Clinical Chemistry

December 2013 Audio Summary

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On the cover this month: Droplet digital PCR. In this new generation of PCR technology, droplet microfluidic systems are used to create and analyze nanoliter to picoliter droplets, which enables simple digital PCR workflows that yield highly sensitive mutation detection within complex DNA mixtures. This issue of *Clinical Chemistry* contains two original research articles that illustrate the application of digital droplet PCR for measuring cell free nucleic acids. In the first article researchers use this technology to detect *KRAS* mutations in circulating DNA from the plasma of patients with colorectal cancer. In the second article researchers use digital droplet PCR for the rapid quantification of donor DNA in the circulation of transplant recipients as a potential biomarker of graft injury. An accompanying editorial highlights both of these articles.

Multiplex Picodroplet Digital PCR to Detect *KRAS* Mutations in Circulating DNA from the Plasma of Colorectal Cancer Patients
By Valerie Taly, et al.

The authors of this study demonstrated the feasibility of applying digital PCR technology to analyze circulating tumor DNA from liquid biopsies. They employed picoliter droplet technology to enable multiplexing with the concurrent detection of multiple genetic targets. DNA extracted from plasma samples of colorectal cancer patients was analyzed to measure the concentration of the 7 most frequent mutations of *KRAS*. The digital PCR results in plasma from 50 patients were congruent with quantitative PCR characterization of DNA from matched tumors. Noninvasive liquid biopsies could be used for real-time assessment of tumor mutation status without reliance on archival specimens or invasive biopsy procedures.

Digital Droplet PCR for Rapid Quantification of Donor DNA in the Circulation of Transplant Recipients as a Potential Universal Biomarker of Graft Injury
By Julia Beck, et al.

This paper describes the use of digital PCR for detection of circulating graft-derived cell-free DNA in solid organ recipients. Digital PCR simplifies the measurement of this potential rejection biomarker so that it can be routinely used. The high increase of graft cell-free DNA observed in liver rejection makes cell-free DNA a potentially universal biomarker for early detection of rejection in transplantation.

Human miRNome Profiling Identifies MicroRNAs Differentially Present in the Urine after Kidney Injury
By Krithika Ramachandran, et al.

Extracellular microRNAs have been proposed as potentially accurate diagnostic markers of various disease conditions and hold the capacity to translate from bench to clinic. While kidney disease is receiving increased global attention, traditional markers of acute kidney injury suffer from limitations of clinical sensitivity, specificity, and timeliness of diagnosis. The objective of this study was to identify differentially expressed urinary microRNAs as biomarkers for acute kidney injury. This study describes the first clinical evaluation of 1809 microRNAs in human urine and identifies 4 microRNAs: microRNA-21, microRNA-200c,

microRNA-423, and microRNA-4640 as noninvasive and sensitive indicators of kidney damage.

Neutrophil CD64 for Daily Surveillance of Systemic Infection and Necrotizing Enterocolitis in Preterm Infants
By Hugh Simon Lam, et al.

This is a large prospective cohort study in which very low birth weight infants underwent daily surveillance for late onset sepsis with a neutrophil CD64 cell surface marker assay. The study goal was to evaluate whether the CD64 results could facilitate diagnosis of bacterial infection earlier than is possible by means of the clinical presentation. The results indicate that measuring circulating neutrophil CD64 in very low birth weight infants can allow neonatologists to diagnose bacterial infections a mean of 1.5 days before clinical manifestations become evident. Earlier diagnosis in this fashion comes at the expense of a moderate increase in the number of sepsis evaluations performed.

Effect of *CYP3A4*22*, *POR*28*, and *PPARA* rs4253728 on Sirolimus In Vitro Metabolism and Trough Concentrations in Kidney Transplant Recipients
By Jean-Baptiste Woillard, et al.

