

The One

a publication that highlights new tests and announcements from ARUP

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

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Upcoming Trade Shows

NOVEMBER 5-7, 2011

American Association for the Study of Liver Diseases (AASLD); San Francisco, CA

NOVEMBER 17-19, 2011

Association of Molecular Pathology (AMP); Grapevine, TX

DECEMBER 7-9, 2011

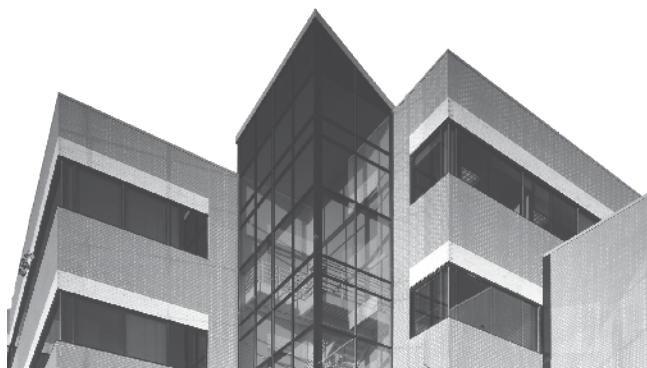
San Antonio Breast Cancer Symposium (SABCS); San Antonio, TX

DECEMBER 12-14, 2011

G-2 Lab Compete; Scottsdale, AZ

FEBRUARY 29-MARCH 2, 2012

American Pain Foundation (APF); San Diego, CA



25TH ANNUAL PARK CITY COMBINED AP/CP PATHOLOGY UPDATE

Jan 30–Feb 3, 2012 | The Canyons Resort, Park City, Utah

Special guest: Brian Rubin, MD, PhD, Cleveland Clinic

For the first time, this conference will combine both anatomic pathology for three days and clinical pathology for two days. This five-day course consists of short lectures and case-oriented discussions lead by distinguished faculty from the University of Utah and guest presenters. Faculty consists of clinicians involved in patient care, clinical and surgical pathologists, and clinical laboratory scientists. Discussion of timely topics by faculty and participants ensures that this course will be informative, interesting, and relevant. Cases are selected to represent common and/or difficult diagnostic problems.

Come enjoy Utah's unparalleled snow and ski terrain while enjoying this educational and informative pathology update.

ACCREDITATION—The University of Utah School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

For more information regarding the update, please contact:

Leita Rogers, (800) 242-2034, leita.rogers@aruplab.com

www.arup.utah.edu/parkcityupdate

GENETICS OF FAMILIAL COLORECTAL CANCER SYNDROMES

Wade Samowitz, PhD; Professor, Department of Pathology, University of Utah School of Medicine; Pathologist, Anatomic Pathology, ARUP Laboratories

There are three main familial colorectal syndromes: familial adenomatous polyposis (FAP), *MYH*-associated polyposis (MAP), and Lynch syndrome. FAP and MAP are characterized by numerous colonic adenomatous polyps. Lynch syndrome (formerly known as hereditary non-polyposis colorectal cancer or HNPCC) is a dominantly inherited syndrome characterized by early onset, usually proximally located colorectal cancer and a variety of extracolonic tumors. It is caused by germline mutations in one of four mismatch repair genes: *MLH1*, *MSH2*, *MSH6*, and *PMS2*.

Learning Objectives:

1. Identify the phenotype and genetic basis for familial colorectal cancer syndromes.
2. Apply the molecular biology of mismatch repair deficiency in the workup of Lynch syndrome.
3. Identify different strategies for Lynch syndrome screening.
4. Identify the phenotype and genetic basis for hamartomatous polyposes.

Visit www.arup.utah.edu and click on Video Lectures to listen to this lecture and earn 1 CEU!

ACOs: WHERE TO BEGIN?

Brian R. Jackson, MD, MS, Medical Director of Medical Informatics, ARUP Laboratories

Dr. Jackson will discuss accountable care organizations (ACO) in relation to healthcare reform during this webinar. He will describe what ACOs are and how they might affect the future of medical laboratories and healthcare throughout the United States. Dr. Jackson's presentation will focus on how to define "value" in a clinical lab context, how to improve that value, and the role of payment reform in driving these changes.

Learning Objectives:

1. Understand how ACOs could view diagnostic processes differently than traditional fee-for-service providers.
2. Understand the potential impact of bundling outpatient lab payments.
3. Envision potential roles for laboratories within ACOs.

Visit www.arup.utah.edu and click on Video Lectures to listen to this lecture and earn 1 P.A.C.E. credit!

Angelman Syndrome: Methylation or *UBE3A* Sequencing

DNA testing to confirm a clinical suspicion/diagnosis of Angelman syndrome

Disease Overview

- Angelman syndrome (AS) is characterized by severe developmental delay or mental retardation, severe speech impairment, gait ataxia and/or tremulousness of the limbs, and unique behaviors with an inappropriately happy demeanor.
- At birth, newborns with AS have normal head circumference and no major birth defects.
- Infants with AS can present with feeding problems and generalized oral-motor incoordination followed by developmental delays at 6–12 months of age.
- Children with AS develop microcephaly by age 2, and seizures and characteristic EEG pattern by age 3.
- Speech impairment is severe, with little to no development of expressive language.
- Most children with AS learn to walk between 30 months and 6 years of age, but 10 percent remain non-ambulatory.
- Unique behaviors may include frequent laughter, happy demeanor, excitability, hand flapping, short attention span, sleep disturbances, and abnormal food-related behaviors.
- Individuals may have dysmorphic features such as flat occiput, occipital groove, wide mouth and protruding tongue, prognathism, and strabismus. Hypopigmented skin, as well as light hair and eye color relative to the family members, may be present.
- Affected adults require assisted living arrangements. Both men and women have normal fertility and near-normal life span.
- There is currently no effective treatment of AS. Management includes antiepileptic drugs to control seizures; orthotic braces or surgery for scoliosis and other orthopedic problems; safe night-time confinement; physical, speech, and occupational therapy; behavioral modifications; individual educational plans; and weight control.

Epidemiology

- Prevalence is approximately one in 15,000, with males and females equally affected.

