

NEWS YOU CAN USE

New Director of Operations

Cynthia Samson, MA, MT(ASCP) SH, CLS, was recently appointed as the new Director of Operations for Anatomic Pathology and Clinical Laboratories. Previously manager of the Pre-Analytic section, she has been with Stanford University Medical Center for five years. We are happy to have Cynthia in her new role as Director of Operations, and as a key member of the Laboratory Leadership Team. She can be reached at csamson@stanfordmed.org or 650-725-9571.

Brief Updates

Influenza A Molecular Testing -

As the current flu season continues, we have adapted our approach to influenza A testing. We still offer a direct fluorescent antibody (DFA) panel for a number of respiratory viruses but the specific influenza A DFA has been replaced by a new reverse-transcriptase real-time PCR method based on an assay developed by the CDC (CODE: FLUAPC). This assay detects all subtypes of Influenza A (including the novel 2009 H1N1), but does not distinguish between them. Coming soon: a rapid PCR method which is specific for the novel strain, which can also detect the most common mutation conferring oseltamivir (Tamiflu) resistance.

hsCRP & LpPLA2 for

Cardiovascular Risk - The use of high-sensitivity C-reactive protein (hsCRP) as a predictor of cardiovascular risk is

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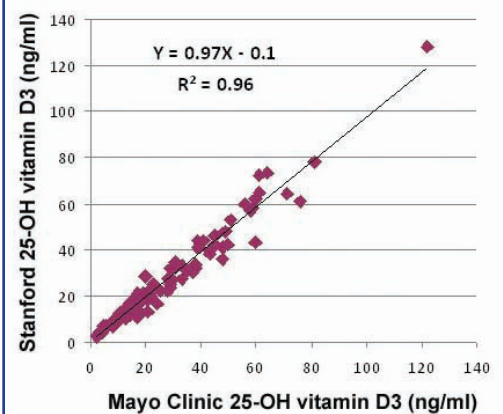
Vitamin D Testing Now In-House

Jim Faix MD & Run Z. Shi PhD - Chemistry & Immunology Section

"Vitamin" D is really a hormone. A precursor, made by the skin in response to ultraviolet irradiation, undergoes molecular rearrangement to form vitamin D. Most of the vitamin D reaching the circulation is hydroxylated in the liver at the C-25 position, producing 25-hydroxy vitamin D (25-OH D). Because it has a long half-life (several weeks), 25-OH D is the best marker of vitamin D status. The most active form of vitamin D is 1,25-dihydroxy vitamin D (1,25-OH₂ D), produced in the kidney, but this form has a very short half-life (several hours).

Although the traditional biological role for vitamin D is believed to be calcium absorption and bone homeostasis, research has shown that it may be involved in many biological processes. It is also clear that, in the wake of avoidance of sun exposure, a significant percentage of the population may be vitamin D deficient. Like most laboratories around the nation, we have seen a marked increase in the number of requests for vitamin D testing. We have recently brought the test for 25-OH D in-house, and will be bringing the test for 1,25-OH₂ D in-house sometime in 2010.

As assays for vitamin D have evolved, moving from competitive protein binding and HPLC to immunoassay and mass spectrometry, the effect of laboratory variability continues to be controversial, especially with regard to recommended cut-off levels. We implemented our assay for 25-OH D (CODE :VD25H) using the new tandem mass spectrometer in the Chemistry & Immunology section of the laboratory and correlated our assay with Mayo Clinic, the reference laboratory we have



Correlation of 25-OH vitamin D3

Correlation of specimens sent to Mayo Clinic Laboratory and also tested at Stanford, showing excellent agreement. Similar agreement was seen when comparing results for 25-OH vitamin D2.

been using for specimens from Stanford Hospital and Clinics (see figure).

The alternative source of vitamin D is the diet. Endogenous vitamin D (and dietary sources of animal origin) is called D3; vitamin D from plant sources is called D2 (there is a slight difference in the side chain attached to the cholesterol nucleus of the molecules). Both are hydroxylated equally (and are believed to be equivalent biologically). We will be reporting both 25-OH D3 and 25-OH D2 levels as well as the total. Because of the excellent correlation, we will continue to use Mayo Clinic's cut-off for total 25-OH vitamin D. When we look at bringing in pediatric requests for vitamin D testing, currently sent to a different reference lab, we will be re-evaluating our entire reference range.

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NEWS YOU CAN USE (CONT.)

controversial, especially because CRP is non-specifically elevated in a variety of infectious and inflammatory disorders. We now offer a new test for hsCRP (CODE: HSCLPA) which includes a reflex to lipoprotein-associated phospholipase A2 (Lp-PLA2) if the hsCRP is elevated. Lp-PLA2 is an inflammatory marker specific for unstable atherosclerotic plaque. Patients with elevations of both hsCRP and Lp-PLA2 have been shown to be at significant risk of both acute coronary syndrome and stroke.

