The Association for
Mass Spectrometry: Applications to the Clinical Lab

MSACL 2015 US
The 7th Annual International Conference of
The Association for
Mass Spectrometry: Applications to the Clinical Lab
San Diego, CA
March 28 - April 1, 2015
Sheraton Hotel & Marina

Dedicated to the Memory of Professor Karl-Siegfried Boos

The Association is a non-membership, non-profit 501(c)(3) tax-exempt California Corporation with the mission of furthering education in the field of mass spectrometry.

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Corporate Sponsors

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Ionics Mass Spectrometry
Scientific Committee

Please take a moment to recognize the members of the Scientific Committee who were pivotal in the development of the MSACL 2015 US Scientific Program.

**Chair**
David Herold, MD, PhD  
*University of California, San Diego/VA Medical Center, San Diego*

**Small Molecule/Toxicology**
Kara Lynch, PhD (Lead)  
*University of California, San Francisco*

Brian Rappold  
*Essential Testing*

Dan Holmes, MD  
*St. Paul’s Hospital*

Jason Sawyer  
*ARUP*

**Metabolomics**
Gary Patti, PhD (Lead)  
*Washington University*

Kyu Rhee, MD, PhD  
*Weill Cornell Medical College*

Mohit Jain, MD, PhD  
*University of California, San Diego*

**Proteomics**
Cory Bystrom, PhD (Lead)  
*Cleveland Heart Lab*

Leigh Anderson, PhD  
*University of Pennsylvania*

Steve Master, MD, PhD  
*University of Pennsylvania*
**Microbiology**

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*University of Washington*

Carey-Ann Burnham, PhD
*Washington University*

Nathan Ledeboer, PhD
*Medical College of Wisconsin*

Pieter Dorrestein, PhD
*University of California, San Diego*

Vanessa Phelan, PhD
*University of California, San Diego*

**Regulations, Standards, Proficiency**

Julianne Botelho, PhD (Lead)
*CDC*

Hubert Vesper, PhD
*CDC*

Victoria Zhang, PhD
*University of Rochester*

**Practical Training**

Rob Fitzgerald, PhD (Lead)
*University of California, San Diego*

Judy Stone, PhD
*University of California, San Diego*
The Association for Mass Spectrometry: Applications to the Clinical Lab

presents

The 2nd Annual European Conference on Clinical Mass Spectrometry

hosted at The Salzburg Congress Center

Plenary Lectures ★ Scientific Sessions
Short Courses ★ Exhibits ★ Corporate Workshops

DEADLINES

May 8, 2015
PODIUM Abstracts
Travel Grant Application

July 15, 2015
POSTER Abstracts

July 24, 2015
Early Bird Registration

Aug 7, 2015
Regular Registration

Aug 31, 2015
Late Registration
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Connect

You are invited to attend our workshops, technical posters and to visit us in booth #21 while at MSACL 2015.

• Time Matters. Simplify Routine Screening Complexity.

Speaker: Jason Lai Ph.D., MBA – Regulated Product Manager
Discover three newly listed Class I medical devices for general clinical use: Thermo Scientific™ Prelude MD™ HPLC, Thermo Scientific™ Endura MD™ mass spectrometer, and Thermo Scientific™ ClinQuan MD™ software. Clinical laboratories can use these devices to build their own lab developed tests (LDT). Various example compounds and workflows will be used to demonstrate the robustness and time efficiency of the new Prelude MD HPLC and Endura MD MS.

For in vitro diagnostic use. Not available in all countries.

FREE workshop • Monday, March 30, 2015 • 1– 2 p.m. • Harbor 2

• Break the Bottleneck – Accelerate Genomics to Proteomics to Bedside with New Informatics Solutions

Speaker: Mazi Mohiuddin – Senior Applications Scientist
An overview of the proteo-genomic data analysis workflows and automation of informatics solutions for clinical research service providers and translational researchers to run routine data analysis workflows in a seamless manner.

For research use only. Not for use in diagnostic procedures.

FREE Workshop • Tuesday, March 31, 2015  7–8 p.m. • Harbor 2

• Employing Translational Research Workflows on LC-HRAM Platform for Detection of Pathogen Induced Cancer in a Human T-Cell Leukemia Virus Type 1 Disease Model

Speaker: Sucharita Dutta Ph.D; Eastern Virginia Medical School
In this research-based workshop, the speaker will discuss the Human T-Cell Leukemia Virus Type 1 (HTLV-1) as the potential factor for the development of an aggressive lymphoma, Adult T-cell Leukemia (ATL). The translational workflow involves standard data-dependent acquisition (DDA) experiments followed by more in-depth pSMART data acquisition methodologies for the most comprehensive global profiling of proteins from exosome samples to exhaustively mine for proteins that show functional significance via pathway analysis.

For research use only. Not for use in diagnostic procedures.

FREE workshop • Wednesday, April 1, 2015 • 1 – 2 p.m. • Harbor 2

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**Noviplex Cards (Lab-on-a-Card Technology)**

A powerful tool used to collect a volumetric sample of plasma from a non-volumetric application of whole blood in just minutes, Noviplex integrates and automates multiple sample preparation steps, and enables in-transit sample preparation. The new Noviplex Duo cards enable multiple plasma spots to be collected from a single blood drop.

**Be sure to attend our lunchtime workshops:**

**Secrets to Successful LC-Tandem MS Implementation:**

*Tips and tricks for Triple Quadrupole Mass Spec Excellence*

Tuesday, 3/31, 1-2 PM, HARBOR 2. Presentation by Kent Johnson, Exceltox Laboratories, LLC.

**Prepare Your Laboratory for the Future:**

*Laboratory-on-a-Card Technology and Ultra-Fast Mass Spectrometry*

Wednesday, 4/1, 1-2 PM, HARBOR 1. Presentation by Fred Regnier, Novilytic Labs

**T-shirts and Giant Microbes will be given to all attendees!**
Mass spectrometry is rapidly advancing as a smart alternative for scores of clinical research applications involving trace level quantitation of multiple compounds such as endocrines, biomarkers, drugs of abuse and many more.

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General Information

**Continuing Education Credits**
Continuing Education credits will be available on the MSACL website for the short courses and the Scientific Sessions through ACCENT by AACC. Go to: msacl.org > CE Credits > ACCENT > MSACL 2015 US

**Conference Badges**
Your badge constitutes your admission pass to the Conference, receptions and the Exhibit Hall. Please display your badge prominently while attending the conference and at all associated functions. If you do not have your badge you will be escorted to the registration desk to get one, or off the premises. If you have an EXHIBITS ONLY badge YOU ARE NOT PERMITTED IN THE SCIENTIFIC SESSIONS, except the Plenary. If you are identified to be flouting this detail, your registration may be revoked without refund.

**Parking**
The visitor parking rate for the Sheraton is: $10 for Day Parking and $18 for Overnight Parking

**Yoga**
Yoga will be held every day at 5:45 - 6:45 AM in Nautilus located down the stairs to the left of Harbor Ballroom 3. MSACL will be providing a limited number of yoga mats.

**Breakfast**
**Short Course Registrants** Breakfast served Sat – Sun in Harbor/Grande Foyer from 6:30-8:00 AM.
**Conference Registrants**: Breakfast served Mon – Wed in the Harbor/Grande Foyer from 6:00-8:00 AM

**Lunch**
**Short Course Registrants**: Saturday and Sunday: (12:00 - 1:00 PM)
Box Lunch is offered in the Harbor/Grande Foyer Saturday and Sunday.
**Travel Grantees go directly to Shoreline Patio on Sunday.**
**Conference Registrants**: Monday and Tuesday and Wednesday: (12:00 - 1:00 PM)
Box lunches will be available for pickup in the Harbor and Bayview Foyers.

**Receptions**
Sun - Tue  4:15 – 7:00 PM @ Exhibit Hall (Grande Ballroom)
The MSACL evening Receptions provide a selection of heavy appetizers and drinks while allowing you the time to commune with exhibitors and fellow compatriots in mass spectrometry.

**Hospitality Receptions**
Sat – Tue: 8:00 to 10:00 PM
Wed: 3:45 to 7:00 PM
@ ShoreLine Patio overlooking the San Diego Marina

**Smoking**
Smoking is prohibited within the conference facility and outside within 50 ft of the building.

**Airport Shuttle**
Travel Distance: Approximately 1.61 km/1.0 miles.
- Complimentary airport shuttle runs every 20 minutes from the San Diego International Airport between the hours of 4:45 AM and 12:00 AM. Outside of these hours guests may request a pickup by phoning the Hotel directly. (619) 291-2900
- The shuttle vans are grey, blue and white and run from the Marina Hotel, Bay and Terminals 1 and 2.

**Tape Recording/Video Recording Policy**
Please observe the MSACL policy which prohibits operation of tape recorders, video recorders, cameras, or camera phones, except for official association equipment, at all conference sessions, in the Exhibit Hall, and during the plenary sessions.
Throughout Conferences MSACL will be videotaping and taking photographs to be used for promotional or educational purposes by MSACL. If you do not wish to appear on camera, please notify the videographer or photographer and your request will be honored.
Presenter Info and Guidelines

Podium Presentations

Location: Harbor Ballrooms 1-3, Marina 6 & Executive Center

- If an individual is unable to present or does not show, the presentation time slot will be left open. **IT WILL NOT BE FILLED BY THE NEXT SPEAKER.** The next speaker will begin presenting at his/her scheduled time.
  - **Back-Up Presenters:** If a presenter does not show a back-up presenter may be called to fill in the open spot. **Session Chairs, please contact registration immediately on determining that a speaker may not show so that efforts may be put in place to locate a back-up speaker.**
- Speakers: Please make an effort to repeat any questions from the audience before answering.
- Podium presentations are ~20 minutes with ~5 minutes for Q&A.
- PC Laptops running Windows 7 & Office 2007 and 365 will be provided.
- Presenters should check-in 30 minutes prior to their Session (NOT their talk) with either the Session Chair or AV Support on-hand to upload their presentation files to the primary presentation laptop.
- Presenters must bring their presentations on thumb (USB) drives for placement on a single presentation computer from which all presenters will access their PowerPoint presentations.
- Laser pointers will be provided.

Poster Presentations

Location: Exhibit Hall

Poster sessions are held on Sunday, Monday and Tuesday.

- Poster attendance is obligatory for 1 hour,
  - 2:00-3:00 PM (Mon & Tue) or
  - 5:00-6:00 PM (Sun, Mon & Tue).
- **2:00 PM Posters**
  - Place on Poster Board before 8:00 AM
  - Attend from 2:00 - 3:00 PM
  - Remove between 7:00 – 7:30 PM
- **5:00 PM Posters**
  - Place on Poster Board before 8:00 AM (or Sunday between 2:00 and 4:15 PM)
  - Attend from 5:00 - 6:00 PM
  - Remove between 7:00 - 7:30 PM
- Maximum Poster dimensions (for each presenter) are 3.5 feet wide x 3.5 feet high.
- Poster Boards are Fabric.
- Poster Pins WILL BE provided.
A personal account of a journey spanning more than 30 years of applied mass spectrometry in a clinical setting is summarized in this lecture. Inspired by a clinician’s account of a child rescued from near death by a revolutionary therapeutic intervention, the author applied chemistry and mass spectrometry to solve an analytical challenge that led to the first front-line diagnostic test performed by MSMS – the analysis of acylcarnitines to recognize and diagnose inherited disorders of fatty acid and branched-chain amino acid catabolism. By applying this method to dried blood spots and adding an additional analytical component to include certain essential amino acids, a novel multiplex assay was developed to screen newborns for over 30 inherited metabolic conditions with a single test. This concept subsequently became the basis of a targeted metabolomics platform that was used to help identify new animal models of metabolic disease by screening the offspring of genetically modified adults. MSMS with UPLC has been widely applied to develop new assays for useful biomarkers of metabolic disease for both diagnosis and therapeutic monitoring. Examples from the author’s laboratory will be used to illustrate the value and scope of these methods.

Precision medicine, also known as personalized medicine, is the term used for the model of health care involving the selection of diagnostic tests that have the potential to guide the physician towards the most efficacious course of treatment for the individual patient. The promise of precision medicine is a reduction in extraneous patient treatment with a concomitant increase in disease management efficacy based around the concept of “the correct drug for the correct reason”. This lecture will look at the role of precision medicine based around mass spectrometry and genetics; specifically the treatment of breast cancer with Tamoxifen and its metabolites. PK/PD relationships and their role in the use of pro-drugs such as Tamoxifen in the face of the various genetic mutations in the CYP family will be defined and the use of individual patient phenotypic metabolite measurements will be examined.
Innovations for Translating Metabolomics into the Clinical Laboratory
Rick Yost
University of Florida
Monday @ 9:15 AM in the Harbor Ballroom

Translating rapid advances in mass spectrometry and metabolomics into the clinical laboratory portends major changes in clinical analysis. Innovations in mass spectrometry, including ion mobility and FAIMS, ambient ionization, imaging mass spectrometry and global metabolomics all offer the opportunity for developing more sensitive, selective and rapid methods for the clinical laboratory. This lecture will include a perspective on the changing landscape for mass spectrometry in the clinical laboratory, insights into instrumental innovations, and examples of clinical applications.

Microfluidic Devices and Microscale Mass Spectrometry: Integration of Miniaturized Technologies for Acquiring Biological Information
J. Michael Ramsey
University of North Carolina at Chapel Hill
Tuesday @ 8:30 AM in the Harbor Ballroom

Mass spectrometry is a label free measurement technique that provides primary structure in addition to quantification, provided the target molecules can be placed into the gas phase as ions. While electrospray ionization has largely solved the problem of converting liquid-borne analytes into the gas phase ions, mass spectrometry has had the burden of being instrumentally complex, costly, and unwieldy.

The primary reason for the size, weight, and cost of mass spectrometers is the vacuum systems conventionally required for their operation. Over the past decade, we have been developing miniaturized mass analyzers that relax the necessary mean-free-path for operation. As a result these instruments can operate at pressures many orders of magnitude higher than conventional instruments, e.g., in the 1 Torr range. We refer to this mode of operation as High Pressure Mass Spectrometry (HPMS). HPMS allows mass spectrometry platforms that have size, weight, and power metrics that are all at least an order of magnitude smaller than the most compact conventional platforms. We have also been involved in integrating nano-electrospray (nESI) functionalities to microfluidic separation devices that yield state-of-the-art separations and nESI efficiency. The coupling of microfluidic nESI devices with HPMS platforms and their potential applications will be discussed in this presentation.

The Rise of Intelligent Machines and What It Means to the Lab and Healthcare
Randall Julian
Indigo BioSystems
Monday @ 9:15 AM in the Harbor Ballroom

Intelligent machines teamed with experts are superior to experts working alone. This will have profound effects on the nature of healthcare delivery. Further, the advance of automation is already having a significant effect on labor markets, and there is no reason to believe healthcare will not be impacted. In this lecture examples of human-machine teams will be given. Also, the impact on society of the increased role of smart machines will be discussed. Comparisons between the first and second machine ages will be used to draw out the consequences, benefits and difficulties we will face as a scientific community.
This year seventy-three (73) Young Investigator Travel Grants were provided. Young Investigator Travel Awards are awarded to support trainees (MD/residents/fellows and PhD - students / post-docs) and young faculty members (less than 4 years since appointment) who have submitted an exceptional abstract. These awards cover conference and short course registration, as well as hotel.

Julia Balog Imperial College London
Harris Bell-Temin University of South Florida
Maria Bergquist Uppsala University
Laura Bernstone University Hospital of South Manchester
Brian Bird Utah Valley University
James Bollinger University of Washington
Tiphaine Cecchini Phelma, Grenoble INP
Crystal Cheung Institute for Bioscience and Biotechnology Research
Kevin Cho Washington University
Didia Coelho Graça University of Geneva
Jennifer Colby University of California, San Francisco
Gonçalo Correia Imperial College London
Lewis Couchman King’s College Hospital
Surendra Dasari Mayo Clinic
Mari DeMarco St Paul’s Hospital & University of British Columbia
Kelly Doyle University of Utah
Joe El-Khoury Yale University
Evelyn Gitau Kemri-Wellcome Trust Research Programme
Germán Augusto Gómez-Ríos University of Waterloo
Mark Gonzalez Washington University in St. Louis
Marianne Hädener University of Bern
Jörg Hanrieder University of Gothenburg
Lidong He University of Utah
Curtis Hedman Wisconsin State Laboratory of Hygiene
Tony Hu Houston Methodist Research Institute
Frances Jackson Imperial College London
Alan Jarmusch Purdue University
Carl Jenkinson University of Birmingham
Caroline Johnson The Scripps Research Institute
Hemamalini Ketha Mayo Clinic
Jo-Il Kim Yonsei University
Yin-Hung Lai Academia Sinica
Hang Li George Washington University
Mark Marzinke Johns Hopkins University
Allison McMullen Washington University in St. Louis
Rafael Montenegro-Burke Vanderbilt University
Dana Ohana Leiden University Medical Centre
Amelia Palermo Laboratorio Antidoping FMSI
Seungman Park Green Cross Laboratories
Sandip Kumar Patel Indian Institute of Technology Bombay
Liz Payne Washington University in St. Louis
Nilini Ranbaduge The Ohio State University
Yue Ren Purdue University
Nathaly Reyes-Garcés University of Waterloo
Alec Saitman University of California, San Diego
Kerry Scott NIST
Christopher Shiea University of California, Irvine
Laura Smy University of Toronto
Zdenek Spacil University of Washington
Sylvia Stopka The George Washington University
Nicole Strittmatter Imperial College London
Linda Switzar Leiden University Medical Center
Kalev Takkis University of Tartu
J. Will Thompson Duke University
Katie Thoren Memorial Sloan Kettering Cancer Center
David Tonoli University of Geneva
Lawrence Tse Medical College of Wisconsin
Shahid Ullah Karolinska Institute, Sweden
Irene van den Broek Leiden University Medical Center (LUMC)
Panagiotis Vorkas Imperial College London
Ryan Walsh University of Colorado, Denver
Dan Wang Cleveland State University
Nan Wang Zhejiang University
Arnaud Wolfer Imperial College London
Gary Woodward University Hospital Birmingham
Jane Yang University of California, San Diego
He Yang University of California, San Francisco
Han-Yin Yang University of Washington
Xin Yi The University of Chicago
Ankit Zalavadia Virginia Commonwealth University
Jiangjiang Zhu University of Washington
Maike Zimmermann University of California Davis
This year, thirteen (13) Lab Director Awards were granted to individuals leading clinical labs. These individuals have had minimal exposure to mass spectrometry and are interested in gaining more understanding of its potential applications.

Neil Anderson Washington University
Charles Beavers Mercy Hospital
Sarah Brown Washington University
Yu Chen Horizon Health Network
Sarah Hackenmueller University of Wisconsin
Thomas Kampfrath Santa Clara Valley Medical Center
Edward Leung University of Chicago

Veronica Luzzi Providence Regional Laboratory
Nang Nguyen Rhode Island State Health Laboratories
Edward Sass University of Alabama in Huntsville
Shu-Chu Shiesh National Cheng Kung University
Nicole Tolan Beth Israel Deaconess Medical Center
Maria Alice Willrich Mayo Clinic

The Lab Director Grants are sponsored by:
The Trainee Grants are sponsored by:

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Short Course Overview

The Short Course Program provides a rapid introduction to topics, issues and techniques. Each course is led by one or more distinguished instructors with expertise in their respective course topics.

LC-MSMS 101
Getting Started with Quantitative LC-MS/MS in the Diagnostic Laboratory
Length: 2 days (Saturday - Sunday)
Location: Seabreeze
Level: 1-2 (Beginner - Intermediate)
Instructor(s): Judy Stone, PhD & Lorin Bachmann, PhD

Is your laboratory under pressure to purchase an LC-tandem MS or is the ROI you wrote last year haunting you now? This short course is designed for attendees implementing quantitative LC-tandem MS for patient testing who have laboratory medicine experience but no mass spectrometry training - CLS bench analysts, supervisors, R&D scientists, and laboratory directors. Theoretical concepts necessary for a robust implementation of clinical mass spectrometry will be presented – but the emphasis is on practical recommendations for:

- LC-MS/MS system purchasing
- site preparation and installation
- defining preliminary MSMS and LC parameters for your first method
- selecting and optimizing sample preparation for your first method
- choosing internal standards, solvents, and water, making reagents and calibrators
- adjusting sample preparation, LC and MSMS parameters to achieve the desired assay performance
- establishing data analysis & review criteria and an interface to the LIS
- pre-validation stress testing and method validation
- preventative maintenance and troubleshooting
- maintaining quality in production.

Our goal is to present just enough theory so you can report high quality results, while opening a window to the depth and complexity of clinical mass spectrometry such that your appetite is whetted to learn more. Previous exposure to the principles of clinical method validation, either didactic or practical, is assumed. A glossary of common LC-MSMS terms/acronyms, and diagrams delineating basic LC and MSMS instrument components and functions will be emailed to attendees a week prior to the beginning of the course. This material will also be addressed at the beginning of the course, but the initial learning curve can be steep and review prior to the course will be beneficial if you have absolutely no previous exposure with LC-MSMS.

LC-MSMS 102
Intro to Clinical MS Method Development
Length: 2 Days (Saturday - Sunday)
Location: Spinnaker
Level: 1-2 (Beginner - Intermediate)
Instructor(s): Robert Kobelski, PhD

This course is designed for the person who will be responsible for implementing, improving, or developing clinical analysis methods using hyphenated mass spectrometry techniques. It will emphasize the basic science associated with chromatographic separation and detection using mass spectrometry with an emphasis on applying that science to produce valid, reliable and robust clinical analysis methods. The course will cover:

- Fundamentals of separation
- Chromatographic theory
- Gas chromatography and how to optimize separations
- Liquid chromatography (HPLC & UHPLC) and how to optimize separations
- Fundamentals of mass spectrometry
- Quadrupole mass analysis
- Time-of-Flight mass analysis
- Orbi-trap mass analysis
- Tandem mass spectrometry (triple quad and Q-TOF)
- Qualitative analysis
- Quantitative analysis
• Quality control and assurance.
Critical analysis of published and hypothetical analysis methods will be used to highlight proper integration of the various aspect of clinical MS analysis.

**LC-MSMS 201**

**Understanding and Optimization of LC-MS/MS to Develop Successful Methods for Identification and Quantitation in Complex Matrices**

*Length: 2 Days (Saturday - Sunday)*  
*Location: Marina 6*  
*Level: 2 (Intermediate)*  
*Instructor(s): Robert D. Voyksner, PhD*

This course is designed for the chromatographer / mass spectrometrist who want to be successful in developing methods, method optimization and solving problems using LC/MS/MS. The course covers the atmospheric pressure ionization (API) techniques of electrospray, pneumatically assisted electrospray and atmospheric pressure chemical ionization (APCI) and atmospheric pressure photo ionization (APPI) using single quadrupole, triple quadrupole, time-of-flight and ion trap mass analyzers. Discussions of sample preparation and chromatography will target method development and optimization for the analysis of “real-world” samples by LC/MS/MS. The course highlights the following topics with respect to optimization a method to achieve the best sensitivity, specificity and sample throughput:

1. Optimization ionization in API techniques,
2. understanding and minimizing matrix suppression,
3. relative merits of various LC column lengths, particle sizes and column diameters for LC/MS/MS analysis,
4. introduction into the interpretation of MS/MS spectra,
5. important issues in LC/MS/MS quantitation, and
6. optimization of an quantitative analysis.

Applications of LC/MS/MS to analyze compounds of clinical interest in biological matrices will be discussed throughout the course to emphasize the topics covered.

**LC-MSMS 301**

**Development and Validation of Quantitative LC-MS/MS Assays for Use in Clinical Diagnostics**

*Length: 2 Days (Saturday - Sunday)*  
*Location: Harbor Ballroom 2*  
*Level: 3 (Advanced)*  
*Instructor(s): Russell Grant, PhD & Brian Rappold*

This 2-day course will briefly introduce the key aspects of the LC-MS/MS experimental workflow and then focus on processes and experimental designs for assay development and analytical validation of assays to be employed within clinical diagnostics.

The first day will describe method development in detail, including how-to guides for initial optimization of mass spectrometry systems, chromatographic development and sample preparation schemes. Techniques and technologies for streamlining analytical performance will also be described. Transitional experiments from development to validation will be discussed in detail to stress test methodologies prior to analytical validation.

Day two will cover all details pertinent in validation of LC-MS/MS analytical workflows. Experimental designs for all aspects of validation, putative acceptance criteria and analytical solutions will be shown. Key validation criteria of selectivity, carry-over, matrix effect, accuracy, precision, linearity, stability and inter-assay correlation will be described using multiple case studies.
This 1-day course will introduce fundamentals of modern methods of sample preparation for clinical mass spectrometry analysis. This course will consider all steps in the analytical process, focusing on sampling and sample preparation in particular. Special emphasis will be given to preanalytics (sampling, transport, storage) of specimens, sample clean-up and elimination of matrix effects in subsequent determinations.

The focus will be on specific sample preparation approaches used prior to LC-MS based clinical analysis and comparison with standard approaches like protein precipitation, ultrafiltration, liquid-liquid and solid-liquid extraction. Theory will also be touched upon with an emphasis on common fundamental features among different groups of extraction techniques. Additionally, the strategies to obtain selectivity and specificity of the assay (e.g. use of aptamers, immunosorbents, molecularly imprinted polymers and other molecular recognition strategies) will be discussed. Furthermore, an overview of sample preparation/sample introduction approaches for direct coupling to mass spectrometers will be presented.

The course will discuss pros and cons of each method and compare their workflows based on the published protocols and collected data to provide guidelines to select the best available solution for different applications. A special focus will be on selection of the method for targeted and untargeted analysis in the view of the method characteristics and the investigated matrix (biofluids, tissue and breath analysis). The course will outline availability of the extraction phases and strategies for their selection. The prospect for using the techniques in high-throughput and automated environments, as well as in vivo and for bedside use (rapid diagnostic tool) will be also presented.

Sampling, preservation and storage issues:
- sample collection approaches
- influence of the container and anticoagulant on the results
- metabolism quenching methods
- effect of storage and transportation conditions on sample stability
- integration of sampling and sample preparation

Sample preparation methods discussed during the course:
- solid phase extraction (SPE)
- micro-solid phase extraction (µ-SPE)
- column switching
- solid phase microextraction (SPME)
- liquid phase microextraction (LPME)
- thin film microextraction (TFME)
- needle trap devices (NTD)
- dried blood spot (DBS)
- extracted biofluid spot (EBS)

Overview of direct sample introduction to mass spectrometer:
- desorption electrospray ionization (DESI)
- surface-enhanced laser desorption/ionization (SELDI)
- matrix-assisted laser desorption/ionization (MALDI)
- paper spray
- nonoelectrospray
- blade spray
- direct analysis in real time (DART)
- modifications of the above technologies
Clinical MS 301

A Comprehensive Review of Clinical Mass Spectrometry Technology & Techniques, including Miniaturization

Length: 2 Days (Saturday - Sunday)
Location: Executive 1
Level: 2-3 (Intermediate - Advanced)
Instructor(s): Jack Henion, PhD

This two-day course presents a comprehensive overview of technology and techniques of analytical mass spectrometry and from that foundation extends into exciting recent developments.

1. Sample preparation
   - Topics: Types of extraction, Objectives of extraction, Prefractionation techniques, Sample types, Issues to consider, Protein precipitation, Liquid-solid extraction, Liquid-liquid extraction, Solid-phase extraction, 96-well format SPE, Mixed mode SPE, On-line SPE, Automation, Micro SPE (pipette tip-based), Ultrafiltration, Affinity techniques, Electrophoretic extraction, Quechers, SISCAPA, Dried blood spots (DBS)

2. Advanced separation techniques
   - Topics: HPLC, HILIC, Porous Graphite Carbon, UPLC NanoHPLC, Capillary Electrophoresis (CE), Differential Mobility Spectrometry (DMS)

3. Ionization techniques for MS
   - Topics: Electrospray ionization (ESI), Nano ESI, Atmospheric pressure chemical ionization (APCI), Atmospheric pressure photoionization (APPI), Matrix assisted laser desorption ionization (MALDI), LAESI, Direct analysis in real time (DART), Desorption electrospray ionization (DESI), Atmospheric sampling analysis probe (ASAP)
   - To Discuss: New ionization techniques which may be used with or without on-line separation science technology such as HPLC, UPLC or capillary electrophoresis (CE). This area has evolved into ambient ionization techniques such as DESI, DART, ASAP, etc. -- examples and comparisons of the potential and pitfalls associated with these techniques will be explored.

4. Mass Analyzers
   - Topics: Quadrupoles, Ion traps, Time-of-Flight (TOF), Orbitraps, Hybrid mass analyzer systems, Ion mobility spectrometers, FTMS
   - To Discuss: Developments and improvements in mass analyzers including linear ion traps, FTMS, time-of-flight (TOF), orbitraps, and accelerator mass spectrometry (AMS), the latter currently being applied to micro-dosing experiments by the pharmaceutical industry. Issues such as full-scan acquisition rates, high-resolution mass spectrometry (HRMS), the importance and usefulness of exact mass measurements for qualitative and quantitative analysis, and the analytical merits compared with modern SRM LC/MS experiments will be discussed with many practical examples and applications. The latter will include clinical chemistry issues, pharmaceutical, food safety, environmental and industrial examples.

5. Imaging and profiling by MS
   - Topics: MALDI, DESI, LAESI, LES
   - To Discuss: The technique of MALDI and its applications to tissue imaging as well as liquid extraction surface analysis (LESA) employing nano-electrospray. Chip-based nanoscale HPLC separations coupled with the benefits of nano-electrospray with a focus on modern strategies to obtain lower detection limits with the benefits of reduced matrix suppression and more uniform analyte response.

6. High resolution MS
   - Topics: Fundamentals, Mass Defects, Isotopic patterns, Mass axis calibration, Types of HRMS systems, Qual/Quan Analysis, Data mining processes, Future directions

7. Miniaturization in MS
   - Topics: Purdue University "Mini 11", Torion, Microsaic, Advion expression

8. Synergistic Integration
   - To Discuss: Developing technologies are likely to be important aspects of modern mass spectrometry and its expansion to new future applications. Ion mobility spectrometry (IMS) and portable mass spectrometers could lead to point-of-care applications in due time. A relevant lecture includes an introduction to the interpretation of CID mass spectra employing a combination of MS/MS data and full-scan exact mass assignments which demonstrates the complimentary benefits of these combined data.
Proteomics 101
**Introduction to Quantitative Proteomics**
Length: 1 Day (Saturday)
Location: Harbor Ballroom 1
Level: 1-2 (Beginner - Intermediate)
Instructor(s): Mike MacCoss, PhD & Michael Bereman, PhD

This course will introduce the researchers to the basics of performing quantitative peptide measurements by mass spectrometry. We will cover the basics of discovery and targeted methods, sample preparation, quality control, sample preparation, and software for method building and data analysis. To prepare participants for performing quantitative proteomics proteomics experiments in their own labs we will focus on the following topics:

- Instrumentation for proteomics
- Basics and challenges of NanoLC
- Introduction to quantitative analysis. Challenges for proteomics measurements
- System suitability and quality control
- Basics of complex protein mixture sample preparation
- Tips and tricks from instructor experience
- Use of Skyline for targeted proteomics method building and data analysis.
- Next generation methods
- Case studies

Proteomics 201
**Clinical Proteomics**
Length: 1 Day (Sunday)
Location: Harbor Ballroom 1
Level: 2-3 (Intermediate - Advanced)
Instructor(s): Andy Hoofnagle, MD, PhD & Cory Bystrom, PhD

This course will explore the background of clinical proteomics and approaches to method development and validation. We will provide the motivation for using mass spectrometry to quantify proteins in clinical research and in clinical care. The promise of mass spectrometry to improve the accuracy and precision of results is only realized with robust methods. In order to prepare participants to begin to develop their own robust methods for quantification we will focus on the following topics:

- Why mass spec for peptides and proteins
- Optimization of digestion and other aspects of the method
- Internal standards
- Calibration
- Immunoaffinity enrichment
- Validation
- Quality control
- Case studies

Metabolomics 301
**Metabolomics**
Length: 1 Day (Sunday)
Location: Executive Center 3
Level: 3 (Advanced)
Instructor(s): Gary Siuzdak, PhD & Gary Patti, PhD

The following topics will be particularly emphasized:

1. metabolomic data streaming
2. biological dependent data acquisition
3. terabyte-scale processing remotely

These topics will be covered in detail and students will be given the opportunity to test related software via hands-on tutorials.

This course is designed for the chromatographer / mass spectrometrist who wants learn about metabolomics. The course covers sample preparation, choice of ionization method and mass spectrometer, as well as data preparation and analysis, structural characterization and biomarker validation. Discussions will
include the optimization of sample preparation for metabolite extraction and protein removal, as well as choosing the most appropriate ionization method and mass spectrometer. The development of LC gradients and MS conditions will be covered. During the course there will be an emphasis on data preparation and analysis, including peak picking, alignment and retention time correction, differential profiling, and multivariate statistics including principal components analysis (PCA). The course also highlights the following topics: metabolite structural characterization using MS/MS for fragmentation data, and accurate mass measurements using FTMS. Further characterization approaches will be discussed, as well as biomarker validation and ultimately the transfer to the clinic.

Toxicology 101

**General Toxicology**
Length: 1 Day (Saturday)
Location: Executive Center 2
Level: 1 (Beginner)
Instructor(s): Jeffery Moran, PhD

The General Toxicology short course has been developed to provide scientists background in general toxicological principles. This series of lectures will review the definition of toxicants and the basic science behind uptake/distribution and biotransformation pathways that lead to excretion. This is important for laboratories charged with developing new testing strategies for drugs and emerging agents of concern when analytical testing strategies are not readily available.

The additional half-day training will use synthetic marijuana aminoalkylindoles as a timely example to emphasize the need for this basic understanding. Aminoalkylindoles are thought to be produced in China and marketed in consumer products now being sold in the US. Humans are being exposed and injured at unprecedented rates, and clinical, forensic, and public health laboratories are now challenged with assaying these new drugs of abuse. When synthetic marijuana first emerged little was known about how these substances were metabolized, and human tests were not available. The information shared in this course reveals the process of developing liquid chromatography tandem mass spectrometry (LC-MS/MS) assays now being used to screen for these drugs.

Toxicology 201

**Mass Spectrometry Toxicology Applications: Method Development and Validation, Troubleshooting, Triaging Instrumentation, Tips, Tricks and Lessons Learned**
Length: 1 Day (Sunday)
Location: Executive Center 2
Level: 1-3 (Beginner - Advanced)
Instructor(s): Marilyn Huestis, PhD & Karl Scheidweiler, PhD

Over the last 15 years, our National Institute on Drug Abuse research tools have transformed from gas chromatography mass spectrometry to liquid chromatography tandem mass spectrometry (LC-MS/MS) to high resolution mass spectrometry (HRMS). This course introduces method development and validation for qualitative and quantitative LC-MS/MS and HRMS analysis through specific examples including cannabinoids quantification in blood, oral fluid and breath, anti-retrovirals quantification in meconium, non-targeted acquisition HRMS for identifying novel psychoactive substances, and determining primary metabolic targets with human hepatocytes and HRMS. We present examples from our laboratory demonstrating these approaches and illustrating potential pitfalls. Important skills are how to select the most appropriate instrument to solve your analytical problem and the required complexity of sample preparation. The new Scientific Working Group for Forensic Toxicology (SWGTOX) Guidelines for Method Validation are demonstrated, and the challenges of troubleshooting LC-MS/MS and HRMS instrument problems are shared. Navigating and mastering the challenges of LC-MS/MS and harnessing its power is the subject of this short course.
Statistics 101

**Breaking up with Excel: A Newbie’s Introduction to the R Statistical Programming Language**

**Length:** 2 Days (*Saturday - Sunday*)

**Location:** Harbor Ballroom 3

**Level:** 1-2 (*Beginner - Intermediate*)

**Instructor(s):** Daniel Holmes, MD & Stephen Master, MD PhD

Have you ever tried to do Deming regression in Excel only to discover that it is not available? Have you had your figure rejected by a journal because the resolution was not good enough? Have you wished that you could figure out a way to stop manually transcribing your LC-MS/MS results into the LIS? Well, your wait is over because this year at MSACL we will be offering a course for complete programming newbies that will help you get going analyzing real data related to LC-MS/MS assay development, validation, implementation and publication. The only background expected is the ability to use a spreadsheet program. The skills that you will acquire will allow you to take advantage of the many tools already available in the R language and thereafter, when you see that your spreadsheet program does not have the capabilities to do what you need, you will no longer have to burst into tears. You will be empowe-R-ed.

The course will be run over two days and time will be evenly split between didactic sessions and hands on problem solving with real data sets. Drs Holmes and Master will adopt a “no student left behind policy”. Students will be given ample time to solve mini problems taken from real life laboratory work and focused on common laboratory tasks. All attendees will need to bring a laptop with the R language installed R Studio interface installed. Students may use Windows, Mac OSX or Linux environments. Both R and R studio are free and open-source. No cash required.

Students should be prepared for learning what computer programming is really like. This may involve some personal frustration but it will be worth it.

**Course Description**

The course will cover:

- The major types of R variables: vectors (numerical, character, logical), matrices, data frames and lists.
- The important classes: numeric, character, list and changing between them
- Importing data from Excel
- Dealing with non-numeric instrument data: the “<10”s and “>1000”s.
- Manipulating your data: sub-setting, which, match, sort, unique, cut
- Simple statistical tests: mean, median, quantiles (normal ranges), t-tests, ANOVA, Wilcoxon.
- Data merges: matching rows between sets
- Exporting data to Excel-like format.
- Non-linear regressions
- Looping: Doing things repeatedly
- Writing your own functions
- Making highly customized graphs: scatter plots, regression lines, histograms, box plots, qq-plots
- Putting it all together projects:
  - Preparing method comparison regression and Bland Altman plots
  - Preparing mass spectrometry data for upload to LIS.

