



Test Bulletin

Effective Wednesday, January 19, 2011

Physician signature required on requisitions

The Centers for Medicare and Medicaid Services (CMS) recently released a final rule requiring physician signatures on **requisitions** for laboratory services beginning Jan. 1, 2011. CMS will focus on developing educational and outreach materials to clarify the details of this ruling during the first quarter of 2011. An implementation date for the ordering provider signature is expected once the educational campaign is fully underway.

This ruling reverses a long-standing policy that “requisitions” used to convey patient and test request information to the laboratory did not need to be signed by the ordering provider as long as the ordering provider documented the laboratory test request in the patient medical record.

ACL will monitor and communicate the CMS educational materials as they become available. ACL also has begun the process of revising its current paper requisitions to include space for an ordering provider signature to ensure readiness with this new requirement within the CMS implementation timeframe.

Further information about this new requirement will be posted on the ACL website at www.acllaboratories.com. Please direct any questions or concerns to Kathy Lindgren, ACL compliance officer, at 800-877-7016, ext. 7916, or 414-328-7916.

ACL Laboratories offers P450 CYP2C19 Gene Mutation

The cytochrome P450 family (CYP) is a large and diverse group of enzymes. The function of most CYP enzymes is to catalyze the oxidation of organic substances, including xenobiotic substances such as drugs. CYPs are the major enzymes involved in drug metabolism and bioactivation, accounting for about 75% of the total drug metabolism.

Impaired drug metabolism causing adverse drug reactions or lack of drug response. Drugs metabolized by CYP2C19 include clopidogrel, S-mephenytoin, diazepam, R-warfarin, some antidepressants (e.g., citalopram, amitriptyline, clomipramine), proton pump inhibitors (e.g., omeprazole, lansoprazole) and antimalarials (e.g., chloroguanide).

Genetic polymorphisms in CYP2C19 are common and can affect therapeutic response to drugs. The enzyme activity is expressed at highly variable levels. Three phenotypes are identified: poor metabolizers (**PM**), intermediate metabolizers (**IM**) and normal metabolizers (**NM**). Phenotype prevalence is 2-6% PM Caucasian, 13-19% PM Asian, 10-20% PM African American; 24-36% IM; Drugs metabolized by this enzyme – approximately 5-10%.

The seven CYP2C19 allelic variants detected in ACL Laboratories genotyping assay provide greater than 98% coverage of the variant alleles found for this gene. The active allele (wild type) of the CYP2C19 gene is designated CYP2C19*1. Homozygous wild-type individuals have a normal metabolizer phenotype (NM). The most common poor metabolizer phenotypes have been identified as CYP2C19*2 and CYP2C19*3. CYP2C19*2 (G681A) and CYP2C19*3 (G636A) each differ from the active CYP2C19*1 by a single nucleotide substitution, which leads to impaired enzyme activity. The allele frequency of CYP2C19*2 has been reported to be as high as 75-85% in Asians and approximately 15% in Europeans and African Americans. The allele frequency of CYP2C19*3 has been reported

continued

CYP 2C19 gene mutations

2C19 allele	Nucleotide change	Enzyme metabolism
*1	None (WT)	Normal
*2	681G>A	Inactive
*3	636G>A	Inactive
*4	1A>G	Inactive
*5	1297C>T	Inactive
*6	395G>A	Inactive
*7	IVS5+2T>A	Inactive
*8	358T>C	Inactive

For additional information, see the CYP2C19 allele nomenclature database at www.cypalleles.ki.se/cyp2c19.htm

to be as high as 6-10% in Asians and is rare in Europeans and African Americans. Other alleles associated with reduced metabolism include CYP2C19 *4, *5, *6, *7 and *8, but these are less frequent in the general population. CYP2C19*4 accounts for approximately 3% of Caucasian poor metabolizers.

ACL Laboratories test identifies seven (7) small nucleotide variants in PCR-multiplex format, providing increased sensitivity and quality performance.

