

# New Procedures

Several new procedures have been made available since the last issue of *LabHorizons* was published. For a complete file of LabCorp's published clinical assays, keep back issues of *LabHorizons* together with your copy of the *Directory of Services and Interpretive Guide*.

## Alpha-Thalassemia ..... 511172

**CPT** 83891; 83900; 83901(x7); 83894; 83912

**Related Information** Beta-Thalassemia

**Synonyms**  $\alpha$ -Thalassemia

**Specimen** Whole blood, amniotic fluid, CVS, buccal swabs

**Volume** 7 mL whole blood, 10 mL amniotic fluid, 20 mg CVS, two buccal swabs

**Minimum Volume** 3 mL whole blood, 5 mL amniotic fluid, 10 mg CVS, two buccal swabs

**Container** Lavender-stopper (EDTA) tube or yellow-stopper (ACD) tube, LabCorp buccal swab kit

**Storage Instructions** Maintain at room temperature or refrigerate at 4° C

**Cause for Rejection** Hemolyzed specimen; quantity not sufficient for analysis; improper container; wet buccal swab

**Use** Alpha-thalassemia is the most common inherited disorder of hemoglobin (Hb) synthesis in the world, with gene frequencies varying between 1% and 98% throughout the tropics and subtropics. Greater than 95% of recognized alpha-thalassemia involves deletion of one or both alpha-globin genes from chromosome 16p13.3. Polymerase chain reaction followed by agarose gel electrophoresis is used to characterize six of the most frequently observed determinants of alpha-thalassemia. The mutations include Southeast Asian (SEA), Thai (THAI), Filipino (FIL), Mediterranean (MED), a 3.7, and a 4.2.

**Limitations** The test is designed for only six common mutations. Other rare mutations are not tested.

**Methodology** Polymerase chain reaction (PCR) and gel electrophoresis

### References

Chong S, et al. Simplified Multiplex-PCR Diagnosis of Common Southeast Asian Deletional Determinants of  $\alpha$ -Thalassemia. *Clinical Chemistry Technical Briefs*. 2000; 46(10):1692-1695.

## Beta-Thalassemia ..... 511174

**CPT** 83891; 83900; 83896(x46); 83912

**Related Information** Alpha-Thalassemia

**Synonyms**  $\beta$ -Thalassemia

**Specimen** Whole blood, amniotic fluid, CVS, buccal swabs

**Volume** 7 mL whole blood, 10 mL amniotic fluid, 20 mg CVS, two buccal swabs

**Minimum Volume** 3 mL whole blood, 5 mL amniotic fluid, 10 mg CVS, two buccal swabs

**Container** Lavender-stopper (EDTA) tube or yellow-stopper (ACD) tube or LabCorp buccal swab kit

**Storage Instructions** Maintain at room temperature or refrigerate at 4° C

**Causes for Rejection** Hemolyzed specimen, quantity not sufficient, improper container, wet buccal swab

**Use** Beta-thalassemia is a hemoglobinopathy disorder due to an alteration in the quality and/or synthesis of the hemoglobin beta chain resulting in microcytic hypochromic anemia, abnormal peripheral blood smear with nucleated red blood cells, and reduced amounts of hemoglobin A (HbA) on hemoglobin analysis. Individuals with beta-thalassemia major have severe anemia and hepatosplenomegaly usually within the first two years of life. Without treatment, affected children have severe failure to thrive and shortened life expectancy. The linear array test offers  $\geq 90\%$  sensitivity for specifically detecting 43  $\beta$ -hemoglobin mutations in all affected population groups worldwide. Thus, the clinical use of this molecular diagnostic test is to test for carrier testing, prenatal diagnosis, and prognosis to predict clinical severity for proper treatment.

**Limitations** The linear array test offers  $\geq 90\%$  sensitivity for 43  $\beta$ -hemoglobin mutations in all affected population groups worldwide. Other mutations will not be detected by this assay.

**Methodology** Polymerase chain reaction line probe hybridization, colorimetric detection

### References

Weatherall DJ. Phenotype-genotype relationships in monogenic disease: Lessons from the thalassemias. *Nature Reviews*. 2002; 2:245-255.

Cao A, Saba L, Galanello R, Rosatelli MC. Molecular diagnosis and carrier screening for beta thalassemia. *JAMA*. 1997; 278:1273-1277.