Statistics 201

**How to Develop Robust Assays Faster Using Free Data Analysis Tools**

**Length:** 2 Days (*Saturday - Sunday*)

**Location:** Marina 5

**Level:** 2-3 (*Intermediate - Advanced*)

**Instructor(s):** Fred Lytle, PhD

Every analytical method development project and every method validation involves data analysis. Everyone involved in method development knows the basic, classical statistics usually applied to problems of precision, accuracy and stability. In real experiments, however, sometimes data are not well behaved. Further, in method development, factors often interact with each other in complex ways making robust method development time consuming and difficult. To develop more robust methods faster, better data analysis tools are needed.
Using free data analysis tools, and real world examples, this course will give participants a solid, understandable description of "classical" statistical methods for processing well-behaved data. We will then show where problems arise with real data such as outliers, unequal variances, etc., and how to use modern robust statistical methods for producing more reliable results. We will show how the basic ideas of regression analysis lead to powerful experimental design techniques that can improve the speed and robustness of method development. Students will be taught how to use the free statistics oriented language "R" to easily perform calculations that are difficult or impossible to do correctly using spreadsheets.

**Topics will include:**
1. Use of classical statistics - strengths and weaknesses
2. Robust methods to improve statistical analysis
3. Applications: QC sample analysis and calibration
4. Analysis of Variance (ANOVA) & experimental design Data

<table>
<thead>
<tr>
<th>ICP-MS 101</th>
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<tr>
<td><strong>Clinical and Research Applications using Inductively-Coupled Plasma Mass Spectrometry</strong></td>
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<tr>
<td>Length: 1 Day (Saturday)</td>
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<td>Location: Executive 4</td>
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<tr>
<td>Level: 1-2 (Beginner - Intermediate)</td>
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<tr>
<td>Instructor(s): Frederick Strathmann, PhD &amp; Carrie Haglock-Adler, MSFS</td>
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This course is designed to provide an introduction to the use of ICP-MS in the clinical laboratory as well as its applications in clinical research. The course will cover the components of current ICP-MS systems as well as the principles of measurement and basics of mass spectrometry as applicable to ICP-MS. An overview of sample preparation techniques, method validation aspects and regulatory considerations will be discussed. In addition, an overview of the clinical context to the most commonly measured elements will be provided. Research applications using ICP-MS as well as hyphenated techniques such as HPLC-ICP-MS, CE-ICP-MS, LA-ICP-MS will be highlighted. A brief overview of the applications of mass cytometry will be presented.

<table>
<thead>
<tr>
<th>Manuscripts 101</th>
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<tr>
<td><strong>Preparing Manuscripts for Publication: Improving Your Chances for Success</strong></td>
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<tr>
<td>Length: 1 Day (Sunday)</td>
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<tr>
<td>Location: Executive Center 4</td>
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<tr>
<td>Level: 0 (General Interest)</td>
</tr>
<tr>
<td>Instructor(s): Thomas Annesley, PhD</td>
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Scientific publication is an important and necessary activity for researchers. Being a good researcher, however, does not automatically make you a good writer. Good science is the foundation of a scientific paper, but how the science is presented also strongly influences whether a paper gets accepted for publication. This session focuses on key elements of writing a scientific paper, starting with the first word put onto a page to the final printed product:

- Choosing the most effective words
- Writing clear sentences
- Developing the proper title
- Creating an informative abstract
- Organizing the introduction
- Emphasizing results versus data
- How to start and end the discussion
- Creating tables and figures
- How to correctly use citations and references
- How to choose the right journal
- How to respond to reviewer comments
- Acceptance does not mean the finish line

**Expected Outcomes:**
After this session, participants will be able to:
1. Bring greater clarity and consistency to a scientific paper;
2. Describe the features that distinguish papers accepted for publication;
3. Organize the major sections of a scientific paper; and
4. Create more effective tables and figures.
Exhibits Summary

The Exhibits officially open at 4:15 PM on Sunday with the Opening Reception in the Grande Ballroom. Exhibits are open for viewing from 10:00 AM to 7:00 PM on Monday and Tuesday.

Below is the Exhibit schedule that also includes events intended to provide focused opportunities for attendees to visit the Exhibits during the Conference.

<table>
<thead>
<tr>
<th>Sunday</th>
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<tbody>
<tr>
<td>9:00 am – 4:15 PM</td>
<td>Exhibitor Set-Up (EXHIBITS CLOSED) – Poster Placement for Sunday Presenters Allowed.</td>
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<tr>
<td>4:15 – 7:00 PM</td>
<td>Opening Reception in Exhibit Hall</td>
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<th>Monday</th>
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<tbody>
<tr>
<td>10:00 – 10:45 AM</td>
<td>AM Coffee Break in Exhibit Hall</td>
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<tr>
<td>12:00 – 1:00 PM</td>
<td>Lunch provided in the Harbor Foyer.</td>
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<tr>
<td>2:00 – 3:00 PM</td>
<td>Posters in Exhibit Hall</td>
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<tr>
<td>2:00 – 3:00 PM</td>
<td>PM Coffee Break in Exhibit Hall</td>
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<tr>
<td>4:15 – 7:00 PM</td>
<td>Reception in Exhibit Hall</td>
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<tr>
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<tr>
<td>4:15 – 7:00 PM</td>
<td>Reception in Exhibit Hall</td>
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<tr>
<td>7:00 PM</td>
<td>END OF EXHIBITS</td>
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<tr>
<td>Midnight</td>
<td>Deadline for removal of Exhibits from Exhibit Hall</td>
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MSACL 2015 US: Exhibit Booth Layout

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Exhibits

Attendee Flow

Attendee Flow

Attendee Flow
Exhibitors

**Agilent Technologies** Booth #16-17
http://www.agilent.com/chem

Agilent Technologies delivers premiere analytical technologies for clinical research ensuring your success from sample prep to final answer. These include a comprehensive portfolio of innovative automation, chemistries, GC, GC/MS, ICP/MS, LC, and LC/MS solutions which enables the identification and quantification of both endogenous and exogenous substances in complex biological matrices with the utmost accuracy and reliability. Coupled with our dedicated global support network, we will get you to your final answer with minimal ramp-up and maximum productivity.

**American Assoc for Clinical Chemistry** Booth #39
http://www.aacc.org

Dedicated to achieving better health through laboratory medicine, the American Association for Clinical Chemistry (AACC) brings together more than 50,000 clinical laboratory professionals, physicians, research scientists, and business leaders from around the world focused on clinical chemistry, molecular diagnostics, mass spectrometry, translational medicine, lab management, and other areas of breaking laboratory science. Since 1948, AACC has worked to advance the common interests of the field, providing programs that advance scientific collaboration, knowledge, expertise, and innovation. For more information, visit www.aacc.org.

**bioMerieux** Booth #47
http://www.biomerieux.com/

bioMerieux provides diagnostic solutions (reagents, instruments, software) which determine the source of disease and contamination to improve patient health and ensure consumer safety. Its products are used for diagnosing infectious diseases and providing high medical value results for cancer screening and monitoring and cardiovascular emergencies.

**Biotage** Booth #02
http://www.biotage.com/

Biotage is a leading provider of sample preparation instrumentation and consumables for a wide range of applications, including pharmaceutical, clinical, forensic, environmental, and agrochemical/food. ISOLUTE® and EVOLUTE® brand solid-phase extraction (SPE) and Supported Liquid Extraction (SLE ) products can be run in either a manual or automated environment. The new RapidTrace+ SPE workstation and TurboVap® Solvent evaporators are ideal for increasing throughput and achieving accurate results. Stop by our booth for the latest innovations and applications for Evaporation and Sample preparation.

**Bruker Daltonics** Booth #26
http://www.bdal.com/

Bruker Daltonics is a leading provider of high performance analytical systems whose innovative and easy-to-use product families encompass a variety of Mass Spectrometers and Gas Chromatography instruments. Bruker utilizes LC/GC/MS based and other MS instruments (ESI-TOFs, Ion Traps, FTMS, MALDI-TOFs, single and triple quadrupole GCMS and ICP-MS) to provide outstanding results for a wide range of small molecule and protein analysis applications. Delivering premium value and backed by decades of Application and Technical Support expertise, Bruker systems enable analytical chemists working in Pharmaceutical, Applied Analytical, Life Science and the Clinical Research laboratories to answer even the most challenging analytical questions.

**Cambridge Isotope Laboratories** Booth #23
http://www.isotope.com

Cambridge Isotope Laboratories, Inc. is the world leader in the manufacture and separation of stable isotopes and stable isotope labeled compounds. CIL offers an array of highly pure compounds that are uniformly or selectively enriched in $^{13}$C, $^{15}$N, D, $^{18}$O or $^{17}$O. Our labeled reagents are used across scientific fields including proteomics, metabolomics, metabolism and environmental applications for quantitative mass spectrometry. CIL's products include SILAC protein quantitation kits, media and reagents, 99% enriched amino acids, MouseExpress® Lys 13C6 mouse feed and mouse tissue, MouseExpress® 15N mouse feed and mouse tissue, Spirulina 15N, intact labeled proteins, growth media for protein expression, cell-free protein synthesis products, environmental contaminants standards for ultratrace analysis, steroids, acylcarnitines, drug metabolites, nucleic acids, lipids and carbohydrates. CIL has GMP capabilities; a majority of substrates can be manufactured to be Q7A compliant.
Cerilliant Booth #14-15
https://www.cerilliant.com/?
Analytical Reference Standards & Certified Spiking Solutions®-Cerilliant offers over 3,000 catalog standards (labeled & unlabeled) including Drugs (pharmaceutical, OTC, & TDM such as hormones, steroids and immunosuppressants), Vitamins, Phytochemicals, Nitroglycerin & by-products, and Environmental Contaminants. Custom services include synthesis, analytical certification, packaging & custom Certified Spiking Solutions®. Cerilliant’s accredited to ISO Guide 34 & ISO/IEC 17025 and certified to ISO 13485 & ISO 9001. Our quality system is compliant to ISO 15194 and incorporates cGMP and GLP. A COA is provided with every product. Call 512-238-9974 or visit www.cerilliant.com.

Chrom Tech Booth #30
http://www.chromtech.com
Distributor of Chromatography consumables, instrumentation and supplies. Featuring: Sample Preparation Products, 96 Well Plates for MS, Columns, Instrument consumables and replacement parts, Pumps, Gas Generators. Featured Suppliers include: Agilent Technologies, Thermo Scientific, Sigma Aldrich, Idex (Upchurch and Rheodyne), Parker Balston, Hamilton, Restek.

ChromSystems Booth #31
http://www.chromsystems.com
Chromsystems is a leading global company providing ready-to-use reagent kits and supplies for routine clinical diagnostics by HPLC and LC-MS/MS, the latter representing the gold standard for many parameters. Our product portfolio includes complete kits, quality controls and multilevel calibrators, all ensuring highly accurate as well as a cost-effective analysis in the laboratory. They enable any laboratory to introduce HPLC and LC-MS/MS methods into their diagnostic routine without prior technical expertise. Analyses can be started immediately and sample preparations require the minimum of laboratory time. Our products are comprehensively validated, in particular LC-MS/MS methods with all widely used tandem mass spectrometers. They are CE-IVD compliant, satisfying regulatory requirements for the laboratory. We combine these high quality products with an excellent support programme and service for our customers.

DPX Labs Booth #24
http://www.dpxlabs.com/
At DPX Labs we believe that your sample preparation should be fast and easy. That is why we have incorporated the benefits of solid-phase extraction into a simple to use pipette tip. The patented Dispersive Pipette Extraction (DPX) tip functions by dispersive SPE, requiring only seconds of mixing within the DPX tip to complete the sample preparation process. Now anyone can rapidly extract samples with high recoveries prior to LC/MS analysis. Whether your laboratory uses a single channel pipettor or a fully robotic liquid handler, there is a DPX tip compatible with your analysis method and throughput. Contact DPX Labs so we can help you eliminate matrix interferences and make ion suppression a thing of the past.

EMD Millipore Booth #37-38
http://www.emdmillipore.com
EMD Millipore provides the innovative solutions you need to advance your research, and more importantly, the support and expertise to utilize them successfully in your lab. You’ll identify more than analytes, target molecules and contaminants. Our full range of water purification products provides accurate lab results, high reliability, low maintenance, predictable and economical running costs and total support. In cellular analysis, protein detection, separation science and membrane filtration, we continue to set the standard for analytical research by providing the highest quality bioanalysis platforms, sample preparation solutions, essential biochemicals, and analytical separation tools.

GERSTEL Booth #18
http://www.gerstelus.com
40+ Years of Chemical Analysis Solutions: GC/MS, LC/MS sample introduction and stand-alone workstations with the most advanced software control available (MAESTRO). MultiPurpose and PrepStation Autosamplers provide maximum versatility and throughput for liquid injection, SPME, Headspace, ALEX, SPE, dpx®, Dynamic HS, ATEX weighing, centrifugation, and SBSE. Twister® performs solventless extraction and ultra-low detection limits. The most versatile Thermal Desorption System available for all sample types. Cooled (PTV) inlet, Olfactory Detection, Multidimensional Heartcutting, Preparative Fraction Collector.
**Golden West Biologicals** Booth #49  

Golden West Biologicals, Inc. addresses the need for quality, cost effective biological raw materials for the development of immunoassays and LC-MS applications. GWB provides manufacturers and laboratories with over 80 products including Vitamin D free human serum, serum for ultra-sensitive testing, HSA, HGG, and RGG. Please visit us at www.goldenwestbio.com.

**Hamilton Robotics** Booth #34  
[http://www.hamiltonrobotics.com](http://www.hamiltonrobotics.com)

Hamilton Robotics is dedicated to the design and manufacture of automated liquid handling workstations. We offer several types workstations for direct sale and OEM. Key to our products is our air displacement pipetting and monitoring technology as well as the software controlling our systems. We believe every laboratory automation project is unique. Our workstations and software serve as a common high precision and flexible base upon which to provide automated solutions. To this end we employ teams of highly skilled and experienced application and hardware customization specialists around the world to provide our customers with unique solutions to automate their assays successfully and within budget. Please come explore our products and contact us to discuss your automated liquid handling needs further.

**Imtakt USA** Booth #36  
[http://www.imtaktusa.com](http://www.imtaktusa.com)

We are advancing HPLC science by creating unique columns with novel chemistries that provide enhanced selectivity and resolution. We offer a wide range of innovative stationary phases compatible with HPLC, UPLC and LC-MS. Our columns have 25-50% lower pressure and excellent batch-to-batch reproducibility. For more information, please visit our website to view our Product Guide and Application Library.

**Indigo Biosystems** Booth #12  

Indigo Biosystems has changed how both industry and academic scientists utilize Mass Spectrometers. Its flagship software, ASCENT, is a next generation CDS, that automatically picks and integrates peaks with incomparable accuracy. Indigo BioSystems is committed to developing computational tools that deliver better science.

**Ion Bench / MS Noise** Booth #40  

IonBench provides furniture for mass spectrometry and HPLC. On display is our IonBench for mass spectrometry, showing industry leading sound suppression, anti-vibration, and space savings advantages of the bench. Custom sizes, features and options make IonBench the first choice for any mass spectrometer installation. IonBench manufactures IonBench LC, elevator benches that enable safer operation and maintenance of stacked HPLC/UPLC systems by electrically raising and lowering the work surface. IonBench LC is transportable, providing versatility, including the ability to connect the HPLC as close to the source as possible. MS Noise manufactures enclosures for vacuum pumps, water chillers, nitrogen generators, and more. Guaranteed sound suppression is 15 dBA, or about 75%. The booth is staffed by FarHawk Marketing Services, distributors for IonBench and MS Noise products in North America.

**IONICS Mass Spectrometry** Booth #32  
[http://www.ionics.ca](http://www.ionics.ca)

IONICS provides complete, integrated solutions including various options for sample preparation, liquid chromatography and high performance mass spectrometry ensuring laboratories are able to establish an efficient LC-MS/MS workflow and obtain reliable results day-after-day. Designed for clinical labs, IONICS 3Q Series triple quads incorporate features such as: modular design, remote diagnostics and optimization and a self-cleaning interface while providing ultra-high sensitivity and day-after-day performance.
IsoSciences Booth #35
http://isosciences.com/
IsoSciences, LLC is a world leader in the custom synthesis of stable isotope labeled vitamins, steroids, drug substances, metabolites and other compounds of interest. IsoSciences is ISO9001 certified and has an extensive catalog of stable isotope labeled standards available for immediate delivery both as solids and as CertiMass™ Reference Standards with exact concentrations for each lot. IsoSciences has added over 200 new products during 2013 including an extensive range of deuterated bile acids and their conjugates, carbon-13 labeled polyphenols such as quercetin, myricetin and kaempferol and the paralytic shellfish poison (PSP) saxitoxin-15N4. Contact us for your stable isotope labeled needs.

ITSP Solutions Booth #25
http://www.ITSPsolutions.com/
ITSP Solutions specializes in consumable products for Sample Handlers for Chromatography. ITSP will be displaying a patented product called ITSP (Instrument Top Sample Prep) that uses the down time of the LC/MS/MS sample handler to prep patient samples using Solid Phase Extraction and Filtration to reduce costs of these expensive techniques, reduce labor, increase throughput and extend the performance of the analytical instrument. ITSP Solutions offers a variety of methods to test for Pain Management, Vitamin D, Immunosuppressant drugs, and other important compounds. Stop by ITSP Solutions’ booth to review the posters and find out how to manually try ITSP in advance of an instrument purchase.

LEAP Technologies Booth #27
http://www.leaptec.com
LEAP has a novel way to infuse samples directly into the mass spec without loading samples onto an injection valve loop or utilizing a pump, simplifying using MS systems as detectors without complex gradient column chromatography. It also applies a DLW-U and special software for low, constant flow rate provided by refined syringe speed control. LEAP’s various off-line or at-line automated sample clean up by SPE and Magnetic Bead prep for plasma, whole blood, urine and other complex media, reduce interferences and signal suppressions when injecting or infusing samples to a mass spec. HDx-3 PAL ™, provides walk-away automation for Hydrogen-Deuterium Exchange experimental workflows. Gazelle18 UHPLC Pump- PAL-Bundle optimizes LC-MS productivity. PAL3 has 2D barcode reader, unattended method and syringe change, multi-valve capability, low-to-no carryover.

LGC Standards Booth #28
http://www.lgcstandards.com
LGC Standards (www.lgcstandards.com) provides products and services to improve measurement and quality control within the laboratory, and is part of LGC, whose LGC Science & Technology Division acts as the UK National Measurement Institute for chemical and bioanalytical measurements. LGC Standards supplies over 100,000 products, including reference materials, pharmaceutical impurity reference standards (produced under ISO Guide 34 accreditation), biological standards and reagents, and proficiency testing. LGC Standards is headquartered in Teddington, Middlesex, UK. Its global centre for excellence in proficiency testing is located in Bury (Greater Manchester). LGC Standards has offices in Brazil, France, Germany, Italy, Poland, South Africa, Spain, Sweden, China, Russia, United Arab Emirates, UK and the US, a joint venture presence in India, and representatives in Bulgaria, Czech Republic, Finland, Hungary, Ireland, the Netherlands, Romania and Turkey.

New England Peptide Booth #44
http://www.newenglandpeptide.com/
New England Peptide (NEP, Gardner, Massachusetts) has designed and produced high quality custom peptides, polyclonal and monoclonal antibodies for research organizations worldwide since 1998. Our chemists and immunological experts have over 100 years of experience and deliver a full range of peptide and antibody services for biotech and pharmaceutical applications. These include custom peptide synthesis, custom peptide arrays, polyclonal antibodies, quantitative proteomics via our NEPTune™ platform, and analytical services such as mass spec and AAA. Learn more at www.newenglandpeptide.com.
Optimize Technologies Booth #13
http://www.optimizetech.com
Optimize Technologies offers a complete line of innovative components and replacement parts for UHPLC, HPLC and LC/MS systems. Products include EXP® Fittings, Filters, Traps and Guards, OPTI-MAX® Check Valves, OPTI-SEAL® Seals, Replacement Pistons, OPTI-GUARD® Guard Columns, OPTI-PAK® Traps, OPTI-SOLV® Filters and OPTI-LYNX™ Quick-Connect packed beds. New products include EXP® hand-tight fittings, UHPLC/MS traps, UHPLC filtration, guard solutions rated to 20,000+ psi and OPTI-TRAPS™ for large molecules, peptides, online desalting and detergent removal. All Optimize EXP® products feature hand-tight holders and EXP® Titanium Hybrid reusable ferrules.

Parker Hannifin Booth #45
http://www.parker.com/fns/balstonlabgasgenerators
Our company manufactures high efficiency gas generators to eliminate high pressure cylinders from the laboratory. Gas generators provide increased safety, free up laboratory space, save money and produce ultra high purity gasses for your laboratory instruments. With a gas generator you are in control. These state-of-the-art gas generators continuously produce ultra-high purity gases for LC/MS, GC, FT-IR, TOC, ICP, AA and other instrumentation. All products are backed by fully staffed field sales and service organizations and one-year warranty. Preventative maintenance programs and extended warranties are available for all Parker Balston products.

PerkinElmer Booth #29
https://www.perkinelmer.com
PerkinElmer, Inc. is a global leader focused on improving the health and safety of people and their environment. PerkinElmer is dedicated to the quality and sustainability of the environment. With our analytical instrumentation, illumination and detection technologies, and leading laboratory services, we focus on improving the integrity and safety of the world we live in.

Phenomenex Booth #05
http://www.phenomenex.com
Phenomenex is a global technology leader committed to developing novel analytical chemistry solutions that solve the separation and purification challenges of researchers in industrial, government and academic laboratories. Phenomenex's core technologies include products for liquid chromatography, gas chromatography, sample preparation, bulk purification chromatographic media, and chromatography accessories and equipment. For more information, visit www.phenomenex.com.

Phytronix Technologies Booth #07
http://www.phytronix.com/
The leader in quantitative ultra-fast high-throughput analysis for mass spectrometry presents the LDTD-96 and LDTD-384 ion sources. These platforms represent a unique shotgun approach that introduces the sample into the mass spectrometer using an ultra-fast Laser Diode Thermal Desorption (LDTD®) process. The LDTD Ion Source technology is a unique solution to increase sample analysis throughput for your application needs.

Promega Booth #46
http://www.promega.com
Contributing to Science, Discovery and More. Founded in 1978, what started as the production of enzymes for researchers has evolved to offering over 3,000 products for a broad array of applications including basic research, drug discovery, forensics and paternity testing, and molecular diagnostics. Quality Mass Spec Grade reagents like Trypsin Gold and Trypsin/Lys-C Mix assure consistent, reliable results in mass spec applications.
Proteome Software Booth #19
http://www.proteomesoftware.com
Proteome Software's Scaffold Suite is the industry standard for MS/MS based proteomics analysis. Scaffold identifies biologically important results by comparing data from Mascot, SEQUEST, Proteome Discoverer, IdentityE, Spectrum Mill, Protein Pilot, Phenyx, OMSSA, X!Tandem, MaxQuant, MS-GF+, Byonic, PEAKS and more. We specialize in statistical models that provide confident identification and quantitation, PTM site assignment, and analysis of labeled compounds (including SILAC). Our intuitive user interfaces incorporate effective color coding and heat mapping. Elements, our newest product, brings many of these valued features to MS/MS based metabolomics analysis. Mass Spectrometry core labs, clinical labs and researchers utilize Proteome Software products as a valuable part of their workflow.

RECIPE Chemicals & Instruments Booth #50
RECIPE was founded in Munich, Germany, in 1982 and is one of the leading companies in HPLC and LC-MS/MS diagnostics today. For mass spectrometry, RECIPE offers CE/IVD labelled ClinMass® LC-MS/MS Complete Kits. Furthermore, several reagents such as ClinMass® Optimisation Mixes and Internal Standards, ClinCal® Calibrators and ClinChek® Controls are available for a reliable and standardised LC-MS/MS analysis. All products are developed and produced in our state-of-the-art production plant in Munich. RECIPE is recognised worldwide as a reliable partner for clinical laboratories and is certified by the quality management standards ISO 9001 and 13485.

Restek Booth #20
http://www.restek.com
A leading innovator of chromatography solutions for both LC and GC, Restek has been developing and manufacturing columns, reference standards, sample preparation materials, accessories, and more since 1985. We provide analysts around the world with products and services to monitor the quality of air, water, soil, food, pharmaceuticals, chemicals, and petroleum products. Our experts have diverse areas of specialization in chemistry, chromatography, engineering, and related fields as well as close relationships with government agencies, international regulators, academia, and instrument manufacturers.

SCIEX Booth #01
http://sciex.com/
SCIEX helps to improve the world we live in. SCIEX LC/MS/MS solutions enable clinical researchers to push the limits of analysis across a wide variety of applications, including quantitation of steroids, vitamin D, immunosuppressants or drugs of abuse, by harnessing the power of mass spectrometry through exceptionally simple-to-use tools. SCIEX offers the most comprehensive portfolio of pre-configured LC/MS/MS methods and software for clinical research and toxicology. All based on the proven reliability of SCIEX systems, including the SCIEX QTRAP® 5500 system, the most sensitive LC/MS/MS system for trace level analysis -- all backed by the most comprehensive service and support organization in the industry. For more information, go to www.sciex.com/clinicalresearch

Shimadzu Booth #11
http://www.shimadzu.com/
Founded in 1875, Shimadzu is a multinational corporation with three major divisions: Medical Diagnostics, Aerospace/Industrial, and Analytical Instruments. The Analytical Division is one of the world’s largest manufacturers of analytical instrumentation, supporting a broad range of applications including life sciences, pharmaceuticals, food safety, environmental, chemicals, and forensics. Shimadzu expanded the scope of its ISO-13485 certification, which covered blood coagulation and automatic clinical chemistry analyzers, to include LCMS and LC instruments. Shimadzu will continue to register medical devices with the FDA, and support the growing demand for LC and LCMS in clinical testing markets. Visit our booth to learn more about new Shimadzu platforms, including our ultra-fast LCMS-8050 triple quadrupole, automated protein digestion workstations and a “lab-on-a-card” technology that generates volumetric plasma from an unmeasured drop of whole blood in minutes.
Sigma-Aldrich Booth #48
http://www.sigma-aldrich.com
Sigma-Aldrich, a leading Life Science and High Technology company, offers a variety of solutions designed to meet the needs of clinical and forensic scientists. The combined expertise from Sigma-Aldrich, Supelco and Cerilliant create a complete solution for the mass spectrometry-based toxicology workflow. This portfolio of tools includes high quality reagents, analytical products and certified reference materials. Our commitment to high quality and reliable delivery is always focused on accelerating our customers' success by ensuring that our products do not interfere with results and keep the laboratory running smoothly.

Spark Holland Booth #06
http://www.sparkholland.com/
Spark Holland is a specialist in front-end HPLC and UHPLC instrumentation for LC/MS. We are an independent company owned privately by Spark personnel. Our business model is largely based on OEM and in that arena we are best known for our prominent position in autosamplers for HPLC, UHPLC, and micro LC. We are also well recognized for our advanced online solid phase extraction (SPE) technology. Recently we added automated Dried Blood Spot extraction to the palette of front-end instruments using our patented Flow-Through Desorption (FTDTM) technology. We aim to provide best in class instrumentation through continuous innovation, ensuring our commitment by spending more than 12% of our revenues on R&D. Plus, we have mastered the demanding art of OEM partnering! Spark Holland is ISO 13485 certified.

SPEware Booth #33
http://www.speware.com
SPEware Corporation brings advanced separation efficiency to the extraction laboratory using micro-particulate Solid Phase Extraction (SPE) paired with Positive Pressure Manifolds. We have 20 years of experience designing, manufacturing and providing Positive Pressure Processors directly to our customer and to resellers. As the original equipment manufacturer, we have perfected the technology of positive pressure for use with our micro-particulate products as well as developed fully automated extraction procedures. SPEware specializes in customized solutions ranging from traditional extraction problems to unique issues that require a high degree of purification and efficient processing. We strive to provide superior customer service and quick turnaround times. We offer a team of experts that includes our scientific advisory board, analytical chemists, and field application scientists in order to deliver innovative solutions to your SPE needs.

Tecan Booth #03
http://www.tecan.com
Tecan is a leading global provider of laboratory instruments and solutions in biopharmaceuticals, forensics, and clinical diagnostics. Had enough of tedious mass spectrometry sample preparation? Tecan offers Freedom EVO®-based end-to-end process automation for even the most challenging protocols, liberating you from the bottleneck of manual sample preparation. Keep up with ever-increasing demands with Tecan Freedom EVO • Solid phase extraction • Liquid liquid extraction • Protein purification AC Extraction Plate™ The Tecan AC Extraction Plate with TICE™ (Tecan Immobilized Coating Extraction) technology revolutionizes your sample preparation routine. A simple pipette and shake sequence, with no filtration, centrifugation or solvent evaporation, is all that is required. The AC Extraction Plate is easily integrated into automated processes, making it a perfect match with Tecan’s Freedom EVO® liquid handling platform.

Thermo Scientific Booth #21-22
http://www.thermoscientific.com
Innovation applied to clinical research and forensic toxicology. Look to Thermo Scientific for continuous innovation in clinical research solutions, including mass spectrometry, chromatography, automated online sample preparation, multiplexing, software and consumables. Whether your lab is large or small. Whether your need is to analyze small molecules or proteins. We have the expertise, products and flexibility to supply the right answer.
Thomson Instrument Co. Booth #08
http://htslabs.com/
Thomson Instrument Company is a leading-edge manufacturer and supplier of consumable products for the Chemistry and Biological fields. Our SINGLE Step Filter Vials (450uL capacity), Nano Filter Vials (10uL minimum sample volume), and eXtreme Filter Vials (>30% particulates) are used in many labs for all your sample preparation needs and are compatible with most standard autosamplers for HPLC, GC, LC/MS. We provide a number of simple standard and custom products to meet our customer’s needs. Please look at our website at www.htslabs.com. We are committed to competitive pricing and quality customer service. Ph: 800-541-4792 or 760-757-8080 Fax: 760-757-9367 E-Mail: folks@htslabs.com

UTAK Laboratories Booth #04
http://www.utak.com
Since 1973, UTAK Laboratories, Inc., has been connecting Research and Commercial Laboratories with the most comprehensive menu of Stock and Custom manufactured Quality Controls available. Our Products offer complete commutability with many methods of evaluation including; Immunoassay, ELISA, HPLC, UHPLC, ICPMS, GC/MS, and LC/MS, TOF, etc. Our entire line of 100% REAL Human Matrix products along with our Specialty Matrix (SMx™) products come together to offer Laboratorys a true 3rd party Quality Control, especially for Laboratory Developed Tests or LDT’s. Ask us about QC for your LDT. UTAK, createCONTROL

Waters Booth #09-10
http://www.waters.com/clinical
Working with clinical and forensic laboratories around the world, we have pioneered an integrated portfolio of MassTrak Solutions that include separations science, mass spectrometry, consumables, laboratory information management software, services and support. This comprehensive approach helps optimize your laboratory processes and ability to implement LC/MS/MS assays. MassTrak Solutions bring the power of Waters technologies, including tandem mass spectrometry, in easy-to-use, cost effective packages addressing clinical research, therapeutic drug monitoring for tacrolimus and everolimus, and forensic toxicology needs. Waters innovations and laboratory solutions deliver better accuracy and precision for your assays and help ensure the quality of results. www.waters.com/clinical

Zef Scientific Inc. Booth #51
http://www.zefsci.com
•Is your Mass Spectrometer showing the uptime that you expect? •Do the different vendors tend to blame each other—or your method—for an issue? •Are you looking for a more harmonized and seamless experience in maintaining your LC-MS/MS? ZefSci is the country’s premier independent LC-MS/MS engineering firm. A network of experienced field service and qualification engineers are strategically positioned nationwide supplying our customers with the highest level of services on AB/Sciex, Thermo, Waters, Agilent, and Shimadzu. 1- Service Contracts 2- Preventative Maintenance 3 - Repair 4- Compliance IQ/OQ/PQ 5- Method validation services.
Discussion Groups: Monday 7:00 – 8:00 PM

Discussion Groups are non-commercial gatherings that are intended to provide an opportunity for like-minded individuals to get together to share their ideas, create common networks of interest or just have a good time while learning a little bit more about clinical mass spectrometry. If you are interested in leading a Discussion Group at a future meeting please feel free to contact Chris Herold at chris.herold@msacl.org.

**MSACL Jeopardy!**  
@ Harbor Ballroom 1  
Lead(s): Robert Kobelski, Jack Henion & Jeff Moran  
MSACL Jeopardy! A fun game of answer & question with topics covering aspects of clinical mass spectrometry. The audience will be divided into three teams who will race to pose questions to the answers provided in the jeopardy, double jeopardy and final jeopardy round.

**Critical Method Development**  
@ Harbor Ballroom 2  
Lead(s): Russell Grant & Brian Rappold  
In memory of Dr. Dr. Karl Siegfried Boos. The discussion group will feature a short “Life in Review” of Professor Karl Siegfried Boos’s contributions to clinical diagnosis. This will be followed with a "Critical Method Review" competition – specifically highlighting sample preparation, a subject near and dear to Professor Boos.

**Interest Group: Young Clinical Mass Spectrometrists**  
@ Harbor Ballroom 3  
Lead(s): David Herold  
The purpose of this discussion group is to create the opportunity for clinical mass spectrometrists, early in their careers, to assemble with the intent of creating and maintaining an interest group with a discernible voice that will communicate directly and effectively with MSACL, MSACL vendors and the clinical mass spectrometry community at large.  
It expected that this group will be fundamental in shaping the future path of clinical mass spectrometry.

**Regulations & Standards**  
@ Spinnaker  
Lead(s): Julianne Botelho & Hubert Vesper  
Open discussion on current regulations and standards for routine clinical laboratories employing mass spectrometry methods.

**Vendor Feedback**  
@ Marina 6  
Lead(s): Sharon McAvoy  
This is your chance as a vendor to provide feedback regarding your preferences for the customization the MSACL conference. This year we will be exploring a new avenue of communication whereby a vendor representative will lead the discussion and then meet independently with MSACL to relay the interests of the vendor body.
**Restek - Marina 6**

**Pain Assays Do Not Have to Be a Big Pain! Analyze 230 therapeutic and drugs of abuse compounds by LC-MS/MS with the Restek Raptor Biphenyl**

*Frances Carroll, LC Application Chemist, Restek Corporation*

- The use of pain management drugs is steadily increasing. As a result, hospital and contract labs are seeing an increase in patient samples that must be screened for a wide variety of drugs to prevent drug abuse and to ensure patient safety and adherence to their medication regimen. The Raptor™ Biphenyl column was developed to complement high-throughput LC-MS/MS analyses. In this workshop, we will present the methodology for a 230 compound multi-class drug and metabolite screen and discuss the challenges one must consider when developing a large screening assay. Topics of the discussion will include mobile phase considerations, isobar resolution, drug interference, and Instrumentation. Optimized chromatography will also be presented for nine separate drug panels for use during confirmation and quantitative analyses.

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**Waters - Seabreeze**

**Enabling Steroid Analysis For Clinical Research**

*Benjamin K. Beppler, TriCore Reference Laboratories, Development and Technology Scientist (1), Heather A. Brown, Ph.D, Senior Clinical Applications Scientist, Health Sciences Diagnostics, Waters Corporation (2)*

- (1) Quantitation of 17β-Estradiol in Serum Using an Aggressive Sample Prep Method
- (2) A LC-MS/MS SPE method for the accurate quantitation of serum testosterone and androstenedione

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**Sigma-Aldrich - Spinnaker**

**Sample Preparation Considerations for Multiplexed MRM LC-MS Protein Assays in the Clinical Laboratory**

*Steve Hunsucker, Ph.D. - Senior Director of Laboratory Operations, Indi (Integrated Diagnostics)*

- Multiplexed protein assays have tremendous potential in clinical diagnostics, in particular measurement of proteins in plasma or serum derived from circulating blood. The dynamic range of protein concentration in these samples, and the dominance of very high abundance proteins such as albumin and immunoglobulins, make measurement of low concentration proteins impossible without some type of enrichment approach. This workshop will discuss the benefits of using Seppro® protein depletion columns in sample preparation for multiplexed LC-MS protein clinical assays.
Thomson Instrument Co - Harbor Ballroom 1

**Improved Sample Preparation of Biological Samples using the Thomson eXtreme Filter Vials® and analysis by LC-MS/MS**

Lisa Wanders, Technical Sales, Thomson Instrument Company

- Sample preparation continues to be a critical factor in the quantitative measurement of biological samples. The goal of this seminar is to discuss how to streamline the sample preparation process of oral fluids and urine utilizing the Thomson eXtreme Filter VialsTM to reduce interferences from the sample matrix and increase analyte recovery. The Thomson eXtreme Filter VialsTM saves time, reduces solvent usage, alleviates the need for expensive consumables and lab equipment. Samples preparation for matrices such as urine and oral fluids will be discussed.

Thermo Scientific - Harbor Ballroom 2

**Time Matters. Simplify Workflow Complexity.**

Jason Lai Ph.D., MBA, Regulated Product Manager - Thermo Fisher Scientific

- Discover three newly listed Class I medical devices for general clinical use: Thermo Scientific™ Prelude MD™ HPLC, Thermo Scientific™ Endura MD™ mass spectrometer, and Thermo Scientific™ ClinQuan MD™ software. Clinical laboratories can use these devices to build their own lab developed tests (LDT). Combined, tools provide laboratories the ability to obtain the quantitative accuracy of LC-MS, easily and confidently. Examples of various compounds and workflows will demonstrate the robustness, stability, and time efficiency of these new class I medical devices.