Genetics

- Angelman syndrome is caused by lack of functional maternal copy of the *UBE3A* gene on chromosome 15q11.2-q 13.
- Ubiquitin protein ligase (*UBE3A*), or E6-associated protein (E6-AP), is an E3 ligase that functions in the E3 complex of the ubiquitin cycle.
- *UBE3A*, which is expressed only from the maternal allele in fetal and adult brain prefrontal cortex neurons, may be regulated through paternally expressed antisense transcript. The *UBE3A* protein controls synaptic function by ubiquitinating and degrading the synaptic protein Arc.

- Disruption of degradation of a number of *UBE3A* substrates is thought to be responsible for the phenotypic effects of AS.
- The etiology of AS is as follows:
 - Maternal deletion involving 15q11.2-q13 (68 percent).
 - Paternal uniparental disomy for chromosome 15 (7 percent).
 - *UBE3A* mutation (11 percent).
 - Imprinting center defect (3 percent).
 - Cytogenetically visible chromosomal translocation (<1 percent).
 - Presently unidentified genetic mechanism (10 percent).
- Determining the molecular mechanism responsible for AS is important for accurate genetic counseling regarding recurrence risk.
- Inheritance varies depending upon the molecular genetic mechanism. *UBE3A* mutations identified by sequencing may be maternally inherited or de novo.
- Offspring of a female carrier of a *UBE3A* sequence mutation are at 50 percent risk for AS.
- A few individuals with AS have been found to have complete or partial *UBE3A* gene deletions.
- Mosaicism for germline *UBE3A* mutations has been reported. As molecular testing cannot exclude maternal germline mosaicism, prenatal testing for the familial *UBE3A* mutation should be offered in subsequent pregnancies to all females who have a child with AS.

Indications for Ordering

- Angelman syndrome by methylation:
 - To establish a diagnosis of AS in individuals with clinical symptoms.
 - DNA methylation analysis identifies approximately 78 percent of individuals with AS and is the most sensitive diagnostic test.
- Angelman syndrome *UBE3A* sequencing:
 - Individuals with clinical symptoms of AS and normal DNA methylation results should undergo *UBE3A* gene mutation analysis.
 - *UBE3A* sequencing identifies approximately 11 percent of individuals with AS.
 - For optimal test interpretation, provide information regarding patient symptoms/manifestations and family history of AS.

Contraindication

- Testing for individuals with a previously identified familial *UBE3A* mutation.
- To test individuals for a specific sequence mutation, it is more cost-effective to order Familial Mutation, Targeted Sequencing (ARUP test code 2001961). A copy of the laboratory report detailing the familial mutation must be provided for targeted sequencing.

Angelman Syndrome: Methylation or *UBE3A* Sequencing, continued

Interpretation

- Angelman syndrome by methylation:
 - Unaffected individuals have a methylated, maternally inherited and an unmethylated, paternally inherited allele detectable by methylation-specific PCR. Absence of the methylated maternal allele is indicative of AS.
 - An abnormal methylation result should be followed by FISH or array CGH to determine if a deletion is present. If a deletion is present, chromosome analysis should be performed to exclude a chromosome rearrangement that may alter recurrence risk.
 - If FISH analysis is normal, DNA polymorphism analysis should be performed to distinguish between paternal UPD and an imprinting defect.
 - If there is no UPD, further DNA studies can determine if an imprinting center deletion is present.
 - Parental testing may be indicated to determine if chromosomal deletions, chromosomal rearrangements, or gene mutations are de novo.
- Angelman syndrome *UBE3A* sequencing:
 - Identification of a known pathogenic *UBE3A* mutation in a symptomatic individual confirms a diagnosis of AS.
 - Lack of an identifiable *UBE3A* mutation in a clinically affected individual reduces but does not rule out AS due to the possibility of an undetectable *UBE3A* mutation or another causative genetic mechanism that was not tested.

Methodology and Limitations

- Angelman syndrome by methylation:
 - Bisulfate conversion and PCR amplification to detect methylation using melting-curve analysis.
 - Other molecular mechanisms resulting in AS will not be assessed.
- Angelman syndrome *UBE3A* sequencing:
 - Bidirectional sequencing of the entire *UBE3A* coding region and intron-exon borders.
 - Analytical sensitivity and specificity of sequencing are 99 percent.
 - Clinical sensitivity of *UBE3A* sequencing is 11 percent for AS syndrome.
 - Rare diagnostic errors can occur due to primer-site mutations.
 - *UBE3A* regulatory region mutations, deep intronic mutations, and large deletion/duplications will not be detected.
 - *UBE3A* variants of unknown clinical significance may be detected by sequencing.

Related Tests

- Chromosome FISH, Metaphase (Angelman Syndrome) (2002299)
- Cytogenomic SNP Microarray (2003414)
- Rett Syndrome (*MECP2*), Full Gene Analysis (0051614)

References

1. Chamberlain SJ, Lalande M. Angelman syndrome, a genomic imprinting disorder of the brain. *J Neurosci* 2010;30:9958–63.
2. GeneTests. <http://www.genetests.org> (accessed on April 4, 2011).
3. Greer PL, et al. The Angelman syndrome protein Ube3A regulates synapse development by ubiquitinating arc. *Cell* 2010;140:704–16.
4. Lossie AC, et al. Distinct phenotype distinguish the molecular classes of Angelman syndrome. *J Med Genet* 2001;38:834–45.
5. Williams CA, Driscoll DJ, Dagli AI. Clinical and genetic aspects of Angelman syndrome. *Genet Med* 2010;12:385–95.

Author

- Tatiana Tvrdik, MS, PhD

For specific collection, transport, and testing information, refer to Angelman Syndrome and Prader-Willi Syndrome by Methylation (2005077) and Angelman Syndrome (*UBE3A*) Sequencing (2005564) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Anti-Endomysial Antibody (EMA) IgG by Indirect Immunofluorescence Antibody (IFA) Assay

Aids in the diagnosis of celiac disease and dermatitis herpetiformis (DH)

Test Highlights

- EMA IgG test by IFA is a semi-quantitative assay for the detection of endomysial antibodies of the IgG class in human serum.