CYP 2C19 genotyping and Plavix – March 12, 2010, statements from the FDA

The FDA requires the following **black-box warning** for Plavix:

- Effectiveness of Plavix depends on activation to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19.
- Poor metabolizers treated with Plavix at recommended doses exhibit higher cardiovascular event rates following acute coronary syndrome (ACS) or percutaneous coronary intervention (PCI) than patients with normal CYP2C19 function.
- Tests are available to identify a patient's CYP2C19 genotype and can be used as an aid in determining therapeutic strategy.
- Consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers.

ACL Laboratories offers Epstein-Barr Virus Quantitative Assay (Test order code EBVQN)

The **Epstein-Barr virus**, also called human herpesvirus 4 (HHV-4), is a cancer-causing virus of the herpes family. Epstein-Barr virus is one of the most common viruses in humans. The virus occurs worldwide and causes infectious mononucleosis (glandular fever). There is also strong evidence that the virus has a primary role in the pathogenesis of multiple autoimmune diseases, particularly dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome and multiple sclerosis. It is also known to cause several lymphoproliferative disorders and cancers, particularly Hodgkin's disease, Burkitt's lymphoma, nasopharyngeal carcinoma, and central nervous system lymphomas associated with HIV infants who become susceptible to EBV as soon as maternal antibody protection (present at birth) disappears. Many children become infected with Epstein-Barr virus, and these infections usually cause no symptoms or are indistinguishable from the other mild, brief illnesses of childhood. In the U.S. and in other developed countries, many persons are not infected with the virus in their childhood years. When infection occurs during adolescence or young adulthood, it causes infectious mononucleosis 35-50% of the time.

Monitoring Epstein-Barr virus DNA levels by quantitative PCR in patients at risk of EBV-associated lymphoproliferative disorders may allow timely recognition of virus reactivation and permit installment of antiviral treatment.

EBVQN is a quantitative molecular test, with a linear range of 500- 500,000 copies/mL.

Assay was validated on plasma and CSF specimens.

ACL Laboratories offers Blood Viral Panel (CMVQN, EVBQN, BKVQN ASSAY) (Test order code BVPQN)

Cytomegalovirus (CMV), a member of the herpes virus family, has the ability to establish lifelong persistent and latent infection following primary exposure. Under certain conditions, the virus can reactivate, resulting in asymptomatic shedding or development of disease. CMV disease is usually restricted to the immunocompromised or immunologically immature host. In transplant patients, infection with CMV from the donor organ or reactivation of latent virus in the recipient can lead to disease development.

BK virus (BKV) is a nonenveloped, double stranded DNA virus of the polyomavirus family that primarily affects immunocompromised individuals. It was first detected in the urine of a renal allograft recipient in whom ureteric stenosis developed and was named based on the initials of the patient (B.K.).¹ The virus is ubiquitous in human populations worldwide.² Infection typically occurs in childhood, with a seroprevalence up to 90% in adults.

BKVN occurs in 1-10% of kidney transplant recipients, usually manifesting in the first year following transplantation and leading to graft loss in 15-80% of BKVN cases within five years. Active replication of BKV in urothelial cells and subsequent tissue damage cause release of BKV into urine and blood that can be monitored by molecular assays. Monitoring of BK viruria and viremia can facilitate early diagnosis of BK replication, guide management of immunosuppressive therapy, and monitor response to intervention.

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Monitoring EBV, CMV, BKV DNA levels by quantitative PCR in patients at risk of virus associated lymphoproliferative disorders may allow timely recognition of virus reactivation and permit installment of antiviral treatment.

BVPQN is a quantitative molecular test, with a linear range of 500 - 500,000 copies/mL for CMV, 500 - 500,000 copies/mL for EBV and 500-100,000,000 copies/mL for BKV.