Phenomenex - Harbor Ballroom 3

**Solutions for Challenging Biological Matrices in Analytical Method Development**

(1) Seyed Sadjadi, Ph.D., Phenomenex (2) Stephanie J. Marin, Ph.D., ARUP Institute for Clinical and Experimental Pathology

- **(1) When SPE is Not Enough: Considerations in developing a drug analysis in whole blood.** We evaluate 7 common pretreatment procedures that hemolyze erythrocytes and precipitate plasma proteins from whole blood. The effectiveness of a procedure was based on overall recovery, response and reproducibility for analytes representative of different drug classes.

- **(2) Optimizing Sample Prep for Quantitation of Buprenorphine and Norbuprenorphine in Meconium.** Meconium, the first stool produced by a newborn, is a complex matrix of materials ingested by a fetus, making it a good specimen to detect in utero drug exposure. Traditionally, meconium is homogenized in a laborious methodology. A simplified method where meconium is homogenized directly in enzyme, hydrolyzed and cleaned up by SPE to extract buprenorphine and norbuprenorphine is explored.

Bruker - Marina 6

**(1) Robustness of the EVOQ Elite in Clinical Research Analysis**

**(2) MALDI-TOF MS in a Public Health Laboratory: Faster Bacterial Identification, Impact of Cost and Staff Savings and Future Applications**

**(3) Use of the Bruker Microflex™ LRF MALDI-TOF MS as a Rapid Screening Platform for Clinical Toxicology**

(1) Dr. Timothy Garrett, Univ. of Florida, Gainesville, FL, (2) Kimberlee A. Musser, PhD, Wadsworth Center, NYSDOH, (3) Christina Wilson, PhD, Purdue University

- **(1) Due to the high sensitivity and selectivity provided by LC-MS/MS, this technology is being adopted in more clinical research applications. This talk will describe the unique design and performance characteristics of the EVOQ LC triple quadrupole system for the analysis of various research assays including Vitamin D, alcohol biomarkers, fructose and steroid profiling.**

- **(2) The cost, staffing and turn-around time benefits with the adoption of the testing will be addressed. Additionally, validation of bacterial and mycobacterial identification and MALDI-TOF MS approaches to molecular serogrouping and assessment of antibiotic resistance will be discussed.**

- **(3) This seminar will highlight the use of the Bruker Microflex™ LRF MALDI-TOF MS as a rapid, reliable tool for qualitative screening and confirmation of toxins and toxicants in clinical diagnostic toxicology cases.**
**Corporate Workshops : Tuesday 7:00 - 8:00 AM**

**Tecan - Marina 6**

**Automation Solutions for Mass Spec Sample Preparation in the Clinical Laboratory**  
*Daniel Leach, Senior Application Scientist*

- Although there have been monumental advances in mass spectrometry (MS) instrumentation in recent years, its unglamorous counterpart, sample preparation, has not enjoyed the same rate of development. This workshop will illustrate how the status quo is changing and will highlight a number of recent advances by Tecan utilizing its world class Freedom EVO liquid handling workstation to develop a variety of protocols designed to handle a broad range of sample types, sample preparation protocols and analytes to meet the needs of the modern analytical clinical laboratory. This includes both vacuum and positive pressure based SPE protocols, tip based cleanup for rapid processing of a small number of samples, sample tracking and LIMS integration.

**ZefSci - Seabreeze**

**Special Considerations for Clinical LC-MS/MS Method Validation**  
*James Byrd, Application Chemist for Zef Scientific*

- CLIA regulations require method validation, but do not specify which tests are required. We will talk about why LC-MS/MS methods may need extra validation steps compared to other high complexity tests.

**IONICS MS - Spinnaker**

**Identification of Potential Biomarkers for Efficacy and Toxicity in Clinical Trials of a Cancer Vaccine using Targeted Metabolomic Profiling**  
*Dr. Devanand Pinto*

- Dr. Devanand Pinto will discuss what his Biomarker Quantification Team at the National Research Council of Canada has accomplished in their pursuit of a faster, more sensitive and more robust method of measuring immune response in cancer vaccine trials. Immune response is typically measured by cytokine profiling and flow cytometry; however, these techniques require extensive sample preparation and, due to the use of antibodies, have limited multiplexing capabilities. The Biomarker Quantification Team investigated the use of targeted metabolomics profiling to study the metabolic profile of 38 patients enrolled in a Phase I/Ib clinical trial for a novel cancer vaccine. This targeted metabolomic profiling approach utilizes an IONICS 3Q 320 which provides the leading sensitivity and advanced multiplexing capabilities necessary for success.
Corporate Workshops: Tuesday 1:00 – 2:00 PM

Waters - Harbor Ballroom 1

**Clinical Research and Biomarker Discoveries, Challenges and Opportunities**

(1) Dr. Amrita K Cheema, Associate Professor & Co-Director-PMSR, Georgetown University Medical Center. (2) J. Will Thompson, Ph.D., Assistant Research Professor, Proteomics and Metabolomics, Duke University

• (1) Supporting the Systems Medicine Paradigm: Metabolomics and Lipidomics Core Technologies for Clinical and Translational Research
• (2) The Utilization of Quality Measures in Experimental Design and Interpretation to Aid Quantitative Clinical Proteomics and Metabolomics Studies

Shimadzu - Harbor Ballroom 2

**Secrets to Successful LC-Tandem MS Implementation - Tips and tricks for Triple Quadrupole Mass Spec Techniques**

Kent Johnson (Excelltox Laboratories, LLC), Chris Gilles (Shimadzu Scientific Instruments)

• From screening to confirmation/quantitative testing, there are many pitfalls that can prevent maximum laboratory efficiency. This workshop will discuss several specific scenarios and offer solutions to everyday challenges in medication monitoring and urine drug testing, including: - Better sample preparation, - Validity Testing (to ensure the sample is human and that it has not been diluted nor adulterated), - Detection of early signs of medication misuse or illicit drug use, - State of the art LC/MS technology, - Delivering quality data on an ultra-fast timescale, and - Simplifying reporting and billing. Please join us for this practical workshop and learn how to operate your laboratory at maximum efficiency. Attendees will receive a lunch, t-shirt and GiantMicrobe!

Agilent Technologies - Harbor Ballroom 3

(1) Development of a Multiplexed MRM LCMS LDT
(2) Challenges in Quantitative Dried Spot Sampling and Analyses

(1) Stephen W. Hunsucker, Ph.D., Integrated Diagnostics (2) Kenneth Lewis, Ph.D., OpAns, LLC

• (1) The potential impact of multiplexed MRM LCMS analysis for clinical diagnostics is enormous and has garnered much attention in recent years. This workshop will discuss the approach for the development of multiplexed MRM LCMS LDT(s) and highlight some of the analytical challenges associated with the design and development of such an LDT.
• (2) The Pediatric Trial Network (PTN) was established to create an infrastructure for investigators to conduct trials that improve child health. Advancement of low volume sampling technologies is being achieved through these trials by demonstrating the concordance of drug concentrations in plasma to Dried Blood Spots (DBS). In this presentation, Dr. Lewis will give a brief description of challenges and remedies encountered in the quantitative analysis of dried spot specimens for small molecule targets.

Biotage - Marina 6

**Advances in Sample Prep for Pain Management: Developing a Simple, Fast Method for Nonpolar Compounds and Polar Metabolites in Urine prior to LC/MS.**

Matthew Slawson, PhD

• Described here is a method utilizing supported liquid extraction for the detection of tapentadol, its glucuronidated, sulfated, and N-desmethyl metabolites; tramadol, its N- and O-desmethyl metabolites; and meperidine and its N-desmethyl metabolite. The method utilizes ISOLUTE SLE+ (Biotage; Charlotte, NC) for sample clean-up followed by analysis by LC-MS/MS. The extraction was optimized to ensure good recovery of both nonpolar parent drug polar metabolites by utilizing a sample pretreatment with a basified brine solution and elution with acidified methylene chloride/isopropanol. These steps ensured that both polar and nonpolar analytes eluted in the same extract. The method has a limit of detection of at least 50 ng/mL and an upper limit of linearity of at least 5,000 ng/mL. The method is simple, fast and offers excellent S:N.
Corporate Workshops: Tuesday 7:00 – 8:00 PM

Thermo Scientific - Harbor Ballroom 2

Break the Bottleneck - Accelerate Genomics to Proteomics to Bedside with New Cloud-computing and Informatics Solutions

Mazi Mohiuddin, Senior Applications Scientist Thermo Fisher Scientific, Biomarker Research Initiatives in MS (BRIMS)

- An overview of the proteo-genomic data analysis workflows and automation of informatics solutions for clinical service providers as well as researchers to run routine data analysis workflows in a seamless manner.

Agilent Technologies - Harbor Ballroom 3

The Agilent StreamSelect LC/MS System – Up To Four Times the Throughput with Outstanding Reliability

Agilent Technologies

- The StreamSelect LC/MS System delivers up to four parallel chromatographic separations to the same triple quadrupole mass spectrometer, with superior robustness and data quality. Intuitive automation software coordinates the completely integrated system, maximizing MS utilization and greatly enhancing throughput and cost-effectiveness.

IONICS MS - Marina 6

Recent Developments in Endocrinology – Diurnal Steroids Fluctuations, Free 25-OH Vitamin D3 & Thyroid Hormone Management

Dr. Steven Soldin

- In this workshop Dr. Steven Soldin from the National Institutes of Health (NIH) will address several recent developments in endocrinology. Topics discussed will include: diurnal fluctuations of steroids, changing trends in management of thyroid patients, the role in measurement of free 25-OH Vitamin D3 and a variety of clinical applications.
Spark Holland - Marina 6

(1) Routine Clinical LC-MS Assays Using Online Sample Preparation: Daily User Experiences and Illustrations
Martijn van Faassen, UMCG, The Netherlands (1). Jack Henion, Rob Sturm and Regina Oliveira, Quintiles BioAnalytical and ADME Labs, Ithaca, NY 14850 (2).

*(1) For reproducible highly sensitive LC-MS/MS assays, sample preparation is pivotal. Several sample prep approaches are available, each with its own (dis)advantages. In our clinical chemistry laboratory different sample prep strategies are used with online sample prep as chosen method. In this presentation we provide an overview of our clinical LC-MS/MS assays using online sample prep and discuss different strategies by focusing on relevant examples. (2) Punching of DBS cards for LC/MS analysis is tedious and too manual for a high sample volume lab. This presentation describes how commercial DBS cards as well as a prototype dried plasma spot (DPS) card can be analyzed on-line in a fully automated fashion using either SRM LC/MS or high res. mass spectrometry techniques for the bioanalytical determination of drugs in micro blood samples.*

Sigma-Aldrich - Spinnaker

Developing the Best Sample Preparation Methods to Ensure Robust LC/MS Analyses in Biological Fluids
Craig R. Aurand, Supelco/Sigma-Aldrich

*(1) Sample preparation continues to be one of the critical factors for effective method development when analyzing biological samples. Too often this portion of the assay is not allocated sufficient attention to ensure a robust analytical method. The goal of this seminar is to discuss several approaches for sample preparation for biological fluids, and to demonstrate the benefit that proper sample clean up can have on an LC/MS based methods. Sample preparation methods for matrices such as plasma and urine will be covered, along with techniques such as solid phase extraction, phospholipid depletion, and enzymatic digestion.*
Corporate Workshops: Wednesday 1:00 – 2:00 PM

Shimadzu - Harbor Ballroom 1

Prepare Your Laboratory for the Future - Laboratory-on-a-Card Technology and Ultra-Fast Mass Spectrometry

Fred Regnier (Novilytic Labs), Scott Kuzdzal (Shimadzu Scientific Instruments)

‣ Please join us for this interactive workshop where you will discover a new technology (Noviplex Duo) that enables multiple plasma extractions and sample preparation steps to be performed from a single blood drop in just minutes. This "lab-on-a-card" technology simplifies and accelerates sample preparation by: - Integrating and automating multiple sample prep steps, - Introducing in-transit sample preparation, - Allowing collection of multiple fractions, - Reducing sample prep time, - Simplifying sample transport, - Reducing chain of custody issues, and - Enabling simultaneous analysis of proteins and metabolites.

Combined with ultra-fast mass spectrometry, these technologies can save your laboratory time, money and resources. Attendees will receive a lunch, t-shirt and GiantMicrobe!

Thermo Scientific - Harbor Ballroom 2

Employing Translational Research Workflow on LC-HRAM Platform for Detection of Pathogen Induced Cancer in a Human T-Cell Leukemia Virus Type 1 Disease Model


‣ Dr. Sucharita Dutta, from Eastern Virginia Medical School will discuss the Human T-Cell Leukemia Virus Type 1 (HTLV-1) as the causative factor for the development of an aggressive lymphoma, Adult T-cell Leukemia (ATL). The translational workflow involves standard data-dependent acquisition (DDA) experiments followed by more in-depth pSMART data acquisition methodologies or the most comprehensive global profiling of proteins from exosome samples to exhaustively mine for proteins that show functional significance via pathway analysis.

SCIEX - Harbor Ballroom 3

1) Lipid Clinical Biomarkers: From Discovery to Quantitation. 2) Implementing LC-TOF-MS/MS in the Clinical Research Arena Developing A Broad Spectrum Test

1) Paul RS Baker, PhD, SCIEX. 2) Kara Lynch, PhD, University of California, San Francisco

‣ 1) Lipid Clinical Biomarkers: From Discovery to Quantitation - This presentation will focus on the general methods used for lipid biomarker discovery will be presented with special focus on the novel use of DMS as a tool orthogonal to chromatography to achieve better qualitative lipid identification. 2) Implementing LC-TOF-MS/MS in the clinical research arena developing a broad spectrum test - This presentation will focus on the pros and cons of assorted screening approaches for maximum compound coverage, minimizing false positive finding and improving confidence in results. As well as, tips for managing challenging cases that include complex samples and matrices.
Monday @ 10:45 AM in Harbor 1
Optimizing Trypsin Digestion
Kevin Meyer - Perfinity Biosciences (kmeyer@perfinity.com)
• Digestion has historically been a significant bottleneck and source of irreproducibility associated with the preparation of certain protein samples for mass spectrometric analyses. This presentation will provide a comparison of the use of recombinant trypsin, pancreatic trypsin, additive/solvent enhanced digestion and elevated temperature methods as they apply to clinically relevant biomarkers including thyroglobulin and C-reactive protein as well as a highly multiplexed assay for apolipoproteins.

Monday @ 11:10 AM in Harbor 1
All that Glitters Is Not the Gold Standard: Calibration of a Sensitive Protein Cleavage-Isotope Dilution Mass Spectrometry (PC-IDMS) Assay for Thyroglobulin
Christopher Shuford - Laboratory Corporation of America (shuforc@labcorp.com)
• A PC-IDMS assay was developed for quantification thyroglobulin in serum with a lower limit of quantification (LLOQ) of 0.2 ng/mL. To enable sensitive and accurate measurement at this LLOQ, external calibration was investigated with multiple sources of thyroglobulin and surrogate matrices lacking endogenous human thyroglobulin, including pooled human sera from remissive thyroidectomy patients (<0.1 ng/mL). Implications of matrix affects were explored as a function of thyroglobulin source and matrix, with consideration to the digestion process. Veracity of the final PC-IDMS assay was investigated by correlation with the FDA-approved Beckman Access® immunoassay and by comparison of two signature peptides.

Monday @ 11:35 AM in Harbor 1
Unraveling Trypsin Digestion, a Continuing Story: Mitigating Matrix Effects for Accurate MS-based Quantification of Serum Apolipoproteins
Irene van Den Broek - Leiden University Medical Center (LUMC) (i.van_den_broek@lumc.nl) -- *Young Investigator Grantee*
• Despite excellent agreement and correlation between LC-MS/MS and immunoturbidimetric quantification of apolipoprotein A-I (apoA-I) in 100 patient sera, matrix effects on digestion completeness have been assigned as a remaining source of inaccuracy. In this study, we describe a detailed optimization of digestion conditions with a particular emphasis on reducing matrix effects on the digestion completeness of apoA-I, while additionally focusing on increasing peptide yield for multiple apolipoproteins (apoB-48, apoB-100, apoC-I, apoC-II, apoC-III, and apoE), and decreasing the digestion time. Effects of automation of the digestion protocol on assay simplicity, imprecision, and throughput will furthermore be discussed.
Monday @ 10:45 AM in Harbor 2

**Measured GFR by Iohexol Clearance: A Pediatric Perspective**

*Shannon Haymond - Northwestern University Feinberg School of Medicine (shaymond@luriechildrens.org)*

» Accurate assessment of glomerular filtration rate (GFR) is critical for diagnosing and managing kidney disease and for safely prescribing and monitoring side effects of nephrotoxic and renally cleared agents. Estimated GFR (eGFR) is commonly calculated using equations based on creatinine and other parameters. Although relatively inexpensive and convenient, these equations are particularly problematic in children and adolescents. As efforts to refine and improve pediatric eGFR continue, there are frequently cases where an accurate method for mGFR is needed. We will describe our experience developing and implementing a semi-automated LC-MS/MS method for measurement of serum iohexol. Select case studies will be reviewed to demonstrate the clinical benefit of mGFR over eGFR in children. Details of the challenges faced in operationalizing an mGFR procedure will also be discussed.

Monday @ 11:10 AM in Harbor 2

**Accuracy of Mass Spectrometry with the Ease of Immunoassay? LC-MS/MS Workflow Improvements for Primary Aldosteronism Screening**

*Daniel Holmes - University of British Columbia (dtholmes@mail.ubc.ca)*

» Uptake of LC-MS/MS is often hampered by perceived and actual workflow challenges and the enticement of walk-away automation offered by immunoassay. Both aldosterone and plasma renin activity (PRA) are difficult analytes with separate sample preparations by LC-MS/MS. We have significantly simplified the sample preparation of PRA and aldosterone to a single process. Comparisons with our current assays was excellent: Aldo-New = 0.99 × Aldo-Current + 10.1 pmol/L, R-sq=0.98 PRA-New = 0.94 × PRA-Current + 0.026 ng/mL/h, R-sq=0.99 Additionally, we present exploratory data on the aldosterone:AngI ratio as an equivalent screening tool to aldosterone:PRA ratios.

Monday @ 11:35 AM in Harbor 2

**The Benefits and Pitfalls of Using MRM3 Detection for the Analysis of Plasma Free Metanephrines by LC-MS/MS**

*Michael Wright - SEALS, Prince of Wales Hospital (mwright_11@yahoo.co.uk)*

» Liquid chromatography coupled to tandem mass spectrometry using multiple reaction monitoring (MRM) is a powerful tool for the quantitation of target analytes in complex matrices. However, this technique lacks specificity when plasma free metanephrines are measured. In this presentation we demonstrate the use of multistage fragmentation (MRM3) to improve the analytical selectivity of plasma free metanephrine service whilst also outlining other considerations that need to be taken into account before introducing this technology to a routine clinical chemistry laboratory.
Monday @ 10:45 AM in Harbor 3  
**Taming Tuberculosis: Metabolic Insights into the Host-pathogen Interface**  
*Kyu Rhee* - *Weill Cornell Medical College* (kyr9001@med.cornell.edu)  
- Despite the advent of anti-infective chemotherapy well over 50 years ago, tuberculosis (TB) remains the bacterial cause of deaths worldwide. Unlike other pathogens, *Mycobacterium tuberculosis* (Mtb), the causative agent of TB, resides in humans as its only known reservoir and host. Mtb has thus evolved within an ultranarrow ecologic niche. Recent evidence has further highlighted metabolism as a key mediator of the host-pathogen interaction. Here, we present recent work highlighting specific elements of the bidirectional metabolic discourse between Mtb and the host.

Monday @ 11:10 AM in Harbor 3  
**Tracing Metabolism in Cancer**  
*Matthew Vander Heiden* - *Koch Institute for Cancer Research at MIT* (mvh@mit.edu)  
- Cells adapt metabolism to meet distinct physiological needs, and metabolic regulation influences tumor progression. To proliferate, cancer cells must adapt metabolism to support anabolic processes and allow the accumulation of biomass. However, those nutrients with the highest consumption by cancer cells are not necessarily the fuels that contribute directly to cell mass. Cell culture provides a system to study how metabolism supports proliferation, but understanding non-proliferating cell populations requires an analysis of metabolism in patients and in tumor tissue. Use of mass spectrometry to trace nutrient use both in cell culture models and mouse cancer models will be presented to provide insight into how metabolism impacts cancer biology.

Monday @ 11:35 AM in Harbor 3  
**Understanding the Metabolic Remodeling of the Heart Under Chronic Stresses**  
*Rong Tian* - *University of Washington* (rongtian@uw.edu)  
- Energy metabolism is essential for maintaining normal cardiac function. Alterations of substrate metabolism, especially glucose and lipid metabolism; are integral to the development of pathological hypertrophy and heart failure. Furthermore, emerging evidence indicates that the branched-chain amino acids (BCAAs) catabolism is significantly changed during the development of cardiovascular and metabolic diseases. For example, plasma accumulation of BCAAs is strongly correlated with insulin resistance and coronary heart disease. Our recent studies describe a reciprocal regulation between glucose and BCAA metabolism in the heart. Investigation of the mechanisms governing the regulatory circuit will provide novel insight on the metabolic regulation of cardiac biology and diseases.
Monday @ 10:45 AM in Marina 6
Rapid Antibiotic Susceptibility Detection Using Mass Spectrometric-Antibiotic Susceptibility Rapid Assay (MS-ASTRA) in Streptococcus Pneumoniae

Lawrence Tse - Medical College of Wisconsin (ltse@mcw.edu) -- *Young Investigator Grantee*

- In this study, we have adapted the Mass Spectrometric-Antibiotic Susceptibility Rapid Assay (MS-ASTRA) technique to determine penicillin resistance in over 50 clinical isolates of Streptococcus pneumoniae in less than 5 hours, compared to the overnight incubation requirement needed by many current methods. This technique uses MALDI-TOF to determine the relative amount of growth in the presence and absence of an antibiotic. Susceptibility data using MS-ASTRA was comparable to broth microdilution techniques (Phoenix, BD, Sparks, MD) with an overall agreement of over 90% and no very major errors.

Monday @ 11:10 AM in Marina 6
Quantitative Assessment of Multifactorial Resistance Mechanisms in Acinetobacter baumannii Using Selected Reaction Monitoring

Tiphaine Cecchini - bioMérieux (tiphaine.cecchini@biomerieux.com) -- *Young Investigator Grantee*

- Multidrug resistant isolates of Acinetobacter baumannii responsible for nosocomial infections are reported increasingly. β-lactam resistance together with overexpression of RND efflux pumps are of particular concern. Following a simple sample preparation, a conventional liquid chromatography coupled with a triple quadrupole mass spectrometer was carried out in sMRM mode to detect in 1 hour both β-lactam hydrolyzing enzyme production (AmpC, OXA, TEM, GES, NDM, VIM, VEB, PER) and efflux pumps overexpression (AdeABC, AdeIJK) in multidrug resistant strains. Resistance phenotype might be deduced from these quantitative data. Correlation of mRNA and protein expression levels will be discussed for the efflux pump.

Monday @ 11:35 AM in Marina 6
Solving a Microbiological Quandary: Does MALDI-TOF Mass Spectrometry Hold the Key to Rapid Detection of Vancomycin-intermediate Staphylococcus Aureus?

Susan Butler-Wu - University of Washington (butlerwu@uw.edu)

- Vancomycin is a mainstay in the treatment of serious infections due to methicillin-resistant Staphylococcus aureus (MRSA). However, vancomycin treatment failure rates are higher in patients with blood-stream infection due to MRSA strains that have a vancomycin-intermediate sub-population (so-called heterogeneous VISA strains or “hVISA”). This subpopulation can often go undetected because it is present at a frequency lower than that what can be detected by standard susceptibility testing methods. In this study, we investigate the potential for MALDI-TOF Mass Spectrometry to rapidly detect VISA and hVISA strains.
Basics of Mass Spectrometry

Monday @ 10:45 AM in Exec Ctr
Session Chair: Rob Fitzgerald - University of California, San Diego

Introduction to Mass Spectrometry
Jane Yang - University of California, San Diego (jyy008@ucsd.edu) -- *Young Investigator Grantee*
- Mass spectrometry is a powerful analytical chemistry tool that detects molecules in the gas phase based on their mass to charge ratio. This session will cover the basic concepts of mass spectrometry, how it works, the components of a mass spectrum, tandem MS (or MS/MS), the relationship between chromatograms and mass spectra, tuning, and what your service representative will do.

Introduction to Ionization Modes in Mass Spectrometry
Imir Metushi - University of California, San Diego (imetushi@mail.ucsd.edu)
- Ionization of analytes into the gas phase is a critical step for accurate mass identification. Various types of ionization methods can be applied for optimal analyte identification. This session will involve a basic introduction to various ionization modes used in mass spectrometry and will discuss the advantages and disadvantages of each.

Introduction to Mass Analyzers: Quadrupole vs. Time-Of-Flight (TOF)
Alec Saitman - University of California, San Diego (asaitman@ucsd.edu) -- *Young Investigator Grantee*
- Choosing a mass analyzer for clinical analysis is an important step in setting up a mass spectrometry laboratory. But what is a mass analyzer? Are all mass analyzers created equal? What types of clinical tests can be validated on each? Each type of mass analyzer has its own benefits and caveats in mass resolution, sensitivity, and dynamic range for small molecule analysis. Choosing a mass analyzer to suit the needs of the clinical laboratory is an important consideration to make. This lecture will focus on quadruple and time of flight (TOF) mass analyzers for identification and quantification of small molecules. This lecture will also describe how these different mass analyzers actually create mass separations and will describe the various clinical applications available to each.
• Session 2 • Track 1 •
Proteomics: Endocrine Assays
Monday @ 3:00 PM in Harbor 1
Session Chair: Leigh Anderson - SISCAPA

Monday @ 3:00 PM in Harbor 1
High Sensitivity Measurement of Parathyroid Hormone Related Protein by LC-MS/MS for Diagnosing Disorders of Calcium Regulation
Mark Kushnir - ARUP Institute for Clinical and Experimental Path (kushnmm@arulab.com)
• Measurement of Parathyroid Hormone related Protein (PTHrP) plays important role in follow-up of patients suspected of hypercalcemia. Concentrations of PTHrP in blood are very low and because commercial PTHrP immunoassays (IAs) are insufficiently sensitive, this result in a high false negative rate (FNR) of IAs and misdiagnosis of patients with elevated calcium. We developed LC-MS/MS method that overcomes drawbacks of commercial IAs and allows accurate measurement of PTHrP. Limit of quantitation of assay is 0.6 pmol/L, sensitivity sufficient to quantify PTHrP in samples of healthy and pathologic individuals. Our data suggest a FNR of one commercial IA of approximately 20%.

Monday @ 3:25 PM in Harbor 1
A High-throughput Mass Spectrometry Multiplexed Assay to Measure Insulin and C-peptide
Steven Taylor - Quest Diagnostics Nichol's Institute (Steven.W.Taylor@questdiagnostics.com)
• We have developed a multiplexed method to measure insulin and C-peptide using an LC tandem mass spectrometry assay. The assay involves enrichment of the peptides from patient sera using 2 different monoclonal antibodies immobilized on magnetic beads and processing on a Hamilton Star robotic liquid handler. Eluted peptides are directly analyzed by LC-MS/MS on an Agilent 6490 triple quadrupole mass spectrometer. The assay has a clinical reportable range from 2.5 to 320 µIU/mL for insulin and 0.11 to 27.2 ng/mL for C-peptide. Intra- and inter-day assay variation is less than 11% for both peptides.

Monday @ 3:50 PM in Harbor 1
Quantitation of Glycated Hemoglobin by MALDI Mass Spectrometry
Stephen Hattan - SimuToF Systems (stephen.hattan@simultof.com)
• MALDI MS of whole blood hemolysates allows the direct quantitation of the total glycated hemoglobin (TGHb) ratios of alpha and beta chains from a single mass spectrum. HbA1c, the standard measure of glycated hemoglobin, can be calculated from TGHb. Sample preparation for this approach is minimal, analysis is rapid, 80 spots in 30 min (20 sec/spot), and hemoglobin variants are also detected. TGHb by MALDI vs. cation exchange HPLC, the reference standard, exhibited linearity (y = 0.79x + 1.4; r² = 0.99) from 1.36% to 17.94% with CVs < 1.66%. MALDI method is better, faster, and less expensive than HPLC.
**Feeling Your Pain: TDM and Pain Medication Analysis**  
*Monday @ 3:00 PM in Harbor 2*  
Session Chair: Katie Thoren - Memorial Sloan Kettering Cancer Center

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**Monday @ 3:00 PM in Harbor 2**  
**Formation of 6-acetylmorphine in Urine Samples with High Morphine Levels During Sample Preparation Involving Enzymatic Hydrolysis**  
*Sihe Wang - Cleveland Clinic (wangs2@ccf.org)*  
- 6-Acetylmorphine (6-AM), an unique metabolite of heroin, is known as a definitive indicator of heroin intake. Due to variable glucuronide conjugation rates between and within individuals, an enzymatic hydrolysis using glucuronidase during sample preparation is frequently used to improve detection sensitivity and consistency. Acetate buffer is the primary choice for preparing enzymatic hydrolysis solution due to the desirable pH. We report here that urine samples with elevated levels of morphine (>100,000 ng/mL) incubated for >12 hours using an acetate buffer prior to LC-MS/MS analysis could form measurable amounts (≥ 5 ng/mL) of 6-AM.

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**Monday @ 3:25 PM in Harbor 2**  
**Antiretroviral Testing: Development and Validation of LC-MS/MS Assays in Unique Specimen Sources to Support Clinical Trials**  
*Mark Marzinke - Johns Hopkins University (mmarzin1@jhmi.edu) -- *Young Investigator Grantee*  
- In order to better understand the pharmacokinetic-pharmacodynamic (PK-PD) relationships of antiretroviral drugs (ARVs) in disease prevention and management, compartmentalized PK studies are required to assess localized drug concentrations. Quantification of ARVs in cervicovaginal secretions (CVS) and rectal fluid (RF) can help determine efficacy at the site of viral transmission. Thus, liquid chromatographic-tandem mass spectrometric (LC-MS/MS) methods for the dual quantification of tenofovir and emtricitabine in CVS and RF have been validated according to the recommendations of the FDA, Guidance for Industry: Bioanalytical Method Validation document. Further, the described work illustrates the considerations for method validation in unique specimen sources.

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**Monday @ 3:50 PM in Harbor 2**  
**To Hydrolyze, or Not to Hydrolyze, that Is the Question for HRMS Pain Management Testing**  
*Kara Lynch - University of California, San Francisco (kara.lynch@ucsf.edu)*  
- The demand for drug testing to monitor patients receiving treatment for chronic pain is continuing to increase. The objectives of this study were to 1) develop and validate a liquid chromatography high resolution mass spectrometry (LC-HRMS) screening panel for pain management testing and 2) evaluate two sample preparation approaches including hydrolysis and directly monitoring conjugated metabolites. HRMS offers a great platform for testing panels of compounds in one analytical run and shows good concordance with traditional MS based methods. Monitoring conjugated metabolites directly was sufficient for most analytes, however, there were 6 analytes missed in the 24 routine samples.
Intestinal Fat Absorption and Systemic Metabolism: Applications of Mass Spectrometry in Discovery Research

Eric Yen - University of Wisconsin-Madison (yen@nutrisci.wisc.edu)

- Triacylglycerol is the storage and transport molecule of fatty acids in animals. Its biosynthesis serves many physiological functions, including the absorption of dietary fat. However, excessive accumulation of triacylglycerol leads to obesity and related metabolic diseases. Acyl CoA:monoacylglycerol acyltransferase (MGAT) mediates one of the two TAG synthesis pathways. Among known MGATs, MGAT2 is highly expressed in the intestine of mice and humans. Findings from genetically engineered mice indicate that MGAT2 modulates the kinetics of fat absorption, the efficiency of energy metabolism, and the propensity to gain weight in response to calorie-dense diets. We are using proteomics as well as lipidomics profiling to explore molecular mechanisms underlying the functions of MGAT2.

Metabolomics by Masses, Metabolomics for the Masses

Nicola Zamboni - ETH Zurich (nzamboni@ethz.ch)

- Metabolism plays a pivotal role in cellular processes by providing building blocks and energy for biosynthesis and participating in decision-making. In many biomedical areas such as nutrition, oncology, toxicology, stem cell research, etc., metabolism is currently regarded as a key driver and a differentiating factor to be exploited in diagnostics and selective therapy. To drive these activities, we developed a flow injection mass spectrometry platform that allows to routinely profile thousands of small molecules in biological extracts in thousands of samples per day. The massive throughput and information provided by our flow injection platform opens new fascinating horizons both in discovery and diagnostics.

Integrative Physiological Analysis of Adipocyte Metabolism

Christian Metallo - University of California, San Diego (cmetallo@ucsd.edu)

- Obesity and metabolic syndrome are characterized by dysfunction in nutrient homeostasis, and adipose tissue can influence systemic metabolism via signaling or direct metabolic activity. Using a combination of oxygen physiology measurements and 13C metabolic flux analysis we have characterized how adipocyte hypoxia regulates amino acid metabolism. While branched chain amino acids (BCAAs) are normally major oxidative substrates for adipocytes, hypoxia elicits profound and lasting effects on glucose and amino acid metabolism such that BCAA catabolism is significantly compromised. These defects in adipose tissue oxygen physiology therefore contribute to the disturbances in amino acid homeostasis observed in the context of obesity.
Taxon-specific Markers for the Qualitative and Quantitative Detection of Bacteria in Human Samples
Nicole Strittmatter - Imperial College London (n.strittmatter12@imperial.ac.uk) -- *Young Investigator Grantee*

• Based on a database of lipid profiles of microorganisms acquired using rapid evaporative ionization mass spectrometry, taxon-specific markers were derived for a variety of bacterial taxa at different levels. These markers were shown to be absent in human lipidome/metabolome and can thus be used to detect and quantify bacteria in human samples as exemplified by the imaging mass spectrometric analysis of human colorectal tissue. The approach can be used to detect certain types of bacteria in arbitrary matrices while maintaining both the untargeted and the spatially resolved nature of the mass spectrometric experiment.

Rapid Bacterial Identification Using a Mass Spectrometry Based Molecular Diagnostics Approach: Evaluation of the Iridica Platform
Alec Saitman - University of California, San Diego (asaitman@ucsd.edu) -- *Young Investigator Grantee*

• Abbott Diagnostics has developed the Iridica platform, capable of producing an accurate bacterial identification in less than 8 hours directly from blood specimens. The technology uses PCR based amplification of bacterial DNA followed by analysis by ESI-TOF mass spectrometry to make accurate identifications. The rapid identification is potentially useful in the clinical treatment and outcome of sepsis patients. In this study, we evaluated the clinical robustness of the Iridica platform by analyzing known positive blood cultures and outdated blood spiked with known bacterial isolates and demonstrated that the Iridica platform rapidly identified bacterial pathogens with good accuracy and precision in less than eight hours.

MALDI Imaging Mass Spectrometry: Providing Molecular Insight at the Host-Pathogen Interface
Jessica Moore - Vanderbilt University (Jessica.L.Moore.1@Vanderbilt.edu)

• Imaging Mass Spectrometry (IMS) provides specific molecular information directly from tissue while preserving spatial fidelity. As mass spectrometric technologies advance, high spatial (<10µm) and high mass resolution (>100,000 Resolving Power) IMS has been achieved. When applied to infected tissue, IMS can provide molecular information at the host-pathogen interface and molecularly define histological features without a priori knowledge of analytes. High mass resolution IMS using MALDI FTICR MS allows for isotopic resolution of protein species up to 10,000 MW and isolation of post-translationally modified epitopes in infectious lesions. Such analyses provide unprecedented capabilities for the study of infectious diseases.
Monday @ 3:00 PM in Exec Ctr

**Basics: Tuning Your Mass Spectrometer-How to Make It Sing!**
*Robert Fitzgerald - University of California, San Diego (rfitzgerald@ucsd.edu)*

- Tuning is fundamental to operating a mass spectrometer and involves mass calibration and mass resolution. This overview will focus on what happens when the mass spectrometer is tuned and introduces basic chemical concepts to help novice users understand what it means when your instrument representative says "I need to tune the mass spectrometer".

Monday @ 3:25 PM in Exec Ctr

**Basics: Compound Specific Tuning**
*Robert Fitzgerald - University of California, San Diego (rfitzgerald@ucsd.edu)*

- After ensuring that your instrument’s resolution and mass accuracy are set appropriately the next step in developing a quantitative LC/MS/MS assay based on multiple reaction monitoring (MRM) is to perform compound specific tuning. The established resolution and calibration files are used to optimize ion source electronics as well as gas flows for the compound of interest. This overview will focus on compound specific tuning and introduces basic concepts to help novice users understand what it means when your instrument representative says "We need to tune the mass spectrometer for your compound".

Monday @ 3:50 PM in Exec Ctr

**Basics: Developing MRM Transitions**
*Robert Fitzgerald - University of California, San Diego (rfitzgerald@ucsd.edu)*

- After optimizing instrument tuning and compound specific tuning parameters the next step in developing a quantitative LC/MS/MS assay based on multiple reaction monitoring (MRM) is to identify appropriate fragment ions and optimize collision voltages for optimal sensitivity. This overview will focus on optimizing MRM transitions and introduces basic concepts to help novice users understand what it means when your instrument representative says "We need to develop MRM transitions for your assay".
Development of Clinical Assays Based on Parallel Reaction Monitoring

Bruno Domon - Luxembourg Clinical Proteomics Center (Bruno.domon@lih.lu)

- Targeted analyses of clinical samples performed on a quadrupole-orbitrap instrument using parallel reaction monitoring showed a significant gain in sensitivity and selectivity. In order to fully leverage the potential of this approach to develop clinical assays, we have designed a new data acquisition scheme. It relies on added internal standards and on-the-fly adjustment of acquisition parameters to drive in real-time the measurement of endogenous peptides (corresponding to proteins of interest) and generate precise and high confidence quantitative results. Applied to the analysis of lung cancer markers in plasma samples, it improved the discrimination of the disease stages and subtypes.