Clinical Background

- Serologic tests for celiac disease (CD) and dermatitis herpetiformis (DH) include endomysial antibodies (EMA), anti-tissue transglutaminase (tTG), and anti-deamidated gliadin peptide (DGP) IgA and IgG assays.
- In individuals with IgA deficiency, the use of IgG assays is recommended for ruling out false-negative results.
- Anti-tTG IgG antibody is currently recommended as a first-line screening serologic test for identifying IgA-deficient individuals who may have CD for biopsy.
- EMA IgG may be of additional diagnostic value in individuals who test negative for anti-tTG IgG.
- EMA IgG testing may also be useful in monitoring adherence to a gluten-free diet (GFD) in IgA-deficient patients with biopsy confirmed CD.

Indications for Ordering

- Clinically suspected CD or DH as a result of the following conditions:
 - Chronic diarrhea without infectious etiology.
 - Family history of celiac disease.
 - Early-onset osteoporosis.
 - Autoimmune disease associated with celiac disease (diabetes mellitus type 1, autoimmune thyroiditis, etc.).
 - Non-autoimmune conditions associated with celiac disease (Down syndrome, Turner syndrome, etc.).
 - Chronic pruritic dermatitis/skin lesions in patient with or without known celiac disease.
- Patient has IgA deficiency or presentation is atypical.

Interpretation

- A positive result alone is not diagnostic; biopsy is recommended for a diagnosis of CD.
- EMA IgG titers may not reliably predict adherence to GFD.
- For diagnosis of DH, a positive result should be followed by a perilesional skin biopsy for DIF.
- Results should be interpreted alongside pemphigoid and pemphigus panel tests or epithelial skin antibody tests to differentiate DH from other immunobullous skin diseases.

Limitations

- In some cases, EMA-positive sera may show the prozone phenomenon, in which antibodies are either very weak or negative at the initial screening dilution. If this occurs, screen sera at higher dilutions.
- Some patients' sera contain anti-smooth muscle antibodies (ASMA), which are reactive with the tissue and interfere with the detection of EMA IgG. In such cases, sera should be further tested at higher dilutions.
- EMA IgG by IFA may be negative in early disease or in patients on a gluten-free diet.
- Test results should be evaluated along with the patient's total clinical history for diagnosis.

Methodology

- The presence of EMA IgG in serum is detected by IFA using monkey esophageal tissue.

Related Tests

- A positive EMA IgG test should be followed by biopsy to confirm diagnosis.

References

1. Craig D, Robins G, Howdle PD. Advances in celiac disease. *Curr Opin Gastroenterol* 2007;23(2):142–8.
2. McGowan KE, Lyon ME, Butzner JD. Celiac disease and IgA deficiency: complications of serological testing approaches encountered in the clinic. *Clin Chem* 2008;54(7):1203–9.
3. Plebani M, Basso D. Diagnostic testing for celiac disease. *JAMA* 2010;304(6):639–40.
4. Kumar V, et al. Tissue transglutaminase and endomysial antibodies—diagnostic markers of gluten-sensitive enteropathy in dermatitis herpetiformis. *Clin Immunol* 2001;98(3):378–82.

Authors

- Anne Tebo, PhD
- Brenda Suh-Lailam, PhD

For specific collection, transport, and testing information, refer to Endomysial Antibody, IgG (2005501) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

BCR-ABL1 T315I Mutation

Detects and quantitates T315I-positive *BCR-ABL1* transcripts in chronic myelogenous leukemia

Clinical Background

- Chronic myelogenous leukemia (CML) is characterized by the *BCR-ABL1* fusion oncogene. Patients with CML typically respond very favorably to treatment with tyrosine-kinase inhibitors (TKIs) such as imatinib.¹ However, a subset of patients will eventually acquire secondary *ABL1* kinase-domain mutations that promote TKI resistance.²
- A subset of patients who develop TKI resistance do so because of the presence of the T315I point mutation in the *ABL1* kinase domain.³
- Detection and quantitation of the T315I point mutation is clinically useful in managing those patients who fail current front-line CML therapies.

Indications for Ordering

- The *BCR-ABL1* T315I mutation has been associated with resistance to TKIs used to treat CML patients. Patients with a T315I mutation may benefit from alternative therapies.

Interpretation

- Not detected: The T315I mutation was not detected.
- Detected: The T315I mutation was detected (percent of mutant allele).

Limitations

- Results of this test must always be interpreted in the context of morphologic and other relevant data, and should not be used alone for a diagnosis of malignancy.
- Samples that do not demonstrate the T315I mutation by this test may still have the mutation but in quantities below the test's limit of detection.
- This test may not accurately detect mutations if present in fewer than 5 percent of CML cells.

Methodology

- Total RNA is extracted, reverse-transcribed into cDNA, and amplified across the *BCR-ABL1* fusion breakpoint.
- Amplicons are subjected to a second PCR reaction targeting *ABL* exon 6 and subsequently pyrosequenced. The percent of mutant allele is reported.

References

1. Druker BJ, et al. Efficacy and safety of a specific inhibitor of the *BCR-ABL* tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 2001;344:1031–7.
2. Jabbour E, et al. Long-term outcome of patients with chronic myeloid leukemia treated with second-generation tyrosine kinase inhibitors after imatinib failure is predicted by the in vitro sensitivity of *BCR-ABL* kinase domain mutations. *Blood* 2009;114:2037–43.
3. Jabbour E, et al. Characteristics and outcomes of patients with chronic myeloid leukemia and T315I mutation following failure of imatinib mesylate therapy. *Blood* 2008;112:53–5.

