Clinical Chemistry

September 2017 Audio Summary

This is the September 2017 issue of *Clinical Chemistry*, Volume 63, Issue 9.

ON THE COVER Woman clutching her painful chest. There are profound differences between the sexes regarding cardiac physiology and the development of heart disease. These differences can affect the concentrations of circulating diagnostic and prognostic biomarkers. Cardiac troponin (cTn) has become the gold standard biomarker for the detection of myocardial infarction and, given the observed differences in the distribution of cTn concentrations between the sexes, expert task forces have endorsed the use of sex-specific cut-offs for high-sensitivity (hs) cTn assays. However, does the use of sex-specific hs-cTn 99th percentiles really affect clinical management and improve outcome prediction compared to a single 99th percentile for both men and women? Are we adding unnecessary complexity to the decision-making process? This issue of *Clinical Chemistry* contains a review of the published studies investigating the interrelation between hs-cTn and sex, diagnostic classification and outcome prediction in different settings.

Synthetic Circulating Cell-free DNA as Quality Control Materials for Somatic Mutation Detection in Liquid Biopsy for Cancer
By Rui Zhang, et al.

The authors of this study developed a set of synthetic cell-free DNA quality control materials which are comprised of spike-in cell-free DNA carrying somatic mutations based on micrococcal nuclease digestion, and matched genomic DNA as genetic background. To prove their suitability these synthetic cell-free DNA QC materials were compared with patient derived plasma samples and evaluated in a collaborative study that encompassed 11 laboratories. The results of analysis of these materials showed strong agreement with those of patient-derived plasma samples, including the size profile of cell-free DNA and the quality control metrics of the sequencing data. The synthetic cell-free DNA QC materials were successfully applied across a broad range of laboratories, methodologies, and informatics techniques. Synthetic cell-free DNA QC materials can be utilized as optimal quality controls in test performance assessments for circulating tumor DNA somatic mutation detection.

Technical Stability and Biological Variability in MicroRNAs from Dried Blood Spots: A Lung Cancer Therapy-Monitoring Showcase
By Mustafa Kahraman, et al.

Although there are abundant scientific data on microRNAs, circulating microRNAs are not measured in clinical routine. The first step of the workflow, collecting blood from patients, is known to have a substantial influence on diagnostic result. The authors of this study examined the use of dried blood spots as an inexpensive alternative for measuring microRNAs in a stable and reproducible manner. The microRNA profiles were found to be consistent and independent of environmental factors. In a proof-of-concept study employing dried blood spot samples from patients receiving different lung cancer therapies the study demonstrated that biological variation of microRNA profiles significantly exceeded the technical variation. The study authors propose a stable workflow for profiling of whole microRNomes based on samples collected from dried blood spots that will help facilitate the translation of this approach to clinics.

United States and European Multicenter Prospective Study for the Analytical Performance and Clinical Validation of a Novel Sensitive Fully Automated Immunoassay for Calcitonin
By George J Kahaly, et al.

Sensitive, reliable assays for serum calcitonin are warranted for an accurate diagnosis of medullary thyroid cancer. This study evaluated the analytical performance and validated a novel, electrochemiluminescence immunoassay for calcitonin. Reference cut-offs were derived from large collectives of healthy controls in Europe and the US. Diagnostic sensitivity and specificity were 100% and 96.4%. Compared to available calcitonin assays, lower limits of detection, quantification, and lower CVs were noted. Excellent analytical performance, low inter-individual variability, and low impact of confounders for increased calcitonin concentrations in non-medullary thyroid cancer patients revealed the clinical utility of the investigated assay to be appropriate.

Analytically Sensitive Protein Detection in Microtiter Plates by Proximity Ligation with Rolling Circle Amplification
By Tonge Ebai, et al.

This research was focused on developing a new enhanced sensitive and specific tool for protein detection in serum, plasma, or blood. There is a need to increase the specificity and sensitivity of the traditional methods for detecting proteins. The authors of this study set out to adapt the proximity ligation assay and rolling circle amplification into the instruments used in clinics and routine diagnostic laboratories. They developed assays for low abundance cytokines and were able to demonstrate increased sensitivity and dynamic range for a set of proteins over ELISA. This work suggests that low abundance proteins can be detected and this capability represents a unique tool for early disease diagnosis.

Reducing Artifactual EGFR T790M Mutations in DNA from Formalin-Fixed Paraffin-Embedded Tissue by Use of Thymine-DNA Glycosylase
By Hongdo Do, et al.

False positive EGFR T790M mutations have been reported in formalin-fixed lung tumors. This study reports two major findings. First, the cytosine of the EGFR T790 position which is part of a CpG dinucleotide is methylated at high levels in normal tissues and lung tumors. Secondly, because cytosine and 5-methycytosine undergo deamination to uracil and thymine causing artifactual EGFR T790M mutations, enzymatic removal of the resultant U:G and T:G mismatches with uracil- and thymine-DNA glycosylase markedly reduces the artifactual EGFR T790M mutations. This is the first report of the use of thymine-DNA glycosylase to reduce sequence artifacts in formalin-fixed DNA.

Identification, Confirmation, and Replication of Novel Urinary MicroRNA Biomarkers in Lupus Nephritis and Diabetic Nephropathy
By Mariana Cardenas-Gonzalez, et al.

Given the current challenges of traditional biomarkers of renal function in identifying underlying diagnoses in chronic kidney disease, the authors of this study set out to identify novel urinary microRNAs associated with diabetic nephropathy and lupus nephritis. In biopsy-confirmed patients they conducted a discovery profiling of 2402 microRNAs, followed by the confirmation, and replication of the differentially expressed microRNAs in two independent patient cohorts. MicroRNA candidates were found to be associated with functional markers and relevant histopathological lesions. Moreover, the candidate microRNAs were able to discriminate between patients with renal disease and either healthy or diseased controls. These biomarkers appear useful to facilitate the non-invasive diagnosis of diabetic nephropathy and lupus nephritis.

The EuBIVAS Project: Within- and Between-Subject Biological Variation Data for Serum Creatinine Using Enzymatic and Alkaline Picrate Methods and Implications for Monitoring
By Anna Carobene, et al.

Estimates of the biological variation of serum creatinine have been obtained from the EuBIVAS cohort using enzymatic and Jaffe methods. Analyses were performed in duplicate within a single run on an ADVIA 2400. The data were subjected to outlier and homogeneity analysis prior to CV-ANOVA. The within-subject biological variation estimates were similar for both methods, and lower than the estimates available online. Between-subject biological variation estimates were similar to historical biological variation data. The analytical coefficient of variation calculated was 1.1% for the enzymatic and 4.4% for Jaffe methods. The serum creatinine biological variation estimates obtained in the current study pose a more stringent performance specification than previously identified. The Jaffe method failed to meet the imprecision goal, raising questions regarding its future use.