Evaluation of Different Mass Spectrometry Data Acquisition and Analysis Strategies for Amyloidosis Typing

Han-Yin Yang - University of Washington (hyyang@uw.edu) -- *Young Investigator Grantee*

- Laser microdissection couple with mass spectrometry (LDMS) based amyloidosis diagnosis method has been a better alternative to immunohistochemistry method. However, several aspects need to be considered in the assessment of a reliable diagnosis platform. Here, we apply different normalization approach to correct for the variances in LD capture quantities and sample preparation. In addition, we quantify the difference between samples using peptide and protein levels, and discuss the diagnosis ability of different quantification approaches. Preliminary results show data-independent acquisition followed by intensity based method provides more evidence for diagnosis.

Development of an Automated Exosome Isolation Procedure for Determining Prostate Cancer Aggressiveness

Alex Rai - Columbia University Medical Center (ajr2170@cumc.columbia.edu)

- Almost 230,000 men are diagnosed with prostate cancer annually, but over 900,000 men undergo biopsy. A biomarker to identify aggressive disease could reduce biopsies and associated morbidity. Exosomes are small (30-200 nm) membranous vesicles secreted by all cells. They harbor biomarkers and can serve as a real-time "liquid biopsy". Proteomics analysis using 1D LC-MS/MS and multidimensional protein identification technology (MudPIT) was performed after trypsin digest. A 44 exosomal-protein signature was delineated to distinguish patients with metastatic disease and those after radical prostactectomy. The 44 proteins can be classified into eight major functions, identifying pathways involved in disease progression & metastasis.
Comparison of CDC's Candidate Reference Method for Serum 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 with Recognized Reference Methods

Ekaterina Mineva - CDC (emineva@cdc.gov)

- Vitamin D status is routinely assessed by measuring serum concentrations of the most stable and abundant vitamin D metabolites, namely, 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2. To assure the accuracy of measurements, worldwide vitamin D standardization activities are ongoing. As part of these efforts, we have developed a candidate reference measurement procedure to support CDC's Vitamin D Standardization Certification Program. We are presenting a comparison of the serum concentrations of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2, measured using CDC's candidate reference method, to target concentrations established by JCTLM-recognized reference methods (University of Ghent and NIST) in various single donor samples and standard reference materials. We will compare method features and highlight performance.

Direct Quantitation via Internal Standard Ratios for LC-MS/MS Assays

Brian Rappold - Essential Testing (brappold@etlab.org)

- Diagnostic MS/MS methods largely utilize calibration curve-based quantitation on a batch basis. Initial forays into internal standard ratio quantitation have been recently proposed. This mode allows for smaller batches, faster run-times and expedited data review while still leveraging the analytical horsepower of mass spectrometric platforms. Additionally, this workflow satisfies the core requirements of quality control under CLIA and various accrediting agencies. These approaches, however, do not address non-linear MS detection, increased imprecision at low responses or noise/baseline variations between analytes and internal standard transitions. This paper shall introduce a highly accurate means of internal standard ratio quantitation via calculation of correction factors across the entire analytical range for a diverse array of analytes and internal standard labeling conditions.

Using Database Mining on Residual Samples to Establish Healthy Reference Intervals for Testosterone Measured by LC-MS/MS

Julia Drees - Kaiser Permanente Regional Laboratory (julia.c.drees@kp.org)

- Establishing reference intervals is challenging for clinical laboratories. In the case of testosterone, clinical laboratories must decide whether to consider the age-related decline in male testosterone a normal phenomenon which should be reflected in the reference intervals. In this study, automated electronic medical record database mining was used to identify residual samples from healthy adult males and females for use in establishing total and calculated free testosterone reference intervals. Total testosterone was measured on an LC-MS/MS assay that demonstrated excellent correlation with the CDC reference method. Data from 292 adult males did not support decreasing reference intervals for total testosterone with increasing age. In contrast, free testosterone was observed to decline.
**Session 3 • Track 3 •**

**Metabolomics 3: Pathways to Lipids**

*Tuesday @ 10:45 AM in Harbor 3*

Session Chair: Mohit Jain - *University of California, San Diego*

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**Tuesday @ 10:45 AM in Harbor 3**

**Discovery and Characterization of Novel Bioactive Mammalian Lipids**

*Alan Saghatelian - The Salk Institute* ([asaghatelian@salk.edu](mailto:asaghatelian@salk.edu))

- Lipidomics led to the identification of a novel class of mammalian lipids which have beneficial metabolic effects – Branched Fatty Acid esters of Hydroxy-Fatty Acids (FAHFAs). A member of this class, Palmitic Acid Hydroxy Stearic Acid (PAHSA) has at least 8 isomers which are present in serum and many mouse and human tissues. And administration of these lipids to mice improved various metabolic parameters. These lipids are of interest as biomarkers and potential therapeutics.

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**Tuesday @ 11:10 AM in Harbor 3**

**Metabolomic Analyses of Subclinical Indicators and Predictors of Disease**

*Clary Clish - Broad Institute* ([clary@broadinstitute.org](mailto:clary@broadinstitute.org))

- We have developed a robust LC-MS-based metabolomics platform that is capable of measuring hundreds of intermediate metabolites in multiple pathways, including glucose and lipid metabolism, and we have applied this technology to profile samples derived from cell culture, body fluids, tissues, and microbes. We have investigated whether plasma metabolite profiles predict future development of diabetes in participants of the Framingham Heart Study. These analyses revealed amino acid and lipid signatures that are predictive of risk and were replicated in an independent, prospective cohort. In addition to these results, findings from recent efforts to find metabolic indicators of pancreatic cancer in will be presented.

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**Tuesday @ 11:35 AM in Harbor 3**

**Credentialing Biologically Relevant Features: Honey, I Shrunk the Metabolome**

*Gary Patti - Washington University* ([gjpattij@gmail.com](mailto:gjpattij@gmail.com))

- In LC/MS-based metabolomics, it is routine to detect thousands of features from the metabolic extract of a biological sample. When the mass-to-charge values of these features are searched exhaustively in available databases, however, about 50% do not return hits. This raises questions about the size of the metabolome and the number of potential unknown structures that have yet to be characterized. We have developed a platform called "credentialing" to identify features in a metabolomic experiment that are of biological origin. In brief, unlabeled and labeled samples are mixed at unique ratios and analyzed together on the basis of isotope spacing and intensity. The credentialing platform enables data reduction and improved identification of bona fide unknowns. The application of credentialing to identify an unknown altered in cancer cells will be discussed.
Three-dimensional Metabolic Imaging of Live Bacterial Colonies by Laser Ablation Electrospray Ionization Mass Spectrometry with Ion Mobility Separation

Hang Li - George Washington University (amy_li@gwmail,gwu.edu) -- *Young Investigator Grantee*

With the rising tide of antibiotic resistant bacterial strains, understanding microbial metabolism is of great importance. Conventional bioanalytical tools often have limited ability to provide spatiotemporal metabolite distributions in microbial colonies. Here, we introduce laser ablation electrospray ionization (LAESI), in combination with ion mobility separation (IMS) and mass spectrometry (MS), to rapidly investigate microbial metabolism. Over one hundred metabolites and lipids were detected by LAESI-IMS-MS from a single bacterial colony within seconds. Three-dimensional images were built from lateral rastering and depth profiling. For example, the m/z 188.2 ion, identified as acetylspermidine, exhibited stronger signal on the perimeter of the colony. Our results show that high throughput metabolic analysis and three-dimensional imaging can be performed by LAESI-IMS-MS.

Harnessing Metabolomics for Microbial Identification in Complex Samples

Vanessa Phelan - University of California, San Diego (vphelan@ucsd.edu)

Current mass spectrometry methods in clinical laboratories for microbial detection are limited to identifying the genus and species. However, it is well established that microbes produce a number of organism specific metabolites to interact with their environments. These metabolites function as toxins, antibiotics, redox active molecules and nutrient scavenging entities. Using newly developed bioinformatics tools, we can begin to evaluate whether specific specialized metabolites are detectable in clinical samples and if those metabolites can provide insight into patient status. Here, we describe the application of MS/MS based molecular networking to identify Pseudomonas aeruginosa metabolites in sputum samples from CF patients.

Direct Microbial Analysis by Ambient Ionization MS: Paper Spray and Swab Touch Spray

Alan Jarmusch - Purdue University (ajarmusc@purdue.edu) -- *Young Investigator Grantee*

Ambient ionization mass spectrometry allows for rapid analysis of bacteria and fungi samples with minimal sample preparation. The biomolecule profiles (e.g. lipids) detected are characteristic and allow for molecular-based identification via multivariate statistics. Paper spray (PS) ionization allowed differentiation of various species of bacteria and yeast, requiring only a minute amount of material applied to a paper triangle. Swab touch spray (STS), a novel method developed for in vivo sampling, allowed direct microbial analysis from swabs. PS and STS methods for microorganism identification can extend into clinical medicine, enhancing current analytical methods with significant impact on patient treatment.
Tuesday @ 10:45 AM in Exec Ctr

Part 1: Short Review of LC-MS/MS Sample Preparation Principles

Julia Drees - Kaiser Permanente Regional Laboratory (julia.c.drees@kp.org)

• This presentation is an introduction prior to an in-depth case study examining sample preparation. The most common types of sample clean-up used in clinical LC-MS/MS will be discussed along with their relative strengths and weaknesses. Ion suppression will be introduced and a robust method to quantify matrix effects and recovery will be explained. Options for automating sample prep will be presented.

Tuesday @ 11:10 AM in Exec Ctr

Part 2a: The Trials and Tribulations of Sample Preparation for Testosterone by LC-MS/MS

Deborah French - University of California, San Francisco (deborah.french@ucsf.edu)

• Use of liquid chromatography-mass spectrometry for analysis of small molecules in clinical laboratories has increased in the past few years. An important component of developing a mass spectrometry method is the sample preparation technique employed in order to sufficiently clean up the sample without sacrificing analyte recovery. A number of references out there detail sample preparation techniques but which one do you choose? This presentation will detail the thought process behind the sample preparation technique choice for measurement of serum total testosterone in one laboratory, including the balancing act of what you want to, and what you can realistically achieve.

Tuesday @ 11:35 AM in Exec Ctr

Part 2b: The Trials and Tribulations of Sample Preparation for Testosterone by LC-MS/MS, CONTINUED

Deborah French - University of California, San Francisco (deborah.french@ucsf.edu)

• Use of liquid chromatography-mass spectrometry for analysis of small molecules in clinical laboratories has increased in the past few years. An important component of developing a mass spectrometry method is the sample preparation technique employed in order to sufficiently clean up the sample without sacrificing analyte recovery. A number of references out there detail sample preparation techniques but which one do you choose? This presentation will detail the thought process behind the sample preparation technique choice for measurement of serum total testosterone in one laboratory, including the balancing act of what you want to, and what you can realistically achieve.
Tuesday @ 3:00 PM in Harbor 1

**Detection of CSF Proteins Using LC-MS/MS: Measurement of Beta-Trace Protein as an Indicator of a CNS Breach**

**Kari Gurtner** - Mayo Clinic (gurtner.kari@mayo.edu)

The presence of CSF in a body fluid, indicative of a CNS breach, is currently detected by gel immunofixation (IFE) and nephelometric quantification. Both methods rely on the detection of a single marker for CSF and have limitations in the presence of serum contamination. Testing for beta-trace proteotypic peptides by LC-MS/MS aims to overcome those limitations. Of 104 body fluids tested, 96% of the samples were in agreement between IFE and LC-MS/MS testing. Peptidyl analysis by LC-MS/MS increases the accuracy of detection of CSF proteins without being impacted by non-CSF contamination or bacterial deglycosylation, offering superior sensitivity and specificity.

Tuesday @ 3:25 PM in Harbor 1

**How to Avoid a Bone Marrow Biopsy when Monitoring Minimum Residual Disease in Multiple Myeloma: Hope for the Future!**

**H. Robert Bergen, III** - Mayo Clinic (bergen.bob@mayo.edu)

Therapeutic effectiveness in multiple myeloma (MM) currently requires monitoring the relevant myeloma cells in a bone marrow sample. Because the plasma cell clones are producing a clonal antibody we sought to identify the antibody the clone was producing directly and monitoring the circulating antibody originating from these clones regardless of location. Utilizing blood plasma where the M-protein is >0.8g/dL we have been able to identify unique tryptic peptides corresponding to immunoglobulin light chain variable regions belonging to each patients clone. This is accomplished on a Q Exactive MS system with PEAKS de novo software and filters based upon abundance. Subsequent blood samples are utilized to measure MRD and the target peptide corresponding to each patients clone is monitored. The results of our analysis of 57 patient samples utilizing this methodology will be illustrated.

Tuesday @ 3:50 PM in Harbor 1

**Targeted Quantitative Mass Spectrometric Immunoassay for Analysis of Serum Amyloid A (SAA) in Human Plasma**

**Olgica Trenchevska** - Arizona State University (olgica.trenchevska@asu.edu)

Proteins can exist as multiple proteoforms in vivo that can have important roles in physiological and pathological states. Presented here is the development and characterization of mass spectrometric immunoassay for quantitative determination of serum amyloid A (SAA) proteoforms. Intra- and inter-day precision and recovery characteristics of the assay were established, yielding CVs<10%. The new assay was benchmarked against existing SAA ELISA, producing 2.2% Altman-Bland bias. We used the assay to determine the individual concentrations of the SAA proteoforms across a cohort of ~ 300 samples, revealing 7 different SAA genetic polymorphic types and a total of 18 different proteoforms.
Tuesday @ 3:00 PM in Harbor 2
Broad Spectrum Drug Screening Using Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry: How Valuable Is Retention Time for Identifying Compounds?
Katie Thoren - Memorial Sloan Kettering Cancer Center (thorenk@mskcc.org) -- *Young Investigator Grantee*

- Using a LC-QTOF mass spectrometer for broad-spectrum drug screening, compounds are identified based on their precursor mass, isotope pattern, retention time and product ion spectra. Ideally, assays would only be based on intrinsic compound parameters so that changes in method conditions would not affect compound identification. Out of these four parameters, retention time information is the most method-dependent. But how valuable is it for compound identification, especially when product ion spectra are available? Using 100 routine clinical urine samples, we compared how well our method identifies compounds with and without the use of retention time information. Ultimately, our goal is to make drug screening methods more universal while still achieving reasonable compound identification.

Tuesday @ 3:25 PM in Harbor 2
Comparing TOF and QTOF for Comprehensive Drug Screening: Do You Really Need Fragmentation Information?
Jennifer Colby - University of California, San Francisco (jennifer.colby@ucsf.edu) -- *Young Investigator Grantee*

- High resolution mass spectrometry (HRMS) is an emerging technique that has been applied to comprehensive drug screening. HRMS instruments, including time of flight (TOF) analyzers, measure mass accurately. Quadrupole TOF (QTOF) mass analyzers can also select precursor ions and produce compound specific fragmentation patterns. We compared the ability of a QTOF instrument to identify drugs in patient samples, including and excluding fragmentation information. We found that including fragmentation patterns improved the assay’s sensitivity by 11% and improved positive predictive value by 25%. Fragmentation patterns increase confidence in compound identification and may allow screening results to be released without confirmatory testing.

Tuesday @ 3:50 PM in Harbor 2
Development, Implementation, and Stress Testing of a Rapid HPLC-HRAMS Screening Method for Detection of Twenty Antiretroviral (ARV) Compounds in Human Serum
Autumn Breaud - The Johns Hopkins University (abreaud1@jhmi.edu)

- The aim of this work was to develop a method for cost-effective and rapid screening for the presence of a panel of ARV drugs using a multiplexed HPLC-HRAMS approach. Validation studies were performed before preparation and analysis of multiple, large batches of study samples. Upon increased throughput of the method, we found that a number of components of the assay required better life cycle definition. We also found a number of challenges associated with the acquisition mode of the instrument when panel components share fragments and when there may be an isotopic peak interference from one precursor mass to another.
Metabolomics 4: High-Throughput & Computational Approaches to Lab Medicine

Tuesday @ 3:00 PM in Harbor 3
Session Chair: Caroline Johnson - The Scripps Research Institute

Metabostasis of the Aging Brain
Gary Siuzdak - The Scripps Research Institute (siuzdak@scripps.edu)
• Brain structure and function are highly dependent upon metabolic homeostasis (metabostasis) however it is unknown how this is affected during the natural aging process. Biochemical characterization of metabostasis would provide important insight into brain metabolism and potential impairment related to metabolic dysfunction as a hallmark of aging. Here we apply cutting-edge, mass spectrometry-based metabolomics to characterize metabolites across anatomical regions of a mouse brain at different stages of the life cycle. This temporal overview of metabostasis integrated with protein expression data and metabolite magnetic resonance imaging further validated and help define regional brain metabolism during the healthy aging process.

High Throughput Monitoring of Small Molecules in Human Plasma
Mohit Jain - University of California, San Diego (mjain@ucsd.edu)
• Both internal and external environmental factors are critical modulators of human disease through the introduction of small molecules into circulating plasma. Traditional LC-MS based approaches for assessing plasma small molecules are limited in their throughput. In our talk, we will introduce new approaches for high throughput measure of plasma small molecules using automated in-line SPE with direct infusion mass spectrometry, and the association of small molecules with human disease.

Lipidomics of Eicosanoids Highlights Infection and Inflammation Progression
Edward A. Dennis - University of California, San Diego (edennis@ucsd.edu)
• The largest numbers of distinct molecular species in cellular metabolism are the lipids where tens of thousands of distinct molecular species exist in cells/tissues. We have developed novel liquid chromatographic-mass spectrometric based lipidomics techniques termed “CLASS” to solve lipidomics problems, often in the context of an overall omics analysis of immunologically-activated macrophages integrating transcriptomics, proteomics, and metabolomics of lipid metabolites. Our laboratory has developed a robust and comprehensive approach to the lipidomics analysis of hundreds of fatty acids, acylethanolamines and inflammatory eicosanoids. We have built on our previous application of lipidomic analysis to characterize “synergistic” cellular lipid signaling of Toll-like (TLR) and purinergic receptors in stimulated macrophages as models of bacterial infection and inflammation.
Tuesday @ 3:00 PM in Marina 6
PANEL: Diagnostic Gaps in Infectious Diseases: A Proposal for an Interdisciplinary Approach to Develop Mass Spectrometry Methods to Find the Bug and Treat the Host
Carey-Ann Burnham et al. (see Long Abstract) - Washington University (cburnham@path.wustl.edu)
• The application of Mass Spectrometry in the clinical microbiology laboratory has been limited mainly to the identification of microorganisms growing in culture. However, there are a large number of technological gaps in both the diagnosis and management of infections that could potentially make ideal candidates for MS-based solutions. In this panel discussion, three clinical microbiologists will present clinical problems and/or unmet diagnostic needs in the field of clinical microbiology. A panel of experts in MS will then respond to each need presented, weighing in on the potential feasibility of a MS-based analytical solution. The major objective of this session is to foster interdisciplinary collaborations to improve both the diagnosis and management of infections using novel MS-based assays.

Tuesday @ 3:25 PM in Marina 6
PANEL: Diagnostic Gaps in Infectious Diseases: A Proposal for an Interdisciplinary Approach to Develop Mass Spectrometry Methods to Find the Bug and Treat the Host
Susan Butler-Wu et al. (see Long Abstract) - University of Washington (butlerwu@uw.edu)
• The application of Mass Spectrometry in the clinical microbiology laboratory has been limited mainly to the identification of microorganisms growing in culture. However, there are a large number of technological gaps in both the diagnosis and management of infections that could potentially make ideal candidates for MS-based solutions. In this panel discussion, three clinical microbiologists will present clinical problems and/or unmet diagnostic needs in the field of clinical microbiology. A panel of experts in MS will then respond to each need presented, weighing in on the potential feasibility of a MS-based analytical solution. The major objective of this session is to foster interdisciplinary collaborations to improve both the diagnosis and management of infections using novel MS-based assays.

Tuesday @ 3:50 PM in Marina 6
PANEL: Diagnostic Gaps in Infectious Diseases: A Proposal for an Interdisciplinary Approach to Develop Mass Spectrometry Methods to Find the Bug and Treat the Host
Andy Hoofnagle et al. (see Long Abstract) - University of Washington (ahoof@washington.edu)
• The application of Mass Spectrometry in the clinical microbiology laboratory has been limited mainly to the identification of microorganisms growing in culture. However, there are a large number of technological gaps in both the diagnosis and management of infections that could potentially make ideal candidates for MS-based solutions. In this panel discussion, three clinical microbiologists will present clinical problems and/or unmet diagnostic needs in the field of clinical microbiology. A panel of experts in MS will then respond to each need presented, weighing in on the potential feasibility of a MS-based analytical solution. The major objective of this session is to foster interdisciplinary collaborations to improve both the diagnosis and management of infections using novel MS-based assays.
Tuesday @ 3:00 PM in Exec Ctr

**Part 1: Short Review of LC Method Development Principles**

*Julia Drees - Kaiser Permanente Regional Laboratory (julia.c.drees@kp.org)*

- This presentation will introduce the principles of reverse-phase and HILIC high performance liquid chromatography (HPLC) method development and address common problems such as column overload, injection matrix/mobile phase mismatch, and poor ionization. Best practices and maintenance suggestions will be discussed.

Tuesday @ 3:25 PM in Exec Ctr

**Part 2a: Serum Aldosterone: An LC Method Development Case Study for a Difficult Analyte**

*Grace van der Gugten - Provincial Health Services Authority (GvanderGugten@providencehealth.bc.ca)*

- For the most part, LC-MS/MS assay development and validation is, and should be, a within-laboratory project. However, the new user can find assay development a daunting task. We will describe the LC method development for one of our more challenging to measure endogenous steroids: serum/plasma aldosterone. We will discuss selection of mobile phases, column selection, gradient program development, interference testing, and addition of cortisol to the method. Importantly, we will cover unexpected workflow challenges and continual improvements and discoveries we have made while the assay has been in clinical production.

Tuesday @ 3:50 PM in Exec Ctr

**Part 2b: Serum Aldosterone: An LC Method Development Case Study for a Difficult Analyte**

*Grace van der Gugten - Provincial Health Services Authority (GvanderGugten@providencehealth.bc.ca)*

- For the most part, LC-MS/MS assay development and validation is, and should be, a within-laboratory project. However, the new user can find assay development a daunting task. We will describe the LC method development for one of our more challenging to measure endogenous steroids: serum/plasma aldosterone. We will discuss selection of mobile phases, column selection, gradient program development, interference testing, and addition of cortisol to the method. Importantly, we will cover unexpected workflow challenges and continual improvements and discoveries we have made while the assay has been in clinical production.
Wednesday @ 8:30 AM in Harbor 1
Identification of Insulin Like Growth Factor 1 (IGF1) Variants Using High-Resolution Accurate-Mass Mass Spectrometry (HRAM-MS)
Hemamalini Ketha - Mayo Clinic (ketha.hemamalini@mayo.edu) -- *Young Investigator Grantee*
• We demonstrate how high-resolution-accurate-mass mass spectrometry (HRAM-MS) identifies IGF1 variants (V-IGF1) that immunoassays identify as wild type IGF1. IGF1 measured with immunoassay-measured-IGF1 (IGF1-IA) in patients with V-IGF1 are higher than with IGF1-MS. In 15 out of 2480 (0.6%) patients half of IGF1-IA was identified as V-IGF1. Of note, one patient with V-IGF1 was receiving recombinant growth hormone (rGH) for treatment of Noonan Syndrome with a protein tyrosine phosphatase 11 (PTPN11) mutation. Identification and accurate quantitation of V-IGF1 is of clinical value especially for patients receiving GH therapy, those exhibiting GH resistance or IGF1 mutations. This presentation will describe the identification, characterization of V-IG1.

Wednesday @ 8:55 AM in Harbor 1
Mass Spectrometry of Hemoglobin Variants
Jane Yang - University of California, San Diego (jyy008@ucsd.edu) -- *Young Investigator Grantee*
• Hemoglobin variants caused by a single nucleotide polymorphism are mutant forms of hemoglobin that typically result in a single amino acid substitution, such as hemoglobin S. Two classic techniques used in the clinical laboratory, cation exchange (CE)-HPLC and capillary zone electrophoresis (CZE), are oftentimes insufficient to identify specific variants. In addition to the CE-HPLC and CZE results, we use both top-down and bottom-up mass spectrometry (MS) approaches to confirm the identities of hemoglobin variants at the intact subunit and peptide levels, by using mass differences and fragmentation spectra to predict the amino acid substitution and location, respectively.

Wednesday @ 9:20 AM in Harbor 1
Analysis of Hemoglobin Variants by Top-down Mass Spectrometry
Didia Coelho Graça - University of Geneva (didia.coelhograca@unige.ch) -- *Young Investigator Grantee*
• A high-resolution top-down mass spectrometry method was developed for the detection of mutated hemoglobin chains using selected diagnostic product ions for data interpretation. This procedure brings more precise information about the considered hemoglobin variants (proteoforms) than any current protein analysis methods used for hemoglobin disorders diagnosis. The method was successfully applied to the analysis of hemoglobin β chain variants carrying various single point mutations and an Aγ-β fusion protein. The results showed that the developed data analysis process allows fast and reliable interpretation of top-down electron transfer dissociation mass spectrometry data by non-expert users in the clinical area.
One SLE to Measure Them All: Two-part Elution for Analysis of Urinary HIAA, HVA and VMA

Zlatuse Clark - ARUP Laboratories (zlatuse.d.clark@aruplab.com)

- The monoamine acids 5'-hydroxyindoleacetic (HIAA), homovanillic (HVA), and vanillylmandelic (VMA) are metabolites of serotonin and the catecholamine neurotransmitters. Laboratory measurement of these acids in urine is used to assess overproduction of the parent amines due to neuroendocrine tumors. We developed and validated a method for HIAA, HVA, and VMA using SLE-based sample preparation and LC-MS/MS analysis. Development of the sample preparation method required significant diversion from manufacturer guidelines, but resulted in the use of a single product to extract all analytes. Sequential two-part elution of the analytes yielded cleaner extracts and higher recoveries. The final method is robust and fast.

Elimination of Matrix Effects Using Mixed-mode SPE Plate for High Throughput Analysis of Free Arachidonic Acid in Plasma by LC-MS/MS

Jerry Wang - Bonna-Agela Technologies (qunjie_wang@agela.com.cn)

- The matrix effects on the analysis of arachidonic acid in plasma on LC-MS/MS was investigated by comparing various sample pretreatment methods including protein precipitation, LLE, single-mode SPE, and mixed-mode SPE. The results indicated the last one with Cleanert MAS-M gave better results in terms of eliminating matrix effect of phospholipids and proteins in plasma. The optimized method was applied on real human plasma. The study demonstrated that a mixed-mode SPE plate could be adapted to high throughput sample pretreatment of hydrophobic analytes in plasma which are usually co-eluted with phospholipids and proteins on reversed phase HPLC column.

Ultra-rapid, Fully-automated Plasma Clozapine and Norclozapine Analysis Using AC Extraction Plate Technology and Flow-injection MS/MS

Lewis Couchman - King’s College Hospital (lewis.couchman@nhs.net) -- *Young Investigator Grantee*

- Therapeutic drug monitoring (TDM) of plasma clozapine and N-desmethylclozapine (norclozapine) is well-established. Fully-automated sample preparation using novel AC Extraction Plate$^*$ technology and automated liquid handling (both Tecan Schweiz AG) combined with flow-injection MS/MS of extracts minimises analysis times. The data capture time was 5 seconds per sample. Results were comparable to those produced using manual extraction and LC-MS/MS. Use of deuterated internal standards compensated for matrix effects for both analytes. The principle embodied in this approach may have wide application.
Preliminary Study on Clinical Application of Metabolomics for Laboratory Diagnosis of Inborn Errors of Metabolism
Qin Sun - Baylor College of Medicine (qsun@bcm.edu)

To meet the challenge of integrating metabolomic test in diagnostic labs, we developed a rapid metabolomic workflow to analyze plasma from patients with a confirmed inborn error of metabolism (IEM). Analysis was performed using a non-targeted multi-mass spectrometry platform. The analytes detected encompass a number of classes of important small molecule biomarkers such as fatty acids, acylcarnitines, amino acids, bile acids, carbohydrates, lipids and nucleotides, etc. Metabolic profiling was able to correctly diagnose 20 of the 21 disorders. In an additional set of prior unresolved cases, metabolomic analysis detected disturbances that pointed to a genetic disorder or assisted in the interpretation of concurrent DNA analysis. In summary we have demonstrated the clinical utility in the diagnosis of IEMs as well as functional confirmation of genetic and genomic results of uncertain significance.

High-Performance Chemical Isotope Labeling LC-MS for Clinical Metabolomics
Liang Li - University of Alberta (liang.li@ualberta.ca)

In clinical metabolomics, both targeted and untargeted analyses are being used for monitoring the metabolic changes associated with a disease. However, there are still several technical challenges in untargeted metabolome profiling. In particular, it is difficult to detect, quantify and identify a large number of metabolites that are present in a metabolomic sample with a wide range of concentrations and diverse physicochemical properties. In this presentation, a high-performance chemical isotope labeling liquid chromatography mass spectrometric platform for quantitative and comprehensive metabolome profiling of biological systems will be described. Some selected applications of this platform for clinical metabolomics studies including disease biomarker discovery will be presented.

Metabolic Profiling of Developing Infants: Term vs. Preterm at Birth
Frances Jackson - Imperial College London (f.jackson12@imperial.ac.uk) -- *Young Investigator Grantee*

Advances in neonatal medicine have seen an increase in the survival of babies born preterm. This has resulted in a significant burden to health and education services with increased risk for several childhood and later life diseases. To understand these conditions, urine and stool samples from infants born term (> 37 weeks gestation) or preterm (24 - 36 weeks gestation) at birth have been analysed using UPLC-MS techniques. Samples were taken from birth until up to 3 months of age giving us the metabolic profiles of infant’s development. This will provide insight and identify biomarkers which may be prognostic of developmental conditions including adverse health outcomes that manifest later in life.
High-throughput and Reproducible Workflows to Prepare Human Plasma Samples for Proteomic Analysis

Jennifer van Eyk - Advanced Clinical Biosystems Institute, Cedars Sinai (Jennifer.VanEyk@cshs.org)

- We have implemented an automated peptide preparation protocol on a liquid handling workstation (Biomek NXP) coupled with an SRM workflow using triple quadrupole mass spectrometer (QTRAP® 6500 system). The complete analysis had a coefficient of variation of less than 10% based on assessment of the internal beta-galactosidase standard. We present automated high-throughput workflows with sample processing and enrichment robotics for accurate and reproducible large-scale analysis of biological/clinical samples. This workflow can be applied to biomarker validation, drug response monitoring, disease state and progress monitoring.

Automated High-throughput Clinical Proteomics Workflow Using Hydrogel Nanotrap Particle Technology and Mass Spectrometry Analysis

Matthew Rosenow - Translational Genomics Research Institute (mrosenow@tgen.org)

- The functionality of a high-throughput automated preparation workflow that incorporates a novel nanoparticle technology is presented. Serum samples were spiked with cytokines and other small cell signaling proteins, and processed using an automated system. Levels of the protein spikes used to generate response curves ranged from the pg/mL to ng/mL levels and were within the physiological relevant levels of the proteins. SRM mass spectrometry analysis using corresponding isotopically labeled peptides, show low total process CV’s at the various levels, and show the physiological relevant concentration of the proteins to be within the linear region of the response curves. This study demonstrates a simple clinical proteomics workflow that does not require antibody-based target enrichment or complex sample processing that is also capable of producing clinical grade process CVs.

Nanoporous Substrates-Enabled, Functional Mechanism-Based Method for the Early Diagnosis in Cancer Diseases

Tony Hu - Houston Methodist Research Institute (yhu@houstonmethodist.org) -- *Young Investigator Grantee*

- Circulating peptides have been recognized as useful signatures that can be traced to cancer-specific metabolic or post-translational modification events at early-stage tumor progression. We have established “Nanotrap” to effectively fractionate blood peptides with little to no sample processing. By coupling this technique to advanced mass spectrometry, we can bypass the limitation of current proteomic technologies, by “amplifying” the amount of small peptides extracted from blood samples. Our cutting-edge nanotechnologies coupled with advanced mass spectrometry and customized biostatistical analysis facilitated the functional mechanism-driven peptide biomarker studies for revealing the early events associated with the signature mutations or pathways in tumor progression.
Session Chair: Timothy Collier - Cleveland HeartLab, Inc.

Part 1: Introduction to Clinical Proteomics
Timothy Collier - Cleveland HeartLab, Inc. (Tcollier@clevelandheartlab.com)

- This session is directed toward attendees new to mass spectrometry and its use for the measurement of proteins and peptides (proteomics) in the clinical laboratory. It will be presented in three parts with questions and discussion welcomed after each. Part I of this session will focus on the definition of proteomics within the broader context of systems biology. We will also discuss how information gathered from proteomic measurements can offer clinical insight into a patient's state of health, compared and contrasted to genetic, metabolite, and other small molecule diagnostics.

Part 2: Introduction to Clinical Proteomics
Timothy Collier - Cleveland HeartLab, Inc. (Tcollier@clevelandheartlab.com)

- A mass spectrometer is more than a box with specimens going in and a numbers coming out. This part of the session will discuss a few of the most used types of mass spectrometers in the clinical laboratory and how they perform their measurements in the context of proteomic diagnostics. This includes: (1) Protein/Peptide ionization, (2) Guiding ions through the instrument, (3) Fragmentation, (4) Detection, and (5) Databases and Data Interpretation Tools.

Part 3: Introduction to Clinical Proteomics
Timothy Collier - Cleveland HeartLab, Inc. (Tcollier@clevelandheartlab.com)

- The final part of the session will feature examples from the literature of diagnostics employing mass spectrometry based proteomics, highlighting unmet clinical needs addressed in the development of such assays. The session will conclude with an overview of new techniques and technologies that present opportunities for further advancement in the field.
Wednesday @ 10:45 AM in Harbor 1
Trials and Triumphs in the Development of a Quantitative Assay for Amyloid-beta Peptides
Mari Demarco - St Paul's Hospital & Univ. British Columbia (mdmrco@mail.ubc.ca) -- *Young Investigator Grantee*
• While Alzheimer's disease has a defined pathology on autopsy, in vivo diagnosis is challenging—particularly in early stages of disease when treatment opportunities are greatest. Toward the development of an in vivo diagnostic model, cerebrospinal fluid biomarkers amyloid-beta and tau proteins have been extensively studied and are now included in research diagnostic criteria. Historically, quantitation of amyloid-beta peptides has relied on immunometric techniques. Herein we describe our antibody-free LC-MS/MS workflow to quantitate amyloid-beta peptides in cerebrospinal fluid. We will also present a method comparison between LC-MS/MS and a commonly used commercial ELISA, as well as results from a case-control study.

Wednesday @ 11:10 AM in Harbor 1
A Mass Spectrometric Immunoassay Coupled to Selected Reaction Monitoring Reveals Novel Relationships Between Plasma PCSK9 and Metabolic Phenotypes in Patients
Benoit Coulombe - Institut de recherches cliniques de Montréal-IRCM (Benoit.Coulombe@ircm.qc.ca)
• Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a key regulator of blood low density lipoprotein cholesterol (LDL-C) levels and circulates in various forms that affect PCSK9’s function and thereby LDL-C levels. Commercial PCSK9 ELISA assays do not allow discrimination between the various forms of the protein. We have developed and applied a robust and sensitive Mass Spectrometric ImmunoAssay coupled to Selected Reaction Monitoring assay for the absolute quantification of all PCSK9 domains and a posttranslational modification in plasma of two distinct human cohorts. This assay revealed novel relationships between PCSK9 and metabolic phenotypes, as compared to classical ELISA assays.

Wednesday @ 11:35 AM in Harbor 1
Development of a Reference Measurement System for Urine Albumin
Ashley Beasley Green - National Institute of Standards and Technology (ashley.beasley@nist.gov)
• Urinary excretion of albumin is a major diagnostic and prognostic marker of renal dysfunction and cardiovascular disease; therefore, accurate measurement of urine albumin is vital to clinical diagnosis. To address urine albumin measurement precision, we have developed the following components of the urine albumin reference measurement system: a multiplexed candidate reference measurement procedure that utilizes isotope dilution-mass spectrometry (ID-MS) and multiple reaction monitoring (MRM) to quantify urine albumin; a primary reference material to be used as a calibrator for higher-order urine albumin methods; and a secondary reference material to be used as a matrix-based quality control for commercially-available urine albumin assays.
Steroidomic Footprinting Based on UHPLC Qualitative and Quantitative High-resolution MS for the Evaluation of Endocrine Disrupting Chemicals in H295R Cells

David Tonoli - University of Geneva (david.tonoli@unige.ch) -- *Young Investigator Grantee*

- UHPLC coupled to high-resolution MS approaches were devised for the simultaneous untargeted screening and quantification of a selected subset of steroids in H295R cell culture supernatant exposed to different concentrations of a potential endocrine disrupting chemical, triclocarban (TCC). Chemically-driven feature selection with database matching followed by multivariate analysis led to a selection of the most important steroids perturbed by TCC. This approach indicates that TCC affects an early step in steroid biosynthesis. This strategy was devised to be compatible with high-throughput screening and stratification of potential endocrine disruptors.

