



# Saint Luke's Regional Laboratories Clinical Laboratory Letter



February 2010

## C. difficile Toxin PCR Testing Guidelines

Due to the complexity of PCR tests for *C. difficile* toxin testing, guidelines for frequency of testing have recently been published (Ann Intern Med. 2009;151:176-179). These guidelines are designed to limit the false diagnosis of *C. difficile* infection in patients that are colonized with the organism. It is suggested that only patients with 3 or more loose stools per day for at least 1-2 days be tested.

Saint Luke's Regional Laboratories will adopt the following testing criteria, effective immediately:

- Only one stool specimen per patient per day will be tested.
- In the event of a positive test result, no further specimens will be accepted for 10 days.
- Only loose or soft stool specimens will be accepted for testing (no formed stool specimens).

In addition, "test of cure" is imprudent, as the PCR test will remain positive for several days to weeks following treatment.

## Vitamin D Insufficiency in Kansas City

A decade ago, physicians ordered vitamin D levels to assess bone health. Recent epidemiological studies have suggested that vitamin D deficiency may play an important role in the pathogenesis of cardiovascular disease, diabetes, cancer, multiple sclerosis, rheumatoid arthritis, asthma, periodontal disease and depression. Accordingly, demand for 25(OH) vitamin D testing has skyrocketed. Saint Luke's Regional Laboratories (SLRL) received orders for 1710 vitamin D levels in 2007, 11,331 in 2008 and 26,024 in 2009.

The Third National Health and Nutrition Examination Survey (NHANES III) reported the prevalence of vitamin D deficiency in the United States to be between 25% and 75% of adults (Bone 2002;30:771-7). Recently SLRL reviewed the results of the vitamin D measurements performed in 2009.

Vitamin D Level	Number	Percent
<25 ng/mL	6469	25%
<30 ng/mL	10,221	39%
>80 ng/mL	175	0.6%

Reference range was 25-80 ng/mL. However, most experts believe that the optimal concentration of 25(OH) vitamin D is at least 30 ng/mL because this is the threshold for elevation of parathyroid hormone. Using the latter cutoff, 39% of Kansas Citians, who were tested, were vitamin D insufficient, while less than 1% of people had levels above the upper limit of the normal range. The highest observed value was 327 ng/mL.

The cost of performing vitamin D testing in 2009 exceeded \$550,000. Given the high incidence of vitamin D insufficiency and the low risk of adverse effects from vitamin D supplementation, a more practical clinical strategy might be to simply recommend vitamin D supplementation without ordering testing.

## Cord Blood Program in Second Year

Saint Luke's Public Cord Blood Program, the first in the Kansas City region, has been in operation for more than one year. In association with St. Louis Cord Blood Bank (SLCBB) at SSM Cardinal Glennon Children's Medical Center, 432 cord blood units have been collected from mothers delivering their babies at Saint Luke's Hospital.

Umbilical cord blood (UCB) contains stem cells that are being used in therapies for more than 70 diseases, including cancer and blood disorders. UCB units stored in public cord blood banks are available world-wide for patients in need. Collections from a second hospital, Saint Luke's South, are scheduled to begin in March. Further expansion is anticipated in the future.

Expectant mothers pre-register to donate UCB by completing a personal/family medical history questionnaire and IRB-approved consent forms. The program Nurse Coordinator reviews the history and, if all criteria are met, notifies the obstetrician of the anticipated collection. Trained and certified obstetricians and nurses work together in the collection procedure, which includes a maternal blood draw for infectious disease testing and documentation of clinical perinatal data. After delivery, the cord is antiseptically cleansed and the blood is collected by venipuncture while the placenta is *in utero*. Collections are also performed following C-sections.

UCBs that meet initial criteria are processed by Saint Luke's Cell Processing Laboratory to remove red cells and reduce volume. They are then packaged, labeled, and cryopreserved in liquid nitrogen. Samples are tested for total nucleated cell count, CD34 stem cell count, viability, ABO and Rh type, HLA type, colony forming units, hemoglobinopathy, and bacterial and fungal contamination. UCB units meeting final banking criteria are transferred to the SLCBB for long-term storage and distribution.

Saint Luke's entrance into cord blood banking comes at a time when the use of cord blood stem cells for the treatment of various diseases is increasing. FDA has recognized cord blood banking's coming of age and published guidances in October 2009 requiring the licensure of UCBs as a biologic product; UCB units meeting newly defined standards for safety, purity, potency, and effectiveness will qualify for licensure. By October 2011, public cord blood banks will be required to complete the biologics license application and inspection process. UCBs meeting FDA criteria will then bear FDA-approved labeling as a prescription drug product.

### **CD5/CD10-Negative B-Cell Chronic Lymphoproliferative Disorders**

Flow cytometry is routinely used for the immunophenotypic characterization of B-cell chronic lymphoproliferative disorders (B-CLPD). The following table lists the most common B-CLPD immunophenotypes.

<b>B-CLPD</b>	<b>Immunophenotype</b>
Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)	CD5 positive CD23-positive
Mantle cell lymphoma (MCL)	CD5-positive CD23-negative
Follicular lymphoma, Diffuse large B cell lymphoma, Burkitt lymphoma (FL/DLBL/BL)	CD10-positive
Hairy cell leukemia (HCL)	CD11c positive CD25 positive CD103 positive
Other *	CD5 negative CD10-negative

\*Other includes marginal zone lymphoma (MZL), lymphoplasmacytic lymphoma (LPL) and atypical versions of the B-CLPD listed above.

The CD5/CD10-negative immunophenotype is frequently encountered and often poses the greatest diagnostic challenge due to its non-specificity for a particular B-CLPD. These cases are especially problematic when they are identified in blood or bone marrow in the absence of identifiable lymph node or tissue involvement.

In a multicenter retrospective study of CD5/CD10-negative B-CLPD cases, researchers analyzed 156 newly diagnosed CD5/CD10-negative cases between 1990 and 2003 (Am J Hematol 2008; 83:349-54). Despite a multifaceted diagnostic approach, which included morphology, immunophenotype and cytogenetics a specific diagnosis of probable splenic MZL or LPL was determined in only 31% of cases. Although a definitive diagnosis was not possible in the majority of cases, a few prognostic features were identified. Increased LDH, hemoglobin < 11g/dl and splenomegaly predicted shorter treatment-free time. Elevated LDH and age >60 years were associated with shortened overall survival.

An accompanying editorial (Am J Hematol. 83:347-348, 2008) suggested that even though a diagnosis cannot be made with certainty in the majority of CD5/CD10-negative cases, the prognostic approach may be preferable to trying to make a specific diagnosis when additional tissue or lymph node specimens cannot be obtained.