## DPD 5-Fluorouracil Toxicity ..... 511176

**CPT** 83891; 83898; 83892; 83894; 83912

**Synonyms** 5FU; Dihydropyrimidine Dehydrogenase

**Specimen** Whole blood, buccal swabs

**Volume** 7 mL whole blood, 4 buccal swabs

**Minimum Volume** 3 mL whole blood, 2 buccal swabs

**Container** Lavender-stopper (EDTA) tube or yellow-stopper (ACD) tube, LabCorp buccal swab kit

**Causes for Rejection** Hemolyzed specimen; quantity not sufficient; wet buccal swab; improper container

**Storage Instructions** Maintain specimen at room temperature, or refrigerate at 4°C.

**Use** Dihydropyrimidine dehydrogenase (DPD) metabolizes 5-fluorouracil (5-FU), a widely prescribed medication for the systemic treatment of multiple cancers, including those of the breast, head, neck, ovary and gastrointestinal tract. When DPD enzyme activity is low, the stomatitis, diarrhea, dermatitis, myelosuppression, neurotoxicity and cardiotoxicity associated with 5-FU treatment can be severe, and in rare cases, fatal. The prevalence of DPD deficiency in the general population is approximately 2%. This assay identi-

fies individuals who are DPD deficient, and who may be susceptible to 5-fluorouracil toxicity.

**Limitations** This procedure detects only the presence of the wild-type, or mutant, allele for the DPD IVS14+1G>A mutation. This procedure may be considered by Medicare and other carriers as investigational and, therefore, may not be payable as a covered benefit for patients. Other mutations will not be detected by this assay.

**Methodology** Polymerase chain reaction (PCR), restriction enzyme digestion and gel electrophoresis

**Additional Information** Variability in response (efficacy and toxicity) to 5-fluorouracil (5-FU) chemotherapy has been linked to the rate limiting enzyme in the drug's catabolic pathway, known as dihydropyrimidine dehydrogenase (DPD). DPD deficiency results in excessive amounts of 5-FU available to be anabolized to its active metabolite and is relatively undetectable by clinical observation prior to 5-FU administration. Extensive studies have associated both profound and partial deficiency in DPD activity with severe unanticipated toxicity after 5-FU administration. Numerous studies genotyping DPD-deficient patients, their family members, and healthy individuals have shown that the splice-site mutation (IVS14+1G>A) is the most characterized and frequently observed allele associated with decreased DPD enzyme activity. Screening for the presence of this mutation in the Caucasian population showed frequencies of 0.91% homozygous and 1.8% heterozygous for the IVS14+1G>A allele.

**References**

van Kuilenburg ABP, Muller EW, Haasjes J, et al. Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: Frequency of the common IVS14+1G>A mutation causing DPD deficiency. *Clin Can Res.* 2001 May; 7:1149-1153.

Lee A, Ezzeldin H, Fourie J, Diasio R. Dihydropyrimidine dehydrogenase deficiency: Impact of pharmacogenetics on 5-fluorouracil therapy. *Clin Adv Hematol Oncol.* 2004 Aug; 2(8):527-532.

**UGT1A1 Irinotecan Toxicity ..... 511200**

**CPT** 83891; 83909; 83898; 83912

**Synonyms** Camptosar®; Irinotecan

**Specimen** Whole blood

**Volume** 7 mL

**Minimum Volume** 3 mL

**Container** Lavender-stopper (EDTA) tube or yellow-stopper (ACD) tube

**Storage Instructions** Maintain specimen at room temperature, or refrigerate at 4°C.

**Cause for Rejection** Hemolyzed specimen; quantity not sufficient for analysis; improper container

**Use** Irinotecan (Camptosar®) is used or is under evaluation in a broad spectrum of solid tumors, and is often prescribed for treating patients with metastatic cancer of the colon or rectum, especially when 5-fluorouracil treatment has not been entirely successful. Severe toxicity (grade 4 neutropenia) is commonly observed in cancer patients receiving irinotecan who carry the *UGT1A1*\*28 allele, also called (TA)<sub>n</sub>. This test result will provide valuable information to physicians prior to initiating or modifying treatment or supplementing treatment with additional drugs.

**Limitations** This procedure may be considered by Medicare and other carriers as investigational and, therefore, may not be payable as a covered benefit for patients. The current test will only test the TATA box polymorphism of the *UGT1A1* gene. The other polymorphisms of this gene will not be detected.

**Methodology** Polymerase chain reaction (PCR), capillary electrophoresis

**References**

Rouits E, Boisdron-Celle M, Dumont A, Guerin O, Morel A, Gamelin E. Relevance of different UGT1A1 polymorphisms in irinotecan-induced toxicity: A molecular and clinical study of 75 patients. *Clin Cancer Res.* 2004 Aug 1; 10(15):5151-5159.

Innocenti F, Undevia SD, Iyer L, et al. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol.* 2004 Apr 15; 22(8):1356-1359.

Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: A balanced polymorphism for regulation of bilirubin metabolism? *Proc Natl Acad Sci USA.* 1998 Jul 7; 95(14):8170-8174.

# Announcements

## Continuing Medical Education (CME) Opportunities

LabCorp® is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor continuing medical education (CME) for physicians. In addition, LabCorp is accredited by the ASCLS PACE® program, Florida Department of Health-Board of Clinical Laboratory Personnel, and the state of California

Department of Health Services as a provider of continuing medical education for laboratory personnel. CME titles currently available are listed below, along with publication numbers and expiration dates.

Title	Publication N°	Expiration Date
Inherited Venous Thrombosis	L1104-0504-1	May 1, 2006
Alpha <sub>1</sub> -Antitrypsin Deficiency: A Critical Component of Chronic Obstructive Pulmonary Disease	L1106-0804-1	August 1, 2006
Screening for Aneuploidy in the First Trimester	L1145-0805-2	November 1, 2006
Clinical Significance and Assessment of 25-OH Vitamin D	L1162-0305-1	February 1, 2007
Human Papillomavirus and Cervical Cancer: An Update	L1173-0605-1	June 1, 2007
When Good Food Makes You Sick, Part 1: Celiac Disease	L1192-1005-1	September 1, 2007
When Good Food Makes You Sick, Part 2: Food Allergy	L1208-0106-1	February 1, 2008

For more information, or for copies of any of the available materials, please contact the LabCorp CME office at 336-436-4990. In addition,

the CME articles listed above may also be accessed and printed at [www.labcorp.com/cme/index.html](http://www.labcorp.com/cme/index.html).

## Change in Methodology for Homocysteine

LabCorp's **Homocysteine, Plasma** test (706994) is currently referred to Specialty Laboratories. On **April 3, 2006**, Specialty will convert this test to a new methodology.

The methodology currently used by Specialty to perform this test demonstrates a significant positive bias—10% to 40% on average—when compared to the methodology previously used by LabCorp, which employed DPC Immulite 2000 instrumentation. The new

method to which Specialty is converting correlates with the DPC Immulite methodology as well as with high-pressure liquid chromatography (HPLC).

Specialty Laboratories will include a comment on its report to indicate this change in methodology as well as the lower prevalence of high values. Please contact your local LabCorp representative if there are questions.

## Vitamin D, 25-OH Reference Interval Change

Effective March 20, the reference interval for **Vitamin D, 25-OH** (081950) will change to 32-100 ng/mL. This significant change reflects the fact that traditional vitamin D, 25-OH reference intervals were based on a statistical analysis of the distribution of vitamin D, 25-OH levels in ostensibly healthy people in the US.

Recent studies have revealed that many otherwise healthy individuals actually have suboptimal circulating vitamin D, 25-OH levels.

The current consensus is that levels in excess of 32 ng/mL are required for optimal health. With this reference interval change, LabCorp's interval will reflect this fact.

For a more detailed review of this subject, please refer the CME article entitled "Clinical Significance and Assessment of 25-OH Vitamin D" available at the CME link at [www.LabCorp.com](http://www.LabCorp.com).

## Revised HBV Prenatal and Newborn Recommendations

The Advisory Committee on Immunization Practices (ACIP) at the Centers for Disease Control and Prevention recently issued revised recommendations for HBV testing and immunization in prenatal and newborn care. ACIP recommendations for prenatal care manage-

ment include (among other things) testing all pregnant women for hepatitis B surface antigen (HBsAg) during each pregnancy.<sup>1</sup> LabCorp makes available the following to assist providers in addressing these recommendations:

### **Prenatal Profile I With Hepatitis B Surface Antigen . . . . . 202945**

**CPT** 85025; 86592; 86762; 86850; 86900; 86901; 87340

**Profile Includes** This profile also includes ABO grouping and Rh typing; antibody screen (includes ID and titer of all irregular antibodies detected); CBC with differential; HBsAg; rubella antibodies (IgG); syphilis serology (if positive, confirmation is performed by MHA-TP at an additional charge)

**Specimen** Serum, whole blood, blood films

**Container** One 10-mL red-stopper (non-gel-barrier) tube, two 5-mL lavender-stopper (EDTA whole blood) tubes, two clean glass slides, and one 10-mL gel-barrier tube

**Collection** Gently invert lavender-stopper tubes immediately to mix specimen with anticoagulant. Make two fresh blood films on glass slides. Centrifuge only the gel-barrier tube within 45 minutes of venipuncture.

**Storage Instructions** Refrigerate the red-stopper, gel-barrier, and lavender-stopper tubes. Maintain blood films at room temperature for as long as 48 hours.