Meconium Fatty Acid Ethyl Ester, Ethyl Glucuronide, and Ethyl Sulfate Sensitivity and Specificity to Detect Maternal Drinking During Pregnancy

Sarah Himes - Quest Diagnostics Nichols Institute (sarah.k.himes@questdiagnostics.com)

- Meconium fatty acid ethyl esters (FAEE), ethyl glucuronide (EtG), and ethyl sulfate (EtS) were quantified by liquid chromatography-tandem mass spectrometry in the same meconium sample (n=107). Moderate-substantial agreement between maternal self-reported drinking after 19 weeks gestation and meconium EtG >30 ng/g was observed (kappa: 0.57, 95% CI 0.41-0.73). This marker and associated cutoff was superior to a 7 FAEE sum >2 nmol/g and all other individual and combination marker cutoffs. With meconium EtG >30 ng/g as the standard and maternal self-report beyond 19 weeks gestation as the test condition, 82% sensitivity and 75% specificity were observed. These data indicate maternal alcohol consumption in the second half of pregnancy is best represented by meconium EtG >30 ng/g. We recommend meconium EtG as a better alcohol marker than FAEE for identifying prenatal alcohol exposure.

The Utility of High Resolution Mass Spectrometry in Identifying Potential Chemical Culprits of Synthetic Cannabinoid Epidemics

Roy Gerona - University of California, San Francisco (Roy.Gerona@ucsf.edu)

- We report the use of LC-QTOF/MS in the rapid identification of novel synthetic cannabinoids associated with three epidemics in the last three years. Effective collaboration between an analytical toxicology lab, Poison Centers, DPH, DEA and the CDC along with Cayman Chemical, a reference standard provider, in one series of cases allowed the effective use of both non-targeted and targeted screening workflows to identify the novel drug common to patient samples that is associated with the toxidrome observed.
Wednesday @ 10:45 AM in Harbor 3

A Bioinformatic Package for Automated Targeted and Global Profiling Analysis of Large Direct Infusion Mass Spectrometry Datasets

**Gonçalo Correia** - Imperial College London (g.dos-santos-correia12@imperial.ac.uk) -- *Young Investigator Grantee*

- Direct infusion mass spectrometry based methods (DIMS) are being increasingly deployed for high-throughput and cost effective analysis of large sample sets. However, computational tools for automated data pre-processing and analysis tailored for this particular type data are almost nonexistent. We present a new open-source package written in the Python programming language for the automated pre-processing and analysis of DIMS data. It contains modules for pre-processing, targeted quantification and untargeted/profiling analysis. Its development was prompted by the ongoing DIMS analysis of more than 10,000 24 hour urine collection samples from the INTERMAP study.

Wednesday @ 11:10 AM in Harbor 3

Metabolomics and Mitochondria in Human Diabetic Nephropathy

**Kumar Sharma** - University of California San Diego and the Veteran (kusharma@ucsd.edu)

- We have taken a urinary-based targeted metabolomic approach to identify biomarkers for diabetic nephropathy. We identified that a panel of metabolites that are characteristic of patients with diabetes and reduced renal function. These metabolites comprise a large network of interacting pathways and largely indicate a reduction in mitochondrial function. Several novel therapeutic agents provide renoprotection and are able to stimulate mitochondrial biogenesis and increase urinary levels of the metabolomic panel of diabetic kidney disease. These studies suggest that metabolomics is a powerful platform to identify new therapeutic targets and to monitor drug development for diabetic complications.

Wednesday @ 11:35 AM in Harbor 3

Pre-diagnostic Biomarkers and Clinical Biochemistry of Lung Cancer

**William Wikoff** - UC Davis Genome Center (wrwikoff@ucdavis.edu)

- The potential for metabolomics to yield blood-based biomarkers relevant to lung cancer screening and early detection has not been previously investigated. We applied an untargeted metabolomics approach to identify pre-diagnostic biomarkers using sera from a large patient cohort in a blinded discovery mode from current or former heavy smokers, resulting in a single, highly significant potential biomarker. Blinded validation was performed using an independent set of samples and matched controls. Tissue level changes were investigated by comparing normal to matched biopsy tissue from the same patient. Metabolomics revealed key perturbations in multiple pathways associated with early stage lung adenocarcinoma.
Wednesday @ 10:45 AM in Marina 6

Improved Turnaround Time for Identification of Blood Culture Isolates Using MALDI-TOF MS to Assay "Scum", or Brief Outgrowths of Blood Culture Broths

Mark Gonzalez - Washington University in St. Louis (mgonzalez@pathology.wustl.edu) -- *Young Investigator Grantee*

- Prompt and appropriate treatment for bloodstream infections results in improved patient outcomes; this is facilitated by early identification of the causative pathogen. MALDI-TOF MS offers an accurate and rapid method for microbial organism identification. To further expedite identification of bloodstream pathogens, MALDI-TOF was performed on early subculture growth, or "scum" growth, from positive blood cultures, prior to the formation of isolated colonies. We found that this easy, rapid, and cost effective method resulted in a significant reduction in time for microorganism identification for yeast, non-fermenting Gram-negative bacteria and Enterobactericeae isolated from blood culture specimens.

Wednesday @ 11:10 AM in Marina 6

Identification of Nocardia Species by MALDI-TOF Mass Spectrometry

Brian Bird - ARUP Laboratories (brian.bird@aruplab.com) -- *Young Investigator Grantee*

- MALDI-TOF mass spectrometry for identification of Nocardia species remains challenging. However, routine sample preparation on young cultures using an up-to-date commercial database minimally augmented with custom spectra allowed 82% (64 of 78), 89% (8 of 9), and 94% identification to species, complex, and genus level, respectively. This indicates that special sample preparation and custom databases may be unnecessary for routine Nocardia spp. identification.

Wednesday @ 11:35 AM in Marina 6

Evaluation of Sample Preparation Methods, Instrumentation, and Databases for the Identification of Rapid Growing Mycobacterium spp... Using MALDI-TOF MS

Allison McMullen - Washington University in St. Louis (amcmullen@path.wustl.edu) -- *Young Investigator Grantee*

- We evaluated different sample preparation methods, instrumentation platforms (VITEK MS and Bruker Biotyper), and databases for MALDI-TOF MS identification of 64 rapid growing Mycobacterium spp. (RGM) cultivated on solid media. Bruker Biotyper correctly identified 62/64 isolates. No identification was obtained for two isolates. The VITEK MS provided no result for one isolate and one incorrect identification (Saramis database) and no results for four isolates (v3.0 database). The VITEK-MS sample preparation required less hands-on time and had high confidence values/scores for the RGM isolates tested. MALDI-TOF MS will be able to expedite the identification of RGM from clinical specimens.
Metabolomics for Newbies

Wednesday @ 10:45 AM in Exec Ctr

Caroline Johnson & Julijana Ivanisevic - The Scripps Research Institute (johnsonc@scripps.edu) -- *Young Investigator Grantee*

Part 1: In this course we will introduce mass spectrometry-based metabolomics. We will cover the importance of metabolomics in science research, the diversity of the metabolome, and challenges of global metabolomics. Mass spectrometry technologies will be covered and include electrospray ionization mass spectrometry coupled to liquid chromatography which is making a significant impact in metabolomics. We will cover untargeted and targeted approaches, data processing, including profile alignment, statistical analysis and metabolite identification. To follow on from the basics of metabolomics, the biomedical applications of metabolomics will be discussed for biomarker discovery, pharmacometabolomics and integration of other –omic technologies with metabolomics. This course is designed to introduce the non-expert to metabolomics and its potential applications of the field.

Wednesday @ 11:10 AM in Exec Ctr

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Metabolomics for Newbies

Caroline Johnson & Julijana Ivanisevic - The Scripps Research Institute (johnsonc@scripps.edu) -- *Young Investigator Grantee*

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The Development of a Proteomic Wellness Assay Using Dried Blood Spots: Moving Clinical Protein Diagnostics Towards Personalized Reference Ranges

James Bollinger - University of Washington - Genome Sciences (jgb2@uw.edu) -- *Young Investigator Grantee*

- Dried blood spot (DBS) sampling represent an attractive technique suitable to meet the demands of both personalized medicine and widespread epidemiological research. We describe method development parameters for the multiplexed analysis of a panel of clinically relevant proteins in DBS samples via selected reaction monitoring. For each protein target, we derive a set of optimal peptides for tandem MS analysis and demonstrate the value of our approach by designing and validating an SRM assay for the multiplexed quantitation of a set of 50 proteins within DBS samples using a single protein as an internal standard.

In Pursuit of ‘Normal Baselines’: Longitudinal Measurement of Protein Biomarkers in Dried Blood Spots

N. Leigh Anderson - SISCAPA Assay Technologies (leighanderson@siscapa.com)

- Precise, longitudinal measurement of protein biomarkers in dried-blood-spots (DBS) is an attractive option for providing preventative, personalized medicine at reasonable cost. Here we present an automated SISCAPA workflow for multiplexed measurement of 20 proteins in longitudinal DBS samples collected from 10 individuals. By reducing the total workflow CV for all proteins below their ‘normal’ biological coefficient of variation, we were able to define the ‘normal’ range for each analyte in the longitudinal DBS samples. The data suggests that each individual possesses a unique ‘protein-fingerprint’ that if monitored longitudinally can provide invaluable insight into the person’s state of wellness and/or disease.

Systematic Comparison of Internal Standard Platforms for Absolute Protein Quantification of Cytokines by MRM-MS

Kerry Scott - NIST (kerry.bauer@nist.gov) -- *Young Investigator Grantee*

- Protein quantification methods based on the principle of multiple reaction monitoring can provide absolute protein quantification values. These methodologies are broadly applicable to clinical biomarkers, systems biology, and the pharmaceutical industry; which require accurate and precise protein abundance measurements. An evaluation of three conventional internal standards (AQUA peptides, QconCAT constructs, and PSAQ proteins) for quantitative accuracy, precision, and inherent advantages and limitations was conducted. A strategy for methodological improvement to current internal standard platforms was also investigated. The results of these studies as well as take home lessons will be presented.
Wednesday @ 2:30 PM in Harbor 2
A Novel Method for Plasma Metanephrine Analysis Using the Waters Unispray Ionisation Technique
Joanne Adaway - University Hospital South Manchester (jo.adaway@uhsm.nhs.uk)
• Measurement of plasma metanephrines is useful in the diagnosis of paragangliomas, but many assays require a large volume of plasma due to poor assay sensitivity, and often require lengthy sample preparation. We developed a method using the Waters Online Solid Phase Extraction manager coupled to a Waters Xevo TQS with a Unispray source that required only 50 µL of sample. Validation was carried out according to FDA guidelines and found to be acceptable, with an LLOQ of 18.75 PMol/L for metanephrine and 20.2 PMol/L for normetanephrine. We believe that this method is suitable for use in a busy clinical laboratory.

Wednesday @ 2:55 PM in Harbor 2
Recurrent Need for a Robust Method for Measuring T3/rT3 by LC-MS/MS: An Exercise in Madness or a New Beginning for a Misused Marker?
Julie Ray - ARUP Laboratories (julie.ray@aruplab.com)
• Even though measurement of reverse T3 (rT3) remains controversial in the diagnosis of hypothyroidism, the test continues to attract attention for managing an underactive thyroid in the integrative medical community. We present an evaluation of our sample preparation method for measuring T3/rT3 by LC-MS/MS and the frequent fouling of the mass spectrometer associated with it. Removal of pigmentation in 30-40% samples (suspected from bilirubin), with the use of 1% formic acid in dichloromethane helped maintain cleanliness of the instrument. A new application for the measurement of rT3 and T3 was tested in the CSF (cerebro spinal fluid) of brain injury patients. Preliminary results indicated that measurement of rT3 may have utility in brain injury in addition to the diagnosis of hypothyroidism.

Wednesday @ 3:20 PM in Harbor 2
Performance of Symmetric Dimethylarginine Against Other Markers of Kidney Function
Joe El-khoury - Yale University (joe.el-khoury@yale.edu) -- *Young Investigator Grantee*
• Glomerular filtration rate (GFR) is the best overall index of kidney function. Knowledge of GFR is essential to the diagnosis, classification, and management of kidney disease. Serum measurements of cystatin C (cysC), creatinine and their equations are the most widely used indirect markers, while symmetric dimethylarginine (SDMA) is an emerging marker. The objective of this study was to compare the performance of SDMA with cysC, creatinine and their equations to direct GFR measurement by radioactive iothalamate. A total of 40 subjects were included in this study. A published LC-MS/MS method was used for measuring SDMA. CysC and creatinine were measured by Roche Cobas 8000 (Indianapolis, IN). CysC showed the best overall correlation with GFR (r=0.92) and highest AUC (0.923) for kidney donor eligibility, followed by SDMA (r=0.87, AUC=0.882) then creatinine (r=0.76, AUC=0.767).
Wednesday @ 2:30 PM in Harbor 3

**Metabolic Reprogramming and Lipid Distribution Protects Gclm KO Mice from Alcohol Induced Steatosis**

*Srujana Golla* - National Cancer Institute, NIH (srujana.golla@nih.gov)

- The present study elucidated the protective role of antioxidant glutathione against chronic alcohol induced steatosis using the integrated analysis of metabolomics and lipidomics. The biochemical adaptation and lipid distribution patterns of wild-type and gamma-glutamylcysteine synthetase (Gclm)-null mice on chronic alcohol exposure were analyzed by robust UPLC coupled with ESI-QTOF mass spectrometry. The data matrix generated was further analyzed by multivariate data analysis using SIMCA 13+ software for identifying differential makers underlying the disease. The depleted glutathione was shown to impair de novo fatty acid and alters lipid distribution patterns in the Gclm-null mice upon alcohol exposure.

Wednesday @ 2:55 PM in Harbor 3

**Metabolomics Study of Premature Labor: Combined Supercritical Fluid Chromatography and Liquid Chromatography Coupled with Ion Mobility-Mass Spectrometry**

*Rafael Montenegro-Burke* - Vanderbilt University (rafael.montenegro@vanderbilt.edu) -- *Young Investigator Grantee*

- The cause of preterm birth is not entirely understood, and several factors (maternal age, infections, etc.) can influence the gestation period. Therefore, discovering a set of delivery date predictive biomarkers would improve the diagnosis of preterm risk pregnancies. In order to address this, an unbiased, untargeted metabolomics study of both term and preterm amniotic fluid samples was performed. The combination of liquid chromatography (LC) and supercritical fluid chromatography (SFC) coupled to ion mobility-mass spectrometry (IM-MS) demonstrates the great advantage of higher metabolome coverage, which increases the probabilities of detecting significant differences between sample groups in complex biological samples.

Wednesday @ 3:20 PM in Harbor 3

**In vivo Global Isotope Metabolomics Implicates the Arginase Pathway in Ischemic Retinopathy**

*Caroline Johnson* - The Scripps Research Institute (johnsonc@scripps.edu) -- *Young Investigator Grantee*

- Proliferative diabetic retinopathy (PDR) is the most severe form of diabetic retinopathy, here, global isotope metabolomic analysis was used to investigate metabolism to understand the ocular metabolic landscape. Analysis of vitreous humor from patients revealed an upregulation of arginine metabolism in PDR patients compared to non-diabetic controls. The oxygen-induced-retinopathy (OIR) mouse model revealed similar dysregulation. Validation by targeted metabolomics and the analysis of a second set of patient samples confirmed the upregulation of the same metabolites. The metabolic fate of U-15N-arginine determined in the OIR model, revealed a predominance of the arginase pathway to form proline. These results indicate that in PDR, the arginase pathway predominates, decreasing the availability of arginine for NO synthesis, a metabolite required for adequate endothelial cell function.
Clinical Applications of Top-down Proteomics
Daojing Wang - Newomics, Inc. (wang@newomics.com)

- The penetration of MS-based proteomics, particularly top-down proteomics, into the in vitro diagnostics market has remained low. MS-based platform has to achieve the: 1) sensitivity, 2) throughput; and 3) robustness, comparable to or even better than those of ELISA in order to find wider clinical acceptance. We have developed the silicon-based monolithic multinozzle emitter array (MEA) chip for high-sensitivity and high-throughput nanoflow-LC-ESI/MS. In this talk, I will present new MEA chip-enabled workflows and assays, for direct top-down proteomics analysis of sub-microliter volumes of human blood samples, and demonstrate the utilities of our assays in diagnosis and monitoring of several diseases.

Rapid Quantitative Biomedical Testing with Ambient Mass Spectrometry
Jentaie Shiea - National Sun Yat-sen University (jetea@fac.nsysu.edu.tw)

- Ambient mass spectrometry is a technique that operates the ionization source under ambient conditions to characterize analytes with minimum or no sample pretreatment. However, applying AMS for accurately quantitative analysis is still a problem, since the sample quantity is inconsistent for each analysis. In this study, an AMS technique - thermal desorption-electrospray ionization/mass spectrometry combined with solid-phase microextraction was developed for quantifying trace drugs in biological fluids. SPME/TD-ESI/MS contains features from both SPME and TD-ESI/MS, including simple and fast extraction, concentration, and characterization. Since the quantity of analytes extracted by SPME is dependent on the surface area of the SPME fiber, a combination of SPME with AMS makes it possible to perform rapidly quantitative analysis for certain types of drugs and chemical compounds in biofluids.

Challenges in Rapid Evaporative Ionization of Breast Tissue: A Novel Method for Real-time MS Guided Margin Control During Breast Surgery
Julia Balog - Imperial College London (j.balog@imperial.ac.uk) -- *Young Investigator Grantee*

- A novel setup based on Rapid Evaporative Ionisation Mass Spectrometry has been developed for the analysis of heterogeneous breast tissue. This technique is suitable with almost 100% accuracy for the separation of normal breast tissue, benign alterations (fibroadenoma) and different breast cancers. The novel setup enables both the use of cutting and coagulation electrosurgical settings, allowing us to record and analyze REIMS data throughout the whole breast surgery. The technique can be used for MS guided breast surgery, likely reducing the number of positive margins and therefore decreasing local regional cancer recurrence and necessary re-operations.
Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is quickly becoming the primary method of microorganism identification in many clinical laboratories. It is rapid, accurate, and cost-effective. In addition, the increased species-level resolution made possible by using this method, especially for Gram-positive bacilli and infrequently encountered taxa, is helping to inform clinicians and laboratorians about the biology and clinical significance of many bacterial species. In this session, we will present (interactive) case studies that demonstrate both the diagnostic pitfalls and the clinical utility of MALDI-TOF MS and "interesting" results acquired as a result of widespread use of this identification method.
Posters by Topic

Please Find *Extended Poster Abstracts* under Posters by Day/Time (starting on page 67)

**Fundamentals : General**

Fundamentals : General | Sunday 5:00 PM Poster #29

*Selection of Internal Standards for LC-MS/MS Applications*

*Uma Sreenivasan* - Cerilliant

Fundamentals : General | Sunday 5:00 PM Poster #58

*Using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry to Study the Distribution of Human Neutrophil Peptides in Tears*

*Hung Su* - National Sun Yat-sen University

Fundamentals : General | Sunday 5:00 PM Poster #65

*A LC-MS/MS Method for the Measurement of Testosterone Undecanoate and Dihydrotestosterone Undecanoate*

*Andrew Leung* - Los Angeles Biomedical Research Institute

Fundamentals : General | Sunday 5:00 PM Poster #80

*Column and Solvent Considerations that Contribute to the Success of LC-MS Analyses*

*Maricar Dube* - EMD Millipore

Fundamentals : General | Monday 5:00 PM Poster #27

*Addition of Solid Phase Extraction to Opiate Sample Preparation for UPLC-MS/MS*

*Hanan Mohammad* - University of North Carolina Hospitals

Fundamentals : General | Monday 2:00 PM Poster #58

*Differential Breast Cancer Glycosylation Detected by Gas Chromatography Nodal Glycan Analysis*

*Shayesteh R. Ferdosi* - Arizona State University

Fundamentals : General | Monday 5:00 PM Poster #69

*A Comprehensive Study for Validation of a LC-MS/MS Method for the Simultaneous Determination of Four Immunosuppressive Drugs in Whole Blood*

*Erdim Sertoglu* - Ankara Mevki Military Hospital, Anittepe Dispensary

Fundamentals : General | Monday 5:00 PM Poster #71

*Cortisol Measurement in Urine: LC-MS/MS Method Validation and Preliminary Clinical Application*

*Serkan Tapan* - Gulhane School of Medicine

**Fundamentals : Metabolomics**

Fundamentals : Metabolomics | Sunday 5:00 PM Poster #43

*Brain Region Mapping Using Global Metabolomics*

*Julijana Ivanisevic* - Center for Metabolomics, TSRI

Fundamentals : Metabolomics | Monday 5:00 PM Poster #17

*An Interactive Digital Pathway Map: A Resource for Interpreting Metabolomic Data*

*Nick Spittler* - Washington University in St. Louis

Fundamentals : Metabolomics | Monday 2:00 PM Poster #74

*Global Mapping of Nutrient Utilization by Untargeted Metabolomics*

*Liz Payne* - Washington University in St. Louis
Complete Annotation of the Untargeted, LC-MS Based Metabolomic Analysis of Escherichia coli
Nathaniel Mahieu - Washington University, St. Louis

Rapid Detection of Microbial Resistance to Lactam Antibiotics by LC-MS/MS
Michael Jarvis - SCIEX

Proteomics and Metabolomics Analysis of Patients Sera Revealed Activation of Anti-Oxidative Pathways in Vivax Malaria
Sandip Kumar Patel - Indian Institute of Technology Bombay

Protein Disulfide Bond Mapping Using Online LC–Electrochemistry–MS Applied to the Characterization of notch3 Protein Fragments
Linda Switzar - Leiden University Medical Center

Tandem Mass Spectrometry Analysis of Urinary Glycosaminoglycans as Biomarkers for Mucopolysaccharidose Patients
Pamela Lavoie - CRCHUS-Fleurimont Université de Sherbrooke

UDP-Galactose-4'-epimerase Activity Determination in Red Blood Cells by LC-MS/MS
Stephen Welna - Mayo Clinic
Inborn Errors of Metabolism | Sunday 5:00 PM Poster #27
Tandem Mass Spectrometric Determination of Atypical 3-beta-Hydroxy-delta-5-bile Acids in Patients with 3-beta-HSD Deficiency
Kenneth Setchell - Cincinnati Children's Hospital Medical Center

Inborn Errors of Metabolism | Sunday 5:00 PM Poster #54
Development and Implementation of Amino Acid Quantitation by LC-MS/MS at BC Children's Hospital
Andy De Souza - BC Children's Hospital

Inborn Errors of Metabolism | Sunday 5:00 PM Poster #68
Urinary Glucose Tetrasaccharide Assay Using Rapid Ultraperformance LC-MS/MS for Pompe Disease
Youngwon Nam - Seoul National University Hospital

Inborn Errors of Metabolism | Sunday 5:00 PM Poster #77
Development of a Direct Assay of Iduronate-2-sulfatase for Mucopolysaccharidosis Type II (Hunter Syndrome) Using UPLC-MS/MS
Kyunghoon Lee - Seoul National University College of Medicine

Inborn Errors of Metabolism | Sunday 5:00 PM Poster #82
Ceramide Trihexosides and Sulfatides Quantitation in Urine by LC-MS/MS
Jean Lacey - Mayo Clinic

Inborn Errors of Metabolism | Monday 2:00 PM Poster #12
Measurement of Lymphocyte Argininosuccinate Synthetase Activity by Tandem Mass Spectrometry
Shu-Chu Shiesh - National Cheng Kung University

Inborn Errors of Metabolism | Monday 2:00 PM Poster #40
Innovations in Newborn Screening for Inborn Metabolic Disorders in Dried Blood Spots
Zdenek Spacil - University of Washington

Inborn Errors of Metabolism | Tuesday 5:00 PM Poster #15
Triplex Tandem Mass Spectrometry Assays for Screening of 3 Lysosomal Storage Disorders in a Korean Population
Sung Eun Cho - LabGenomics Clinical Laboratories

Inborn Errors of Metabolism | Tuesday 2:00 PM Poster #34
Comparison of Non-derivatization and Derivatization Tandem Mass Spectrometry Methods for Analysis of Amino Acids and Acylcarnitines in Dried Blood Spot
Xiaolei Xie - Thermo Fisher Scientific

Metabolomics

Metabolomics | Sunday 5:00 PM Poster #55
Targeted Serum Metabolite Profiling for Colorectal Cancer Progression Monitoring
Jiangjiang Zhu - University of Washington

Metabolomics | Sunday 5:00 PM Poster #67
A Pipeline for Untargeted UPLC-MS Profiling to Expand Tissue Metabolome Coverage – Optimization of Chromatographic Separation and Tissue Extraction
Panagiotis Vorkas - Imperial College London

Metabolomics | Sunday 5:00 PM Poster #79
LC-MS/MS Method After Derivation for the Determination of L- and D- Isomers of 2-hydroxyglutarate in Biological Fluids: Application as Biomarker of IDH Mutation
Vianney Poinsignon - Gustave Roussy
Rapid, Simultaneous Analysis of Urinary Catecholamines and Metanephrines by Mixed-mode SPE and HILIC LC-MS/MS
Jonathan Danaceau - Waters Corporation

Skyline for Small Molecules: A Flexible Tool for Cross-platform LC-MS/MS Method Creation and Data Analysis for Metabolomics
J. Will Thompson - Duke University

A Metabolomic Approach to Identify Potential Therapeutic Biomarkers for Diabetic Foot Ulcers
Chia-ni Lin - Chang Gung Memorial Hospital

Metabolic Phenotyping Reveals a Lipid Mediator Response to Ionizing Radiation
Giuseppe Astarita - Georgetown University

Targeted Oxylipin Profiling for Clinical Diagnostic: A Novel Insight in Ventilator Associated Pneumonia
Arnaud Wolfer - Imperial College London

X13CMS and IsoMETLIN: Platforms for Isotope-Based Metabolomics
Kevin Cho - Washington University

Comprehensive Human Fecal Metabolome Analysis Using Chemical Isotope Labeling LC-MS
Nan Wang - Zhejiang University

Development of Chemical Isotope Labeling LC-MS for Human Serum Metabolome Profiling from Dried Serum Spots
Liyan Liu - University of Alberta

Patrick Caron - CHU de Quebec Research Center

A Data Independent LC-MS Based Method for a Multi-omic Approach to Investigate Obesity Treatment Within a Mouse Model
Giuseppe Astarita - Waters Corporation

A High Throughput LC-MS/MS Method for the Analysis of Multiple Vitamin D Analytes in Serum and Placenta Using Supportive Liquid-liquid Extraction
Carl Jenkinson - University of Birmingham

Metformin Treated Wistar Rats Demonstrate Remarkable Alterations in Lipid and Bile Acid Plasma Levels
Panagiotis Vorkas - Imperial College London

A Novel High Resolution Metabolite MS/MS Spectral Library for Accurate Metabolite Identification in Human Biofluids
Annette Michalski - Bruker Daltonics
## Microbiology/Virology

**Identification of Staphylococcus Aureus by Shotgun Spectral Matching**  
*Dana Ohana* - Leiden University Medical Center

**MALDI-TOF-MS and MS-ASTRA Assay Development for the Generation of Biotyper Reference Spectra and Antibiotic Sensitivity Determination for Category B Bacteria**  
*Tara Kenny* - USAMRIID

**Highly Sensitive Screening for Antibiotic Resistance Using Parylene-matrix Chip**  
*Jong-Min Park* - Yonsei University

**Multiple Reaction Monitoring Assay for Identification of Borrelia burgdorferi**  
*Crystal Cheung* - Institute for Bioscience and Biotechnology Research

**High Throughput Pulse-chase Analysis of Metabolite Turnover in Microorganisms Followed by LAESI Mass Spectrometry**  
*Sylwia Stopka* - The George Washington University

**An Illustrative Example of the Need for Ongoing Clinical Microbiology Competency in the Era of MALDI-TOF MS Microorganism Identification: Neisseria spp.**  
*Neil Anderson* - Washington University School of Medicine

## Molecular Diagnostics

**Pharmacodynamic Strategy for Monitoring the Extent of Immunosuppression: Requirement of a Kinase Inhibitor for Measuring Calcineurin Phosphatase Activity**  
*Sylvia Sanquer* - Hôpital Necker-Enfants Malades

**Utility of the Microflex LT Platform in the Development of Serum Proteomic Companion Diagnostic (CDx) Tests in NSCLC**  
*Nicholas Dupuis* - Biodiesix, Inc

**Swab Touch Spray - Mass Spectrometry for Direct Analysis of Bacteria and Drugs in Oral Fluid**  
*Karen Cesafsky* - Purdue University

**Personalized Chemotherapy Through the Combination of Microdosing and Accelerator Mass Spectrometry**  
*Maike Zimmermann* - University of California, Davis

## New Advances

**Quantification of Multiple Therapeutic mAbs in Serum Using microLC-ESI-Q-TOF Mass Spectrometry**  
*David Barnidge* - Mayo Clinic

**Mass Spectrometry and Stable Isotope Labeling for Quantitative Analysis of Ribosomal RNA Modifications**  
*Anna Popova* - The Scripps Research Institute
New Advances | Sunday 5:00 PM Poster #73
PhoTorrent Atmospheric Pressure Photo Ionization (APPI) Source to Achieve High Efficiency Photo Ionization on Testosterone and 25-OH Vitamin D3
Ellie Majdi - IONICS Mass Spectrometry

New Advances | Sunday 5:00 PM Poster #76
Exploring the Potential of the Last Generation UHR-Q-TOF for Rapid Generation of Accurate Information on Proteoforms Distribution and Relative Abundancy
Nicolai Bache - Bruker Daltonics

New Advances | Monday 2:00 PM Poster #6
Direct Mass Spectrometry Analysis of Wet Biofluid Samples UsingSlug-Flow Microextraction
Yue Ren - Purdue University

New Advances | Monday 5:00 PM Poster #53
Usability Study of a New HPLC, a New Tandem MS and a New Data Processing Software
Jason Lai - Thermo Fisher Scientific

New Advances | Monday 2:00 PM Poster #64
Using MALDI-TOF MS to Screen for Monoclonal Proteins in Serum
Mindy Kohlhagen - Mayo Clinic

New Advances | Tuesday 2:00 PM Poster #2
Standardized and Quantitative Metabolic Phenotyping of Bile Acids in Blood - An International Inter-laboratory Ring Trial Test
Ralf Bogumil - BIOCRATES Life Sciences AG

New Advances | Tuesday 5:00 PM Poster #19
Preliminary Experience with the Waters Unispray™ Source
Brian Keevil - University Hospital of South Manchester

New Advances | Tuesday 5:00 PM Poster #53
Strategies for the Direct Coupling of Solid Phase Microextraction (SPME) to Mass Spectrometry: Applications in the Clinical Lab
Germán Augusto Gómez-rios - University of Waterloo

Occupational and Environmental Health

Occupational and Environmental Health | Sunday 5:00 PM Poster #5
Development of a Sensitive Analytical Method for Serum Bisphenol A Using LC-MS/MS
Dae-Hyun Ko - Asan Medical Center

Pain Management

Pain Management | Sunday 5:00 PM Poster #22
The Positive Inconsistent in Urine Drug Testing at a Community Specialty Pain Clinic
Nguyen Nguyen - Soloniuk Pain Center

Pain Management | Sunday 5:00 PM Poster #72
Sensitive Measurement of HU-210 from Oral Fluids via LC-MS/MS: A Fully Automated SPE Sample Preparation and MS Sensitizing Derivatization Process
Qi Huang - Quantalytical Labs, Inc

Pain Management | Monday 2:00 PM Poster #4
Development of a Whole Blood Microsampling Bioanalytical Method for the Analysis of Opiates with a Goal of Point-of-Care Therapeutic Drug Monitoring
Daniel Kassel - SciAnalytical Strategies, Inc.

Pain Management | Monday 2:00 PM Poster #22
Improved Method for the Analysis of a Pain Management Supplemental Panel in Urine Using the Thomson EXtreme Filter Vials® by LC-MS/MS
Lisa Wanders - Thomson Instrument Company
High Throughput Screening and Confirmation of 41 Pain Panel Drugs in Oral Fluid by an Integrated On-Line Extraction UHPLC-MS/MS System  
*Louis Maljers* - Bruker

Development and Validation of an Opioid LC-MS/MS Assay: Evaluation of Different β-glucuronidase Enzymes and Protein Precipitation Plates  
*He Yang* - University of California, San Francisco/SF General Hospital

A Fast, Sensitive, and High-throughput LC-MS/MS Assay for Benzodiazepines/Z-Drugs/Barbiturates  
*Hui Qiao* - IONICS Mass Spectrometry

A Fast Polarity Switching LC-MS/MS Analysis of Benzodiazepines and Barbiturates  
*Joshua (Sha) Ye* - IONICS Mass Spectrometry

Validation of an Automated Method to Remove β-Glucuronidase from Hydrolyzed Pain Management Urine Samples  
*Shahana Huq* - Phenomenex

Development of a Rapid Mass Spectrometry Based Screen of Tricyclic Antidepressants as an Alternative to Immunoassay Screening  
*Erin C. Strickland* - Ameritox, Ltd.

High-throughput Targeted Screening and Definitive Method for Barbiturate Drugs in Urine Using LDTD-MS/MS with Ultra-fast Analysis at 9 Seconds Sample to Sample  
*Pierre Picard* - Phytronix Technologies, Inc

Paper Spray Ionization - Tandem Mass Spectrometry for Quantification of Prescription Drugs in Oral Fluid  
*Karen Cesafsky* - Purdue University

The Analysis of Fentanyl and Its Analogues in Human Urine by LC-MS/MS  
*Landon Wiest* - Restek Corporation

A Novel 6x5 Peptide Mixture for Instrument Performance Monitoring  
*Michael Rosenblatt* - Promega Corporation

Characterization of Stable Isotope Labeled Insulin-Like Growth Factor-1 for Use as an Internal Standard in Quantitative High-Resolution MS Workflows  
*Kevin Ray* - Sigma-Aldrich Corporation

Value Assignment of Vitamin D Metabolites in Vitamin D Standardization Program (VDSP) Serum Samples  
*Karen Phinney* - National Institute of Standards and Technology
Proficiency, Regulations, Standards | Monday 5:00 PM Poster #65
Accuracy Evaluation of High- and Low-density Lipoprotein Cholesterol Assays in Clinical Laboratories by Comparison with Isotope Dilution Mass Spectrometry
*Misuk Ji* - Konkuk University School of Medicine

Proficiency, Regulations, Standards | Monday 5:00 PM Poster #67
Value Assignment of Candidate Standard Reference Material® 3949 Folate Vitamers in Frozen Human Serum by Isotope-Dilution LC-MS/MS
*Johanna Camara* - National Institute of Standards and Technology

Proteomics

Proteomics | Sunday 5:00 PM Poster #4
Two-dimensional Liquid Chromatography Coupled to High-resolution Mass Spectrometry for Mapping LKB1 Dependent Signaling Networks in Non-small Cell Lung Cancer
*Nilini Ranbaduge* - The Ohio State University

Proteomics | Sunday 5:00 PM Poster #6
Free Urinary Light Chain Analysis Assisted via Proteolytic Cleavage and LC-MS/MS
*Jesse Seegmiller* - University of Minnesota

Proteomics | Sunday 5:00 PM Poster #9
Evaluation of Two LC-MS/MS Thyroglobulin Assays Performance in the Presence of Anti-thyroglobulin Autoantibodies
*Brian Netzel* - Mayo Clinic, Rochester, Minnesota

Proteomics | Sunday 5:00 PM Poster #20
Profiling of the Mucosal Metabolome by the DESI-MS of Medical Swabs – New POC Diagnostic Approach for Infections, Dysbiosis and Immunological Diseases
*Zoltan Takats* - Imperial College London

Proteomics | Sunday 5:00 PM Poster #26
Proteomic Platform for Comprehensive and Quantitative Urinary Proteomes
*Garwin Pichler* - Max Planck Institute of Biochemistry

Proteomics | Sunday 5:00 PM Poster #39
The Measurement of Food-intake and Nutrient Absorption in C. elegans by Quantitative Mass Spectrometry
*Elizabeth Valentine* - The Scripps Research Institute

Proteomics | Sunday 5:00 PM Poster #53
Mass Spectrometric Quantification of Enriched Microglia Using a Metabolically-labeled Immortalized Cell Mix
*Harris Bell-Temin* - University of South Florida

Proteomics | Monday 5:00 PM Poster #41
Targeted Quantitation of 1-84 Parathyroid Hormone (PTH) by SID-MRM Mass Spectrometry
*Cheng Zhao* - Abbott Laboratories

Proteomics | Monday 2:00 PM Poster #82
Identifying the Proteome of Different Mycobacterial Species Using Orbitrap™ Mass Spectrometry
*Suraj Saraswat* - ARUP Lab

Proteomics | Tuesday 5:00 PM Poster #31
Rapid and Robust Plasma Proteomics Platform for Clinical Settings
*Philipp Emanuel Geyer* - Max Planck Institute of Biochemistry

Proteomics | Tuesday 5:00 PM Poster #33
A Hybrid Approach to Proteomic Sequencing of Immunoglobulins
*Natalie Castellana* - Digital Proteomics LLC
Proteomics | Tuesday 2:00 PM Poster #50
Proteomic and Metabolic Changes in Malnutrition
Evelyn Gitau - Kenri Wellcome Trust Research Programme

Proteomics | Tuesday 2:00 PM Poster #52
Comparison of SDVB-Monolithic & Bead-based Columns Used in Nanoflow LC-MS for Proteomic Study
Sung-Fang Chen - National Taiwan Normal University

Proteomics | Tuesday 2:00 PM Poster #60
Phosphoproteomic Analysis by HAMMOC Enrichment and LC-MS/MS
Ting-Yu Wei - National Taiwan Normal University

Proteomics | Tuesday 2:00 PM Poster #62
Differential Proteomic Analysis of PLC/PRF/5 Cell Lines Treated with Various Anti-cancer Drugs by iTRAQ Labeling and Mass Spectrometry
Shih-Hua Huang - National Taiwan Normal University

Proteomics | Tuesday 2:00 PM Poster #64
Analysis of COL6A Proteins as a Potential Therapeutic Marker for Ullrich Muscular Dystrophy and Bethlem Myopathies
Sunhee Jung - Seattle Children's Hospital Research Institute

Proteomics | Tuesday 2:00 PM Poster #68
Mapping c-Src Phosphorylation Sites as Potential Disease Biomarkers
Kunhong Xiao - Duke University Medical Center

Sample Prep & Automation

Sample Prep & Automation | Sunday 5:00 PM Poster #7
Effect of Enzyme Source, Form and Hydrolysis Conditions on the Conversion of Glucuronide Drug Metabolites in Urine to Parent Drugs by β-Glucuronidase
Craig Aurand - Supelco

Sample Prep & Automation | Sunday 5:00 PM Poster #11
Rapid Quantitative Analysis of 25-OH Vitamin D2 and D3 in Patient Serum Using a Novel Weak Anion Exchange Disposable Pipette Extraction (DPX-WAX) and LC-MS/MS
Gary Woodward - University Hospital Birmingham

Sample Prep & Automation | Sunday 5:00 PM Poster #13
Optimization and Validation of Cannabinoid Metabolite Confirmation in Urine Using LC-MS/MS and Biotage EVOLUTE EXPRESS AX SPE Cartridges
Heather Hochrein - UC San Diego Health System

Sample Prep & Automation | Sunday 5:00 PM Poster #28
Quantitation of 17β-Estradiol in Serum by LC-MS/MS: Achieving 2 pg/mL Sensitivity Using an Aggressive Sample Preparation Procedure
Matthew Myer - TriCore Reference Laboratories

Sample Prep & Automation | Sunday 5:00 PM Poster #33
Sample Preparation: The Achilles Heel of Rapid Mass Spectral Analysis
Fred Regnier - Purdue University

Sample Prep & Automation | Sunday 5:00 PM Poster #34
Multiplexed Analysis of apo A1, apo B and apo E in Normo- and Hyper-triglyceridemic Specimens Using the Automated SISCAPA-MRM Workflow
Selena Larkin - SISCAPA Assay Technologies

Sample Prep & Automation | Sunday 5:00 PM Poster #42
FFPE Protein Recovery and Optimization for Proteomics Analysis
Patrick Vanderboom - Mayo Clinic
An Automated Sensitive Measurement of Estrone and 17β-Estradiol from Human Plasma on LC-MS Using Solid-Phase Extraction and MassBoost Derivatization
*Emmanuel Chanco* - SPEware Corporation

**Sample Prep & Automation | Sunday 5:00 PM Poster #78**

**iST Sample Preparation for High Throughput Clinical Proteomics**
*Nils Kulak* - Max Planck Institute of Biochemistry

**Sample Prep & Automation | Monday 5:00 PM Poster #7**

**Determination of Testosterone in Serum by Automated Sample Preparation and Ultra-fast LDTD-MS/MS in a Cross Validation Study with Real Patient Samples**
*Alex Birsan* - Phytronix Technologies, Inc.