The BVPQN assay is based on a real-time PCR format in which viral DNA is amplified by PCR and detected by fluorescently-labeled hybridization probes

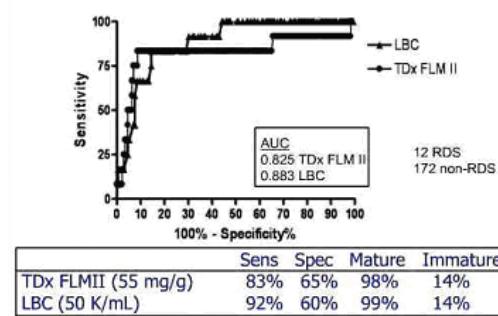
ACL Laboratories discontinues Fetal Lung Maturity Test (Test order code FLMAT)

ACL Laboratories will discontinue the Fetal Lung Maturity Test (test order code FLMAT). ACL Laboratories offers Lamellar Body Counts – Fetal Lung Maturity Test (test order code LAME) as a means of assessing fetal lung maturity. Lamellar body counts is more reproducible than other fetal lung maturity tests currently available.

Lamellar bodies are surfactant containing lamellated structures secreted by the type II cells in the alveoli. They are released into the amniotic fluid from the fetal lungs in increasing quantities that parallel surfactant production. Surfactant and lamellar bodies appear in the amniotic fluid at 28 to 32 weeks and increase exponentially as gestation progresses. Measuring amniotic fluid lamellar bodies assists estimation of fetal lung development and risk of Respiratory Distress Syndrome (RDS) during weeks 32 to 36 of gestation. By week 37 of gestation and beyond, the risk of RDS is so low that laboratory assessment of fetal lung maturity is rarely indicated.

Lamellar body counts have similar sensitivity and specificity to the fluorescent polarization surfactant/albumin ratio (TDx FLM II test currently performed at ACL).

Clinical Utility of LBC vs TDx FLM II



Amniotic fluid samples for lamellar body count testing are stable for 12 hours at ambient temperature and 48 hours refrigerated. Frozen samples are unacceptable for lamellar body counts. As with other fetal lung maturity testing, the presence of blood and meconium may affect results. Amniotic fluids contaminated with blood may have artificially decreased lamellar body counts. Samples with meconium contamination may have artificially increased lamellar body counts.

ACL Laboratories converts to Siemens Advia Centaur Electrochemiluminescence Methodology for cancer antigen tests

ACL Laboratories will analyze cancer antigen 15.3 and cancer antigen 19.9 using Siemens Advia Centaur Electrochemiluminescence methodology. This methodology is the most widely accepted method for testing these cancer markers.¹

Assay comparison will be performed on all patients that were monitored prior to Jan. 19, 2011, using ARUP's Roche electrochemiluminescence methodology.

The chart below provides an overview of the changes occurring utilizing the new instrumentation.

Cancer antigen 15.3

Change parameter	Old methodology	New methodology
Specimen requirements	1.0 ml refrigerated	2.0 ml frozen
Reference ranges	0-31 U/ml	0-32 U/ml

Cancer antigen 19.9

Change parameter	Old methodology	New methodology
Specimen requirements	1.0 ml frozen	2.0 ml frozen
Reference ranges	0-37 U/ml	0-35 U/ml

¹Based on 2009 College of American Pathologists survey participation data.

Reference laboratory information added to ACL's Directory of Services

Effective immediately, a field titled "Reference Labs" will be viewable in ACL's Directory of Services. This field will be completed when ACL does not perform testing and refers testing to another certified laboratory. The laboratory name and test code will appear in this field.

There will also be additional information added under "Reimbursement Guidelines" in the Directory of Services, when necessary:

1. If a test could be billed directly from another laboratory other than ACL.
2. When the cost of testing may not be covered by a patient's insurance, may result in higher copays/deductibles, and/or may require insurance pre-authorization. Patients should contact their insurance provider with specific questions.