Authors

- Todd Kelley, MD
- Jonathan Schumacher, MS

For specific collection, transport, and testing information, refer to *BCR-ABL1*, T315I Mutation Detection, Quantitative (2004924) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Ehlers-Danlos Syndrome Kyphoscoliotic Form, Type VI (*PLOD1*) Sequencing and Deletion/Duplication

To confirm a suspected diagnosis of Ehlers-Danlos Syndrome VI or determine carrier status

Disease Overview

- Ehlers-Danlos syndrome kyphoscoliotic form, also known as type VI (EDS VI or VIA), is a connective tissue disorder characterized by kyphoscoliosis at birth or within the first year of life, severe neonatal hypotonia, thin hyperextensible and bruisable skin, atrophic scarring, joint hypermobility, and scleral fragility leading to increased risk for rupture of the globe. There is an increased risk for rupture of medium size arteries, and individuals with severe kyphoscoliosis are at increased risk for respiratory compromise.
- EDS VI is caused by deficiency of lysyl hydroxylase, an enzyme important in the formation of collagen cross-links.
- EDS VI can be diagnosed in the following ways:
 - Increased ratio of deoxypyridinoline to pyridinoline crosslinks (Dpyr:Pyr) detected in urine.
 - Decreased lysyl hydroxylase activity; less than 25 percent of normal in fibroblasts.
 - Identification of two pathogenic procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (*PLOD1*) gene mutations.
- EDS kyphoscoliotic form is sometimes described as EDS VIA to differentiate it from EDS VIB, a rare condition with similar clinical features but normal lysyl hydroxylase activity.
- Management of individuals with EDS VI involves regular follow-up with several specialties, including orthopedic surgery, physical therapy, cardiology, and ophthalmology. Appropriate management helps prevent or minimize serious disease complications.

Epidemiology

- Incidence is approximately one in 100,000; carrier frequency is estimated at one in 150.

Genetics

- Autosomal recessive inheritance with variable expressivity.
- The *PLOD1* gene is the only gene associated with lysyl hydroxylase deficiency or EDS VIA.
- A common 8.3kb gene duplication, located between introns 9 and 16 (exons 10–16), is responsible for approximately 20 percent of pathogenic mutations.

Indications for Ordering

- To confirm causative mutations in a symptomatic individual with an increased Dpyr:Pyr ratio.
- To determine carrier status of at-risk family members when the familial mutation is unknown.

Contraindications for Ordering

- Prenatal testing.
- The biochemical screen (ARUP test code #0080351) should be ordered as a first-line test to determine whether an individual may be affected with EDS VI.

Additional Ordering Notes

- If there is a family history of EDS VI and the specific familial mutation(s) has already been identified, testing can be performed on at-risk family members by contacting ARUP's genetic counselor and requesting targeted sequencing for the familial mutation(s).

Interpretation

- The detection of two pathogenic *PLOD1* mutations on opposite chromosomes predicts EDS VI.
- When one or no *PLOD1* mutations are detected in a clinically affected individual, the patient may have *PLOD1* mutation(s) undetectable by this assay. Thus, medical management should rely on clinical and biochemical findings.
- Sequencing may detect *PLOD1* mutations of unknown clinical significance.

Methodology

- PCR followed by bidirectional sequencing of the entire coding region and intron-exon boundaries of the *PLOD1* gene.
- Multiplex ligation-dependent probe amplification (MLPA) to detect large *PLOD1* coding region deletions/duplications, including the common 8.3kb duplication of exons 10–16.
- Clinical sensitivity is unknown.
- Analytical sensitivity and specificity are 99 percent.

Limitations

- Rare diagnostic errors may occur due to primer- or probe-site mutations.
- Regulatory region mutations and deep intronic mutations will not be detected.
- Large deletions/duplications of exon 9 will not be detected. Large deletions/duplications of exons 1, 3, 5, and 19 may or may not be detected based on the breakpoints of the rearrangement.
- The breakpoints of large deletions/duplications will not be determined.

Related Test

- Ehlers-Danlos Syndrome Type VI Screen—Biochemical (HPLC) (0080351) test to determine whether an individual may be affected; carrier status cannot be determined with this assay.

References

1. GeneTests: Ehlers-Danlos syndrome kyphoscoliotic form. <http://www.genetests.org> (accessed on June 1, 2011).
2. Yeowell HN, Walker LC. Mutations in the lysyl hydroxylase 1 gene that result in enzyme deficiency and the clinical phenotype of Ehlers-Danlos syndrome type VI. *Mol Genet Metab* 2000;71(1–2):212–24.
3. Walker LC, et al. Heterogeneous basis of the type VIB form of Ehlers-Danlos syndrome (EDS VIB) that is unrelated to decreased collagen lysyl hydroxylation. *Am J Med Genet* 2004;131(2):155–62.
4. Heikkinen J. Duplication of seven exons in the lysyl hydroxylase gene is associated with longer forms of a repetitive sequence within the gene and is a common cause for the type VI variant of Ehlers-Danlos syndrome. *Am J Hum Genet* 1997;60:48–56.

Authors

- Pinar Bayrak-Toydemir, MD, PhD
- Marzia Pasquali, PhD
- Amanda Openshaw, MS, LCGC

For specific collection, transport, and testing information, refer to Ehlers-Danlos Syndrome, Type VI (*PLOD1*) Sequencing and Deletion/Duplication (2005559) and Ehlers-Danlos Syndrome, Type VI (*PLOD1*) Deletion/Duplication (2005555) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Hereditary Persistence of Fetal Hemoglobin (HPFH), 8 Mutations

Detects 8 common beta globin gene cluster deletions resulting in HPFH

Disease Overview

- Hemoglobin (Hb) is a tetrameric molecule that reversibly binds oxygen in red blood cells. It consists of two proteins expressed from the alpha globin gene cluster and two from the beta globin cluster. The expression of genes within these clusters is developmentally regulated and results in production of embryonic, fetal, and adult hemoglobin forms.
- By 6 months of age, a shift from gamma globin to beta globin (*HBB*) gene expression occurs, reducing the amount of fetal hemoglobin (Hb F; $\alpha_2\gamma_2$) produced so that the major form of hemoglobin present is Hb A ($\alpha_2\beta_2$).
- Although residual amounts of Hb F are produced throughout life, the majority of healthy adults have less than 1 percent Hb F.
- Hereditary persistence of fetal Hb (HPFH) results from mutations within the beta globin gene cluster that alter normal hemoglobin switching.
- Delta/beta thalassemia and HPFH are inherited conditions characterized by increased Hb F production; they are distinguished using hematologic and molecular analyses. Delta/beta thalassemia and some forms of HPFH are caused by deletions within the beta globin gene cluster.
 - Heterozygotes for delta/beta thalassemia deletions have moderate elevation of Hb F (5–20 percent) and hypochromic, microcytic anemia. Non-equal distribution of Hb F among red blood cells is observed (heterocellular HPFH).
 - Heterozygotes for HPFH-associated deletions typically have high levels of Hb F (up to 30 percent) with normal red blood cell indices. Equal distribution of Hb F among red blood cells is observed (pancellular HPFH).
- Elevation of Hb F in adults can occur due to acquired conditions (e.g., pregnancy, anemias, or leukemias).
- Elevated Hb F has no clinical significance in healthy individuals; however, HPFH can be beneficial in patients with sickle cell disease or beta thalassemia, as increased Hb F leads to milder phenotypes.
- HPFH has traditionally been diagnosed hematologically by the percentage of Hb F present and the distribution among red blood cells, but molecular diagnosis is most definitive.