**Sample Prep & Automation | Monday 5:00 PM Poster #11**

**Evaluation of Methylisothiazolinone (MI) Extraction from Sunscreen Using Supported Liquid Extraction Prior to GC/MS Analysis**
*Lee Williams* - Biotage GB Limited

**Sample Prep & Automation | Monday 2:00 PM Poster #34**

**Quantitative Analysis of IGF-1 Using Online Digestion Coupled to the Triple Quad LCMS-8050**
*David Colquhoun* - Shimadzu Scientific Instruments

**Sample Prep & Automation | Monday 2:00 PM Poster #56**

**Multi-channeling LC-MS/MS Forensic Methods for High-Throughput Urine Screening to Detect Buprenorphine and Ethanol Use**
*Joseph Di Bussolo* - Thermo Fisher Scientific

**Sample Prep & Automation | Monday 2:00 PM Poster #72**

**Extraction of Buprenorphine and Norbuprenorphine from Urine Samples Using New Nbe™ (Narrow Bore Extraction) Columns: Fully Automated Sample Preparation**
*Emmanuel Chanco* - SPEware Corp.

**Sample Prep & Automation | Monday 2:00 PM Poster #76**

**Rapid Quantification of Free and Glucuronidated THC-COOH in Human Urine Using Coated Well Plates and Column-switching LC-MS/MS**
*Marianne Hädener* - University of Bern

**Sample Prep & Automation | Monday 2:00 PM Poster #78**

**Enhancing Ion Abundances and Spatial Homogeneity of Glycans by Regulating the Substrate Temperature in MALDI MS: A Physical Chemistry Perspective**
*Yin-Hung Lai* - Genomics Research Center, Academia Sinica

**Sample Prep & Automation | Tuesday 5:00 PM Poster #1**

**HPLC-UHPLC Hybrid 2D Platform for LC-MS Analysis of Biological Samples. Back to the Future**
*Eduard Rogatsky* - Albert Einstein College of Medicine

**Sample Prep & Automation | Tuesday 5:00 PM Poster #3**

**Optimization and Validation of Online SPE-UHPLC-MS/MS for Trace Level Quantitation of Bisphenol A Analogues in Human Urine**
*Wei Zou* - EHLB, California Department of Public Health

**Sample Prep & Automation | Tuesday 5:00 PM Poster #11**

**Converting a Liquid-liquid Extraction Method for Vitamin D to a 96-well Plate Supported-liquid Extraction Format: A Case Study with Real Patient Plasma Samples**
*Katerina Sadilkova* - Seattle Children’s Hospital

**Sample Prep & Automation | Tuesday 2:00 PM Poster #12**

**Sample Preparation of Three Steroids for Quantitative Determination by LC-MS/MS – Comparison of Two Extraction Procedures**
*Dave van Staveren* - Tecan Schweiz AG
Comparison of 25-hydroxy Vitamin D Extraction Using Supported Liquid Extraction and Phospholipid Depletion Plate Technology Prior to LC-MS/MS Analysis
Lee Williams - Biotage GB Limited

Analysis of Aldosterone in Plasma for Clinical Research Using Automated Extraction
Heather Brown - Waters Corporation

Improved Method for the Analysis of 31 Drugs of Abuse/Pain Management Panel in Oral Fluid Samples Using the Thomson EXTreme® Filter Vials by LC-MS/MS
Nadine Koenig - Health Networks

Comparison of Different Liquid-liquid Sample Preparations for LC-MS/MS Assays of Total Serum Testosterone Measurements
Yuyong Ke - EndoCeutics, Inc

A Rapid Sample Preparation Method for Quantitative Analysis of Cortisol in Saliva and Urine by LC-MS/MS
Daniel Zhou - Stanford Health Care

High Throughput Determination of Multiple Drugs in Plasma and in Blood Using Solid Phase Microextraction
Nathaly Reyes-garces - University of Waterloo

Simple Sample Preparation for Measuring Methylmalonic Acid in Blood Serum by LC-MS
Joseph Di Bussolo - Thermo Fisher Scientific

Catecholamine Analysis: Evaluation of Method Optimization to Improve Sensitivity and Reduce Limits of Quantitation Using LC-MS/MS
Adam Senior - Biotage GB Limited

Simultaneous Quantification of 17-β-oestradiol and Oestrone in Human Plasma by LC-MS/MS
Sherry Gregory - Thermo Fisher Scientific

Versatile Platform for Fully Automated Sample Preparation of Forensic Whole Blood for LC-MS Analysis
Brian Rasmussen - University of Copenhagen

Fast, Simple Method for the Analysis of Benzodiazepines in Meconium and an Interlaboratory Method Comparison
William Brewer - University of South Carolina

Small Molecule Analytes

Effective Monitoring for Enantomeric Forms of Methamphetamine and Related Compounds by LC-MS
Carmen Santasania - Supelco/Sigma-Aldrich

Quantitation of Ganciclovir in Human Serum by UPLC-MS/MS
Katerina Sadilkova - Seattle Children's Hospital
A Five-Minute Analysis that Separates 25-hydroxyvitamin D from Its C3 Epimer
Katherine Rogers - Yale New Haven Hospital

LC-MS Quantitative Analysis of Fat Soluble Vitamins in Blood
Lauren Frick - Agilent Technologies, Inc

Medicinal Cannabis and the Need for Enhanced Cannabinoid Profiling
Scott Kuzdzal - Shimadzu Scientific Instruments

The Analysis of Vitamin D and Metabolites in Plasma by LC-MS/MS
Paul Connolly - Restek Corporation

Quantitative Analysis of 25-Hydroxy-Vitamin D in Serum Using LC/QQQ and LC/Q-TOF
Jeff Keever - Agilent Technologies, Inc

Development of High Sensitivity Micro-LC-MS/MS Method for Estradiol in Human Serum without Derivatization
Xin Yi - The University of Chicago

Simultaneous Determination of Methylmalonic Acid and Homocysteine in Plasma by LC-MS/MS
Petra Prochazkova - SPADIA Lab, a.s.

Sensitive Analysis of Serum 5alpha-Dihydrotestosterone by 2D-LC-MS/MS
Bingfang Yue - NMS Labs

Quantitative Analysis of a Glyburide Analogue, a Potential NLRP3 Inhibitor, Using Micro-sampling, Hybrid Solid Phase Extraction and LC-MS/MS
Ankit Zalavadia - Virginia Commonwealth University

A Fast Analysis of Low Level Estrogens in Serum by Bruker EVOQ Elite LCMS
Zicheng Yang - Bruker

Profiling Sialylation Status of Macrophage Upon Cell Activation
Dan Wang - Cleveland State University

LC-MS Quantitative Analysis of Water Soluble Vitamins in Blood
Lauren Frick - Agilent Technologies, Inc

Comparison of Different Whole Blood Sample Pretreatment Methods for Targeted Analysis of Basic Drugs
Seyed Sadjadi - Phenomenex, Inc.

High Throughput Measurement of Five Tobacco-specific Nitrosamines in Urine by Automation and Liquid Chromatography–mass Spectrometry
Baoyun Xia - Centers for Disease Control and Prevention
Small Molecule Analytes | Monday 5:00 PM Poster #31
Small Molecule Analysis Using MALDI-TOF MS with Solid Nanostructure Matrices
Jo-Il Kim - Yonsei University

Small Molecule Analytes | Monday 5:00 PM Poster #35
Mass-Directed Isolation and Profiling of Small Molecule Analytes with SFC
Lu Dai - Theravance Biopharma US, Inc.

Small Molecule Analytes | Monday 5:00 PM Poster #37
Use of 96-well Pipetting Workstation for Liquid-liquid Extraction of Adrenal Steroids
Geoffrey Rule - ARUP Laboratories

Small Molecule Analytes | Monday 5:00 PM Poster #39
LC-MS Quantitative Analysis of 25-Hydroxy-Vitamin D, 1, 25-Dihydroxy-Vitamin D, and their Isobars in Serum
Jeff Keever - Agilent Technologies, Inc

Small Molecule Analytes | Monday 2:00 PM Poster #42
Rapid, Simultaneous Analysis of Plasma Catecholamines and Metanephrines by Mixed-mode SPE and HILIC LC-MS/MS
Sherri Naughton - Waters Corporation

Small Molecule Analytes | Monday 5:00 PM Poster #45
A Rapid and Accurate LC-MS/MS Method for the Analysis of Nicotine, Nicotine Metabolites, and Minor Tobacco Alkaloid in Urine
Shun-Hsin Liang - Restek Corporation

Small Molecule Analytes | Monday 2:00 PM Poster #48
Accuracy Evaluation of Three Routine 25-hydroxyvitamin D Assays by Comparing with LC-MS/MS
Yeo-Min Yun - Konkuk University School of Medicine

Small Molecule Analytes | Monday 5:00 PM Poster #49
Signal Enhancement in HPLC-ESI-MS/MS Analysis of Spironolactone Metabolites Using HFIP and NH4F as Eluent Additives
Kalev Takkis - University of Tartu

Small Molecule Analytes | Monday 5:00 PM Poster #51
Bioanalytical UPLC-MS/MS Method Development and Validation for Measuring Penicillins in Human Blood Plasma– Analyte Stability Issues
Karin Kipper - Institute for Infection and Immunity

Small Molecule Analytes | Monday 5:00 PM Poster #55
Evaluation of Bench-top Quadrupole Orbitrap Ultra High Resolution MS for Use in Clinical Research for Rapid Quantitative Analysis of Vitamin D in Human Plasma
Mindy Gao - ThermoFisher Scientific

Small Molecule Analytes | Monday 2:00 PM Poster #62
A Novel Analytical Method to Analyze Phosphatidylcholine in Human Breath Using UHPLC-MS/MS
Shahid Ullah - Karolinska Institute

Small Molecule Analytes | Monday 5:00 PM Poster #75
1,25-di-hydroxy Vitamin D Analysis by LC-MS: Optimization for Sample Prep Automation and Medium Throughput Lab
Dave van Staveren - Tecan Schweiz AG

Small Molecule Analytes | Monday 2:00 PM Poster #80
Investigation of Arachidonic Acid and Its Metabolites as Biomarkers for Potential Efficacy Endpoints for Monoacylglycerol Lipase Inhibition
Kimberly Navetta - Pfizer Global Research and Development
Detection and Quantitation of Exemestane, Letrozole and Anastrozole in Human Serum by LC-MS/MS and Atmospheric Pressure Chemical Ionization
Julia Addiss - Quest Diagnostics, Inc.

Validation of a Reliable LC-MS/MS Method for Analysis of Five Steroids Simultaneously in Clinical Laboratory
Atecla Alves - Universidade de São Paulo/HCFMUSP

Altered Adrenal and Gonadal Steroids Biosynthesis in Patients with Burn Injury
Maria Bergquist - Uppsala University

Separation and Quantification of Serum L- and D-2-hydroxyglutarate Enantiomers by LC-MS/MS Following Derivatisation
Laura Bernstone - University Hospital of South Manchester

Analysis of Sex Steroids in Urine by LC-MS/MS
Maria Ospina - Centers of Disease Control and Prevention

HDL and Total Cholesterol Analysis by LDTD-MS/MS Analysis in 7 Seconds Per Sample with Cross-validation Data from a Clinical Laboratory Using Real Patient Serum Samples
Alex Birsan - Phytronix Technologies, Inc

A UPLC-MS/MS Method for the Analysis of Plasma Mycophenolic Acid for Clinical Research
Michelle Wills - Waters Corporation

Development of a Consolidated LC-MS/MS Assay for Quantification of Voriconazole, Posaconazole and Teriflunomide
Pratistha Ranjitkar - University of Washington

Determination of Plasma Catecholamines by LC-MS/MS for Clinical Research
Linda Cote - Agilent Technologies

Improved Detection of 17ß-Estradiol and Estrone in Serum Through Derivatization with Dansyl Chloride Utilizing LC-MS/MS Technology
Andre Szczesniewski - Agilent Technologies

An Improved Platform for the Recovery and Analysis of Cannabinoids from Dried Blood Samples
James Hill - Spot On Sciences

Determination of Plasma Renin Activity by LC-MS/MS for Clinical Research
Linda Cote - Agilent Technologies

Measurement of Urinary Serotonin for Clinical Research, Using Mixed Mode SPE and a High-strength Silica PFP Column
Sherri Naughton - Waters Corporation
Small Molecule Analytes | Tuesday 5:00 PM Poster #45
A Rapid and Sensitive LC-MS/MS Method for the Analysis of Free Thyroid Hormones
Shun-Hsin Liang - Restek Corporation

Small Molecule Analytes | Tuesday 5:00 PM Poster #47
LC-MS/MS Measurement of Urinary 2,3-dinor-11β-Prostaglandin F2α in Patients with Systemic Mastocytosis: Improved Diagnostic Accuracy Compared to ELISA
Alan Lueke - Mayo Clinic Rochester

Small Molecule Analytes | Tuesday 2:00 PM Poster #54
Evaluation of a Method for Forensic Quantitative Screening of Over 120 Drugs of Abuse on a Triple Quadrupole Mass Spectrometer
Kristine Van Natta - Thermo Fisher Scientific

Small Molecule Analytes | Tuesday 5:00 PM Poster #57
Online Analysis of Immunosuppressants in Whole Blood with the Evoq Triple Quad
Rafaela Martin - Bruker Daltonik GmbH

Small Molecule Analytes | Tuesday 2:00 PM Poster #76
A Simplified, Rapid LC-MS/MS Assay for Serum and Salivary Creatinine
Laura Bernstone - University Hospital of South Manchester

Small Molecule Analytes | Tuesday 2:00 PM Poster #80
Development of a High-throughput UPLC-MS/MS Method for Medroxyprogesterone Acetate (MP 17 Acetate) Quantification in Human Plasma
Pamela Hummert - Johns Hopkins University School of Medicine

Tissue Imaging & Analysis | Sunday 5:00 PM Poster #3
Stable Isotope Nanostructure Imaging Mass Spectrometry to Monitor Tumor Metabolism
Michael Kurczy - The Scripps Research Institute

Tissue Imaging & Analysis | Sunday 5:00 PM Poster #25
Dual Polarity Mass Spectrometric Imaging from Single Tissue Sections with Desorption Electrospray Ionization (DESI) Mass Spectrometry
Darwin Asa - Waters Corporation

Tissue Imaging & Analysis | Monday 5:00 PM Poster #19
Determination of Concentration and Distribution of Doxorubicin in Lungs by in vivo and in Situ Solid Phase Microextraction
Barbara Bojko - University of Waterloo

Tissue Imaging & Analysis | Tuesday 2:00 PM Poster #6
Multimodal Imaging Mass Spectrometry for Probing Aβ-Plaque Pathology
Jörg Hanrieder - Gothenburg University

Tissue Imaging & Analysis | Tuesday 5:00 PM Poster #23
Multi-mode Desorption Electrospray Ionization (DESI) Mass Spectrometry Imaging at Different Pixel Resolutions for Human Tissue Imaging
Khalid Khan - Waters Corporation
### Tissue Imaging & Analysis

**Tuesday 2:00 PM Poster #26**
**Desorption Electrospray Ionization (DESI) for Tissue Imaging on a Time-Of-Flight (TOF) Mass Spectrometer**  
*Darwin Asa - Waters Corporation*

**Tuesday 2:00 PM Poster #66**
**Imaging Analysis of Metals, Lipids, and Proteins in Biological Tissues via Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry**  
*Christopher Shiea - Kaohsiung Medical University*

**Tuesday 5:00 PM Poster #81**
**Biomarker Candidates Discovery of Myocarditis Using MALDI Imaging Mass Spectrometry**  
*Jungju Seo - Korea Basic Science Institute*

### Toxicology

**Sunday 5:00 PM Poster #12**
**Forensic Drugs Screening Analysis by HPLC Coupled to QTOF Mass Spectrometry: Comparison to a Routine EMIT, HPLC, GC/NPD and GC/MS Workflow**  
*Curtis Hedman - Wisconsin State Laboratory of Hygiene*

**Sunday 5:00 PM Poster #14**
**LC-MS/MS Analysis of Pain Management Drugs and their Polar Metabolites Utilizing Supported Liquid Extraction for Sample Pretreatment**  
*Matthew Slawson - ARUP Institute for Clinical and Experimental Pathology*

**Sunday 5:00 PM Poster #23**
**A Simplified, Mixed-mode Sample Preparation Strategy for Urinary Forensic Toxicology Screening by LC-MS/MS**  
*Jonathan Danaceau - Waters Corporation*

**Sunday 5:00 PM Poster #30**
**A Novel Separation for the Bath Salts Using a Multi-Mode Reversed-Phase Column**  
*Peter Simms - Lux Laboratories*

**Sunday 5:00 PM Poster #32**
**LC-MS Analysis of Phytocannabinoids and their Metabolites in Urine, Oral Fluid and Blood**  
*Rory Doyle - Agilent Technologies, Inc*

**Sunday 5:00 PM Poster #35**
**Hepatic Metabolism of Licochalcone A, a Chalcone from Licorice (Glycyrrhiza Inflata)**  
*Lingyi Huang - University of Illinois at Chicago*

**Sunday 5:00 PM Poster #37**
**LC-MS Analysis of Phytocannabinoids and their Metabolites in Urine, Oral Fluid and Blood**  
*Rory Doyle - Agilent Technologies, Inc*

**Sunday 5:00 PM Poster #45**
**In Vitro Human Metabolism of Designer Cathinones: LC-MS/(MS) Metabolites Identification and Characterization for Doping Control Purposes**  
*Amelia Palermo - Laboratorio Antidoping FMSI*

**Sunday 5:00 PM Poster #46**
**The Analysis of Common Drugs of Abuse in Human Urine by LC-MS/MS**  
*Frances Carroll - Restek Corporation*

**Sunday 5:00 PM Poster #47**
**Experience and Lessons Learned Developing a Comprehensive Toxicology Panel for Pain Management and Beyond**  
*Ping Wang - Houston Methodist Hospital*
Optimization of Automated Online SPE-LC-MS/MS Used in Pain Management Drug Monitoring  
Mark Hayward - Assurance Scientific Laboratories

Cost Advantage and Improved Accuracy of Medication Compliance by a Qualitative Time-Of-Flight Mass Spectrometry and Immunoassay-based Screen in Pain Management  
Kelly Doyle - ARUP, University of Utah

Ethanol Metabolites by Paper Spray Ionization: Method Development in Negative Ion Mode  
Maria C Prieto Conaway - Thermo Fisher Scientific

In Oral Fluid 7-Aminoclonazepam Is Superior to Clonazepam for Detection of Clonazepam Use  
James Flood - Massachusetts General Hospital

LC-MS/MS Method for Quantitative Analysis of Gabapentin and Pregabalin in Serum or Plasma  
Stephen Merrigan - ARUP

Drug Excretion into Breast Milk: Are All Drugs Contraindicated for Breastfeeding?  
Joshua (Sha) Ye - IONICS Mass Spectrometry

LC-MS/MS Study of 25-OH Vitamin D2 and D3 with Perkin Elmer Vitamin D Kit Using Both Derivatized and Non-derivatized Methods  
Hui Qiao - IONICS Mass Spectrometry

New Acquisition and Processing Tools for Targeted and Unknown Screening Approaches in Clinical Research and Forensic Toxicology  
Benedicte Duretz - ThermoFisher

Automated Targeted Screening of Benzodiazepines in Urine Using LDTD-MS/MS at 400 Samples Per Hour Rate  
Pierre Picard - Phytronix Technologies, Inc

Development of a Rapid LC-MS/MS Method for Hair Cortisol Determination to Assess the HPA Axis  
Laura Smy - The Hospital for Sick Children

The Development of an LC-MS/MS Screening Method for 104 Targeted Compounds in Whole Blood, Using Library Searching on a QTRAP Mass Spectrometer  
Heather Singletary - Metro Nashville Police Department-Crime Lab

A Novel and Fast Workflow for Forensic Toxicological Screening and Quantitation Using QTOF LC-MS/MS System  
Xiang He - SCIEX

Comparison of Accurate Mass MS/MS Acquisition and Processing Techniques on Forensic Toxicological Screening  
Michael Jarvis - SCIEX
Toxicology | Monday 2:00 PM Poster #20
**Enzyme Hydrolysis Using a Novel Recombinant β-Glucuronidase for Pain Management Urine Drug Testing**
*Agnes Cua - Precision Toxicology*

Toxicology | Monday 2:00 PM Poster #32
**Is It Noroxymorphone or Nornaloxone, and Why Should You Care?**
*Stephanie Martin - ARUP Institute for Clinical and Experimental Pathology*

Toxicology | Monday 2:00 PM Poster #38
**Comprehensive and Extended LC-MS Analysis of 166 Various Drugs and their Metabolites in Urine, Oral Fluid and Blood**
*Rory Doyle - Agilent Technologies, Inc*

Toxicology | Monday 2:00 PM Poster #44
**Comparison of Tacrolimus Quantification Using the Waters MassTrak LC-MS/MS Assay with the Abbott Architect Immunoassay**
*Imir Metushi - University of California, San Diego - CALM*

Toxicology | Monday 2:00 PM Poster #46
**The Analysis of Synthetic Cannabinoids and their Metabolites in Human Urine by LC-MS/MS**
*Frances Carroll - Restek Corporation*

Toxicology | Monday 2:00 PM Poster #50
**Using LC-MS/MS Urine Drug Testing to Identify Licit and Illicit Drug-Use in a Community-based Patient Population**
*Adam Ptolemy - Gamma-Dynacare Medical Laboratories*

Toxicology | Monday 5:00 PM Poster #77
**Rapid Mass Spectrometry Based Urine Drug Screening of 27 Antipsychotic and Antidepressant Medications**
*Jeffrey Enders - Ameritox, Ltd.*

Toxicology | Monday 5:00 PM Poster #79
**RapidFire-Based Screening of THCA: Comparison with Established Methods**
*Jennifer Hitchcock - Ameritox, Ltd.*

Toxicology | Tuesday 5:00 PM Poster #5
**Measurement of Human Urinary Organophosphate Pesticide Metabolites in a Clinical Setting**
*Stephen Donovan - NMS Labs*

Toxicology | Tuesday 2:00 PM Poster #10
**LC-MS Analysis of Nicotine and Its Metabolites in Urine, Oral Fluid and Blood**
*Julie Cichelli - Agilent Technologies, Inc*

Toxicology | Tuesday 2:00 PM Poster #44
**Detection of Ethyl Glucuronide and Ethyl Sulfate in Urine by Hydrophilic Interaction Liquid Chromatography (HILIC)-MS/MS**
*Maricar Dube - EMD Millipore*

Toxicology | Tuesday 2:00 PM Poster #58
**Fast and Confident Identification of Drugs and their Metabolites Using Ion Trap LC-MSn Analysis and a Library of >4,500 Compounds**
*Rafaela Martin - Bruker Daltonik*

Toxicology | Tuesday 5:00 PM Poster #65
**Clinical and Forensic Monitoring of Zopiclone Through the Use of a Degradation Product**
*Anna Miller - MedTox Laboratories, LabCorp*

Toxicology | Tuesday 5:00 PM Poster #69
**Quantification of Drugs for Drug-Facilitated Crimes in Human Urine by LC-MS/MS**
*Claudio De Nardi - Thermo Fisher Scientific*
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<td>Lidong He - Department of Chemistry, University of Utah and ARUP Institute for Clinical and Experimental Pathology</td>
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<td>Haoyue Zhang - Duke Medicine Biochemical Genetics Laboratory</td>
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Combine HDX-MS and NMR for Polycystin-2 C-terminal Tail Structural Characterization
Yifei Yang - Yale University

Various OTHER | Tuesday 2:00 PM Poster #74
Quantitative Omics Strategies for Investigating the Oral Microbiome in Dental and Systemic Diseases
Anna Merrill - University of Wisconsin
Posters by Day: SUNDAY

Sunday 5:00 PM
Poster #1 in Exhibit Hall

**UPLC-MS/MS Multiplex Methodology for Creatine Synthesis and Transport Disorders, Triple H Syndrome and OTC Deficiency**

*Christiane Auray-Blais - Université de Sherbrooke (christiane.auray-blais@usherbrooke.ca)*

- We aimed to increase the number of treatable disorders screened by the Mass Urinary Screening Program in the Province of Quebec. Creatine synthesis and transport disorders, Triple H syndrome and Ornithine transcarbamylase deficiency (OTC) were targeted by selecting specific urinary biomarkers: creatine, guanidineacetate, uracil, orotic acid and creatinine. A rapid multiplex methodology was developed and validated to analyze these biomarkers from urine samples dried on filter paper. This efficient methodology demonstrates the feasibility of mass or high-risk screening urine samples for early, pre-symptomatic detection and treatment of these inborn errors of metabolism.

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Sunday 5:00 PM
Poster #2 in Exhibit Hall

**Tandem Mass Spectrometry Analysis of Urinary Glycosaminoglycans as Biomarkers for Mucopolysaccharidose Patients**

*Pamela Lavoie - CRCHUS-Fleurimont Université de Sherbrooke (pamela.lavoie@usherbrooke.ca)*

- Mucopolysaccharide disorders are the result of primary defects in lysosomal enzymes. The primary objectives of this research project were to evaluate the GAG urinary excretion and the relationship with disease severity, response to treatment and the impact of co-morbidities in mucopolysaccharidoses. We thus devised a quantitative methodology for the analysis of urine samples using a tandem mass spectrometry multiplex approach. Dermatan sulfate, heparan sulfate, keratan sulfate, and chondroitin sulfate disaccharides were specifically targeted. Our results show an efficient differentiation between MPS patients and controls. High sensitivity and good resolution allow the detection of small quantities of urinary GAGs.

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Sunday 5:00 PM
Poster #3 in Exhibit Hall

**Stable Isotope Nanostructure Imaging Mass Spectrometry to Monitor Tumor Metabolism**

*Michael Kurczy - The Scripps Research Institute (kurczy@scripps.edu)*

- Metabolic variation in tumor cells is tightly coupled to cancer pathology and progression. While it is reasonable to consider a tumor as a homogeneous mass of similar cells for metabolomic analysis these structures are in reality a diverse population of cells in different metabolic states. We have developed a method to monitor anaerobic glycolysis within tumors with single cell spatial resolution to address the unavoidable averaging that occurs in traditional extraction based methods. This analysis utilizes nanostructure imaging mass spectrometry (NIMS), with ESI LC-MS/MS, to map the conversion of stable isotope-labeled glucose to lactate (13C3).

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Sunday 5:00 PM
Poster #4 in Exhibit Hall

**Two-dimensional Liquid Chromatography Coupled to High-resolution Mass Spectrometry for Mapping LKB1 Dependent Signaling Networks in Non-small Cell Lung Cancer**

*Nilini Ranbaduge - The Ohio State University (ranbaduge.1@osu.edu) -- *Young Investigator Grantee**

- Liver kinase B1 (LKB1) is a tumor suppressor gene that encodes for a key regulator kinase in cells. Somatic mutations of this gene lead to loss of its expression, which is commonly found in 30-40% of non-small cell lung cancer. However, the understanding of the role of LKB1 loss in the development of lung cancer is unclear. Online 2D LC-MS/MS is used to study LKB1-directed phosphorylation events at protein level that may reveal these interactions. It is the goal here to provide a comprehensive understanding of the underlying molecular biological changes specific to LKB1 loss, and to provide a clearer understanding of the process by which LKB1 loss leads to lung cancer.
Development of a Sensitive Analytical Method for Serum Bisphenol A Using LC-MS/MS

Dae-Hyun Ko - Asan Medical Center (daehyuni1118@gmail.com) -- *Young Investigator Grantee*

- We developed a sensitive assay for serum BPA. BPA in serum were extracted with toluene and derivatized with dansyl chloride. After derivatization, the samples were analyzed by liquid chromatography-tandem mass spectrometry. The assay was linear in a range from 0.05 ng/mL to 5.0 ng/mL. Within-run and between-run imprecision were within acceptable level. The median BPA concentration was 0.044 ng/mL in male (range: 0.003 to 0.551 ng/mL) and 0.029 ng/mL (range: under detection limit to 0.055 ng/mL) in female. Our method provides a valuable tool for public health studies about BPA exposure.

Free Urinary Light Chain Analysis Assisted via Proteolytic Cleavage and LC-MS/MS

Jesse Seegmiller - University of Minnesota (jseegmil@umn.edu)

- In 1847, Dr. Bence Jones described excess protein in the urine of a patient with cancer. Noting that the protein, later discovered to be immunoglobulin light chains, precipitated when warming urine from 40 to 60°C, he thus developed the first method for detecting a cancer marker in bodily fluids. Today, light chains are characterized or measured by clinical labs using techniques such as gel electrophoresis and immunoassay. Recent literature has questioned the validity of quantitative results measured by immunoassay. Therefore we developed a liquid chromatography tandem mass spectrometry method to measure κ and λ free light chains, which employed proteolysis.

Effect of Enzyme Source, Form and Hydrolysis Conditions on the Conversion of Glucuronide Drug Metabolites in Urine to Parent Drugs by β-Glucuronidase

Craig Aurand - Supelco (craig.aurand@sial.com)

- β-Glucuronidase (β-D-glucuronide glucuronosohydrolase) enzymes play an important role in the analysis of biological fluids for the presence of drug metabolites for drug screening and drug metabolism studies. Although there are numerous β-glucuronidase enzymes available, each enzyme has optimum conditions to which hydrolysis of glucuronide metabolites can be effectively conducted. Variables such as β-glucuronidase enzyme concentration, digestion pH, incubation time and temperature all play an important role for effective hydrolysis of glucuronide metabolites. In this study, critical variables are evaluated for determining an appropriate β-glucuronidase enzyme and incubation conditions.

Effective Monitoring for Enantomeric Forms of Methamphetamine and Related Compounds by LC-MS

Carmen Santasania - Supelco/Sigma-Aldrich (carmen.santasania@sial.com)

- Methamphetamine is a powerful CNS stimulant widely abused due to the increased mental alertness and suppression of fatigue it produces. The L-isomer is used legally in several over the counter medicines. L-methamphetamine is also a metabolite of therapeutic drugs such as selegiline. Immunoassay does not differentiate between the legal and illicit versions and a positive result is seen if either form is detected. A chiral LC-MS method is presented on urine samples for methamphetamine. Sample recoveries and detection limits are presented. The method will be used to determine other related enantomeric compounds, similar in structure, to methamphetamine.
Evaluation of Two LC-MS/MS Thyroglobulin Assays Performance in the Presence of Anti-thyroglobulin Autoantibodies

Brian Netzel - Mayo Clinic, Rochester Minnesota (netzel.brian@mayo.edu)

- Thyroglobulin (Tg) measurement by MS is attractive due to its insensitivity to Tg autoantibodies (TgAB) interference, a problem observed with immunoassays (IA) and radioimmunoassays (RIA). Standardization of Tg-MS assays is paramount for clinical adaptation. Two Tg-MS assays were compared across Tg and TgAB concentration ranges. Mixtures controlling Tg and TgAB levels were run to assess effects in IA/RIA vs. Tg-MS. Tg-MS methods correlated well (Slope = 1.1; R² = 0.97), with minimal variation in Tg concentration (-0.2 to +0.2-fold) while IA showed significant deviation from expected Tg concentrations (-0.3 to -7-fold, and +0.3 to +10.7-fold in IA and RIA, respectively).

Cut-off Validation of Newborn Screening by Tandem Mass Spectrometry with Application of Worldwide Collaborative Project

Seungman Park - Green Cross Laboratories (freenuri78@gmail.com) -- *Young Investigator Grantee*

- Cut-off validation of newborn screening test (NST) of metabolic disorders is important to reduce false negative and false positive cases. We reviewed NST data and adjusted cut-off values and interpretation criteria. Presumptive cut-off values of 10 amino acids and 30 acylcarnitines were estimated by calculation of mean, standard deviations (SD), cumulative percentiles, and positive rate of 148,534 neonates born in 2013-2014. New cut-off values were then decided in consideration of reduction for false negative rate. We believe that our new cut-off is enough to detect inborn error metabolism and reduce false negative rate. Our experience for cut-off validation will be helpful for other laboratories doing newborn screening test.

Rapid Quantitative Analysis of 25-OH Vitamin D2 and D3 in Patient Serum Using a Novel Weak Anion Exchange Disposable Pipette Extraction (DPX-WAX) and LC-MS/MS

Gary Woodward - University Hospital Birmingham (gary.woodward@uhb.nhs.uk) -- *Young Investigator Grantee*

- The increased clinical interest in vitamin D analysis has meant an increased demand on hospital laboratories, requiring quicker and more efficient analysis methods. To this end, we present a novel and rapid method for the extraction of vitamin D2 and D3 using disposable pipette extraction with weak anion exchange (DPX-WAX) and LC-MS/MS detection, evaluated against conventional liquid-liquid extraction in routine patient samples. The limits of detection were determined to be >10 and >5 µM for Vit D2 and D3, respectively. The average recovery was 103% with a mean CV of 13.6 and 7.6% for vitamin D2 and D3, respectively. Coefficients of determination (R²) were greater than 94% between the extraction techniques, with a proportional bias of 1.09-1.12%. These results indicate that DPX-WAX is effective for vitamin D analysis in routine laboratories.

Forensic Drugs Screening Analysis by HPLC Coupled to QTOF Mass Spectrometry: Comparison to a Routine EMIT, HPLC, GC/NPD and GC/MS Workflow

Curtis Hedman - Wisconsin State Laboratory of Hygiene (curtis.hedman@slh.wisc.edu) -- *Young Investigator Grantee*

- Recent advances in QTOF hardware and software have increased the feasibility of applying this technology to routine forensic drugs screening. To prove this, ten blinded whole blood samples previously analyzed by EMIT, HPLC-UV, GC/NPD and GC/MS workflows were analyzed by HPLC-QTOF-MS/MS with both TOF-IDAMS/MS and TOF-MS/MS-All with SWATH™. With an estimated 50% reduction in analysis time, simpler sample preparation, and higher confidence especially with high resolution accurate mass MS/MS library matching, HPLC-QTOF-MS/MS detected nearly all of the 106 compounds detected by EMIT, HPLC, GC/NPD and GC/MS, and also detected five compounds missed in the original screening analysis.
Optimization and Validation of Cannabinoid Metabolite Confirmation in Urine Using LC-MS/MS and Biotage EVOLUTE EXPRESS AX SPE Cartridges

Heather Hochrein - UC San Diego Health System (hhochrein@ucsd.edu)

- Solid Phase Extraction (SPE) has shown to be an effective process for sample clean-up and concentration of THC, which is a common illicit found in clinical urine samples. We present an initial validation of the Biotage EVOLUTE EXPRESS AX cartridge for THC-COOH, which omits a pre-conditioning step and allows for direct elution into auto-sampler vials. We also validated previously published strategies to reduce non-specific adsorption of this analyte. Results were comparable to a previously validated SPE product and techniques adopted in this method provided for a robust and precise extraction procedure for analysis by LC-MS/MS.

