This is the January 2016 issue of *Clinical Chemistry*, Volume 62, Issue 1.

On the cover this month, Collision, by Dina Greene. This image, by AACC member Dina Greene, was selected to highlight our 2016 special issue devoted to clinical mass spectrometry and its prominence in laboratory medicine. Dina Greene describes this artistic creation as follows: "In clinical chemistry arguably one of the most abstract methods to visualize is mass spectrometry. Collision was created with the intention of translating the textbook explanation of this powerful analytical technique into a visual representation using encaustics, a wax-based medium, and oil paint. The image captures a snapshot of the quadrupole from an ion's perspective." Indeed, just like art, mass spectrometry has both power and beauty, as you will discover as you read the wide variety of articles contained in this special issue.

High-Resolution Mass Spectrometry for Characterizing the Metabolism of Synthetic Cannabinoid THJ-018 and Its 5-Fluoro Analog THJ-2201 after Incubation in Human Hepatocytes
Xingxing Diao et al.

New synthetic cannabinoids constantly emerge on the drug market and are not detectable by standard drug testing. Using high-resolution mass spectrometry, the authors of this study characterized the metabolism of two novel synthetic cannabinoids, THJ-018 and THJ-2201 in human hepatocytes and proposed optimal metabolite markers for documenting their intake. These studies provide THJ-018 and THJ-2201 targets to be included in a new urine drug testing panel. The analysis strategy by high-resolution mass spectrometry and hepatocytes also is applicable for further studies of newly emerging novel psychoactive substances.

Comparison of Information-Dependent Acquisition on a Tandem Quadrupole TOF vs a Triple Quadrupole Linear Ion Trap Mass Spectrometer for Broad-Spectrum Drug Screening
Katie L. Thoren et al.

Liquid chromatography high-resolution mass spectrometry offers several advantages over nominal mass, selected-reaction-monitoring-based techniques for broad-spectrum drug screening. In order to determine if high-resolution mass spectrometry with untargeted data collection could be used as an alternative to selected-reaction-monitoring -based methods, the authors of this study developed a broad-spectrum drug screen on a quadrupole time-of-flight instrument and compared its performance to a triple quadrupole linear ion trap method. Using 100 routine clinical urine samples, they compared the methods' ability to identify drugs using library searching. Overall, the performance was similar enough that they conclude quadrupole time-of-flight could serve as an alternative for quadrupole linear ion trap in general unknown screening.

Measurement by a Novel LC-MS/MS Methodology Reveals Similar Serum Concentrations of Vitamin D Binding Protein in Blacks and Whites
Clark M. Henderson et al.

Previous studies estimating the concentration of free and bioavailable vitamin D have relied on immunoassays that give differing results for vitamin D binding globulin. In this study the authors developed and validated a novel LC-MS assay to measure vitamin D binding globulin and used it to reveal that plasma

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concentrations of vitamin D binding globulin appear to be similar in blacks and whites. The assay could permit more robust assessments of free and bioavailable vitamin D in future epidemiological studies.

Automated Multiplex LC-MS/MS Assay for Quantifying Serum Apolipoproteins A-I, B, C-I, C-II, C-III, and E with Qualitative Apolipoprotein E Phenotyping
Irene van den Broek et al.

This paper describes the development and provisional validation of an LC-tandem mass spectrometry assay for quantification of six serum apolipoproteins and assessment of apoE phenotypes. The aim of the LC-tandem mass spectrometry assay was the implementation into clinical routine to improve cardiovascular disease risk assessment. Sample preparation was optimized and automated. Calibration was traceable to protein standards of apoA-I, apoB, apoC-I, apoC-II, apoC-III, and apoE. In addition, specific peptides were included to differentiate between total apoB and apoB-100 and between apoE2, apoE3 and apoE4 isoforms. Validation of the automated LC-tandem mass spectrometry assay demonstrated excellent analytical quality. Follow-up work will focus on clinical validation and implementation into routine clinical chemistry.

An Empirical Approach to Signature Peptide Choice for Selected Reaction Monitoring: Quantification of Uromodulin in Urine
Qin Fu et al.