Recent studies have identified new candidate gene polymorphisms related to cytochrome P450 3A activity or calcineurin inhibitor dose requirements in kidney transplant recipients. The authors of this study investigated the impact of these polymorphisms on the in-vitro metabolism of sirolimus by liver microsomes as well as on-sirolimus trough concentrations and rates of adverse events in 113 kidney transplant recipients. The *CYP3A4*22* allele resulted in a moderate but significant decrease of sirolimus in vitro metabolism but only the *POR*28* allele affected patient trough concentrations, and this did not have any effect on the dose administered. These polymorphisms do not seem to substantially influence the pharmacokinetics of sirolimus or the occurrence of sirolimus adverse events in kidney transplant recipients.

Oral Fluid Cannabinoids in Chronic Cannabis Smokers during Oral Δ9-Tetrahydrocannabinol Therapy and Smoked Cannabis Challenge
By Dayong Lee, et al.

The frequency of oral fluid drug testing is increasing in clinical practice, but research on oral fluid cannabinoids is limited after oral dosing with Δ9-tetrahydrocannabinol (also known as THC). This study investigated oral fluid cannabinoid disposition during 4 5-day oral THC sessions and following smoked cannabis challenge on the last day of each medication session. Parent cannabinoid concentrations continued to decrease over time, whereas the THC metabolite carboxy-THC showed dose-dependent concentration changes. Higher cannabinoid concentrations following active oral THC versus placebo suggest a compensatory effect of THC tolerance on smoking topography. The findings demonstrate cannabinoid oral testing to be a promising monitoring tool in oral THC pharmacotherapy.

Cannabinoids in Exhaled Breath following Controlled Administration of Smoked Cannabis
By Sarah K. Himes, et al.

In this study, the amounts of Δ9-tetrahydrocannabinol (also known as THC), 11-nor-9-carboxy-THC, and cannabinol were simultaneously quantified in breath following controlled cannabis smoking to characterize the time course and window of detection of breath cannabinoids. Breath specimens were collected from chronic and occasional cannabis smokers. THC was the major cannabinoid detected in breath; no specimen contained 11-nor-9-carboxy-THC and only 1 contained cannabinol. Breath cannabinoids have a short detection window of 0.5-2h after cannabis smoking, coinciding with possible impairment 1-2h after smoking, making this alternative matrix potentially applicable for "driving under the influence" and “for cause” workplace cannabinoid drug testing; however, the driving impairment window extends beyond the breath cannabinoid detectability.

Diagnosing Diabetes Mellitus: Performance of Hemoglobin A_{1c} Point-of-Care Instruments in General Practice Offices
By Una Ørvim Sølvik, et al.

Hemoglobin A_{1c} has been recommended for the diagnosis of diabetes when it is measured by hospital laboratory instruments, but not when measured by point of care instruments. Results from 13 hemoglobin A_{1c} external quality assurance surveys from hospital laboratory instruments and from general practice offices using point-of-care instruments were compared against the recommended analytical quality specifications for the use of hemoglobin A_{1c} in diabetes diagnosis. A large proportion of general practice offices using the point-of-care instruments from Afinion and DCA fulfilled the analytical quality requirements for diagnosing diabetes. Their analytical quality was comparable to hospital laboratory instruments, and, in circumstances where this is true, point-of-care hemoglobin A_{1c} methods performed in selected general practice offices can be recommended for use in the diagnosis of diabetes.

Troponin T and N-Terminal Pro–B-Type Natriuretic Peptide: A Biomarker Approach to Predict Heart Failure Risk—The Atherosclerosis Risk in Communities Study
By Vijay Nambi, et al.

Heart failure is projected to have the largest increases in incidence among the various cardiovascular diseases in the coming decades. In this paper the authors characterize the value of two biomarkers (troponin and NT-pro B-type natriuretic peptide) in the prediction of heart failure. Their study demonstrates that the biomarkers improve heart failure prediction when added to clinical heart failure prediction models. Additionally, a simple model that included age, race, gender, and the biomarkers and can be incorporated in a lab report was found comparable to clinical risk prediction models. These results will help the development of better estimates of heart failure risk in both clinical practice and clinical trials.