Epidemiology

- Eight deletions of varying size involving the beta globin gene cluster have been reported to result in pancellular HPFH.
 - HPFH-1 (African): described in Africans and African-Americans
 - HPFH-2 (Ghanaian): described in Africans and African-Americans
 - HPFH-3 (Asian Indian)
 - HPFH-4 (Southern Italian)
 - HPFH-5 (Italian)
 - HPFH-6 (Vietnamese)
 - HPFH-7 (Kenyan)
 - SEA-HPFH (Southeast Asian): identified in Cambodian, Vietnamese, and Chinese populations

- The incidence of HPFH in the general population is unknown but is more frequent in the populations described above. Together, HPFH-1 and HPFH-2 are found in ~0.1 percent of African-Americans in the southeastern United States.

Genetics

- Two different molecular mechanisms can result in HPFH:
 - Deletional: HPFH results from specific large deletions in the beta globin gene cluster involving *HBB*.
 - Non-deletional: HPFH is caused by point mutations in the promoters of the gamma globin genes (*HBG1* and *HBG2*).
- The presence of an HPFH deletion may complicate the diagnosis of sickle cell disease or beta thalassemia, especially in infancy when the major form of Hb present is Hb F.
- The presence of an HPFH deletion can also mask beta thalassemia trait by ameliorating the hematological findings typically present.
- Other genetic modifiers of Hb F levels have been identified.

Indications for Ordering

- Diagnostic testing in individuals with elevated levels of Hb F (relative to the individual's age).
- Carrier testing for individuals with a family history consistent with HPFH.
- For optimal test interpretation, please submit a Hemoglobinopathy/Thalassemia Patient History Form detailing clinical findings, family history, and ethnicity.

Interpretation

- Negative: If none of the eight common deletions associated with HPFH was identified, HPFH has not been excluded, as point mutations, rare HPFH deletions, and delta/beta thalassemia deletions are not identified by this assay.
- Heterozygous: When one copy of a deletion associated with HPFH is identified, this predicts the persistent elevation of Hb F in all erythrocytes.
- Homozygous or compound heterozygous: If two deletions associated with HPFH are identified, individuals typically have Hb F levels approaching 100 percent and mild erythrocytosis.

Methodology

- Multiplex PCR and gel electrophoresis to detect eight common deletions associated with HPFH:
 - HPFH-1 (g.5174452_5259368del84917)
 - HPFH-2 (g.5180404_5263982del83579)
 - HPFH-3 (g.5215683_5265453del49771)
 - HPFH-4 (g.5217940_5260078del42139)
 - HPFH-5 (g.5246023_5258951del12929)
 - HPFH-6 (g.5193975_5273259del79278)
 - HPFH-7 (g.5247860_5270651del22792)
 - SEA-HPFH (g.5222878_5250288del27411)

Hereditary Persistence of Fetal Hemoglobin (HPFH), 8 Mutations, continued

- Clinical sensitivity and specificity are unknown.
- Analytical sensitivity and specificity for the mutations tested is over 95 percent.

Limitations

- Only the eight targeted deletions associated with HPFH will be detected. Point mutations or rare deletions that cause HPFH or delta/beta thalassemia will not be identified.
- Other genetic modifiers of Hb F levels will not be assessed.
- This test is unable to differentiate homozygosity for an HPFH deletion from compound heterozygosity for an HPFH deletion and a rare globin gene cluster 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

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For specific collection, transport, and testing information, refer to Hereditary Persistence of Fetal Hemoglobin (HPFH), 8 Mutations (0040227) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

Marfan Syndrome (*FBN1*) Sequencing and Deletion/Duplication

To confirm a clinical suspicion/diagnosis of Marfan syndrome

Disease Overview

- Marfan syndrome (MFS) is a systemic connective tissue disorder characterized by a variety of clinical manifestations, including ocular, skeletal, and cardiovascular findings.
- According to a 2010 revised Ghent nosology for Marfan syndrome (MFS), to confirm a clinical diagnosis of MFS in an individual without family history of MFS one must meet any of the following criteria:
 - Aortic root dilatation or dissection and ectopia lentis.
 - Aortic root dilatation or dissection and a pathogenic *FBN1* mutation.
 - Aortic root dilatation or dissection and at least seven points scored for the following systemic findings:
 - Wrist and thumb sign (three points)
 - Wrist or thumb sign (one point)
 - Pectus carinatum (two points) or excavatum (one point)
 - Hindfoot deformity (two points)
 - Pneumothorax (two points)
 - Dural ectasia (two points)
 - Acetabular protrusion (two points)
 - Scoliosis or thoracolumbar kyphosis (one point)
 - Reduced upper/lower segment ratio and increased arm/height ratio in persons with no severe scoliosis (one point)
 - Reduced elbow extension (one point)
 - Skin striae (one point)
 - Myopia (one point)
 - Mitral valve prolapse (one point)
 - Characteristic facial features (one point).
 - Ectopia lentis and an *FBN1* mutation previously reported to be associated with cardiovascular disease.
 - Diagnosis of Shprintzen-Goldberg syndrome (SGS), Loeys-Dietz syndrome (LDS,) and Ehlers-Danlos syndrome IV vascular type (vEDS) has been excluded.
- A clinical diagnosis of MFS in an individual with a family history of MFS and with an excluded diagnosis of SGS, LDS, and vEDS, is based on the presence of any of the following:
 - Ectopia lentis.
 - Systemic findings scoring seven points or higher.
 - Aortic root dilatation or dissection.
- MFS is characterized by high clinical variability and age-dependent penetrance.
- The size of an affected individual's aortic root should determine echocardiogram surveillance frequency and the timing of possible prophylactic surgery. Assessment of the aortic root in children should be performed at least annually.
- Annual ophthalmology exams are essential to detect ectopia lentis, cataract, glaucoma, and retinal detachment. Skeletal manifestations such as scoliosis and pectus deformity should be followed by orthopedic specialists.
- Individuals with MFS should avoid contact sports, exercise to exhaustion, and isometric activities.
- Pregnancy increases risk for aortic root dilatation and dissection, especially when initial aortic root diameter is greater than 4.0 cm at the onset of pregnancy.
- The average life expectancy of properly managed individuals with MFS is similar to the general population.
- *FBN1* mutations are also associated with type I fibrillinopathy, conditions with an increased risk of aortic dilatation/dissection related to MFS, ranging from early lethal disease in neonatal MFS to isolated features of MFS and near-normal phenotype. These conditions include:
 - Mitral valve prolapse syndrome (MVPS) (mitral valve prolapse, pectus excavatum, scoliosis, and mild arachnodactyly).
 - Autosomal dominant familial ectopia lentis (bilateral ectopia lentis and sometimes scoliosis).
 - MASS syndrome (myopia, mitral valve prolapse, borderline aortic enlargement, and skin and skeletal features of MFS).
 - Weill-Marchesani syndrome 2 (ectopia lentis, brachydactyly, joint stiffness, and short stature).
 - Shprintzen-Goldberg syndrome (craniosynostosis, arachnodactyly, brachycephaly, pectus deformities, scoliosis, mental retardation, and, more rarely, aortic root dilatation).
- *FBN1* mutations have also been detected in patients with MFS phenotype and a severe congenital lipodystrophy with a neonatal progeroid-like appearance.
- Neonatal MFS is typically diagnosed within 3 months of life with symptoms of atrioventricular valve dysfunction, pulmonary emphysema, joint contractures, crumpled ears and loose skin, and death within the first 2 years of life.
- Regular cardiologic follow-up is advised for all patients diagnosed with ectopia lentis syndrome, MASS syndrome, and MVPS, as the incidence of aortic dilatation increases with age.

