Clinical Chemistry

February 2014 Audio Summary

This is the February 2014 issue of *Clinical Chemistry*, Volume 60, Issue 2.

On the cover this month: *Baseball bat, ball, glove*. Baseball, endearingly called America's Pastime, is played by millions of children and adults every year. Coaches teach each player to follow the advice of the sportswriter Henry Grantland Rice, who wrote, "It's not whether you win or lose, it's how you play the game." Some individuals believe that this piece of advice has at its core the belief that "losing while playing fair is better than winning by cheating." Unfortunately, the desire to win at all costs has caused athletes to cheat by doping to enhance sport performance for centuries. The issue of doping arises again this month as the 2014 Winter Olympics begin. Currently, urine and blood are the only matrices authorized for antidoping testing by the World Anti-Doping Agency. Although the usefulness of urine and blood is proven in antidoping testing, issues remain for monitoring some drug classes and in cases involving drugs prohibited only in competition. The alternative matrix oral fluid may offer solutions to some of these issues. In this month's issue of *Clinical Chemistry* two scientists from the National Institute of Drug Abuse discuss the present state of knowledge, advantages and limitations of oral fluid testing, and the research needed to advance oral fluid testing as a viable alternative for antidoping testing.

Flexible Micro Spring Array Device for High-Throughput Enrichment of Viable Circulating Tumor Cells
By Ramdane A. Harouaka, et al.

In this article the authors introduce a flexible micro spring array device that implements an innovative design to enrich circulating tumor cells directly from whole blood samples. The polymer springs act as effective filtration structures at the micro scale to isolate cancer cells based on their size and deformability, independent of antigen expression. Flexibility, pressure regulation, and a high porosity design preserved cell viability, while allowing full tubes of blood to be processed in under 10 minutes. Tumor cells were successfully isolated from clinical samples obtained from advanced cancer patients and analyzed for the presence of microclusters and a marker for epithelial to mesenchymal transition.

Influence of PCR Reagents on DNA Polymerase Extension Rates Measured on Real-Time PCR Instruments
By Jesse L. Montgomery and Carl Thomas Wittwer

Measurement of the speed of DNA extension on a template, i.e., DNA polymerization, has been difficult and requires radioactivity. The authors of this article present a solution to this problem. Using common real-time PCR instruments, the authors have introduced a method to measure DNA extension and study the effect of common PCR reagents on extension. Since PCR is used in all of molecular diagnostics, and the findings from the study are surprising, this tool should enable others to improve diagnostic testing.

Comparison of mRNA Splicing Assay Protocols across Multiple Laboratories: Recommendations for Best Practice in Standardized Clinical Testing
By Phillip Whiley, et al.

This article covers the accurate diagnostic testing of breast cancer susceptibility. The study was undertaken in response to the knowledge that many current tests fail to identify diseases that cause changes in the structure of cancer gene products. The authors approached this problem by comparing the methods performed by multiple laboratories in an international consortium. The results showed that controlling certain aspects of these methods was critical for the analytical sensitivity and accuracy of the test. These results will have implications for the design of all tests analyzing the effects of gene defects on the structure of the gene product.

Calcineurin Activity Assay Measurement by Liquid Chromatography–Tandem Mass Spectrometry in the Multiple Reaction Monitoring Mode
By Lynn Carr, et al.

The measurement of calcineurin phosphatase activity, in addition to blood drug concentrations, has been proposed to improve immunosuppression monitoring in transplant patients treated with cyclosporine or tacrolimus. In response to the current absence of a simple, robust, and high-throughput assay, the authors developed and validated a novel calcineurin activity assay that used HPLC coupled to multiple reaction monitoring tandem mass spectrometry to measure dephosphorylation of a synthetic peptide substrate. The high level of standardization, rapidity, and reliability of the method allowed the authors to record, in a clinical context, calcineurin activity in large cohorts of transplanted patients under calcineurin inhibitor therapy.