LC-MS/MS Analysis of Pain Management Drugs and their Polar Metabolites Utilizing Supported Liquid Extraction for Sample Pretreatment

Matthew Slawson - ARUP Institute for Clinical and Experimental Path. (matthew.slawson@aruplab.com)

- Presented is a method utilizing SLE followed by UPLC-MS/MS for the detection of tapentadol, tramadol, meperidine and their polar metabolites. The extraction was optimized to ensure recovery of parent drugs and metabolites by incorporating a pretreatment with a basified brine and methylene chloride/isopropanol solution followed by an acidified methylene chloride/isopropanol elution. This ensured all analytes eluted in the same extract. The method has a LOD at least 50 ng/mL and a ULOL of 5,000 ng/mL for tapentadol, tramadol and their metabolites. The LOD for meperidine and normeperidine is 2 and 5 ng/mL, respectively with a ULOL of 1000 ng/mL. The method offers excellent S:N compared to other sample preparation methods.

UDP-Galactose-4'-epimerase Activity Determination in Red Blood Cells by LC-MS/MS

Stephen Welna - Mayo Clinic (welna.stephen@mayo.edu)

- UDP-galactose-4'-epimerase (GALE) activity can be measured by the use of a rapid LC-MSMS separation of UDP-glucose and UDP-galactose that allows direct quantification of enzymatic products and is an improvement upon recently published enzyme assays using LC-MS/MS. Seven abnormal results were identified in red blood cells (RBC) during a blinded sample exchange with Emory University; 3 true positive and 4 carriers. Intra and inter assay precision was assessed at four activity levels and CVs were <6% and <10% respectively (N=20). A reference range evaluation has yielded a mean of 16.8 nmol/h/mg Hb (min-max = 7.1-36.8, stdev = 4.8, N=173). Demonstration of reduced GALE activity in RBC is a useful diagnostic test following an abnormal newborn screening result for galactosemia.

Quantification of Multiple Therapeutic mAbs in Serum Using microLC-ESI-Q-TOF Mass Spectrometry

David Barnidge - Mayo Clinic (barnidge.david@mayo.edu)

- Therapeutic monoclonal immunoglobulins (mAbs) are an important class of drugs used to treat diseases ranging from autoimmune disorders to B cell lymphomas. Here we demonstrate the simultaneous quantification of four therapeutic mAbs in serum using the abundance of the kappa light chain from each IgG kappa mAb. We also report the concentration of each therapeutic mAb in pharmaceutical preparations using IgG specific proteotypic peptides. Our results show that microLC-ESI-Q-TOF mass spectrometry is a robust platform for monitoring multiple therapeutic mAbs in a single assay.
Sunday 5:00 PM  
Poster #17 in Exhibit Hall

**Quantitation of Ganciclovir in Human Serum by UPLC-MS/MS**  
*Katerina Sadilkova - Seattle Children’s Hospital (katerina.sadilkova@seattlechildrens.org)*

- Ganciclovir is an antiviral drug administered for the prevention and treatment of cytomegalovirus disease in immunocompromised patients. Ganciclovir concentration levels measured by this assay are used in pharmacokinetic calculations to obtain dosing information using area-under-the-curve analysis of serum concentrations following ganciclovir administration. We developed and validated an ultra-performance liquid chromatography- mass spectrometry method to measure the concentration of ganciclovir in human serum. Sample preparation, UPLC-MS/MS conditions, linearity, precision, patient samples correlation, recovery, stability, matrix effect and interference were demonstrated. This assay can be used in the clinical setting for therapeutic drug monitoring and pharmacokinetic studies of ganciclovir.

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Sunday 5:00 PM  
Poster #18 in Exhibit Hall

**A Novel 6x5 Peptide Mixture for Instrument Performance Monitoring**  
*Michael Rosenblatt - Promega Corporation (mike.rosenblatt@promega.com)*

- Performance monitoring and standardization of LC-MS/MS instrumentation continues to be challenging across all MS laboratories. Towards this end, we have prepared a peptide mixture and produced a novel software tool (PReMiS ™) that reports on LC column performance and MS instrument parameters (including sensitivity and dynamic range). In this current study we will use the reagent and software to optimize LC and MS methods, compare instruments across multiple laboratories and, compare different instrument types. The absolute sensitivity of the instruments (neat and spiked into complex mixtures) as well as a comparison of the peptide separation on multiple reversed phase columns will be presented. The reagent/software combination has also been critical for monitoring trends in instrument parameters.

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Sunday 5:00 PM  
Poster #20 in Exhibit Hall

**Profiling of the Mucosal Metabolome by the DESI-MS of Medical Swabs – New POC Diagnostic Approach for Infections, Dysbiosis and Immunological Diseases**  
*Zoltan Takats - Imperial College London (z.takats@imperial.ac.uk)*

- Medical swabs are standard sampling devices in the diagnostics of various diseases, but also used for DNA collection. Processing protocols for swabs are narrowly targeted (e.g. PCR testing for Chlamydia) and also time consuming with reporting times of 3-4 days. Alternatively, medical swabs can also be analysed by DESI-MS. The mass spectrometric data – acquired in the timeframe of 20-30 s after sampling – features mostly metabolite- and lipid-type constituents including bacterial secondary metabolites and human inflammatory mediators. The spectral information was successfully used for the identification of infectious agents and stratifying allergy patients in case of pharyngeal and nasal mucosa, respectively.

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Sunday 5:00 PM  
Poster #21 in Exhibit Hall

**Pharmacodynamic Strategy for Monitoring the Extent of Immunosuppression: Requirement of a Kinase Inhibitor for Measuring Calcineurin Phosphatase Activity**  
*Sylvia Sanquer - Hôpital Necker-Enfants malades (sylvia.sanquer@gmail.com)*

- An approach for measuring the immunosuppressants effects on their cellular targets, such as calcineurin for calcineurin inhibitors, has been developed in order to improve the monitoring of immunosuppression after transplantation. The determination of calcineurin activity consists in the direct measurement of the dephosphorylation of a phosphorylated substrate of calcineurin, the RII substrate. None of the methods described so far have discussed the possibility of rephosphorylation of the substrate. Here, we demonstrate the importance of such an influence on the measurements of calcineurin activity for monitoring the extent of immunosuppression after transplantation. Accordingly, we report the development and the validation of a LC-MS/MS assay for measuring calcineurin activity in biological samples wherein a kinase inhibitor is present in the assay reaction mix.
The Positive Inconsistent in Urine Drug Testing at a Community Specialty Pain Clinic

Nguyen Nguyen - Soloniuk Pain Center (nguyen@soloniuk.com)

- Urine Drug Testing (UDT) data assists clinicians develop a treatment plan, tailor specific drug panels, determine UDT testing frequency and validate medication therapy compliance or diversion in initiating and ongoing opioid therapy. A recent retrospective review of Soloniuk Pain Center UDT results showed that 28.8% of UDT in Redding, CA, and 36.0% of UDT in Red Bluff, CA were noncompliant. For this study, 480 consecutive samples were analyzed using the Waters liquid chromatography tandem mass spectrometer (LC-MS/MS). Out of the 50 drugs/metabolites, 28 were accounted for all of the inconsistent results. Out of the 14 drugs found in both clinics, the 6 common inconsistent positives with the highest incident percentages were THC (20.8%), morphine (13.4%), codeine (10.1%), lorazepam (9.4%), tramadol (6.7%), and oxycodone (5.4%).

A Simplified, Mixed-mode Sample Preparation Strategy for Urinary Forensic Toxicology Screening by LC-MS/MS

Jonathan Danaceau - Waters Corporation (jonathan_danaceau@waters.com)

- A simplified mixed-mode sample preparation method has been developed for the extraction and LC/MS/MS analysis of a forensic toxicology panel. The entire SPE procedure is reduced from 6 steps to three by eliminating conditioning and equilibration steps and combining both wash procedures into a single step. This enables a panel of opioid drugs, amine stimulants, benzodiazepines and other drugs of abuse to be extracted in a single method with recoveries that exceed 90% for 95% of the compounds, and limits of detection that ranged from 1-10 ng/mL.

A Five-Minute Analysis that Separates 25-hydroxyvitamin D from Its C3 Epimer

Katherine Rogers - Yale New Haven Hospital (Katherine.rogers@ynhh.org)

- Measurement of 25-hydroxyvitamin D (25OHD) is useful for the nutritional assessment of a patient’s vitamin D status. LC-MS/MS methods that measure 25OHD are considered the “gold standard”, but are susceptible to interference from the C3-epimer of 25OHD. Reported methods that separate the C3-epimer from 25OHD tend to involve tedious sample preparation and/or lengthy chromatography. In this study, we achieve our goal of developing a LC-MS/MS method that separates 25OHD from its C3 epimer in under 5 minutes and involves simple sample preparation for routine clinical use.

Dual Polarity Mass Spectrometric Imaging from Single Tissue Sections with Desorption Electrospray Ionization (DESI) Mass Spectrometry

Darwin Asa - Waters Corporation (darwin_asa@waters.com)

- Over the years Desorption Electrospray Ionization (DESI), an ambient ionization technique, has been applied to Mass Spectrometry Imaging (MSI) to allow for the direct analysis of surfaces at atmospheric pressure. Here we demonstrate that using standard DESI analysis conditions combined with a SYNAPT G2-Si, it is possible to analyze a tissue section in one ionization mode followed by a second experiment acquired in the opposite ionization mode and access a wealth of molecular information from a single tissue section without the need to alter the imaging analysis conditions.
Proteomic Platform for Comprehensive and Quantitative Urinary Proteomes

Garwin Pichler - Max Planck Institute of Biochemistry (pichler@biochem.mpg.de)

Urine is a desirable body fluid for clinical research as it can be obtained non-invasively in large quantities from every patient. Here we build on recent developments in our group in the sample-preparation workflow to enable reproducible, parallelized and sensitive processing of urine proteins. We identified a total of 3284 proteins in urine and calculated the LFQ values of 2200 proteins in average per sample, with MS-signals spanning 6 orders of magnitude. Remarkably, we identified 1354 proteins, which have not been reported in urine before. Median coefficient of variation (CV) was 27% for technical replicates, which is excellent for label-free shotgun proteomics. Our developments contribute to a robust and sensitive high-throughput urine proteomics platform which we hope will open urine proteomics to routine, quantitative analysis of patient samples in clinical settings.

Tandem Mass Spectrometric Determination of Atypical 3-beta-Hydroxy-delta-5-bile Acids in Patients with 3-beta-HSD Deficiency

Kenneth Setchell - Cincinnati Children’s Hospital Medical Center (Kenneth.Setchell@cchmc.org)

3β--Hydroxy-delta-5-C27-steroid oxidoreductase (HSD3B7) deficiency, a progressive cholestatic liver disease, is the most common genetic defect in bile acid synthesis. Early diagnosis is important because patients respond to oral primary bile acid therapy, which targets the negative feedback regulation for bile acid synthesis to reduce the production of hepatotoxic 3-beta-hydroxy-delta-5-bile acids. A tandem mass spectrometry method is described for the measurement of 3-beta-hydroxy-delta-5-bile acid sulfates in urine applicable to the diagnosis and accurate monitoring of responses to primary bile acid therapy in HSD3B7 patients.

Quantitation of 17β-Estradiol in Serum by LC-MS/MS: Achieving 2 pg/mL Sensitivity Using an Aggressive Sample Preparation Procedure

Matthew Myer - TriCore Reference Laboratories (matthew.myer@tricore.org)

A method for the quantitation of 17β-Estradiol in human serum, suitable for application on a LC-MS/MS clinical system, is examined. The sample preparation procedure includes a liquid-liquid extraction and derivatization in dansyl chloride, followed by a solid phase extraction. This procedure ensures maximally efficient extraction of the analyte from the serum while significantly reducing matrix interferences in order to achieve the clinically necessary 2 pg/mL sensitivity. The linear range of the assay is demonstrated as 2-5000 pg/mL with a typical patient CV of <7% and excellent calibration agreement (<2% bias) with the Center for Disease Control’s Hormone Standardization (HoSt) program.

Selection of Internal Standards for LC-MS/MS Applications

Uma Sreenivasan - Cerilliant (uma_sreenivasan@cerilliant.com)

Internal standards are utilized across a wide range of clinical mass spectrometry applications including therapeutic drug monitoring, newborn screening, endocrinology, and pain management testing. The ability of an internal standard to improve the accuracy of analyte quantitation depends upon proper internal standard selection and critical assessment and mitigation of the challenges that arise due to internal standard use. Examples will be presented to illustrate the importance of label placement, isotopic distribution and purity, impact of natural isotope abundance, cross talk, and scrambling.
A Novel Separation for the Bath Salts Using a Multi-Mode Reversed-Phase Column

Peter Simms - Lux Laboratories (psimms@kozmary.com)

- Analysis and separation of bath salts mephedrone, methylene and MDPV was achieved using a multi-mode reverse phase column. The separation was obtained using the aqueous normal phase (ANP) mechanism. Bath salts were separated in four minutes using an isocratic mobile phase. The separation was effected by the concentration of acetonitrile and the pH of the aqueous buffer. Increasing the organic modifier concentration caused an increase in retention time. Increasing the pH caused a decrease in retention time. Using these parameters, a rapid and simple method was developed to quantitate the bath salts.

LC-MS Quantitative Analysis of Fat Soluble Vitamins in Blood

Lauren Frick - Agilent Technologies, Inc (lauren.frick@agilent.com)

- Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) are suited for rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of the relevant fat soluble vitamins. This analytical method uses a simple offline sample preparation in blood. The described method achieves the required sensitivity and is capable of quantitating the vitamins over their relevant dynamic range. Excellent reproducibility was observed for all compounds (CV < 15%). All calibration curves displayed linearity with an R2 > 0.995.

LC-MS Analysis of Barbiturates in Urine, Oral Fluid and Blood

Julie Cichelli - Agilent Technologies, Inc (julie.cichelli@agilent.com)

- Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. A sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of barbiturates by QQQ. Using simple sample preparation techniques in urine, oral fluid, and blood, and chromatographic configurations achieves the required sensitivity and separation and is capable of quantitating the drugs over their relevant dynamic range. Excellent reproducibility was observed for all drugs (CV < 15%). All calibration curves displayed linearity with an R2 > 0.995.

Sample Preparation: The Achilles Heel of Rapid Mass Spectral Analysis

Fred Regnier - Purdue University (fregnier@purdue.edu)

- This presentation will describe a microfluidic membrane system in which human plasma is extracted from a drop of blood by capillary action and a 2.5 uL sample aliquot prepared for MS analysis through internal standard addition, immunosorbent binding, and chemical modifications, all within a single membrane card without an energy source. This allows self sampling at remote sites via a finger-stick, a substantial degree of sample preparation within a single membrane bearing card, and dry transport of prepared samples to an analytical laboratory on a collection disc smaller than a postage stamp.

Multiplexed Analysis of apo A1, apo B and apo E in Normo- and Hyper-triglyceridemic Specimens Using the Automated SISCAPA-MRM Workflow

Selena Larkin - SISCAPA Assay Technologies (selenalarkin@siscapa.com)

- Measuring LDL and HDL cholesterol has been the gold standard method for risk estimation in cardiovascular disease (CVD). However, multiple reports indicate that measuring the ratio of apolipoprotein-B100 to apolipoprotein-A1 provides a more accurate risk assessment, especially in patients suffering from hyperlipidemia. Apolipoprotein-E is emerging biomarker for CVD. Here, we present a multiplexed, automated SISCAPA assay for precise measurement of apoA1, apoB100 and apoE both in liquid specimens (e.g. serum) and in dried-blood-spots. We aim to explore the value of longitudinal sample collection for monitoring lipid profile and ultimately cardiovascular health on an individual basis.
Hepatic Metabolism of Licochalcone A, a Chalcone from Licorice (Glycyrrhiza Inflata)

Lingyi Huang - University of Illinois at Chicago (hly0917@gmail.com) -- *Young Investigator Grantee*

- Licochalcone A is a chalcone natural product that has been isolated from roots of the licorice species, Glycyrrhiza inflata. It shows several bioactivities in vitro. Since little information is available concerning the human metabolism of licochalcone A, we carried out preclinical in vitro hepatic metabolism studies. Several Phase I metabolites of licochalcone A were observed and characterized using high resolution LC-MS/MS with accurate mass measurement. While testing for the formation of possible reactive metabolites using human liver microsomes, the cofactor NADPH and the biological nucleophile GSH, several GSH conjugates were detected and characterized using UHPLC-MS/MS. These results indicate that licochalcone A not only can form GSH conjugates due to the reactivity of its alpha,beta-unsaturated ketone structure but also through the formation of electrophilic metabolite.

Medicinal Cannabis and the Need for Enhanced Cannabinoid Profiling

Scott Kudzdzal - Shimadzu Scientific Instruments (sakudzal@shimadzu.com)

- Medicinal cannabis refers to the use of cannabis as a therapy to treat diseases and/or alleviate symptoms. The cannabis industry is projected to be an $8B industry by 2018. With this growth has come an explosion in cannabis testing labs. This presentation will provide an overview of medicinal cannabis, including grow operations, dispensaries and a focus on testing laboratories. Cannabinoid profiling was performed using LCMS and GCMS. A case study involving a 10 year-old epileptic child with leaky gut syndrome and summary of his experiences with CBx oils will be presented. Future opportunities for MS-based testing will be discussed.

LC-MS Analysis of Phytocannabinoids and their Metabolites in Urine, Oral Fluid and Blood

Rory Doyle - Agilent Technologies, Inc (rory.doyle@agilent.com)

- Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analyte’s. A sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of Phytocannabinoids and their metabolites by QQQ. Using simple sample preparation techniques in urine, oral fluid and blood and chromatographic configurations achieves the required sensitivity and separation and is capable of quantitating the drugs over their relevant dynamic range. Excellent reproducibility was observed for all drugs (CV < 15%). All calibration curves displayed linearity with an R2 > 0.995.

Proteomics and Metabolomics Analysis of Patients Sera Revealed Activation of Anti-Oxidative Pathways in Vivax Malaria

Sandip Kumar Patel - Indian Institute of Technology Bombay (sandipsinghpatel@gmail.com) -- *Young Investigator Grantee*

- Oxidative damage of platelets plays a crucial role in the pathogenesis of thrombocytopenia found in Plasmodium vivax malaria. In this study serum samples from patients diagnosed with vivax malaria and healthy controls from different endemic regions of India were investigated using different gel-based (2DE and 2D-DIGE) and MS-based quantitative proteomics (iTRAQ and label-free LC-MS/MS) and metabolomics approaches. In our study, hemopexin, ceruloplasmin, and superoxide dismutase-1 were found to be up-regulated in malaria patients. In metabolomics analysis we have identified oxidative stress markers like nitrotyrosine, tyrosine etc. in malaria patients. Altered with severity differential expression of multiple serum proteins, antioxidative enzymes and oxidation protein products cumulatively represent the oxidative stress status of the malaria patients and reflect severity of infection.
**The Measurement of Food-intake and Nutrient Absorption in C. elegans by Quantitative Mass Spectrometry**

*Elizabeth Valentine - The Scripps Research Institute (erval@scripps.edu)*

- Directly measuring food intake in C. elegans remains challenging, despite it being a preferred genetic model system for studying food-related behaviors. Our “pulse-feeding” method measures food intake by feeding heavy isotope labeled E. coli “food” and then quantifying nutrient absorption by mass spectrometry. The pulse-feeding assay allows absorption of food into the proteome and changes in protein synthesis to be monitored. We show that serotonin induced feeding leads to an increase in protein synthesis in a serotonin receptor dependent manner. The pulse-feeding assay can be an important tool for comparisons of the effects of genetic and pharmaceutical perturbations on eating.

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**Mass Spectrometry and Stable Isotope Labeling for Quantitative Analysis of Ribosomal RNA Modifications**

*Anna Popova - The Scripps Research Institute (popova@scripps.edu)*

- Quantitative Mass Spectrometry (qMS) platform has been developed to perform global profiling of modifications in ribosomal RNA. Using qMS, we have carried out mechanistic studies aimed to characterize temporal and functional relationships between individual RNA modification steps and the ribosome assembly process in bacteria. Now, we are advancing the technology to quantitatively monitor RNA modifications in yeast and human ribosomes. This will be instrumental in defining the key roles ribosomal modifications play in fundamental biological processes of ribosome biogenesis and translation, and to understand what defects in RNA modification machinery are implicated in hereditary diseases and cancer.

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**Quantitative Analysis of Human Tear Fluid by MALDI-TOF MS**

*Ryan Walsh - University of Colorado, Denver (ryan.walsh@ucdenver.edu) -- *Young Investigator Grantee* *

- Identifying biochemical markers for disease is a difficult endeavor. It requires methods that can quantify multiple components simultaneously, are high throughput, cost effective and deliver high precision and accuracy. Ideally, the same methods should also be applicable in a routine clinical setting. We and others are exploring the potential of matrix-assisted laser desorption/ionization (MALDI) for these applications. We demonstrate that precise results (CVs 1-2%) are achievable under ideal conditions, but that in a practical setting, factors such as the selection of internal standard, sample handling and ion suppression mean that CV’s are more typically 10% or greater. Some of the most important factors in determining reproducible and accurate results are illustrated and discussed by way examples.

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**FFPE Protein Recovery and Optimization for Proteomics Analysis**

*Patrick Vanderboom - Mayo Clinic (vanderboom.patrick@mayo.edu)*

- Mayo Clinic has amassed a large archive of Formalin Fixed Paraffin Embedded (FFPE) tissue blocks from a number of different diseases. Protein profiling of such FFPE tissues offers a valuable opportunity to obtain new information regarding the molecular mechanisms of disease. However, the analysis of proteins from FFPE samples is both complex and challenging. With this in mind, we have designed a study to systematically investigate multiple different sample preparation methods to optimize a workflow for the proteomic analysis of FFPE tissues.
Brain Region Mapping Using Global Metabolomics

Julijana Ivanisevic - Center for Metabolomics, TSRI (julijana@scripps.edu)

- Historically, studies of brain metabolism have been based on targeted analyses of a limited number of metabolites. Here we present an untargeted mass spectrometry-based metabolomic strategy that has successfully uncovered differences in a broad array of metabolites across anatomical regions of the mouse brain. The NSG immunodeficient mouse model was chosen because of its ability to undergo humanization leading to numerous applications in oncology and infectious disease research. Metabolic phenotyping by hydrophilic interaction liquid chromatography and nanostructure imaging mass spectrometry revealed both water-soluble and lipid metabolite patterns across brain regions. This study helps define regional homeostasis for the normal mouse brain to give context to the reaction to pathological events.

The Analysis of Vitamin D and Metabolites in Plasma by LC-MS/MS

Paul Connolly - Restek Corporation (paul.connolly@restek.com)

- Vitamin D deficiency has been linked to an increased risk for many chronic diseases including diabetes, heart disease, and some cancers. Vitamin D exists in two forms, vitamin D2 and vitamin D3. Each undergoes metabolism to form 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3. For accurate determination of vitamin D levels in the blood, it is important to distinguish between these metabolites and separate them from matrix interferences. The Raptor™ ARC-18 column combines the speed of superficially porous particles with the resolution of highly selective USLC® technology to produce a simple, rugged method for the determination of vitamin D metabolites in plasma.

In Vitro Human Metabolism of Designer Cathinones: LC-MS/(MS) Metabolites Identification and Characterization for Doping Control Purposes

Amelia Palermo - Laboratorio Antidoping FMSI (amelia.palermo@uniroma1.it) -- *Young Investigator Grantee*

- Designer cathinones are synthetic molecules obtained through the modification of the chemical structure of cathinone, a potent stimulant with pharmacological properties closely resembling those of amphetamine. These stimulants, which are becoming increasingly available mostly on non official marketing channels (i.e. websites), were specifically developed to circumvent the current regulatory restriction but, at the same time, they may remain “invisible” at the analytical controls. In this study we have in vitro investigated the human phase I and phase II metabolism of designer cathinones with the aim to evaluate the most appropriate analytical markers for their administration. On the basis of our results, the hydroxylated and demethylated metabolites represent the best analytical markers to detect the intake of designer cathinones.

The Analysis of Common Drugs of Abuse in Human Urine by LC-MS/MS

Frances Carroll - Restek Corporation (frances.carroll@restek.com)

- The use of liquid chromatography coupled with mass spectrometry (LC-MS/MS) in forensic toxicology labs has increased significantly over the years. LC-MS provides sensitivity, speed, and the ability to simplify sample preparation. The Raptor™ Biphenyl column was developed to complement high-throughput LC-MS/MS analyses by combining the increased efficiency of superficially porous particles with the resolution of Ultra Selective Liquid Chromatography™ technology. A simple dilute and shoot method was developed for 10 common drugs of abuse and their metabolites using a Raptor™ Biphenyl 5µm column. The low back pressure of the 5µm particle column allows even conventional 400 bar LC systems to take advantage of this high speed separation with a total analysis time of 5 minutes.
Experience and Lessons Learned Developing a Comprehensive Toxicology Panel for Pain Management and Beyond

Ping Wang - Houston Methodist Hospital (pwang@houstonmethodist.org)

- We aim to set up a comprehensive toxicology testing program to meet the diverse clinical needs for urine drug testing. An LC-MS/MS method that quantitates 78 drugs and metabolites in urine with a simple dilute-and-shoot sample preparation was established. Method comparison using patient and proficiency testing samples demonstrated that this assay was sensitive and accurate. Urine drug screens are triaged to either immunoassays for emergent toxicity assessment or to mass spectrometry screening and quantitation for risk stratification and regulatory compliance. A comprehensive report is generated after testing detailing the quantity of each drug and metabolite, and provided with an interpretation.

Optimization of Automated Online SPE-LC-MS/MS Used in Pain Management Drug Monitoring

Mark Hayward - Assurance Scientific Laboratories (Mark.Hayward@ITSPsolutions.com)

- A systematic approach was taken to optimize the combined reverse phase (RP) solid phase extraction (SPE), RP liquid chromatography (LC), and mass spectrometry / mass spectrometry (MS/MS) conditions to achieve the following goals: maximize automation / minimize labor and cycle time, maximize applicability to the widest range of drugs encountered in pain management (PM), and scale the combination of sample size and SPE / LC separations to pre-concentrate the sample sufficiently to make all of the drugs (even the challenging ones) relatively easy to measure on a routine basis. Total automation of SPE-LC-MS/MS (urine in vial/tube/plate to results) with a 5 minute cycle time was achieved, and all drugs were readily measured across the full relevant concentration range. The process of achieving balance in all the parameters and scaling / optimizing the separations will be described.

Quantitative Analysis of 25-Hydroxy-Vitamin D in Serum Using LC/QQQ and LC/Q-TOF

Jeff Keever - Agilent Technologies, Inc (jeff_keever@agilent.com)

- Liquid chromatography triple quadrupole (QQQ) and quadrupole time-of-flight (Q-TOF) mass spectrometry (LC/MS/MS) are suited for rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of 25-hydroxy-vitamin D2 and D3 by QQQ and by Q-TOF. Using simple sample preparation techniques and chromatographic configuration achieves the required sensitivity and is capable of quantitating the compounds over their relevant dynamic range. Excellent reproducibility was observed for all compounds (CV < 15%). All calibration curves displayed linearity with an R2 > 0.995.

Cost Advantage and Improved Accuracy of Medication Compliance by a Qualitative Time-Of-Flight Mass Spectrometry and Immunoassay-based Screen in Pain Management

Kelly Doyle - ARUP, University of Utah, Department of Pathology (kelly.doyle@path.utah.edu) -- *Young Investigator Grantee*

- Qualitative screens for pain management testing are an effort to provide a less-expensive, faster, and comprehensive evaluation of medication compliance and drug abuse. This study of a definitive TOF-MS and immunoassay-based workflow challenges the wide standing belief that quantitation is essential for urine drug compliance testing. Our data demonstrates that the hybrid screen is superior in confirming compliance per prescription (226/302 vs. 205/302), identifying substance abuse (97 vs. 71), and has a substantial cost advantage (41% cost savings), when compared to a conventional immunoassay-based screen reflexed to quantitation by LC-MS/MS.
**Ethanol Metabolites by Paper Spray Ionization: Method Development in Negative Ion Mode**

*Maria C Prieto Conaway - Thermo Fisher Scientific (mari.prieto@thermofisher.com)*

- Paper spray is a direct ionization technique that simplifies the mass spectrometric analysis of compounds from biological fluids without time consuming sample preparation and chromatography. Paper spray technology is therefore attractive for compound screening and quantitation in forensic toxicology. The sample collection and storage in a simple paper cassette is attractive for the shipment of samples to the forensic toxicology laboratory. In this work, we develop protocols in negative ion mode for the screening of ethanol metabolites (ethyl sulfate and ethyl glucuronide) in urine by coupling paper spray technology to a new generation triple stage quadrupole (TSQ) mass spectrometer (MS).

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**Mass Spectrometric Quantification of Enriched Microglia Using a Metabolically-labeled Immortalized Cell Mix**

*Harris Bell-Temin - University of South Florida (harris.belltemin@gmail.com) -- *Young Investigator Grantee*

- Isobaric labeling requires a high speed, high resolution MS/MS scan for reporter ion quantification; this is not an option on all instruments. In order to quantify ethanol induced changes to microglial activation, we created a simulated proteome of enriched microglia using heavy metabolically-labeled immortalized cells to approximate protein expression in enriched microglia. Microglia from chronically ethanol exposed mice and control mice were mixed with equal amounts of the immortalized cell mix and analyzed on an Orbitrap XL. Over 2,500 proteins and 9,000 peptides were identified from the microglia, and of these 81% of proteins and 62% of peptides were successfully quantified in the control and ethanol treated groups. The immortalized cells proved to be a successful tool to quantify in vivo protein levels with earlier generation high resolution instrumentation.

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**Development and Implementation of Amino Acid Quantitation by LC-MS/MS at BC Children’s Hospital**

*Andy De Souza - BC Children’s Hospital (andy.desouza@cw.bc.ca)*

- Quantitative analysis of amino acids in biological fluids is utilized in the diagnosis of amino acid disorders, a class of inborn errors of metabolism. The most widely used method for amino acid analysis is ion-exchange chromatography coupled with post-column ninhydrin derivitization. This technique was utilized by our laboratory, but the combination of low throughput and increasing sample numbers led to a backlog and unacceptable turn-around-times. Thus began our endeavor to develop a high throughput liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for amino acid quantitation. The method was validated and, as of Spring 2014, implemented LC-MS/MS for plasma amino acid analysis. In this presentation, we will compare our current platform with our previous methodology, with respect to method performance, testing capacity, turn-around-times, and laboratory workflows.

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**Targeted Serum Metabolite Profiling for Colorectal Cancer Progression Monitoring**

*Jiangjiang Zhu - University of Washington (jzhu6@uw.edu) -- *Young Investigator Grantee*

- Colorectal cancer (CRC) is one of the most prevalent cancers worldwide, and a major cause of human morbidity and mortality. In addition to early detection, close monitoring of disease progression in CRC can be critical for patient prognosis and treatment decisions. In this study we applied a targeted LC-MS/MS metabolic profiling approach using serial serum samples to monitor CRC patient disease progression. A PLS-DA model using a panel of 5 metabolites was established, and excellent model performance (sensitivity=0.83, specificity = 0.94, AUROC=0.91) was obtained, superior to traditional biomarker CEA. Our results suggest the potential usefulness of metabolic profiling for CRC disease progression monitoring.
**Development of High Sensitivity Micro-LC-MS/MS Method for Estradiol in Human Serum without Derivatization**

**Xin Yi** - *The University of Chicago* (xyi5@bsd.uchicago.edu) -- *Young Investigator Grantee*

- Reliable measurement of low concentration of estradiol (E2) in human blood samples has always been a challenge in clinical laboratories. However, there are considerable demands to accurately measure serum E2 at very low concentrations (< 5 pg/mL) in postmenopausal women, men, and pediatric patients. Mass spectrometry-based methods have been increasingly used as the method of choice in clinical laboratories for measuring E2. However, most of them require large sample volume and involve complex sample preparation. Our study aims to develop a high sensitivity method using Micro-LC-MS/MS to reliably measure E2 concentrations below 3 pg/mL using low sample volume and without derivatization.

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**Protein Disulfide Bond Mapping Using Online LC–Electrochemistry–MS Applied to the Characterization of notch3 Protein Fragments**

**Linda Switzar** - *Leiden University Medical Center* (l.switzar@lumc.nl) -- *Young Investigator Grantee*

- Disulfide bonds are crucial for the structure and biological function of proteins. For example in CADASIL (Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy), genetic mutations in the NOTCH3 gene result in disruption of the triply-paired cysteine structure of the corresponding notch3 protein which leads to aggregation. Protein analysis with a bottom-up approach inherently lacks information on the presence and connectivity of disulfide bonds. We have developed an LC-Electrochemistry-MS system that enables online reduction and characterization of disulfide bonds. The approach was optimized using standard proteins and has been applied to notch3 protein fragments.

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**Using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry to Study the Distribution of Human Neutrophil Peptides in Tears**

**Hung Su** - *National Sun Yat-sen University* (impossible122@yahoo.com.tw) -- *Young Investigator Grantee*

- Natural antibiotics are produced as part of the innate immune response in plants and animals. Defensins are important biological antibiotics that are regulators of inflammation and play important roles in the immune response. Additionally, defensins contribute to the antimicrobial action of granulocytes and mucosal host defense in the skin against foreign bodies. Defensins are divided into many types according to their structures. One type known as human neutrophil peptides (HNPs) has a broad spectrum of antimicrobial activity and a high abundance in mammalian epithelia and granulocytes, especially in inflamed or infected tissues. This study explores the distribution and physiological mechanisms of HNPs in human tears.

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**Identification of Staphylococcus Aureus by Shotgun Spectral Matching**

**Dana Ohana** - *Leiden University Medical Center* (d.ohana@lumc.nl) -- *Young Investigator Grantee*

- Staphylococcus aureus is a Gram-positive bacterium that can be pathogenic when expressing specific toxins. Production of altered penicillin binding proteins causes resistance to most active antibiotics. Rapid characterization of virulence and resistance factors may aid in providing clues for treatment of individual patients. A standard bottom-up, shotgun proteomics method was applied on 12 different isolates of S. aureus to create a reference database of spectral libraries with one library from each isolate. Additional isolates, treated as unknowns, were then searched against all reference libraries for sample identification based on relative numbers of spectra finding a match the different spectral libraries.

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**The Role of ICP-MS in Clinical Applications**

**Sherry Gregory** - *Thermo Fisher Scientific* (sherry.gregory@thermofisher.com)

- Trace elemental analysis of urine, blood or serum in clinical research by ICP-MS is demonstrated to be a powerful tool for answering questions on the implication of exposure to metals via food, drugs or the environment at large.
**Development and Validation of a Robust Method to Measure Chromium and Cobalt in Whole Blood of Patients with Metal Implants Using Dual Reaction Mode ICP-MS**

_Brooke Katzman_ - Mayo Clinic (katzman.brooke@mayo.edu) -- *Young Investigator Grantee*

- While all prosthetic implants bear risks, metal-on-metal (MoM) implants have unique hazards associated with their use. In MoM implants, friction between component parts results in wear causing the release of metal particles from the device into the space surrounding the implant. Metal ions (i.e. chromium and cobalt) from the device or from the metal particles enter the bloodstream. In accordance with FDA recommendations, we developed a method using a NexION 350 inductively coupled plasma-mass spectrometer (ICP-MS) to quantitate chromium and cobalt in EDTA anti-coagulated whole blood from patients with MoM implants. Chromium and cobalt ions were separated from polyatomic interferences using the dynamic reaction cell (DRC) and the kinetic energy discrimination (KED) modes, respectively. The preliminary data highlight the exceptional specificity and sensitivity of this method.

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**Using LC-ESI-Q-TOF of Immunoglobulin Light Chains to Resolve Ambiguous Serum Protein Electrophoresis Cases**

_John Mills_ - Mayo Clinic (mills.john2@mayo.edu) -- *Young Investigator Grantee*

- Monoclonal gammopathies are diagnosed by detecting a monoclonal immunoglobulin (M-protein) at levels exceeding the polyclonal background. In clinical practice, M-proteins are routinely detected by protein gel electrophoresis (PEL) and immunofixation electrophoresis (IFE). Occasionally, disease modifying factors and artifacts result in PEL and IFEs which appear to have M-proteins but clinical history and other laboratory testing suggest these could be artifacts. Recently, we have described a LC-ESI-Q-TOF method which can profile the mass distribution of serum immunoglobulin light chains with greater resolution of immunoglobulins which greatly aids in the resolution of gel ambiguities.

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**Utility of the Microflex LT Platform in the Development of Serum Proteomic Companion Diagnostic (CDx) Tests in NSCLC**

_Nicholas Dupuis_ - Biodesix, Inc (nicholas.dupuis@biodesix.com)

- Clinical oncology assays using mass spectrometry have exclusively been run on platforms designed for use in research laboratories. Here, we employ the microflexTM LT (LT), a MALDI-TOF MS which is a component of Bruker’s FDA cleared MALDI Biotyper CA system, for development of a CDx test. In this work, we evaluate the LT through comparative studies with similar RUO platforms, utilizing samples from NSCLC patients. In summary, the LT appears to be suitable for measurement of NSCLC serum proteomic profiles and inclusion of the LT in CDx test development will expand utility of MALDI-based testing in the clinic.