Epidemiology

- Prevalence of MFS is one in 5,000 to one in 10,000.

Genetics

- Autosomal dominant inheritance; 25 percent of cases are de novo.
- Rarely, somatic or germline mosaicism has been reported.
- Fibrillin 1 gene, *FBN1*, located on chromosome 15q21.1, is the only gene known to be associated with MFS.
- Fibrillin 1 glycoprotein and other extracellular proteins form microfibrils that contribute to elastic fibers; support the eye lens, nerves, and muscles; and play a role in transforming growth factor-beta (TGF- β) regulation.
- Reduced or abnormal *FBN1* leads to disturbed microfibril structure, increased TGF- β signaling, and MFS pathogenesis.
- The pathogenicity of *FBN1* mutations is complex and involves dominant negative effects as well as haploinsufficiency.

Marfan Syndrome (*FBN1*) Sequencing and Deletion/Duplication, continued

- The revised Ghent nosology has defined causal *FBN1* mutations as follows:
 - Mutations previously shown to segregate with disease in MFS families.
 - De novo mutations that are nonsense, in- or out-of frame deletions/insertions, splice-site mutations, missense mutations involving cysteine residues or affecting epidermal growth factor-like (EGF) consensus sequence, and missense mutations absent in 400 ethnically matched controls.
- Few phenotype-genotype correlations have been found.
- Patients diagnosed with neonatal MFS carry *FBN1* mutations located predominantly within exons 24–32; mutations identified in this region in patients of all ages are generally associated with severe prognosis.
- Exon 64 frameshift *FBN1* mutations have been reported in three patients with Marfan phenotype, generalized lipodystrophy, and progeroid facial appearance.
- Large genomic deletions of regulatory elements have been reported in individuals with MFS or MFS spectrum disorders, including MASS phenotype.

Indication for Ordering

- To confirm a diagnosis of MFS.
- To determine the specific *FBN1* mutation in a known affected individual.
- To determine disease status in children or other at-risk family members of affected relatives.

Contraindication

- Testing for individuals with a previously identified familial *FBN1* mutation. To test individuals for a specific *FBN1* mutation, it is more cost-effective to order Familial Mutation, Targeted Sequencing (ARUP test code 2001961) and provide a copy of the laboratory report detailing the familial mutation.
- Prenatal testing for an unknown *FBN1* mutation. Pathogenic *FBN1* mutation of an affected family member must be identified before prenatal testing can be performed.

Interpretation

- Identification of a known pathogenic *FBN1* mutation in a symptomatic individual predicts the presence of MFS or an *FBN1*-related disorder. Clinical phenotypes may vary.
- Lack of an identifiable *FBN1* mutation in a clinically affected individual decreases, but does not exclude, a diagnosis of MFS. Medical management should rely on clinical findings and family history.

- *FBN1* sequence variants of unknown clinical significance may be detected by sequencing.

Methodology

- PCR and bidirectional sequencing of the *FBN1* coding regions and intron-exon boundaries.
- Multiplex ligation-dependent probe amplification (MLPA) for large deletions/duplication analysis of the *FBN1* gene.
- Analytical sensitivity and specificity of sequencing and MLPA are 99 percent. Clinical sensitivity is dependent on the accuracy of the clinical diagnosis and ranges between 70 and 93 percent for sequencing and is unknown for MLPA.

Limitations

- Deep intronic mutations and some regulatory region mutations are not detected.
- Large deletions/duplications of exons 11, 12, 21, 23, 28, 33, 38, 40, 49, 52, 60, and 62 will not be detected.
- Breakpoints of large *FBN1* locus and intragenic deletions/duplications will not be determined.
- Rare diagnostic errors may occur due to primer- or probe-site mutations.
- Mutations in genes other than *FBN1* are not evaluated.

