Clinical Chemistry

April 2018 Audio Summary

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ON THE COVER Matthew McQueen. A gentle Scotsman, McQueen initially made his mark as an accomplished debater who won the most coveted prize in all of British debating. Through debating, McQueen saw in himself abilities he did not know he had. After finishing his medical degree, he decided to pursue a PhD while working full time in the clinic. His research overturned assumptions about how cardiac enzymes could be measured. A continuously energetic man who gave up his clinical work in 2014, he still directs the Clinical Trial Research Laboratory and Biobank at Hamilton General Hospital. If that is not enough, he wrote a master's thesis in history in 2015. Matt McQueen has the tendency to attribute his accomplishments to serendipity. But anyone who reads this month's "Inspiring Minds" feature will see that there is much more to the life of this man than serendipity.

Comparison of Generic Fluorescent Markers for Detection of Extracellular Vesicles by Flow Cytometry
By Leonie de Rond, et al.

A generic fluorescent marker to label all extracellular vesicles is desirable. Here the authors evaluated five generic markers for detection of extracellular vesicles in plasma by flow cytometry. None of the markers labeled all—and only—extracellular vesicles. The highest concentrations of extracellular vesicles were detected by lactadherin and side scatter triggering, and a choice between these alternatives would depend primarily on the analytical sensitivity of the flow cytometer used. The lack of specificity of generic markers and the analytical sensitivity of the flow cytometer should be taken into account by all scientists using flow cytometry to detect extracellular vesicles.

Specific Substrate for the Assay of Lysosomal Acid Lipase
By Sophia Masi, et al.

Lysosomal acid lipase deficiency can now be treated with enzyme replacement therapy. In this study the authors have developed the first substrate for this enzyme that can be used to measure its enzymatic activity in dried blood spots.

Measurement of Lipoprotein-Associated Phospholipase A2 by Use of 3 Different Methods: Exploration of Discordance between ELISA and Activity Assays
By Celalettin Topbas, et al.

Lipoprotein Associated Phospholipase-A2 (Lp-PLA2) is used as a biomarker to assess risk of coronary heart disease. The well-documented discordance between the measured concentration and activity of this enzyme is unexplained. Here the authors have developed mass spectrometry-based assays to quantify serum Lp-PLA2 activity and concentration, and investigated their correlation with commercial assays. LC-MS/MS method measured higher concentrations of Lp-PLA2 than commonly used immunoassays and the results compared well with enzymatic activity. Further investigation revealed that immunoassays suffer from interferences due to Lp-PLA2-lipoprotein complexes. This study illustrates the advantages of quantitative LC-MS/MS for measurement of Lp-PLA2 concentration or activity in clinical applications.

Allele-Specific Droplet Digital PCR Combined with a Next-Generation Sequencing-Based Algorithm for Diagnostic Copy Number Analysis in Genes with High Homology: Proof of Concept Using Stereocilin
By Sami S. Amr, et al.

Copy number variants cannot be easily detected in medically relevant genes that have substantial homology to other genomic sequences. Here the authors describe an approach combining next generation sequencing-copy number variant calling and allele specific droplet digital PCR for diagnostic copy number variant detection in such genes. As a proof of concept, the study demonstrates the technical performance and clinical utility in a hearing loss gene, STRC, that has ~99% identity with its pseudogene, pSTRC. This approach markedly contributed to the diagnostic yield in a cohort of 517 patients with hearing loss. More importantly, this approach was reliable, affordable, and potentially scalable to any other gene with similar issues.

Advanced Whole-Genome Sequencing and Analysis of Fetal Genomes from Amniotic Fluid
By Qing Mao, et al.

In this study the authors sequenced the whole genomes of 31 fetuses using amniotic fluid. To do so they developed novel methods for obtaining whole genome sequencing data from small amounts of cell-free DNA. Whole genome sequencing was possible from cell-free DNA and the results were highly accurate and reproducible. The authors conclude that whole genome sequencing can be used on amniocentesis samples. Improved databases will help better understand the results.

Prognostic Implications of Multiplex Detection of KRAS Mutations in Cell-Free DNA from Patients with Pancreatic Ductal Adenocarcinoma
By Min Kyeong Kim, et al.

This study evaluated quantitative multiplex KRAS mutations in cell-free DNA from 106 patients with pancreatic ductal adenocarcinoma. The results showed that KRAS mutation concentration and fraction abundance have prognostic implications for progression-free survival, particularly in the resectable group by both univariate and multivariate analysis. Moreover, the KRAS mutation concentration when combined with CA19-9 concentration showed additive predictive value for overall survival. These data suggest that multiplex KRAS mutation detection using cell free DNA has prognostic value in resectable pancreatic ductal cancer patients.

Resolution of Spurious Immunonephelometric IgG Subclass Measurement Discrepancies by LC-MS/MS
By Jessica Grace van der Gugten, et al.

To investigate discrepant immunonephelometric IgG subclass test results, the authors of this study designed a mass spectrometric gold standard method for the individual IgG subclass concentrations to act as a comparator. Immunonephelometric methods from two vendors showed abnormalities in patient samples with high IgG4 concentrations. Multivariate linear regression demonstrated that the immunonephelometric measurement values for IgG2 (or IgG1) were best predicted by an equation combining the mass spectrometric measurement of IgG2 (or IgG1) and IgG4. These results may reflect unique physicochemical properties of the IgG4 subclass, which can non-specifically bind with immunoglobulins and could therefore be incidentally measured in an immunonephelometric method.