Clinical Chemistry

March 2018 Audio Summary

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ON THE COVER Black Sheep. As a black sheep stands out in a crowd of white sheep, a noncommutable reference material stands out among a group of clinical samples. A black sheep would be a poor choice for calibrating the color of wool for a pale herd. Similarly, a noncommutable reference material is a poor choice for calibrating measurement procedures since these procedures will then produce different results for clinical samples. This issue of *Clinical Chemistry* includes 3 Special Reports from the IFCC Working Group on Commutability that advance our understanding of commutability of reference materials with clinical samples and how commutability can be assessed.

HDL Particle Measurement: Comparison of 5 Methods
By Robert Matera, et al.

Five methods for measuring various-size HDL subfractions were compared in 98 patient samples. Vertical auto profiling measured cholesterol in large and medium/small HDL; ion mobility directly measured HDL particle numbers in large, intermediate, and small HDL; nuclear magnetic resonance calculated total lipid content in large, medium, and small HDL particle subfractions; native 2-dimensional gel-electrophoresis measured apoA-I in large, medium, and small HDL particle subfractions; and preβ-1-HDL, and preβ-1-ELISA measured apoA-I in preβ-1-HDL. There were strong discordances among the assays in measuring HDL subfractions. Nuclear magnetic resonance agreed poorly with the other methods in measuring large HDL, especially in individuals with low HDL cholesterol. Similarly, there was strong discordance in preβ-1-HDL measurements between the ELISA and native 2-dimensional gel-electrophoresis assays.

The Biological Variation Data Critical Appraisal Checklist: A Standard for Evaluating Studies on Biological Variation
By Aasne K Aarsand, et al.

The Biological Variation Data Critical Appraisal Checklist, abbreviated BIVAC, verifies whether publications on biological variation have included all elements that may impact the veracity of associated biological variation estimates. In the BIVAC, publications are rated as A, B, C, or D indicating descending compliance for 14 BIVAC quality items. Application of the BIVAC to 128 biological variation publications on enzymes, lipids, kidney, and diabetes-related measurands identified deficiencies in study detail and delivery, in particular for statistical analysis such as outlier analysis and variance homogeneity testing. Biological variability data from BIVAC compliant studies can be combined to deliver global biological variation estimates for safe clinical application.

Effect of Acute Coronary Syndrome Probability on Diagnostic and Prognostic Performance of High-Sensitivity Cardiac Troponin
By Patrick Badertscher, et al.

There is uncertainty in clinical practice regarding troponin testing in patients with low acute coronary syndrome probability. In this international diagnostic study including 3828 patients presenting to the emergency department with acute chest discomfort, this study investigated the role of high-sensitivity cardiac troponin in patients with quantified low acute coronary syndrome probability. Diagnostic and prognostic accuracy of high-sensitivity cardiac troponin remained very high in patients with acute chest discomfort and low acute coronary syndrome probability when appropriately applied as a quantitative marker. The universal standard of care using clinical assessment and the 12-lead ECG in conjunction with high-sensitivity cardiac troponin also should be applied in patients with low acute coronary syndrome probability.

Improved Detection of HER2 by a Quasi-Targeted Proteomics Approach Using Aptamer–Peptide Probe and Liquid Chromatography–Tandem Mass Spectrometry
By Weixian Zhou, et al.

HER2-positive breast cancer is a particularly aggressive type of the disease. To date, much evidence has indicated that the management of HER2-positive breast cancer can be greatly aided by early diagnosis so as to provide the corresponding therapy in time. However, current available assays for HER2 detection often lack the necessary specificity and reproducibility. In this study, the authors developed a quasi-targeted proteomics assay and applied it to the determination of HER2 levels in the HER2-positive breast cancer cells BT474 and SK-BR-3, and the HER2-negative breast cancer cells MDA-MB-231 and MCF-7, and 36 pairs of human breast primary tumors and adjacent normal tissue samples. The results were compared with those obtained by immunohistochemistry and fluorescence in situ hybridization. The values provided by the new assay were more accurate and applicable for establishment of reference intervals and further substratification.

In Situ Detection and Quantification of AR-V7, AR-FL, PSA, and KRAS Point Mutations in Circulating Tumor Cells
By Amin El-Heliebi, et al.

Numerous studies show the importance of liquid biopsies such as AR-V7 detection in castration-resistant prostate cancer or KRAS mutations in pancreatic cancer. Nevertheless, widespread application is hampered due to technical difficulties. This study reports an in situ padlock probe approach allowing visualization and quantification of clinically relevant transcripts like AR-V7 or KRAS-mutations directly in circulating tumor cells. Analysis showed that 71% of castration-resistant prostate cancer-patients had detectable AR-V7 expression and 40% of pancreatic cancer patients had KRAS-mutations. The technology is fast, applicable to several tumor entities, and can be combined with multiple circulating tumor cell-enrichment platforms, thereby adding clinically relevant information beyond simple circulating tumor cell enumeration.