Related Tests

- Marfan Syndrome, *FBN1* Sequencing (2005589)
- Marfan Syndrome, *FBN1* Deletion/Duplication (2005580)
- Familial Mutation, Targeted Sequencing (2001961)

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For specific collection, transport, and testing information, refer to Marfan Syndrome, *FBN1* Sequencing and Deletion/Duplication (2005584) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

MPL W515 Quantitative Mutation Analysis

Detects and quantitates *MPL* codon 515 mutations in primary myelofibrosis (PMF) and essential thrombocythemia (ET)

Clinical Background

- *BCR-ABL1*-negative chronic myeloproliferative neoplasms include polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF).
- More than 50 percent of ET and PMF patients harbor the *JAK2* V617F activating mutation; a subset of ET cases (5–10 percent) have an activating mutation in codon 515 of the thrombopoietin receptor, *MPL*.
- Mutations in exon 10 of the myeloproliferative leukemia virus oncogene (*MPL*) are present in approximately 5 percent of patients with PMF and ET.¹
- *MPL* encodes a transmembrane receptor tyrosine kinase that acts as a receptor for thrombopoietin (TPO), a glycoprotein cytokine responsible for thrombopoiesis. The most common *MPL* mutations are the W515K and W515L mutations, which have been shown to activate the *JAK/STAT* signaling pathway in the absence of TPO and contribute to the oncogenic phenotype.² The vast majority of patients with *MPL* mutations test negative for the *JAK2* V617F mutation yet possess a phenotype consistent with a myeloproliferative neoplasm.³
- Detection and quantitation of *MPL* mutations can be used in the diagnosis and monitoring of myeloproliferative neoplasms and are suggestive of either PMF or ET in a subset of *JAK2*-unmutated patients.

Indications for Ordering

- Detection of *MPL* mutations in *JAK2*-negative patients with myeloproliferative neoplasms.
- This assay can also be used to monitor for response or clonal expansion in *MPL*-mutated tumors following treatment.

Interpretation

- Not detected: A W515 mutation was not detected.
- Detected: A W515 mutation was detected (percent of mutant allele).

Limitations

- Results of this test must always be interpreted in the context of morphologic and other relevant data, and should not be used alone for a diagnosis of malignancy.
- Samples that do not demonstrate W515 mutations by this test may still have a mutation, but in quantities below the test's limit of detection.
- This test may not accurately detect mutations if present in fewer than 5 percent of granulocytes.

Methodology

- Genomic DNA is purified from enriched granulocytes and subjected to PCR amplification of *MPL* codon 515 followed by pyrosequencing analysis.
- The ratio of wild-type to W515-mutated alleles is reported.

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For specific collection, transport, and testing information, refer to *MPL* codon 515 Mutation Detection by Pyrosequencing, Quantitative (2005545) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

von Willebrand Disease, Type 2 (VWF) Sequencing Selected Exons or Platelet-Type von Willebrand Disease (GP1BA), 4 Mutations

To confirm a diagnosis of von Willebrand disease (VWD), types 2A, 2B, 2M, or 2N, or platelet-type VWD

Disease Overview

- von Willebrand factor (VWF) is a large multimeric glycoprotein that plays a critical role in primary hemostasis.
- The main functions of VWF are binding factor VIII to protect it from premature proteolytic degradation, binding sub-endothelial collagen at the site of vascular damage, causing platelet recruitment via the platelet *GP1BA* receptor, and facilitating clot formation.
- Common symptoms of von Willebrand disease (VWD) include mucocutaneous bleeding after brushing or flossing teeth, unexplained bruising, prolonged repeated nosebleeds, menorrhagia, and prolonged bleeding following childbirth, trauma, or surgery.
- Initial evaluation for VWD includes a panel of tests to evaluate VWF antigen (VWF:Ag) and activity (ristocetin cofactor activity: VWF:RCo), as well as factor VIII activity. Additional tests may also be necessary for diagnosis and subtyping.
- Treatment for VWD is best achieved at a comprehensive bleeding-disorder program. Two common treatments include desmopressin, which releases stored VWF, and clotting factor concentrates containing both VWF and factor VIII. In patients who are intolerant or do not respond to desmopressin, clotting factor concentrate is required. Patients often benefit from fibrinolytic inhibitors (to treat or prevent bleeding episodes) and hormonal treatments (to decrease menorrhagia).
- VWD is subclassified by whether disease is caused by a decreased amount of VWF or the presence of structurally or functionally abnormal VWF.
 - Type 1, caused by a partial deficiency of VWF, accounts for 70 percent of cases and is associated with mild mucocutaneous bleeding.
 - Type 2, caused by structurally or functionally abnormal VWF, accounts for 25 percent of cases, and its clinical presentation is highly variable.
 - Type 3, caused by complete absence of VWF, accounts for <5 percent of cases and is associated with severe mucocutaneous and musculoskeletal bleeding.
- VWD type 2 is further subdivided into 2A, 2B, 2M, and 2N subtypes. It is useful to distinguish these subtypes, as therapeutic recommendations vary among the groups. Subtype frequency in the Caucasian population is 2A>2N>2M>2B.
 - 2A causes mild to moderate mucocutaneous bleeding and is variably responsive to desmopressin.
 - 2B causes mild to moderate mucocutaneous bleeding. Thrombocytopenia may be present due to the enhanced ability of VWF to bind platelet receptor *GP1BA*, causing removal of the platelet/VWF complex. Symptoms may worsen with severe infection, surgery, pregnancy, or desmopressin treatment.
 - 2M causes mild to moderate mucocutaneous bleeding and rarely responds to desmopressin.
 - 2N causes symptoms similar to mild hemophilia A, as both disorders result from reduced factor VIII activity; mutations affect the ability of VWF to bind and protect factor VIII. Desmopressin only treats minor bleeding; severe bleeding requires concentrate containing both VWF and factor VIII.

- Platelet type VWD (PT-VWD or pseudo-VWD) is caused by *GP1BA* mutations and is not considered a type of VWD. Clinical presentation is often indistinguishable from VWD type 2B, but VWD type 2B is caused by mutations in *VWF*.

Prevalence

- VWD affects one in 100 to one in 1,000 individuals.

