Clinical Chemistry

August 2014 Audio Summary

This is the August 2014 issue of *Clinical Chemistry*, Volume 60, Issue 8.

On the cover this month: *Model of Human Hemoglobin*. Imagine the days when theses and dissertations were required to be written in Latin. In his 1825 *Commentatio de vera materiae sanguini purpureum colorem impertientis natura*, Johann Friederich Engelhart proposed the molecular mass of hemoglobin to be an astonishingly large 16000 Daltons per iron. No respectable scientist of the time believed that molecules could be that large. We now know that hemoglobin has a mass of ~64000 Daltons, is the major oxygen-carrying component of blood, and can bind with glucose to form glycated hemoglobin (most commonly measured as Hb A_{1c}). Because Hb A_{1c} is routinely measured for diagnosing diabetes and prediabetes, as well as monitoring mean glycemia, it is important that Hb A_{1c} results be reliable. But how well do existing Hb A_{1c} assays perform? This month's issue of *Clinical Chemistry* contains the results from 2 studies of Hb A_{1c} assay performance, plus an accompanying editorial that discusses both of the studies.

Three of 7 Hemoglobin A1c Point-of-Care Instruments Do Not Meet Generally Accepted Analytical Performance Criteria

By Erna Lenters-Westra and Robbert J. Slingerland

In 2009 the authors of this study investigated the conformance of 8 Hb A_{1c} point-of-care instruments with accepted analytical performance criteria. Since that time instruments have improved and new devices have become available on the market. These factors led the authors to conduct a second study reported here. The Clinical and Laboratory Standards Institute protocols EP-5 and EP-9 were applied to investigate imprecision, accuracy, and bias. Of the analyzers evaluated, the Afinion, DCA Vantage, Cobas B101, and B-analyst met the generally accepted performance criteria for Hb A_{1c}. The Quo-Test, Quo-Lab, and InnovaStar met the criteria for precision but not for bias. These data suggest that proficiency testing should be required for users of Hb A_{1c} point-of-care assays to assure quality.

Utilization of Assay Performance Characteristics to Estimate Hemoglobin A1c Result Reliability

By Alison Woodworth, et al.

The utilization of a newly endorsed Centers for Medicare and Medicaid Services individualized quality control plan supports performing optimal QC based on risk assessment. This study compares the combined influence of allowable total error goals, a routine quality control practice, and assay performance characteristics of 6 instruments across 4 academic medical centers on the risk of reporting unreliable Hb A1c results. Considerable differences were observed in the probability of reporting unreliable Hb A1c results between different National Glycohemoglobin Standardization Program-certified platforms. Risk estimates for Hb A1c methods in individual laboratories can be utilized to assess the residual risk of unreliable Hb A1c results.

Quantification of Serum Immunoglobulin G Subclasses by Use of Subclass-Specific Tryptic Peptides and Liquid Chromatography–Tandem Mass Spectrometry
By Paula M. Ladwig, et al.

This study describes a liquid chromatography-tandem mass spectrometry method that can multiplex all 4 subclasses along with total immunoglobulin G, or IgG, utilizing either IgG subclass peptide-specific stable isotope labeled internal standards or a surrogate digest standard for quantification. Serum was combined with labeled internal peptide standards and intact purified horse IgG. Samples were denatured, reduced, alkylated, digested, and analyzed by liquid chromatography-tandem mass spectrometry for each IgG subclass and total. Limits of detection and quantification, linearity, imprecision, and accuracy were determined for all subclasses along with total IgG. Total IgG and IgG subclasses can be quantified by liquid chromatography-tandem mass spectrometry with comparable precision, sensitivity, and accuracy to nephelometry.

Immunoextraction–Tandem Mass Spectrometry Method for Measuring Intact Human Chorionic Gonadotropin, Free β-Subunit, and β-Subunit Core Fragment in Urine
By Getachew A. Woldemariam and Anthony W. Butch

Human chorionic gonadotropin, or hCG, stimulates testosterone production, and its use in males is prohibited in most sports. Major forms of hCG that appear in urine are intact hCG, free β-subunit and β core fragment. Antidoping laboratories use immunoassays to measure urinary hCG, and the reactivity with different forms of hCG varies widely among immunoassays. The authors of this study have developed a sequential immunoextraction method with liquid chromatography-tandem mass spectrometry detection for quantification of the major forms of HCG in urine. This assay can be used to establish reference intervals in nondoping male athletes for improved doping control.

Revision of the Troponin T Release Mechanism from Damaged Human Myocardium
By Karin Starnberg, et al.

The myocardial damage marker cardiac troponin T often remains increased for over a week in patients with acute myocardial infarction. It is presumed this sustained increase results from slow degradation of myofibrils and release of irreversibly bound cardiac troponin T from necrotic cardiomyocytes. The data of this study indicate that the fraction of cardiac troponin T that can be dissociated is likely affected by local plasma flow and is substantially larger in vivo than previously reported. These findings open the possibility that the sustained increase in cardiac troponin T after acute myocardial infarction may result in part from a slow washout of cardiac troponin T that interacts reversibly with tropomyosin in myofibrils.

Use of Copy Number Deletion Polymorphisms to Assess DNA Chimerism
By Damien Luis Bruno, et al.

The authors of this study describe a novel approach for measuring cell-free DNA chimerism using ubiquitous copy number deletion polymorphisms, which allows for highly sensitive and accurate quantification of "non-self" cell-free DNA against a zero background. This approach is likely to attract widespread interest, as it offers a new approach to noninvasive monitoring of transplant health using a relatively inexpensive methodology, which can be used on a frequent basis. The authors envisage another important use for quantification of fetal cell-free DNA fraction in maternal plasma.

Liquid Chromatography High-Resolution Time of Flight Analysis: Investigation of MS^E for Broad-Spectrum Drug Screening
By Nandkishor S. Chindarkar, et al.

High resolution mass spectrometry analysis with MS^E, a method for tandem mass spectrometry data acquisition using alternating low-energy collision-induced dissociation and high-energy collision-induced dissociation, obtains precursor and fragment ion information using an untargeted approach. The authors investigated use of MS^E for broad spectrum drug screening. Overall, MS^E was able to confirm 92% of true positives with the help of fragment ion information. The confirmation rate increased to 98% when the requirement for a fragment ion was dropped but produced a higher number of false positive results. This manuscript is important to the toxicology community because it evaluates different criteria for identifying unknowns. The authors conclude that including a fragment ion is essential for unequivocal identifications using high-resolution mass spectrometry.