Digital PCR: A Sensitive and Precise Method for KIT D816V Quantification in Mastocytosis
By Georg Greiner, et al.

The analytically sensitive detection and precise quantification of KIT D816V in blood and bone marrow is important for diagnosis and clinical management of patients with systemic mastocytosis. Here the authors performed a validation study of digital PCR for KIT D816V on 302 samples from 156 patients with mastocytosis. Digital PCR was found to be highly sensitive, specific, and precise in detecting KIT D816V, showed high concordance and no systematic deviation compared with allele-specific quantitative real-time PCR, and accurately predicted clinically relevant endpoints in systemic mastocytosis. Thus, digital PCR appears suitable as a new method for KIT D816V testing in mastocytosis.

Rapid Diagnosis of Tick-Borne Illnesses by Use of One-Step Isothermal Nucleic Acid Amplification and Bio-Optical Sensor Detection
By Ji Yeun Kim, et al.

The authors of this study compared a one-step isothermal nucleic acid amplification with bio-optical sensor detection, abbreviated iNAD, with real-time PCR tests in patients with suspected tick-borne illness, including 15 severe fever with thrombocytopenia syndrome and 21 scrub typhus. The sensitivity and specificity of iNAD for severe fever with thrombocytopenia syndrome were 100% and 86%, respectively, while those of real-time PCR were 75% and 95%. The sensitivity and specificity of iNAD for scrub typhus were 100% and 90%, respectively, while those of real-time PCR were 67% and 95%. The iNAD technique appears to be useful to rapidly rule out tick-borne illness.

Leukocyte Counts Based on DNA Methylation at Individual Cytosines
By Joana Frobel, et al.

This study describes an alternative method for white blood cell counts based on epigenetic analysis, abbreviated Epi-Blood-Count. For various subsets of leukocytes, specific CG dinucleotides were identified that are exclusively non-methylated in the respective cell types. DNA methylation levels at these sites were analyzed by pyrosequencing and implemented into deconvolution algorithms. The Epi-Blood-Count reached similar precision as conventional methods. This method also can be applied to frozen samples and very small volumes of blood. In addition, the authors designed and validated a method to determine absolute cell numbers based on a non-methylated reference DNA.

Unbiased Approach to Counteract Upward Drift in Cerebrospinal Fluid Amyloid-beta 1–42 Analysis Results
By Betty M. Tijms, et al.

Several cohorts have demonstrated gradual increases in CSF amyloid values measured by the Innotest assay over the past two decades. The authors of this study realigned year-specific cut-points for amyloid, utilizing Gaussian mixture modeling as an unbiased method to remove drift from the data. Taking year-specific cut-points as a reference, uncorrected data led to misclassification of amyloid abnormality in 15% of individuals. The approach taken in this study removed drift effects, and improved sensitivity estimates at the cost of specificity. This approach optimizes the use of CSF biomarker data collected over a long time period and might be useful to align amyloid data across cohorts.

Plasma Steroid Metabolome Profiling for Diagnosis and Subtyping Patients with Cushing Syndrome
By Graeme Eisenhofer, et al.

In this study the authors assessed whether pituitary, ectopic, and adrenal subtypes of Cushing syndrome were characterized by distinct plasma steroid profiles that might assist diagnosis. Cushing syndrome was tested for in 222 patients, among whom disease was excluded in 138 and confirmed in 84 (51 pituitary, 12 ectopic, 21 adrenal subtypes). A panel of 15 plasma steroids was measured by LC-tandem MS in all patients. Patients with pituitary, ectopic and adrenal subtypes of Cushing syndrome showed distinct steroid profiles that also differed from individuals without disease. Thus, LC-tandem MS steroid profiling may offer a single test for screening and subtyping patients with Cushing syndrome.

Low- and High-renin Heart Failure Phenotypes with Clinical Implications
By Noemi Pavo, et al.

The role of active-renin-concentration for guiding therapy in the presence of renin angiotensin system blockade in stable chronic heart failure with reduced ejection fraction remains to be established. The authors of this study measured active-renin-concentration and angiotensin profiles in heart failure patients with reduced ejection fraction who were on optimal renin-angiotensin blockade treatment. Active-renin-concentration strongly correlated with the summed concentrations of the Angiotensin I and II peptides independent of therapy mode. Thirty percent of patients showed active-renin-concentrations within the lower/normal range. Active-renin-concentration did not correlate with NT-proBNP or New York Heart Association stages of heart failure. The angiotensin I and II peptides were profoundly diminished for the low renin as compared with the high renin phenotypes in heart failure patients with reduced ejection fraction. The active-renin-concentration dependent phenotypes in this patient group offer a potential rationale for adaptive therapeutic interventions.