ACIP recommendations also include universal vaccination of infants beginning at birth, immunoprophylaxis of infants born to HBsAg-positive women and infants born to women with unknown HBsAg status, routine vaccination of previously unvaccinated children and

adolescents, and vaccination of previously unvaccinated adults at increased risk for infection.<sup>2</sup> Full details on these recommendations can be found at [www.cdc.gov/mmwr/PDF/rr/rr5416pdf](http://www.cdc.gov/mmwr/PDF/rr/rr5416pdf).

1. Ward J, Rodewald L. [Letter] Atlanta, Ga: Centers for Disease Control and Prevention; 2006 Jan 18:[1-3].

2. Centers for Disease Control and Prevention. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP); Part 1: Immunization of Infants, Children, and Adolescents. *MMWR*. 2005;54(N° RR-16).

# Updates to the *Directory of Services and Interpretive Guide*

Test Name	Number	Field/Change
<b>BCL2-IGH Gene Rearrangement</b>	<b>480566</b>	<b>Collection</b> Specimens should arrive in the laboratory within 96 hours of collection. Please record the date and time of collection on the test request form. <b>Causes for Rejection</b> Sample greater than 96 hours old; clotted blood
<b>Bloom Syndrome, DNA Analysis</b>	<b>512145</b>	<b>Limitations</b> This test only detects the 2281del6ins7 mutation that is responsible for Bloom syndrome in the Ashkenazi Jewish population. This test is not appropriate for non-Ashkenazi Jewish individuals. This procedure may be considered by Medicare and other carriers as investigational and, therefore, may not be payable as a covered benefit for patients.
<b>Chromosome Analysis, Amniotic Fluid with Reflex to CGH Constitutional</b>	<b>052200</b>	<b>Methodology</b> In situ cell culturing of amniocytes to investigate numerical and/or structural chromosome abnormalities. If chromosome analysis is normal, testing will reflex to comparative genomic hybridization: Competitive hybridization of differentially labeled patient and known normal DNA to bacterial artificial chromosomes or BACs (434 features).
<b>Estimate Glomerular Filtration Rate (eGFR)</b>	<b>100768</b>	<b>Reference Interval</b> Female: 60.0-128.0 mL/minute Male: 60.0-137.0 mL/minute
<b>Hepatitis C Virus (HCV) FibroSure</b>	<b>550123</b>	<b>Special Instructions</b> Please provide age and gender of patient on the test request form.
<b>Platelet Factor 4</b>	<b>500126</b>	<b>Additional Information</b> Platelet factor 4 (PF4), a 30,000 Dalton high-affinity heparin-binding protein, is produced in megakaryocytes and stored in platelet alpha granules. <sup>1</sup> It is secreted by stimulated platelets and its plasma biological half-life is less than five minutes. It constitutes 5% of the protein found in circulating platelets. It is a member of the chemokine family of proteins. Platelet factor 4 is released from activated platelets and, in vivo, it is stored both in the endothelium and on hepatocyte surfaces. The primary function of platelet factor 4 is to neutralize the anticoagulant effect of heparin. Heparin neutralization occurs by the binding of platelet factor 4 to heparin at sites that are different from heparin's antithrombin binding site. Heparin therapy induces the release of platelet factor 4 from endothelial cells, and levels are markedly increased during the initiation of therapy but diminish with time as stores are exhausted. In vivo release of PF4 occurs with platelet hyperactivity and this may result from platelet interaction with subendothelial structures, artificial surfaces, atherosclerotic plaques, and thrombin. Increased levels of platelet factor 4 are observed in a variety of clinical states that are associated with activation of platelets. These include inflammatory or infectious diseases, disseminated intravascular coagulation, shock, polycythemia vera, cerebrovascular disorders, extracorporeal circulation, diabetes, cardiovascular disease, renal disease, cancer, and during the post-operative period. Endothelial cell injury also may result in increased levels of platelet factor 4. Decreased platelet factor 4 levels are observed in patients with gray platelet syndrome, a type of platelet storage pool defect. Increased levels of platelet factor 4 indicate in vivo or in vitro platelet activation. Accelerated platelet turnover can result in platelet factor 4 levels that are 10 times normal. Elevated platelet factor 4 levels may accompany increased levels of beta thromboglobulin indicating platelet activation.

1. Adcock DM, Jensen R, Johns CS, Macy PA. *Coagulation Handbook*. Austin, Texas: Esoterix Coagulation; 2002.

**Note:** For the most up-to-date test information, please consult the electronic *Directory of Services and Interpretive Guide* (e-DoS) at [www.LabCorp.com](http://www.LabCorp.com). Questions regarding *LabHorizons* should be addressed to David Carrozza ([carrozd@labcorp.com](mailto:carrozd@labcorp.com)).