In this article the authors present an empirical workflow for identifying surrogate peptides to quantify proteins by mass spectrometry. Experimental validation was indicated because some otherwise promising peptides have yielded inaccurate and inconsistent results. The workflow proposed by the authors involves the relative quantification of 10 or more candidate peptides in a representative collection of biological samples, followed by the identification of peptides with correlated mass spectrometry peak areas. This workflow was used to identify a set of correlated signature peptides for the quantification of uromodulin in urine. A single reaction monitoring assay targeting these peptides yields results that are consistent with those obtained using a commercial ELISA.

A Novel N-Tetrasaccharide in Patients with Congenital Disorders of Glycosylation, Including Asparagine-Linked Glycosylation Protein 1, Phosphomannomutase 2, and Phosphomannose Isomerase Deficiencies
Wenyue Zhang et al.

This study reports the discovery and validation of a novel N-glycan for diagnosis of congenital glycosylation disorder-Ia, Ib and Ik, the three most common glycosylation disorder subtypes, using mass spectrometry methods. The rational was that analyzing complex glycans with high resolution by mass spectrometry would provide a superior diagnostic tool for congenital disorders of glycosylation, an important set of clinical conditions largely under diagnosed at a biochemical level.

LC-MS/MS Measurement of Parathyroid Hormone–Related Peptide
Mark M. Kushnir et al.

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In this article the authors describe a novel LC tandem mass spectrometry method for measurement of parathyroid hormone related peptide. Prior methods for parathyroid hormone related peptide are insufficiently analytically sensitive and specific, which has led to an assumption that parathyroid hormone related peptide is not present in blood in healthy individuals. The results showed that parathyroid hormone related peptide is a normal constituent in circulating blood and the existing commercial radioimmunoassay substantially underestimates the concentrations of parathyroid hormone related peptide, thus causing false-negative results in samples from patients suspected of hypercalcemia. These observations suggest a link between increased concentrations of parathyroid hormone related peptide in lean postmenopausal women and higher incidence of osteoporosis.

Multiplexed Quantification of Proglucagon-Derived Peptides by Immunoaffinity Enrichment and Tandem Mass Spectrometry After a Meal Tolerance Test
Anita Y.H. Lee, et al.

This report describes an immunoaffinity micro flow liquid chromatography-mass spectrometry method for the simultaneous measurement of proglucagon derived peptides GLP-1[7-36], GLP-1[9-36], oxyntomodulin and glucagon, as well as the successful application to the study of a meal challenge. The application of mass spectrometry to the measurement of these peptide hormones is important because (1) the measurements are derived from a single sample, (2) mass spectrometry based assays are inherently more selective than the traditionally employed laboratory-based assays, and (3) this is the first demonstration of an assay with the analytical selectivity and sensitivity to measure oxyntomodulin in healthy human subjects in a clinical study.

LC-MS/MS for Identifying Patients with CYP24A1 Mutations
Hemamalini Ketha, et al.

In this article the authors describe an LC-tandem mass spectrometry method for measuring serum 24,25 dihydroxy vitamin D and the clinical utility of 25 hydroxy D to 24,25 dihydroxy D ratio for diagnosing hypercalcemia mediated by cytochrome P24A1 mutations. Increased ratios were observed in patients with CYP24A1 gene mutations. The 25 hydroxy D to 24,25dihydroxy D ratio was measured in 91 healthy males and females. Based on data on patients with confirmed CYP24A1 mutations, a ratio >99 could identify patients who were candidates for genetic testing for cytochrome P24A1 mutations. Measurement of 25 hydroxy D to 24,25 dihydroxy D ratio should be useful for diagnosing cytochrome P24A1 mutation-mediated hypercalcemia.

Clonotypic Light Chain Peptides Identified for Monitoring Minimal Residual Disease in Multiple Myeloma without Bone Marrow Aspiration
H. Robert Bergen, III, et al.