Clostridium difficile toxin testing - *C. difficile* is an anaerobic bacterium which produces severe diarrhea and colitis, usually in patients previously treated with antibiotics. Several weeks ago, we replaced cell culture cytotoxicity neutralization for *C. difficile* with a real-time PCR assay for the toxin B gene (CODE: CDTPCR). This new approach is more rapid and more sensitive for the diagnosis of *C. difficile*-associated diarrhea. It is only performed on liquid stool specimens (unless the patient has toxic megacolon) and cannot be used for pediatric patients (less than 1 year of age), who may harbor *C. difficile* but are asymptomatic.

Hepatitis C Virus Genotyping - Physicians treating patients who are infected with HCV benefit from knowing the viral genotype before starting therapy, as this determines duration of therapy and predicts the likelihood of treatment response. We recently brought this test in-house (CODE: HCVGE) using sequence analysis. The assay targets the core region of the HCV genome and provides both genotype and subtype information.

LABletter

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Using Hemoglobin A1c To Diagnose Diabetes

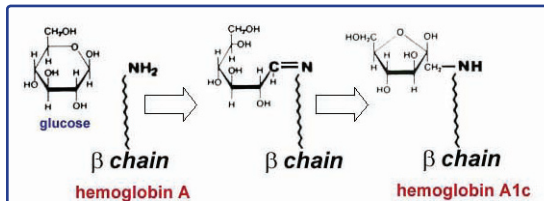
Jim Faix MD - Chemistry & Immunology Section

The diagnosis of diabetes mellitus has always depended on the detection of elevated plasma glucose levels. Cut-offs for fasting specimens or specimens drawn after glucose challenge are based on epidemiological studies linking the risk of significant microvascular complications (primarily retinal disease) to threshold glucose levels.

Accurate measurement of plasma glucose is not as easy as many people think. Instrument bias, diurnal variation, and pre-analytic factors (especially *in vitro* glycolysis by red blood cells) may complicate its use for diabetes screening. The most practical way to measure the average plasma glucose over time is glycated hemoglobin. Given the average red blood cell lifetime, glycated hemoglobin is a measure of mean blood glucose over the preceding 6-8 weeks. Hemoglobin A1c represents most of the glycated hemoglobin (see figure). As everyone knows, this fraction has been the target for managing glycemic control in known diabetic patients for many years. Could hemoglobin A1c be used to diagnose diabetes?

An international panel of experts actually considered making this recommendation when they revised the glucose cut-offs a few years ago. The same threshold effect that showed increased risk of microvascular disease above a fasting glucose of 126 mg/dl (or post-challenge glucose of 200 mg/dl) was seen with hemoglobin A1c greater than 6.5%. However, the panel did not believe that the assay for hemoglobin A1c was sufficiently standardized at that time.

Now, however, different commercial assays for hemoglobin A1c, whether they use chromatography or immunoassay approaches, are harmonized to the standardized procedure



Hemoglobin A1c

When a glucose molecule bumps into a free amino group (like the one on the terminal valine of the hemoglobin beta chain), it forms a stable fructosamine. The amount of fructosamine formation (producing hemoglobin A1c) is a reflection of the mean blood glucose over the preceding few weeks. A level of 8% or less is generally considered "good" glycemic control. The American Diabetes Association has recently recommended using hemoglobin A1c as a screening test for diabetes, instead of plasma glucose, with a diagnostic cut-off of 6.5%.

used in the original Diabetes Control and Complications Trial (DCCT), thanks to the National Glycohemoglobin Standardization Program. Also, an international working group has developed primary reference materials and proposed a new reference method, using tandem mass spectrometry.

Consequently, the panel has recently proposed that hemoglobin A1c replace plasma glucose as the diagnostic test for diabetes. Major advantages of this paradigm shift include the fact that the patient no longer needs to be fasting, and the inconvenience of glucose tolerance testing becomes a thing of the past. Beginning next month, we will change the reference range for hemoglobin A1c to <6.5% to reflect this new recommendation.

Hemoglobin A1c should not be used for screening in pregnant patients; the traditional glucose challenge approach should be used. Also, patients in whom altered red blood cell turnover may be present (e.g. hemolysis, major bleeding or recent transfusion) should be stabilized before using hemoglobin A1c as a screen for diabetes.

Mean Plasma Glucose for Diabetes Monitoring – Many patients do not fully understand the significance of hemoglobin A1c results, and struggle to relate these periodic values to their daily self-monitoring of blood glucose. We now offer a new test for hemoglobin A1c (CODE: HA1C) specifically designed for monitoring known diabetic patients. It includes the estimated mean glucose level over the past several weeks based on the patient's hemoglobin A1c level. This result (sometimes called eAG as an analogue of eGFR) will be calculated using the new equation generated by the recent study designed to address controversy over standardization of hemoglobin A1c assays.