Clinical Chemistry

February 2018 Audio Summary

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**ON THE COVER** Total laboratory automation. Facing the pressure of doing more work with frozen or decreasing staffing resources, laboratories are increasingly considering laboratory automation in planning for future growth and workflow requirements. Although still expensive, a variety of laboratory automation solutions are available. To address the difficulties and benefits involved in implementing and sustaining total laboratory automation in a clinical laboratory setting, we include in this issue of *Clinical Chemistry* a Q&A in which 5 experts share their perspectives on laboratory automation and provide real-world advice based on their experiences at their respective facilities.

**Protein Turnover Measurements in Human Serum by Serial Immunoaffinity LC-MS/MS**
By Vahid Farrokhi, et al.

The half-life of proteins of clinical and therapeutic significance is frequently a sensitive parameter in mechanistic pharmacokinetic and pharmacodynamic modeling of biotherapeutics to predict a clinical dosing regimen or in biomarker investigations. A clinical pulse-chase study is presented based on serial immunoaffinity and mass spectrometry to measure physiologically relevant protein turnover enabling a more efficient use of limited sample quantities. Turnover of multiple human serum proteins such as tumor necrosis factor receptor superfamily member 12A, tissue factor pathway inhibitor, soluble interleukin-1 receptor-like protein, and muscle specific creatine kinase are reported from a single sample set. This work extends the application of clinical pulse-chase studies for efficient and reproducible protein turnover measurement.

**Planning Risk-Based SQC Schedules for Bracketed Operation of Continuous Production Analyzers**
By James O. Westgard, et al.

This paper considers how statistical quality control, or SQC strategies, can be planned on the basis of traditional quality management principles and new guidance from CLSI on risk management and SQC. A nomogram was developed to simplify the estimation of patient risk based on Parvin's MaxE(Nuf) parameter for the maximum estimated number of unacceptable final patient test results. Multi-stage SQC procedures were planned to provide bracketed SQC operation by including a Critical Control Point SQC "startup" design along with a "monitor" design. For high quality methods, that is those that have high observed Sigma-Metrics, multi-stage SQC designs can be implemented to implement bracketed SQC that will minimize the risk of patient harm.

**Multiplex Gene Expression Profiling of in-vivo Isolated Circulating Tumor Cells in High-Risk Prostate Cancer Patients**
By Athina N Markou, et al.

Molecular characterization of circulating tumor cells, or CTCs, is important in selecting patients for targeted treatments. The authors of this study present results on the in vivo isolation of CTCs combined with downstream molecular analysis at the RNA level. Because the captured CTCs immediately are lysed in Trizol to stabilize the nucleic acids, this approach was less susceptible to pre-analytical errors resulting from the instability of CTCs in peripheral blood during transportation of samples to central labs. The findings of this study suggest that this combined approach of in vivo isolation of CTCs with downstream molecular RNA analysis holds promise to provide a high-throughput, specific, and ultrasensitive approach for multiplex liquid biopsy based molecular diagnostics.

**Deactivated CRISPR Associated Protein 9 for Minor Allele Enrichment in Cell-Free DNA**
By Amin Aalipour, et al.

Cell-free DNA, or cfDNA, diagnostics are a new paradigm of disease management, but have limited sensitivity for rare allele detection. While enriching for rare alleles can improve sensitivity, current methods lack generalizability and applicability to point mutations. Leveraging the modular, single base-pair specific CRISPR/Cas9 system, the authors of this study introduce a dCas9-based method capable of efficient multiplexed allele enrichment. Notably, in a cohort of 18 non-small cell lung cancer patient-derived cfDNA samples, enrichment enabled detection of 8 out of 13 otherwise undetectable mutations. This work represents an important application of the CRISPR/Cas9 system and is a significant advance in the sensitivity of cfDNA diagnostics.

**Multiple Hotspot Mutations Scanning by Single Droplet Digital PCR**
By Charles Decraene, et al.

To fulfill the need for universal tools for molecular cancer diagnostics, the authors of this study established two single droplet digital PCR assays detecting all genomic alterations within KRAS and EGFR mutation hotspots, which are of clinical importance in colorectal and lung cancer. They demonstrate that their method is highly sensitive and can be applied to many types of molecular alterations at once while consuming a minimum of the patient sample. This makes it a tool of interest for diagnostic purposes, and optimization of more of this type of ‘universal’ single droplet digital PCR assays targeting other key regions containing mutations used for therapeutic decisions could improve molecular cancer diagnosis.

**Incidental Detection of Maternal Neoplasia in Non-invasive Prenatal Testing**
By Nilesh G Dharajiya, et al.

When a tumor is present at the time of noninvasive prenatal testing, the tumor may contribute to the pool of cell-free DNA rendering the laboratory test result uninformative for fetal abnormalities. During clinical exploration of possible causes, maternal tumors were identified in a subset of at least 40 patients from noninvasive prenatal testing examinations in over 450,000 pregnant patients. To date, this is the most comprehensive report of detection of a variety of maternal neoplastic conditions using genome-wide sequencing of cfDNA for noninvasive prenatal testing. Since noninvasive prenatal testing has been widely adopted in prenatal care, this paper provides useful information to scientists, physicians, and patients.