The most sensitive techniques for measuring minimal residual disease in multiple myeloma require an expensive bone marrow aspirate. In this study the authors have developed a serum based test that uses a high M-spike serum sample for de novo sequencing of immunoglobulin light chain complementary determining regions of the M-protein. These de novo identified tryptic peptides are unique to the plasma cell immunoglobulin clone and can be used to monitor disease. This is

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achieved without a bone marrow aspirate and is more sensitive than immunofixation, immunohistochemistry and multicolor flow cytometry.

Quantification of Anaplastic Lymphoma Kinase Protein Expression in Non–Small Cell Lung Cancer Tissues from Patients Treated with Crizotinib
Todd Hembrough, et al.

Crizotinib, an anaplastic lymphoma kinase and c-ros oncogene 1 inhibitor, has large anti-tumor activity in anaplastic lymphoma kinase-rearranged non-small cell lung cancer. Break-apart fluorescent in-situ hybridization is the current diagnostic test for anaplastic lymphoma kinase-rearrangement, but fluorescent in-situ hybridization is low-throughput and not always reflective of protein concentrations. In this study the authors report a mass spectrometry-based assay for objectively measuring the abundance of anaplastic lymphoma kinase-protein expression in non-small cell lung cancer tissues. In this pilot study, anaplastic lymphoma kinase concentrations correlated with crizotinib response. This assay has the ability to be multiplexed for quantifying anaplastic lymphoma kinase and other clinically relevant proteins. Multiplex screening of patient tissue at the time of initial biopsy maximizes information in limited tissue and provides the clinician with valuable, actionable diagnostic information.

Candidate Reference Measurement Procedure for the Quantification of Total Serum Cortisol with LC-MS/MS
James M Hawley, et al.

Serum cortisol reference measurement procedures and certified reference materials are available to promote routine assay standardization. Despite this, External Quality Assessment schemes still demonstrate a clinically significant bias across routine immunoassays, representing a risk to patient care.
In this article the authors have developed a candidate reference measurement procedure that is free from analytical interferences and exhibits excellent accuracy, imprecision and low measurement uncertainty when validated against a higher-order certified reference material panel listed by the Joint Committee for Traceability in Laboratory Medicine. The method therefore qualifies as a reference measurement procedure. The goal is to use this assay to assign targets to external quality assessment materials, thus allowing participating laboratories to calculate their accuracy and measurement uncertainty.

Planar Functionalized Surfaces for Direct Immunoaffinity Desorption/Ionization Mass Spectrometry
Petr Pompach, et al.

Modern clinical tests are often based on interaction between an analyte of interest and a protein affinity partner. If this partner is an antibody and the analyte is its antigen, the test is commonly referred to as an immunoassay. Typically, the antibody is anchored to a solid surface and the antigen-analyte is selectively enriched from the sample. In this article the authors present an effective method for preparation of antibody-functionalized matrix-assisted laser desorption/ionization plates for immunoassay with mass spectrometry detection. The method is based on ambient ion landing of electrosprayed antibody ions. As a proof of principle the authors developed a fast assay for haptoglobin phenotyping.

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Sulfatide Analysis by Mass Spectrometry for Screening of Metachromatic Leukodystrophy in Dried Blood and Urine Samples
Zdenek Spacil, et al.

The authors of this paper sought a new method that could be used for newborn screening and diagnosis of metachromatic leukodystrophy. They quantified sulfated lipid molecular species in dried blood spots and dried urine spots by tandem mass spectrometry. They found that sulfatides were increased in dried blood and urine spots from metachromatic leukodystrophy patients as compared to non-affected control samples. The new method seems appropriate for newborn screening of metachromatic leukodystrophy.

MALDI-TOF-MS Assay to Detect the Hemizygous 22q11.2 Deletion in DNA from Dried Blood Spots
Lisa J Kobrynski, et al.

In this article the authors describe a novel approach using matrix-assisted laser desorption/ionization -time-of-flight mass spectrometry to measure the hemizygous deletion of the UFD1L gene with DNA from dried blood spots. In the 22q11.2 deletion syndrome there is a deletion of one copy of the genes in this region. This assay technique was found to be sensitive and specific, and could be suitable for use in population based newborn screening for 22q11.2 deletion.

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