calcium-channel antibodies in the Lambert-Eaton syndrome and other paraneoplastic syndromes. *N Engl J Med* 1995; 332:1467–74.
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3. Harper CM, Lennon VA. Lambert-Eaton syndrome. In *Myasthenia gravis and related disorders*, 2nd ed. Kaminski HJ, ed. 2009; Totowa, NJ: Humana Press, 209–25.

For specific collection, transport, and testing information, refer to [Voltage-Gated Calcium Channel \(VGCC\) Antibody \(0092628\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

X-Chromosome Ultra High-Density Microarray, 954 Genes

To detect loss or gain of DNA on the X chromosome in patients with unexplained intellectual disability, autism, and other X-linked genetic conditions

Test Highlights

- The X-array is a custom-designed, X chromosome-specific, ultra high-density oligonucleotide array containing DNA sequences representing specific regions of the human X chromosome. Patient DNA is hybridized to the chip to:
 - Identify unbalanced chromosomal abnormalities (copy-number changes) involving the X chromosome, which are undetectable by conventional chromosome analysis.
 - Define breakpoints for X-chromosome cytogenetic abnormalities identified by conventional cytogenetic methods.
 - Identify very small, exonic-level, intragenic and intergenic deletions and duplications on the X chromosome that may cause specific, X-linked disease in the individual.
- This array uses 720,000 probes, providing very dense coverage of all 954 identified genes located on the X chromosome. At the exonic level, probes are tiled at 15 base-pair (bp) intervals, providing contiguous coverage of each exon of each gene. Probes are spaced 135 bp apart in intronic and flanking regions of each gene, and there is a backbone of probes spaced 525 bp (0.5 kb) apart across the intragenic regions of the chromosome.
- This test will detect copy-number variations within all X-chromosome genes associated with X-linked mental retardation (XLMR) and autism, as well as small, unique deletions and duplications within and between genes associated with other X-linked disorders that may not be identified by conventional sequencing-based technologies.

Clinical Background

- While the X chromosome contains only 4 percent of all human genes, mutations in these genes account for 10 percent of known Mendelian disorders and 27 percent of intellectual disability.
- Small deletions and duplications have been described as causative mutations in many X-linked disorders.
- Mental retardation:
 - Approximately 25–50 percent of intellectual disability/mental retardation/developmental delay (ID/MR/DD) has a genetic basis.
 - X-linked genetic defects are considered to be important causes of mental retardation, based on the observation that significantly more males than females are affected. Mutations on the X chromosome cause up to 30 percent of all inherited ID.
 - XLMR is a highly heterogeneous condition that can be divided into syndromic XLMR and non-syndromic XLMR depending on the presence or absence of physical abnormalities, dysmorphic features, abnormal laboratory findings, and abnormal brain-imaging studies. Approximately two-thirds of XLMR is considered to be non-syndromic, although the distinction between the two forms is blurred, as variable phenotypes are being described for the same genes.

- Other disorders:
 - The X chromosome contains genes that cause many well-described disorders not involving ID/MR/DD. These disorders fall into several general categories, such as hearing loss, hemophilias, immunodeficiency disorders, metabolic disorders, myopathies, neuromuscular disorders, and skin disorders.

Indications for Ordering

- Screening of individuals for microdeletions and microduplications associated with clinically diagnosed X-linked syndromes/clinical phenotypes.
- Screening of individuals with disorders following an X-linked pattern of inheritance.
- Screening of individuals with ID/MR/DD.
- To further characterize X-chromosome abnormalities, including marker and ring chromosomes, deletions or duplications, unbalanced translocations, or apparently balanced de novo rearrangements involving the X chromosome in patients with abnormal phenotypes.

Additional Ordering Notes

- A clinical indication for testing must be provided. A patient sample, along with the Patient History for X-Chromosome Ultra High-Density Microarray Form, which can be found on www.aruplab.com, should be completed and submitted.

Interpretation

- A positive result means that a pathogenic copy-number change was identified.
- A negative result means that no known pathogenic copy-number change was identified.
- A result of unclear clinical significance means that a copy-number change that cannot currently be categorized as either pathogenic or benign was identified.
- Parental testing may be offered free of charge if such testing will be useful for the interpretation of a finding of unclear clinical significance in the patient. However, if parental testing is clinically indicated, such as for recurrence risk, parental testing is available at an additional cost.

Limitations

- This array will not detect numerical X-chromosome changes, such as Klinefelter, Turner, or triple-X syndromes. This technique will only detect copy-number imbalances within the X chromosome. Balanced rearrangements and base-pair changes will not be detected. Copy-number imbalances for areas of high-sequence similarity may not be detected.
- Genomic imbalances smaller than the resolution of this array, regions of the genome not represented on the array, and mosaicism will not be detected.
- A negative result does not exclude the diagnosis of any of the disorders represented on the microarray.

X-Chromosome Ultra High-Density Microarray, 954 Genes, continued

Methodology

- The technique involves DNA extraction, labeling, hybridization, washing, array scanning, analysis, and interpretation.
- Copy-number changes are calculated based on hybridization signal ratios between patient sample and controls.
- This X-array test is run on the NimbleGen custom-designed comparative genomic hybridization (CGH) array with 720,000 oligonucleotide probes mapped to specific regions of the X chromosome.
- At the exonic level, these 720,000 probes are tiled at 15 bp intervals, providing contiguous coverage of each exon of each gene. Probes are spaced 135 bp apart in intronic and flanking regions of each gene, and there is a backbone of probes spaced 525 bp (0.5 kb) apart across the intragenic regions of the chromosome.
- This technique will detect imbalances that are extremely small by cytogenetic standards. It is designed to detect single-exon copy-number changes for all 954 genes on the X chromosome.

Related Tests

- Conventional cytogenetic analysis (karyotyping) will detect large additions, deletions, and rearrangements, including balanced translocations and inversions involving all areas of the genome (including the X chromosome). Conventional cytogenetics generally cannot detect duplications and deletions smaller than approximately 5 Mb (5,000 kb) in size (the average size of a chromosomal band) or larger changes that do not alter the karyotype-banding pattern.
- Whole genome microarray will detect copy-number changes on all other chromosomes in addition to the X chromosome.
- Other molecular techniques (e.g., gene sequencing or other PCR-based assays) are more sensitive than genomic microarray for detecting many intragenic alterations, such as point mutations and very small deletions or duplications (e.g., a single base pair), but are highly specific and restricted to the genetic site or gene of interest.

References

1. Ropers HH and Hamel BCJ. X-linked mental retardation. *Nat Rev* 2005;6:46–57.
2. Lisik MZ and Sieron AL. X-linked mental retardation. *Med Sci Monit* 2008;14(11):RA221–9.

For specific collection, transport, and testing information, refer to [Chromosome Ultra-High Density Microarray, 954 Genes \(2004434\)](#) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.



AN ENTERPRISE OF THE UNIVERSITY OF UTAH
AND ITS DEPARTMENT OF PATHOLOGY

QUESTIONS OR SUGGESTIONS? Contact ARUP Marketing at (800) 242-2787, extension 3635.

ARUP LABORATORIES
500 Chipeta Way
Salt Lake City, UT 84108-1221
Phone: (800) 522-2787
Fax: (801) 583-2712
www.aruplab.com
www.arupconsult.com

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