**Noninvasive Prenatal Diagnosis of Single-Gene Disorders Using Droplet Digital PCR**
By Joan Camunas-Soler, et al.

Prenatal diagnosis of pregnancies at risk of single-gene disorders is currently performed using invasive techniques such as amniocentesis. In this study the authors employed a simple droplet digital PCR protocol for noninvasive prenatal diagnosis of single-gene disorders. The method can be used both when a mutation is shared by both progenitors or for heterozygous compound mutations. This method enables measurement of samples as early as week 11, and with fetal fraction as low as 3.7 +/- 0.3%. This work also highlights the importance of accurately measuring fetal fraction to implement noninvasive prenatal testing of single-gene disorders that can be used in the general population.

**Human Toxicity Caused by Indole and Indazole Carboxylate Synthetic Cannabinoid Receptor Agonists: From Horizon Scanning to Notification**
Simon L Hill, et al.

The authors of this study wished to detect and monitor Novel Psychoactive Substances being abused and causing harm in the UK. They recruited 160 patients with severe features of suspected Novel Psychoactive Substance toxicity presenting to UK emergency departments. They collected clinical data and analyzed biological fluids using non-targeted data-independent LC-MS/MS. Indole and indazole carboxylate synthetic cannabinoids were used to demonstrate the components of a toxicovigilance system. Having anticipated the emergence of these synthetic cannabinoids in the UK, the authors detected them in clinical practice, characterized their toxicity, and notified international agencies. Collaboration between synthetic and analytical chemistry and clinicians is required to understand the harms caused by drug abuse.

**Imputation of Baseline LDL-C Concentration in Patients with Familial Hypercholesterolemia on Statins or Ezetimibe**
By Isabelle Ruel, et al.

Familial hypercholesterolemia is the most common genetic disorder with a prevalence of 1 in 250. Prompt recognition and treatment can prevent cardiovascular disease. It is often underdiagnosed. The authors of this study provide a tool to impute the baseline untreated concentration of LDL cholesterol based on the current lipid-lowering medications. They examined 951 patients with familial hypercholesterolemia who were initiated on statin or ezetimibe and show that the imputation of LDL cholesterol is highly accurate, allowing clinicians to raise awareness of the condition, initiate treatment and cascade screening in first-degree relatives.

**New Insights into Cardiac and Vascular Natriuretic Peptides: Findings from Young Adults Born with Very Low Birth Weight**
By Timothy CR Prickett, et al.

Plasma natriuretic peptides reflect cardiovascular risk in community studies but have not been studied in subjects born with very low birth weight where risk is increased in later life. The results of this study demonstrated that at age 28 years, compared to controls, only C-type natriuretic peptide differed among the many humoral risk factors measured. Associations of C-type natriuretic peptide with cardiovascular risk were all positive whereas B-type natriuretic peptide associations were all negative. The dissociated response likely results from beneficial genetic B-type natriuretic peptide polymorphisms that reduce risk, and an adaptive response to vascular stress raising C-type natriuretic peptide. Combining both measurements may provide a novel index of ideal cardiovascular health.

**Increased Plasma Ferritin Concentration and Low-Grade Inflammation – A Mendelian Randomization Study**
By Ingrid Wahl Moen, et al.

This study combined observational and genetic data on increased ferritin concentration to investigate its influence on low-grade inflammation in 62,537 individuals from the Danish general population. Plasma concentrations of C-reactive protein and complement component 3 served as indicators of inflammatory response. A Mendelian randomization approach was applied, using the homozygous hemochromatosis genotype as an instrument for increased plasma ferritin concentration, to assess causality. Increased plasma ferritin concentration was found associated observationally and genetically with low-grade inflammation, possibly indicating a causal relationship from increased ferritin to inflammation. However, these findings are tempered by the pleiotropism of the hemochromatosis gene associated with its role in immune response, and the incomplete penetrance of the homozygous genotype that could have led to biased estimates of the genetic association.

**Prospective Validation of a Biomarker-Based Rule-Out Strategy for Functionally Relevant Coronary Artery Disease**
By Joan E Walter, et al.

There is a major unmet clinical need for an easily applicable, safe, noninvasive and cost-effective rule-out method for coronary artery disease. Employing the most sensitive measurement technology for high-sensitivity cardiac troponin I currently available, this study aimed to validate a rule-out strategy for inducible myocardial ischemia as pathophysiological hallmark of functionally relevant coronary artery disease. A single very low resting plasma high-sensitivity cardiac troponin I concentration ruled out functionally relevant coronary artery disease with 95% sensitivity in around 10% of patients without known coronary artery disease and additionally was associated with a low risk for myocardial infarction or cardiovascular death during follow up.