



Saint Luke's Regional Laboratories Clinical Laboratory Letter



January 2009

Microbiology Goes FISH-ing

Saint Luke's Health System performs approximately 24,000 aerobic and anaerobic blood cultures per year, of which 10-12% are positive for growth of a micro-organism. The standard approach to positive blood cultures has not changed for many years. All positive blood cultures are Gram stained and sub-cultured for identification of the organism. Blood culture Gram stain results are considered a critical value and are communicated to a caregiver immediately, so that appropriate anti-microbial therapy can be established.

The Gram stain is quite useful in guiding choice of empiric anti-microbial therapy for Gram positive vs. Gram negative organisms. However, the final identification for most Gram positive cocci in clusters is coagulase-negative staphylococci, which are potentially blood culture contaminants for which antibiotics are unnecessary. Coagulase-negative staph accounts for 70% of all staphylococcal isolates from SLHS blood cultures. Furthermore, the Gram stain does not differentiate between Gram negative enteric organisms vs.

Pseudomonas species, which in many cases necessitates very broad-spectrum anti-microbial therapy until the organism is fully identified. The standard time for identification of an organism from a positive blood culture is 1-2 days.

New technology, called PNA FISH, provides more complete information than the Gram stain on the same day a blood culture becomes positive. PNA is a synthetic Peptide Nucleic Acid made up of oligonucleotide bases with a peptide backbone. The PNA probe is fluorescently labeled with tags that bind to species-specific RNA for identification of staphylococci, enterococci, and *Candida*. This allows microbiologists to differentiate *Staphylococcus aureus* from coagulase-negative staphylococci based on a fluorescent stain done directly from a positive blood culture bottle.

Enterococcus faecalis can be differentiated from other enterococcal species that are potentially vancomycin resistant, and *Candida* species can be

distinguished from each other. A new probe that separates *Pseudomonas* from gram negative enterics, including *E. coli* and *K. pneumoniae*, is in development.

Potential benefits of PNA FISH include earlier targeted anti-microbial therapy, as well as avoidance of unnecessary anti-microbials, which may impact the emergence of drug-resistant bacteria. Some published studies have also indicated cost savings due to decreased length of stay and improved patient outcomes.

Saint Luke's Regional Laboratories' Microbiology will begin using PNA FISH technology for identification of staphylococci from blood cultures near the end of January. Initially, testing will occur on Saint Luke's Hospital inpatients only but will soon expand to all SLHS hospitals. Clinicians will see PNA FISH results on positive blood culture reports in addition to the Gram stain result. The PNA FISH stain requires approximately three hours to perform.

Creatine Kinase Reference Range Change

Creatine kinase (CK) is an enzyme that catalyzes the reversible phosphorylation of creatine by ATP. The end product, phosphocreatine, is a readily available energy source for cells. CK is present in many tissues but skeletal and heart muscles contain the highest concentrations. CK released from skeletal muscle accounts for almost all of the CK activity detected in the plasma of healthy individuals. Circulating CK is cleared by degradation in the liver and reticuloendothelial system and has a circulating half-life of 12 hours.

Historically, CK was most often measured to diagnose acute myocardial infarction. In May 2001, the American College of Cardiologists recommended that total CK no longer be included as a cardiac marker. Today, CK is primarily ordered to screen for statin induced myopathy.

A recent study from the Netherlands has convincingly demonstrated that the reference range

should be stratified by gender and race (Am Heart J 2007;154:655-61). Black men have much higher CK levels than non-black men or black women, who have higher levels than white or Asian women. Differences in muscle mass are believed to account for these gender and racial differences.

Saint Luke's Regional Laboratories recently completed its own analysis of CK reference range which confirmed the findings of the Dutch study. Previously, the laboratory has used a reference range of 49 – 397 IU/L for all males and 38 – 234 IU/L for all females. In January, the laboratory will convert to the following reference values.

Population	Reference Range
Black Male	50 – 850 IU/L
Black Female	30 – 430 IU/L
White Male	40 – 425 IU/L
White Female	30 – 160 IU/L

This change should help to prevent the misdiagnosis of myopathy in black individuals. It is also important to remember that CK levels may be even higher in the physically fit, due to their greater muscle mass and that CK levels gradually decline with age or chronic illness due to a reduction in muscle mass.

CYP2C19 Genetic Variants and Decreased Response to Clopidogrel (Plavix)

The CYP2C19 gene encodes for an enzyme involved in the metabolism of a number of drugs as part of the cytochrome P450 system. This enzyme converts the antiplatelet drug clopidogrel (Plavix) to its active metabolite. Two studies published in the December 22, 2008 online issue of the New England Journal of Medicine (NEJM) showed that genetic variants in the CYP2C19 gene lead to a reduced clopidogrel response, thereby increasing the risk for adverse cardiovascular outcomes in some patients.

In the first study, Mega et al. examined the relationship between the plasma concentration of the active drug metabolite, platelet inhibition and the presence of reduced-function CYP2C19 alleles in 162 healthy volunteers who were given clopidogrel. The researchers found that subjects with at least one reduced-function allele had

decreased platelet inhibition and a significantly lower plasma concentration of clopidogrel's active metabolite compared to noncarriers of CYP2C19 genetic variants. In a separate analysis of 1,477 patients in the TRITON-TMI 38 trial (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction 38), the researchers found that patients with acute coronary syndromes who were on clopidogrel therapy and were also carriers of reduced-function CYP2C19 alleles had a 53% increased risk of dying from cardiovascular causes, myocardial infarction or stroke, as well as a threefold increased risk of stent thrombosis compared to noncarriers.

The second NEJM study by Simon et al. also showed an association between CYP2C19 reduced-function alleles and a higher risk for adverse cardiovascular outcomes. In this study, researchers followed 2,208 patients from a nationwide French registry for one year who were taking clopidogrel after having a myocardial infarction. During the follow-up period, 225 patients died and 94 experienced a nonfatal myocardial infarction or stroke. Patients with two CYP2C19 function-reducing alleles (any combination of *2, *3, *4 or *5 loss-of-function alleles) had a 1.98 times higher risk of a subsequent cardiovascular event compared to noncarriers. Additionally, among 1,535 patients who underwent percutaneous coronary intervention while hospitalized, having two reduced function CYP2C19 variants led to a 3.58 increased risk of myocardial infarction, stroke or death.

Based on these results, the authors suggest that genetic screening for reduced-function CYP2C19 alleles may be a cost-effective alternative to the current practice of repeated platelet monitoring for patients on clopidogrel therapy who may have an increased risk for a cardiovascular event. CYP2C19 genotype testing is available through Mayo Medical Laboratories.

Lipase Reference Range Change

On December 16, 2008 the reference range for lipase changed from 22 – 51 U/L to 18 – 51 U/L.