Genetics

- *VWF* is the only gene known to cause VWD, but other conditions may have an indistinguishable phenotype.
 - PT-VWD is phenotypically indistinguishable from type 2B but is caused by *GP1BA* mutations. In fact, both PT-VWD and VWD type 2B are caused by gain-of-function mutations that lead to enhanced binding between plasma VWF and *GP1BA*.
- Both autosomal dominant and recessive forms of VWD exist.
 - Autosomal dominant: all cases of types 2B and 2M and most cases of types 1 and 2A.
 - Autosomal recessive: all cases of type 2N and 3; occasional cases of type 1 and 2A.
 - Most affected individuals carrying a dominant mutation also have an affected parent; the percentage of cases that occur due to de novo mutations is unknown.
 - Typically, dominant mutations are incompletely penetrant when VWF:Ag and VWF:RCo levels are 25–50 IU/dL. Full penetrance can be expected from dominant mutations when VWF:Ag and VWF:RCo levels are <25 IU/dL.
- 80 percent of *VWF* mutations causing autosomal dominant VWD type 2A, 2B, and 2M are located in exon 28; up to 20 percent of mutations causing autosomal recessive type 2A can be detected by sequencing *VWF* exons 11, 12, 14, 15, 16, 24, 25, 51, and 52.
- Although mutations causing VWD type 2N have been identified in *VWF* exons 4, 9, 17, 18, 19, 20, 21, 24, 25, and 27, the sensitivity of such targeted sequencing is unknown.
- Assays for large gene deletions/duplications in *VWF* are not clinically available; furthermore, the percentage of VWD caused by deletions/duplications is unknown.
- Four different mutations in *GP1BA* are responsible for the majority of PT-VWD.

Indications for Ordering

- To confirm a phenotypic diagnosis of VWD type 2A, 2B, 2M, 2N, or PT-VWD.
- To distinguish VWD type 2B from PT-VWD.
- To distinguish VWD type 2N from mild hemophilia A.
- Testing for family members of individuals with known mutations.

Additional Ordering Notes

- If there is a known family history of VWD, please provide the patient's symptoms, the relationship between the patient and the affected family member(s), and the specific *VWF* mutation, if known.
- For familial mutation testing, please order Familial Mutation, Targeted Sequencing (ARUP test code 2001961) and provide a copy of the affected relative's test result detailing the specific mutation identified.

von Willebrand Disease, Type 2 (VWF) Sequencing Selected Exons or Platelet-Type von Willebrand Disease (GP1BA), 4 Mutations, continued

Contraindications

- Testing asymptomatic individuals who have affected relatives without a known pathogenic VWF mutation.
- Targeted testing for a familial VWF mutation in any exon other than 4, 9, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 24, 25, 27, 28, 30, 31, 51, and 52.

Interpretation

- The VWD subtype and inheritance pattern will be provided for pathogenic mutations identified, if known.
- Heterozygotes for known dominant pathogenic VWF mutations are at risk for symptoms of VWD. Since reduced penetrance is observed in VWD, individuals may or may not be symptomatic.
- Heterozygotes for known recessive pathogenic VWF mutations are predicted to be carriers of VWD. Approximately 10 percent of carriers may develop mild symptoms of VWD.
- Compound heterozygotes for known recessive pathogenic mutations are predicted to be affected with VWD.
- If no mutations are detected in an individual with a phenotype consistent with VWD type 2B, consideration should be given to GP1BA sequencing
- If no mutations are detected in VWF exons 4, 9, 17, 18, 19, 20, 21, 24, 25, and 27 in an individual with a phenotype consistent with VWD type 2N, factor VIII gene sequencing is recommended.
- If no mutations are identified, this does not eliminate the possibility of VWD as undetected pathogenic mutation(s) may be present in one of the unsequenced exons, a non-coding region, or the promoter.
- VWF sequencing may identify sequence variants with uncertain clinical significance.

Methodology

- Polymerase chain reaction (PCR) followed by bidirectional sequencing of VWF exons 4, 9, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 24, 25, 27, 28, 30, 31, 51, and 52 depending on the specific VWD test ordered.
 - Analysis of VWD type 2A begins with sequencing exon 28. If no mutations are identified, exons 11, 12, 14, 15, 16, 24, 25, 51, and 52 are subsequently sequenced.
 - Analysis of VWD type 2B involves sequencing exon 28.
 - Analysis of VWD type 2M begins with sequencing exon 28. If no mutations are identified, exons 30 and 31 are subsequently sequenced.
 - Analysis of VWD type 2N involves sequencing VWF exons 4, 9, 17, 18, 19, 20, 21, 24, 25, and 27.
 - Analysis of PT-VWD involves PCR of the GP1BA gene followed by targeted mutation analysis of four common mutations: c.746 G>T, (p. Gly249Val), c.746 G>A (p. Gly249Ser), c.763A>G (p. Met255Val), and c.1306del 27 (p.436-444 del 9).

- Analytical sensitivity and specificity are 99 percent.
- Clinical sensitivity is approximately 80 percent for VWD types 2B and 2M, 99 percent for VWD type 2A, and unknown for type 2N and PT-VWD using the tests as described above. The sensitivity of these tests for other types of VWD is unknown.

Limitations

- VWF mutations, other than those in the exons tested, will not be detected.
- Large VWF deletions/duplications will not be detected.
- No GP1BA mutations, other than the four targeted, are detected by analysis for PT-VWD.
- Rare diagnostic errors may occur due to primer-site mutations.

Related Tests

- von Willebrand Factor Activity Ristocetin Cofactor (vWF:RCo) (0030250)
- von Willebrand Factor Antigen (vWF:Ag) (0030285)
- Factor VIII Activity (0030095)
- von Willebrand Modified Panel (vWF:Ag and vWF:RCo) (0030284)
- von Willebrand Panel (vWF:Ag, vWF:RCo and FVIII activity) (0030125)
- von Willebrand Multimeric Panel (vWF:Ag, vWF:RCo, FVIII activity, and multimers) (0030002)
- von Willebrand Factor Multimers (0092281)
- von Willebrand Panel with Reflex to von Willebrand Multimeric Analysis (2003387)

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For specific collection, transport, and testing information, refer to von Willebrand Disease, Type 2A (VWF) Sequencing Exon 28 with Reflex to Exons 11, 12, 14, 15, 16, 24, 25, 51 & 52 (2005480); von Willebrand Disease, Type 2B (VWF) Sequencing Exon 28 (2005486); von Willebrand Disease, Type 2M (VWF) Sequencing Exons 28, 30 and 31 (2005490); von Willebrand Disease, Type 2N (VWF) Sequencing Exons 4, 9, 17, 18, 19, 20, 21, 24, 25, and 27 (2005494); and von Willebrand Disease, Platelet Type (GP1BA) 4 Mutations (2005476) on the ARUP website at www.aruplab.com. For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.



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