Urinary Cannabinoid Disposition in Occasional and Frequent Smokers: Is THC-Glucuronide in Sequential Urine Samples a Marker of Recent Use in Frequent Smokers?
By Nathalie A. Desrosiers, et al.

Cannabis (or THC) is the most commonly abused illicit drug worldwide. Although phase I cannabinoid metabolite disposition in urine is well documented, much less is known about phase II metabolites. Here the authors recruited 14 frequent and 10 occasional smokers to comprehensively document the disposition of 7 cannabinoids in human urine for 30 hours following ad libitum smoking of a 6.8% Δ^9-tetrahydrocannabinol cigarette. The authors then developed a model to identify recent cannabis smoking by measuring THC-glucuronide in urine in sequentially collected samples. These data will improve urinary cannabinoid interpretation in various drug testing programs.

Newborn Blood Spot Screening for Sickle Cell Disease by Using Tandem Mass Spectrometry: Implementation of a Protocol to Identify Only the Disease States of Sickle Cell Disease
By Stuart J. Moat, et al.

Technologies currently used for newborn screening of sickle disease identify both the disease and carrier states, resulting in large numbers of infants being referred for follow-up. Here the authors developed a screening protocol using tandem mass spectrometry and cutoffs based on the ratio of the variant peptide to wild-type peptide abundance for hemoglobin S, C, D^{Punjab}, O^{Arab}, Lepore, and E peptides. The protocol was robust for routine screening use and could reduce the cost of the screening program by preventing large numbers of carrier infants being identified and avoiding unnecessary follow-up testing and referral for genetic counseling.

False Biomarker Discovery due to Reactivity of a Commercial ELISA for CUZD1 with Cancer Antigen CA125
By Ioannis Prassas, et al.

Using a commercially available assay the authors had previously identified CUB and zona pellucida-like domains protein 1 (known as CUZD1) as a promising new biomarker of pancreatic ductal adenocarcinoma. Their objective in this study was to characterize the specificity of this commercial assay. Proteomic characterization of the antibody specificity, direct mass-spectrometric analyses of immunoreacting biological fluids, and in-house cloning and expression of pure CUZD1 allowed them to establish that instead of CUZD1, this commercial assay recognized a nonhomologous, known cancer antigen (CA125). This documented case of false biomarker discovery highlights the dangers of using commercial kits that have not been thoroughly validated and should encourage the use of more stringent quality assurance procedures.

High-Sensitivity Troponin as a Predictor of Cardiac Events and Mortality in the Stable Dialysis Population
By Hicham Cheikh Hassan, et al.

This study examined the clinical use of fifth-generation high-sensitivity troponin T as a biomarker to predict adverse events in 393 dialysis patients over a 1-year period. Peritoneal dialysis patients were included in the study since they are classically underrepresented. Hemodialysis patients demonstrated a 10% proportional reduction in high-sensitivity troponin concentrations between pre- and postdialysis. High-sensitivity troponin was a good predictor of cardiovascular events and death in both the hemodialysis and peritoneal dialysis patients over a 1-year period. When assays were repeated after 1 year little variation in levels was observed from baseline, even in patients who sustained a cardiac event.

Quality Markers Addressing Preanalytical Variations of Blood and Plasma Processing Identified by Broad and Targeted Metabolite Profiling
By Beate Kamlage, et al.

Metabolomics is a valuable tool with applications in almost all life science areas. The response of the human plasma metabolome to preanalytical variation demands implementation of thorough quality assurance and quality control measures. Tools to detect the effects of both blood and plasma processing are a key for obtaining reproducible and credible results from metabolomics studies. In this article the authors report on the response of the human plasma metabolome to preanalytical variations in a comprehensive mass spectrometry-based metabolomics analysis revealing such quality markers. Common preanalytical confounders resulted in large statistically significant alterations of the analyzed plasma metabolome.