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**In Oral Fluid 7-Aminoclonazepam Is Superior to Clonazepam for Detection of Clonazepam Use**

_James Flood_ - Mass General Hospital (jflood@partners.org)

- The benzodiazepine clonazepam (CLON) requires careful monitoring to minimize abuse and diversion. We screened for both the parent drug CLON and its primary metabolite 7-aminoclonazepam (7AC) using the Orasure Intercept oral fluid (OF) collection device, followed by LC-Tandem MS in more than 800 OF samples from outpatient addiction medicine clinics. 102 samples were found positive for 7AC and/or CLON. 93 (91.2 %) were confirmed by ion ratio to contain 7AC (median, range: 4.15, 0.5-316.7 ng/ml), while only 49 of the 102 (48.0 %) samples were confirmed by ion ratio to contain CLON (median, range: 3.44, 0.1-217.2 ng/ml). No samples were confirmed positive for CLON and negative for 7AC. In the 49 confirmed pairs the median 7AC/CLON concentration ratio was 2.4. 7AC should be the OF analyte measured because of its higher concentration and superior ion ratio confirmation characteristics.
A LC-MS/MS Method for the Measurement of Testosterone Undecanoate and Dihydrotestosterone

Andrew Leung - Los Angeles Biomedical Research Institute (aleung@labiomed.org)

- We developed and validated LC-MS/MS method for measurement of TU and DHTU in serum for a Phase 2 pharmacokinetics study of orally administered TU in men. d21-TU and d21-DHTU were used as internal standards and serum sample processed by liquid/liquid extraction. The LC-MS/MS system used a Shimadzu high-performance LC 20 series system with an Applied Biosystems API 5500 with ESI source, operated in positive ion detection mode. Serum TU and DHTU levels were lower if the TU was administered without food. When administered with a meal, serum TU levels peaked at 4.4 hour which was followed by serum DHTU levels.

LC-MS/MS Method for Quantitative Analysis of Gabapentin and Pregabalin in Serum or Plasma

Stephen Merrigan - ARUP (stephen.d.merrigan@aruplab.com)

- Gabapentin and pregabalin are analogs of the inhibitory neurotransmitter gamma-amino butyric acid. Both analogs can be prescribed as full or partial anticonvulsants for some types of seizures as well as for postherpetic neuralgia and Restless Legs Syndrome (Gabapentin) or for pain from diabetes, shingles, fibromyalgia, or spinal cord injury (pregabalin). Gabapentin and pregabalin are commonly tested to monitor therapeutic efficacy and patient compliance. We developed a 3.5 minute LC-MS/MS method for quantification of gabapentin and pregabalin in serum or plasma. Chromatographic separation and validation data including internal and external method comparison are presented.

A Pipeline for Untargeted UPLC-MS Profiling to Expand Tissue Metabolome Coverage – Optimization of Chromatographic Separation and Tissue Extraction

Panagiotis Vorkas - Imperial College London (p.vorkas09@imperial.ac.uk) -- *Young Investigator Grantee*

- In order to address the challenges of tissue sample analysis and metabolome coverage, a novel pipeline consisting of two consecutive extraction steps (aqueous followed by organic) was developed. Analysis was achieved using two UPLC-MS methodologies: HILIC chromatography for analyzing the aqueous extracts and RP lipid profiling for the organic. Both extraction and separation steps were extensively optimized. This pipeline was successfully applied on tissues including vascular, liver and adipose. The two chromatographic modes proved highly complementary, with >5000 features robustly detected and >250 metabolites structurally assigned and mapped to metabolic pathways covering a wide range of biological and disease processes.

Urinary Glucose Tetrasaccharide Assay Using Rapid Ultraperformance LC-MS/MS for Pompe Disease

Youngwon Nam - Seoul National University Hospital (nyoungwon@gmail.com)

- We evaluated the urinary glucose tetrassaccharide (Glc4) assay using UPLC-MS/MS (SCIEX TQ 6500). Calibration curve were linear over a range from 5 to 500 ¥ìmol/L. Within- and between-day precision CVs were 6.52-14.6% and 11.5-13.2%, respectively. The mean concentration of urinary Glc4 of 27 normal controls and 3 pseudodeficiency patients were 1.5 and 12.1 mmol/mol creatinine, respectively. Urinary Glc4 concentration in a patient with Pompe disease was 171.3 which decreased to 130.9 after enzyme replacement therapy. The urinary Glc4 assay measured by UPLC MS/MS can be a reliable biomarker for diagnosis and therapeutic monitoring of Pompe disease.
LCMS Method for the Simultaneous Determination of Metformin and Miglitol in Plasma: Application to Pharmacokinetic Studies

Mahesh Attimarad - King Faisal University (mattimarad@gmail.com)

* The objective of the current study was to develop a rapid and sensitive LC-MS method for the simultaneous estimation of metformin and miglitol in plasma using voglibose as internal standard. Chromatographic separation of active ingredients was achieved using a Zorbax eclipse C18 (150mm×4.6mmX5μm). The mobile phase constitute 95% ammonium acetate (0.02 mM, pH 6.8) and 5% methanol was pumped at an isocratic flow rate of 0.5 mL/min. The data acquisition was carried out in positive ion mode by Single Ion Monitoring at 130.1 m/z for metformin, 208.1 m/z for miglitol and 268.4 m/z for IS. Simple protein precipitation using higher concentration of acetonitrile was utilized to remove the endogenous materials. The validated method was applied for pharmacokinetic studies. Surprisingly, the pharmacokinetic profiles and parameters observed for both the drugs were comparable.

Simultaneous Determination of Methylmalonic Acid and Homocysteine in Plasma by LC-MS/MS

Petra Prochazkova - SPADIA Lab, a.s. (petra.prochazkova@spadia.cz)

* Methylmalonic acid (MMA) and homocysteine (HCY) belong to sensitive indicators of cobalamine deficiency. Cobalamine is an essential nutrient which plays an important role in the hematopoiesis, in the development of central nervous system (CNS), in the synthesis of DNA and regulation of fatty and amino acids metabolism. The aim of this work was to develop the method for simultaneous determination of MMA and HCY as biomarkers of functional cobalamine deficiency by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The full validation of this method was carried out.

An Automated Sensitive Measurement of Estrone and 17β-Estradiol from Human Plasma on LC-MS Using Solid-Phase Extraction and MassBoost Derivatization

Emmanuel Chanco - SPEware Corporation (luigi.chanco@speware.com)

* Measurements of estrogen levels in plasma are difficult due to their low abundance and lack of ionizable groups. A sensitive method was developed to quantify estrone (E1) and 17β-estradiol (E2) in 100 uL plasma. The process includes solid phase extraction, derivatization via a new reagent, and quantification via LC-tandem mass spectrometry on an API 4000 QTrap MS coupled with an Agilent 1200 HPLC. A linear response to both compounds was observed from 5-500 pg/mL, with an LOQ of 5 pg/mL for estradiol and 10 pg/mL for estrone. Throughput for this method was further improved by coupling it to automated sample preparation.

Sensitive Measurement of HU-210 from Oral Fluids via LC-MS/MS: A Fully Automated SPE Sample Preparation and MS Sensitizing Derivatization Process

Qi Huang - Quantalytical Labs, Inc (qih@unionbiopharma.com)

* HU-210 is reported as 100-800 times more potient than THC. It is a challenging task to detect and properly measure HU210 from a human specimen at low levels of the drug exposure. We would like to report that by using a new MS sensitizing reagent (MB338), we developed an integrated method to quantify HU210 in oral fluid. The process included SPE, derivatization, and LCMSMS quantification via an SCIEX API5000. The range of the quantification linearity is between low pg/mL to ng/mL. This process is also compatible to quantify THC and THCA and may be fully automated.
**PhoTorrent Atmospheric Pressure Photo Ionization (APPI) Source to Achieve High Efficiency Photoionization on Testosterone and 25-OH Vitamin D3**

*Ellie Majdi - IONICS Mass Spectrometry (elliem@ionics.ca)*

- An IONICS PhoTorrent modular APPI source was used to explore APPI detection capabilities of 2 clinical compounds, Testosterone and 25-OH Vitamin D3. Sensitivity as low as 100 ag/µL for Testosterone was achieved, while maintaining excellent linearity to 10 ng/mL. The 25-OH Vitamin D3 gave sensitivity as low as 100 fg/µL with excellent linearity to 100 ng/mL. The PhoTorrentTM APPI source is well suited to flow rates of 100-500 μL/min. The modularity of the PhoTorrent APPI Source facilitates switching from APPI to ESI and APCI, increasing the number and range of compounds that can be analyzed effectively with minimal hardware changes.

**Drug Excretion into Breast Milk: Are All Drugs Contraindicated for Breastfeeding?**

*Joshua (Sha) Ye - IONICS Mass Spectrometry (joshuay@ionics.ca)*

- Although not all drugs may be considered contraindicated while breastfeeding, there remains little data on this topic. The objective of this study is to investigate the risk of methotrexate exposure in nursing infants. We developed a simplified extraction method and an LC-MS/MS method to measure Methotrexate (MTX) and 7-hydroxymethotrexate in breast milk. Patients receiving MTX were recruited. We found that MTX is excreted into breast milk, with the highest concentrations at 1-12 hours post-dose; detectable levels were observed at 48 - 96 hrs. This data provides the foundation to establish a TDM system for measuring drug concentrations in breast milk.

**LC-MS/MS Study of 25-OH Vitamin D2 and D3 with Perkin Elmer Vitamin D Kit Using Both Derivatized and Non-derivatized Methods**

*Hui Qiao - IONICS Mass Spectrometry (huiq@ionics.ca)*

- Quantification of 25-OH Vitamin D2 and D3 is widely used as a means of assessing vitamin D deficiency status because of their clinical significance in a variety of disorders. LC-MS/MS technology has demonstrated superior sensitivity, selectivity, and robustness for simultaneously detecting these Vitamin D metabolites in complex biological matrices. This work presents a rapid, reliable, and accurate LC-MS/MS research method on an IONICS 3Q 120 triple quadrupole mass spectrometer for studying 25-OH Vitamin D2 and D3 with Perkin Elmer Vitamin D kit using both derivatized and non-derivatized methods. Good linearity (coefficients R²>0.993), accuracies (97-102%), and CVs (< 10%) were obtained.

**Exploring the Potential of the Last Generation UHR-Q-TOF for Rapid Generation of Accurate Information on Proteoforms Distribution and Relative Abundancy**

*Nicolai Bache - Bruker Daltonics (nicolai.bache@bruker.com)*

- All along their life cycle, proteins undergo various transformations that can alter their functions while keeping a good part of their primary sequence intact. This multiplication of PTM patterns, alternative splicing forms or products of proteolitic processing cannot be simply resolved with a bottom-up approach, as very few peptides are specific from the given proteoform. In this work we illustrate last-generation UHRQ-TOF’s capability to generate accurate proteoform distribution information within minutes from an highly complex sample, therefore enabling to complement the information obtained with the more traditional bottom-up approaches.
Development of a Direct Assay of Iduronate-2-sulfatase for Mucopolysaccharidosis Type II (Hunter Syndrome) Using UPLC-MS/MS

Kyunghoon Lee - Seoul National University College of Medicine (khlee59023@gmail.com)

Mucopolysaccharidosis type II (Hunter syndrome) is caused by a deficiency in iduronate-2-sulfatase. We developed and evaluated the performance of UPLC-MS/MS with commercially available substrate (4-methylumbelliferyl α-L-idopyranosiduronic acid 2-sulfate) for the detection of Hunter syndrome. All reagents were fully separated within 3 min. No ion suppression was observed. The intra- and inter-assay precisions were 7.9%–10.5% and 4.8–10.2%, respectively. The enzyme activities measured in the DBSs were consistently lower in patients with Hunter syndrome than in normal newborns (P=0.001). Additionally, we performed multiplex tests for five lysosomal storage disease including Hunter, Hurler, Fabry, Pompe, and Gaucher disease.

iST Sample Preparation for High Throughput Clinical Proteomics

Nils Kulak - Max Planck Institute of Biochemistry (kulak@biochem.mpg.de)

Rapid and robust workflows are crucial for day-to-day clinical applications. Especially sample preparation procedures are time consuming and limit the overall technical reproducibility of MS-based proteomics. Here we present the in-StageTip (iST) method for streamlined sample processing of complete proteomes. This simplified 3-step procedure is performed in a single, enclosed volume and allows peptide pre-fractionation in a high-throughput fashion. Applying the procedure to the cancer cell line HeLa allowed us to estimate copy-numbers of 9,667 proteins with excellent reproducibility (R2 = 0.97) in quadruplicates measurements. The in-StageTip method allows high-throughput applications with near complete proteomic coverage of highly complex samples.

LC-MS/MS Method After Derivation for the Determination of L- and D- Isomers of 2-hydroxyglutarate in Biological Fluids: Application as Biomarker of IDH Mutation

Vianney Poinsignon - Gustave Roussy (Vianney.POINSIGNON@gustaveroussy.fr)

Activating mutations of isocitrate dehydrogenase leading to the production of 2-hydroxyglutarate have been described in hematologic malignancies and solid tumors. Therefore, we developed a liquid chromatography tandem mass spectrometry method allowing a rapid, accurate and precise simultaneous quantification of both L and D enantiomers of 2-HGA in human serum for clinical applications as diagnostic and predictive biomarker. A derivatization step with (+)-o,o'-diacetyl-L-tartaric anhydride allowed to separate the two enantiomers without chiral stationary phase, on a C18 column (Agilent ZorbaxSB®, 4.6 x 150 mm, 5 μM) combined to a XevoTQ mass spectrometer with an electrospray ionization (ESI) source. This method was validated according ICH Q2(R1)is linear over the range 0.338-135.04 μmol/L, accurate (bias <5.6% RSD) and precise (repeatability <6.2% and intermediate precision <5.6%).

Column and Solvent Considerations that Contribute to the Success of LC-MS Analyses

Maricar Dube - EMD Millipore (maricar.dube@emdmillipore.com)

Particle packed reversed phase columns are commonly used in LC-MS. Two significant developments in HPLC columns that offer advantages in clinical LC-MS applications will be presented: monolithic columns and hydrophilic interaction chromatography (HILIC). Monolithic columns offer faster analysis without requiring a dedicated UHPLC instrument, and have less matrix sensitivity with biological samples. HILIC columns efficiently separate polar hydrophilic compounds, which are not retained in reversed phase columns. Examples are nucleotides, peptides, metabolites and sugars. The role of LC-MS solvents in the success of LC-MS analyses will also be discussed. It will be shown that contamination and impurities compromise data quality.
Sensitive Analysis of Serum 5alpha-Dihydrotestosterone by 2D-LC-MS/MS

Bingfang Yue - NMS Labs (bingfang.yue@nmslabs.com)

- In humans, circulating androgen 5alpha-dihydrotestosterone (DHT) exerts major biological effects on skin and prostate. DHT is a more potent androgen than testosterone (T) and is the primary androgen in the prostate. The 2D-LC-MS/MS setup allows extensive clean-up and transfers only a small part of elution profile of the 1st dimension containing targeted analyte to the 2nd dimension for high efficiency separation. A simple and sensitive method to accurately quantify DHT in serum by 2D-LC-MS/MS was developed and validated, with a LLOQ of 5 pg/mL and suitable for routine clinical laboratory use.

Ceramide Trihexosides and Sulfatides Quantitation in Urine by LC-MS/MS

Jean Lacey - Mayo Clinic (lacey.jean@mayo.edu)

- We have developed an analytical method to quantify ceramide trihexosides and sulfatides in lipid extracts prepared from human urine. These analytes are useful for the diagnosis of Fabry disease, Metachromatic Leukodystrophy, multiple sulfatase deficiency, sphingolipid activator deficiency, and some cases of mucolipidosis II. This method has greater specificity and sensitivity than our current thin layer chromatography method. Other advantages are markedly decreased sample volume and easy sample preparation. Samples are extracted using chloroform-methanol with appropriate internal standards. Mass spectrometry analysis is performed using liquid chromatography tandem mass spectrometry in positive and negative multiple reaction monitoring mode.
Posters by Day: MONDAY

Monday 5:00 PM
Poster #1 in Exhibit Hall
**Rapid, Simultaneous Analysis of Urinary Catecholamines and Metanephrines by Mixed-mode SPE and HILIC LC-MS/MS**

*Jonathan Danaceau - Waters Corporation (jonathan_danaceau@waters.com)*

- A single extraction and analysis method has been developed for urinary catecholamines and metanephrines. Analytes were extracted using weak cation exchange mixed-mode SPE and analyzed using HILIC LC/MS/MS. Recoveries were good and matrix effects were significantly reduced compared to reverse-phase analysis. Linearity and QC results were excellent for all compounds down to 0.5 ng/mL, with excellent accuracy and precision. This method enables rapid, simultaneous and accurate LC/MS/MS analysis of these challenging compounds without the challenges associated with traditional reversed-phase separation or ion-pairing techniques.

Monday 2:00 PM
Poster #2 in Exhibit Hall
**Quantitative Analysis of a Glyburide Analogue, a Potential NLRP3 Inhibitor, Using Micro-sampling, Hybrid Solid Phase Extraction and LC-MS/MS**

*Ankit Zalavadia - Virginia Commonwealth University (zalavadiaaa2@vcu.edu) -- *Young Investigator Grantee*

- A LC-MS/MS method was developed to quantify a novel inhibitor of the NLRP3 inflammasome that limits myocardial injury after ischemic and non-ischemic injury in mice. Initially, glyburide analogue was extracted from 20-µL of mouse plasma using HybridSPE 96 well plate. Separation and quantification was achieved by RP-LC followed by positive electrospray ionization and selected reaction monitoring (SRM) of the glyburide analogue (369→169) and structural analogue internal standard, glipizide (445→166). The method validation was linear, 1-1000 ng/mL. Precision and Accuracy of the method was ±15%. A cross-validation is underway to employ dried blood spots to enhance the toxicokinetic studies in mice.

Monday 5:00 PM
Poster #3 in Exhibit Hall
**New Acquisition and Processing Tools for Targeted and Unknown Screening Approaches in Clinical Research and Forensic Toxicology**

*Benedicte Duretz - ThermoFisher (benedicte.duretz@thermo.com)*

- Orbitrap instruments have gained in popularity in Forensic and Toxicology Research, offering the possibility to identify new substances in complex matrices. A new scan mode termed vDIA (variable data-independent acquisition) will be described. It provides selectivity and sensitivity comparable to data-dependent MS2 measurements. Processing tools will also be discussed. TraceFinder identifies and confirms substances using the exact mass of the analyte, the isotopic distribution, the fragment ions and the retention times. Identification of unknown compounds can be performed using mzCloud, a unique HRAM MSn spectral database. Structures can be confirmed using Mass Frontier that is capable to automatically generate possible fragments at an expert level.

Monday 2:00 PM
Poster #4 in Exhibit Hall
**Development of a Whole Blood Microsampling Bioanalytical Method for the Analysis of Opiates with a Goal of Point-of-Care Therapeutic Drug Monitoring**

*Daniel Kassel - SciAnalytical Strategies, Inc. (dkassel@scianalytical.com)*

- Knowledge of steady state concentration in chronic opiate therapy is crucial to maintaining drug efficacy, minimizing breakthrough and ensuring proper compliance. To support steady-state measurements, conventional blood sampling by venipuncture is needed and the patient is required to remain at the facility until sampling is complete. DBS sampling can potentially simplify this procedure, allowing at home sampling. We’ve evaluated a new microsampling device that eliminates some of the inherent issues with DBS. The device allows precise collection of 10µL volumes of blood. Commonly prescribed and/or abused opiates were quantified from whole blood sampled onto the microsampling device. Accuracy and precision were evaluated and the data support the potential in the clinical setting.
Monday 5:00 PM
Poster #5 in Exhibit Hall
MALDI-TOF-MS and MS-ASTRA Assay Development for the Generation of Biotyper Reference Spectra and Antibiotic Sensitivity Determination for Category B Bacteria
Tara Kenny - USAMRIID (tara.a.kenny.ctr@mail.mil)
• To aid in the reliability of bacterial identification using the MALDI Biotyper platform, we are generating spectral database entries for over 200 isolates of the Gram-negative facultative intracellular pathogens Burkholderia pseudomallei and Burkholderia mallei. These bacterial strains are inherently resistant to many antibiotics and cause opportunistic infections in immunocompromised individuals. Therefore, in conjunction with our database effort we are testing the applicability of Mass Spectrometric-Antibiotic Susceptibility Rapid Assay (MS-ASTRA) for antibiotic sensitivity testing in Burkholderia isolates. The overall goal is to develop assay conditions which simultaneously identify a bacterial species and screen for sensitivity to antimicrobial drugs.

Monday 2:00 PM
Poster #6 in Exhibit Hall
Direct Mass Spectrometry Analysis of Wet Biofluid Samples Using Slug-Flow Microextraction
Yue Ren - Purdue University (ren64@purdue.edu) -- *Young Investigator Grantee*
• We have previously developed paper spray and extraction spray to analyze dried biofluid for point-of-care analysis. Recently, we introduced a slug flow microextraction method in conjunction with nanoelectrospray for a single-step analysis of wet biofluid samples of low volumes (~5µL). Direct quantitative analysis of therapeutic drugs and illicit drugs in blood and urine sample has been demonstrated with the limits of detection better than 1 ng/mL and RSD better than 15% achieved. Real-time chemical derivatization was incorporated for the detection of anabolic steroids in urine at sub-ppb levels. Furthermore, online monitoring of enzyme function in wet blood has also been enabled using this method.

Monday 5:00 PM
Poster #7 in Exhibit Hall
Determination of Testosterone in Serum by Automated Sample Preparation and Ultra-fast LDTD-MS/MS in a Cross Validation Study with Real Patient Samples
Alex Birsan - Phytronix Technologies, Inc. (a.birsan@phytronix.com)
• A fast and quantitative method for the Testosterone analysis in serum samples is applied to the LDTD-MS/MS with differential ion mobility system. Testosterone is extracted using the new AC extraction plate in an automated 3 step process on a TECAN robot system. Mass spectrometer with ion mobility system (SelexION™) is used for isobaric drug resolution. Validation criteria such as carry over, wet stability and quality controls were successfully evaluated. All analyses were cross-validated and compared with a LC-MS/MS method. The cross-validation results show low percentage differences between the two methods. All samples were analyzed in 9 seconds per samples.

Monday 2:00 PM
Poster #8 in Exhibit Hall
Characterization of Stable Isotope Labeled Insulin-Like Growth Factor-1 for Use as an Internal Standard in Quantitative High-Resolution MS Workflows
Kevin Ray - Sigma-Aldrich Corporation (kevin.ray@sial.com)
• High-resolution mass spectrometry (HRMS) methods for quantitative analysis of intact proteins in clinical samples are becoming more widely adopted. The accurate quantitation of a plasma protein in clinical applications is enabled by early introduction of an internal standard that behaves identically to the native target protein throughout the analytical workflow. Surrogate proteins are typically used as internal standards in HRMS assays, but stable-isotope labeled (SIL) proteins provide a more ideal alternative. We have characterized SIL-N15-IGF1 expressed in E. coli and will demonstrate the use of SIL-IGF1 as an internal standard in a quantitative LC-HRMS method for intact IGF1 in human serum.
**Monday 5:00 PM**  
**Poster #9 in Exhibit Hall**  
**Automated Targeted Screening of Benzodiazepines in Urine Using LDTD-MS/MS at 400 Samples Per Hour Rate**  
*Pierre Picard* - *Phytronix Technologies inc* (p.picard@phytronix.com)  
- Toxicology laboratories use screening methods to obtain fast semi-quantitative screen value for drugs. Lack of specificity of those method demands confirmation of many false positive samples raising cost and time. Uses of LDTD-MS/MS enhance specificity at same throughput. Cross validation with LC-MS/MS validate this method. LC run were adapted to crude sample preparation reducing ionic suppression. Purified beta-glucuronidase enzymes are used for 15 minutes incubation instead of 1 hour. Conventional glucuronidase enzyme incubation is also performed. Complete workflow use Tecan robotic system with 8 channels liquid handler. Two 96 wells plates are process in parallel to feed LDTD-MS/MS system.

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**Monday 2:00 PM**  
**Poster #10 in Exhibit Hall**  
**A Fast Analysis of Low Level Estrogens in Serum by Bruker EVOQ Elite LCMS**  
*Zicheng Yang* - *Bruker* (zicheng.yang@bruker.com)  
- A rapid and sensitive method for the quantification of Estrogens in human serum was developed using Bruker Advance UHPLC coupled to the EVOQ Elite triple quadrupole mass spectrometer system. Excellent sensitivity, linearity and dynamic detection range were obtained. The limited of quantitation (LOQ) was 1pg/mL, 2.5 pg/mL and 5.0 pg/mL for Estrone (E1), 17β-Estradiol (E2) and Estriol (E3), respectively. The low pg/mL level estrogens detection with over four orders of dynamic detection range will cover the clinical research needs. The method run cycle time was 4 minutes.

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**Monday 5:00 PM**  
**Poster #11 in Exhibit Hall**  
**Evaluation of Methylisothiazolinone (MI) Extraction from Sunscreen Using Supported Liquid Extraction Prior to GC/MS Analysis**  
*Lee Williams* - *Biotage GB Limited* (lee.williams@biotage.com)  
- Methylisothiazolinone (MI) has received widespread attention over previous months due to a number of allergic reactions reported from the use of various personal care products such as sunscreens and skin lotions. This poster demonstrates the development of a simple sample preparation protocol using supported liquid extraction columns prior to GC/MS analysis. The final method demonstrates good extraction efficiency, extract cleanliness and acceptable linearity across the required analytical range. Calibration curves were constructed spiking MI into sunscreen from 50-750 ng/mL. Although no internal standard was used, good coefficients of determination (r2) greater than 0.99 were demonstrated.

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**Monday 2:00 PM**  
**Poster #12 in Exhibit Hall**  
**Measurement of Lymphocyte Argininosuccinate Synthetase Activity by Tandem Mass Spectrometry**  
*Shu-Chu Shiesh* - *National Cheng Kung University* (hsieh@mail.ncku.edu.tw) -- *Young Investigator Grantee*  
- We established a liquid chromatography-tandem mass spectrometry method for the measurement of argininosuccinate synthetase (ASS) activity in phytohemagglutinin-activated peripheral lymphocytes and examined the effect of fasting on ASS activity. The assay was based on the measurement of argininosuccinate (ASA) after 1-h incubation with substrates. The mean value of ASS specific activities in activated lymphocytes from healthy subjects (N=10) was 88.3 mU/g (SD 23.8 mU/g). The mean value of ASS activities in patients with citrullinemia (N=3) was 16.6 mU/g (SD 6.7 mU/g). No significant difference in ASS activities was found in subjects with fasting and non-fasting status (66.2 vs. 76.2 mU/g).

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**Monday 5:00 PM**  
**Poster #13 in Exhibit Hall**  
**Development of a Rapid LC-MS/MS Method for Hair Cortisol Determination to Assess the HPA Axis**  
*Laura Smy* - *The Hospital for Sick Children* (L.smy@mail.utoronto.ca) -- *Young Investigator Grantee*  
- With the growing popularity of hair cortisol concentration as a biomarker to assess the HPA axis, we are developing a rapid method for the determination of hair cortisol using UHPLC-MS/MS. Our method uses a 1.7 µm C18 column, with the MS in MRM mode to detect cortisol in a 2-minute run. The calibration curve is linear with a correlation coefficient ≥ 0.998. The LOD is 0.12 ng/g for samples pre-treated with SPE, or 5.4 ng/g for filtered samples. The intra-day coefficient of variation was 2.5%. Further, cortisone and prednisone do not contribute to the cortisol peak with this method.
Monday 2:00 PM  
Poster #14 in Exhibit Hall  
The Development of an LC-MS/MS Screening Method for 104 Targeted Compounds in Whole Blood, Using Library Searching on a QTRAP Mass Spectrometer  
Heather Singletary - Metro Nashville Police Department-Crime Lab (heather.singletary@nashville.gov)  
- In order to detect a large variety of drugs in whole blood, many forensic laboratories incorporate multiple screening assays to cover different drug classes. Our objective was to develop a single LC-MS/MS assay capable of accurately identifying >100 target compounds in less than 10 minutes. We have employed a QTRAP mass spectrometer, which enabled ‘on-the-fly’ acquisition of full-scan MS/MS spectrum for every detected compound, which was searched against a spectral reference library. A cross-method comparison with an outside laboratory demonstrated that our method (i) provided more specific information about compound identity, (ii) provided superior sensitivity, and (iii) detected more compounds.

Monday 5:00 PM  
Poster #15 in Exhibit Hall  
A Novel and Fast Workflow for Forensic Toxicological Screening and Quantitation Using QTOF LC-MS/MS System  
Xiang He - SCIEX (xiang.he@absciex.com)  
- Forensic toxicological screening is challenging partly because of the extensive and evolving compound list. Current detection tools such as immunoassay, LC-UV, and GC-MS either lack the promptness and flexibility to adapt to the new analytes, or require extensive sample preparation methods, or suffer from insufficient sensitivity and specificity. In this study, we aim to (1) develop a sensitive and selective screening workflow in a forensic toxicological setting by utilizing QTOF LC-MS/MS system, IDA-MS/MS acquisition and an MSMSAll approach with the novel SWATH™ acquisition and to (2) to compare two LC methods (6.5-min and 2-min) for throughput/performance evaluation.

Monday 2:00 PM  
Poster #16 in Exhibit Hall  
Comparison of Accurate Mass MS/MS Acquisition and Processing Techniques on Forensic Toxicological Screening  
Michael Jarvis - SCIEX (michael.jarvis@sciex.com)  
- (For research use only, not for use in diagnostic procedures). High resolution mass spectrometry (TOF or ion trap) yields generic methods that can identify some compounds. However, many compounds cannot be identified with MS1 evidence alone. Obtaining MS/MS for every possible compound in a sample can be challenging. Data dependent techniques often miss candidates. Targeted techniques are limited in the number compounds that can be monitored. Data-independent techniques, such as SWATH™ acquisition, are capable of capturing MS1 and MS/MS evidence for all possible candidates. We compared several acquisition techniques, and several data processing techniques, in a toxicological screening scenario.

Monday 5:00 PM  
Poster #17 in Exhibit Hall  
An Interactive Digital Pathway Map: A Resource for Interpreting Metabolomic Data  
Nick Spittler - Washington University in St. Louis (nspittler@wustl.edu)  
- Interpretation of mass spectrometry-based metabolomic data in the context of biochemistry requires a high level of familiarity with metabolic pathways. Metabolomic investigators need to know specific details about the particular pathway of interest and understand how it is integrated within the bigger picture of comprehensive metabolism. Our objective is to provide an all-in-one resource for biochemical interpretation of metabolomic data. Contrary to physical pathway maps, we have created a digital version of comprehensive metabolism that can be viewed at any level. Metabolism can be viewed at the aerial perspective or, with multiple mouse clicks, at the “zoomed-in” molecular level of specific metabolic reactions. This resource can also be used effectively for teaching metabolism in the classroom.
Monday 2:00 PM
Poster #18 in Exhibit Hall
**Profiling Sialylation Status of Macrophage Upon Cell Activation**

*Dan Wang - Cleveland State University* (wangdandan0919@hotmail.com) -- *Young Investigator Grantee*

Sialic acids (SAs) are widely expressed on immune cells and their levels and linkages named as sialylation status may vary upon cell activation related to either physiological or pathological processes. In this study, we globally profiled sialylation status of macrophages upon activation. LC-MS/MS results showed cellular SA increased from 369 to 1.08×10³ ng/mL after cell activation. This result was supported by the increase of α-2,6 linked SAs on the cell surface measured by flow cytometry and confocal microscopy. Results of this work will contribute to a better understanding of the physiological and pathological roles of SAs in the immune system.

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Monday 5:00 PM
Poster #19 in Exhibit Hall
**Determination of Concentration and Distribution of Doxorubicin in Lungs by in vivo and in Situ Solid Phase Microextraction**

*Barbara Bojko - University of Waterloo* (bbojko@uwaterloo.ca)

Currently available sample preparation methods for tissue analysis are mainly based on the biopsy therefore are too invasive for repetitive analysis of living systems and too time consuming and laborious to deliver results in real time. Solid phase microextraction (SPME) addresses the abovementioned issues. In this work we used in vivo and in situ SPME for determination of concentration of the chemotherapeutic agent in lung of living pigs and in human lungs obtained from the deceased donors, during In Vivo Lung Perfusion. This results showed feasibility of the method to provide fully quantitative data with good spatial and temporal resolution.

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Monday 2:00 PM
Poster #20 in Exhibit Hall
**Enzyme Hydrolysis Using a Novel Recombinant β-Glucuronidase for Pain Management Urine Drug Testing**

*Agnes Cua - Precision Toxicology* (agnes@PrecisionTox.com)

A novel recombinant β-glucuronidase (IMCSzyme), known to have higher activity than traditional abalone β-glucuronidase, was investigated. The IMCSzyme successfully hydrolyzed the glucuronide drug metabolites in patient samples, in an assay that is faster and uses less enzyme. Codeine, morphine, cyclobenzaprine, amitriptyline and naloxone showed higher quantitation following hydrolysis with IMCSzyme, indicating more complete conversion than was previously obtained. Our study demonstrates that the recombinant β-glucuronidase (IMCSzyme) is a viable and cost-effective alternative to current hydrolysis procedures using abalone β-glucuronidase.

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Monday 5:00 PM
Poster #21 in Exhibit Hall
**LC-MS Quantitative Analysis of Water Soluble Vitamins in Blood**

*Lauren Frick - Agilent Technologies, Inc* (lauren.frick@agilent.com)

Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) are suited for rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of the relevant water soluble vitamins. This analytical method uses a simple offline sample preparation in blood. The described analytical method achieves the required sensitivity and is capable of quantitating of the vitamins over their relevant dynamic range. Excellent reproducibility was observed for all compounds (CV < 15%). All calibration curves displayed linearity with an R² > 0.995.
Improved Method for the Analysis of a Pain Management Supplemental Panel in Urine Using the Thomson eXtreme Filter Vials® by LC-MS/MS
Lisa Wanders - Thomson Instrument Company (lisa.wanders@htslabs.com)

This improved sample preparation method allows for the quantitative measurement of 17 drugs in a supplemental pain panel in urine. The urine samples were prepared using the eXtreme|FV®, followed by LC/MS/MS analysis. The most critical aspects of reliable urine analysis are the reduction of interferences from the sample matrix and analyte recovery. Traditionally, SPE, SLE and centrifugation have been used to reduce matrix interference prior to analysis. However, these techniques are time consuming, adversely impact recovery, use large amounts of solvent and are expensive. The improved sample preparation method using the Thomson eXtreme|FV® allows for the analysis of 17 drugs.

Comparison of Different Whole Blood Sample Pretreatment Methods for Targeted Analysis of Basic Drugs
Seyed Sadjadi - Phenomenex, Inc. (seyeds@phenomenex.com)

Whole blood presents a complete specimen for analysis of many drugs, therapeutic or illicit. This specimen type also poses significant challenges in pretreatment prior to extraction and eventual analysis by any method(s). We examined several common sample pretreatment procedures that hemolyze the blood and precipitate the plasma proteins. The resulting clarified samples were then treated by a SPE method designed for basic drugs (e.g. amphetamines, natural and synthetic opiates, benzodiazepines, and PCP). The pretreatment techniques were then evaluated based on the overall recovery, response and reproducibility for each analyte at two concentration levels.

Skyline for Small Molecules: A Flexible Tool for Cross-platform LC-MS/MS Method Creation and Data Analysis for Metabolomics
J. Will Thompson - Duke University (will.thompson@duke.edu) -- *Young Investigator Grantee*

This presentation will demonstrate the initial implementation of the Skyline software package for the creation of custom LC-MS/MS methods for small molecule analysis using several classes of metabolites. The software includes the ability to define precursor and product ions based on empirical formula or m/z, define collision energy specifically by molecule or based on a linear equation, and define expected retention time. As with peptide data, Skyline reads in the raw data from all major instrument vendors and allows native instrument method export. Thus the package eases the translation and validation of targeted metabolomics methods between instruments and laboratories.

A Metabolomic Approach to Identify Potential Therapeutic Biomarkers for Diabetic Foot Ulcers
Chia-ni Lin - Chang Gung Memorial Hospital (chianilin@cgmh.org.tw)

Diabetic foot ulcer (DFU) is a common complication of diabetes and may lead to amputation of the lower extremity. To prevent the progression of DFU is imperative because of the great impact in an individual’s life and a significant burden to the healthcare system. We used a combined direct injection and UPLC-MS/MS assay to quantify 186 metabolites in DFU patients (n=55) and diabetic patients (DM) without complications (n=57). PCA and PLS-DA analyses enable a clear differentiation of DMF and DM patients based on changes of their amino acids and acylcarnitines. These metabolites hold the potential to be therapeutic markers.
Monday 2:00 PM
Poster #26 in Exhibit Hall
**Metabolic Phenotyping Reveals a Lipid Mediator Response to Ionizing Radiation**
*Giuseppe Astarita - Georgetown University (giuseppe_astarita@waters.com)*

- Exposure to ionizing radiation has dramatically increased in modern society, raising serious health concerns. The molecular response to ionizing radiation, however, is still not completely understood. Here we screened mouse serum for metabolic alterations following an acute exposure to gamma radiation using a multi-platform, mass-spectrometry-based strategy. A global, molecular profiling revealed that mouse serum undergoes a series of significant molecular alterations following radiation exposure. We identified and quantified bioactive metabolites belonging to key biochemical pathways and eicosanoids, which could be utilized as an indicator of radiation exposure and as novel target for therapeutic intervention. Monitoring such a molecular response to radiation exposure might have implications not only for radiation pathology but also for countermeasures and personalized medicine.

Monday 5:00 PM
Poster #27 in Exhibit Hall
**Addition of Solid Phase Extraction to Opiate Sample Preparation for UPLC-MS/MS**
*Hanan Mohammad - University of North Carolina Hospitals (hanan.mohammad@unchealth.unc.edu) -- Young Investigator Grantee*

- In this study, we are assessing the inclusion of solid phase extraction (SPE) in the preparation of patient’s urine samples for opiate testing using UPLC/MS-MS method. We found comparable recoveries of most opiates using Dilute and shoot (D/S) only versus SPE. The extraction efficiency for all compounds ranged from 83.5-110%. Minimal matrix effect was observed using SPE except for buprenorphine at the high creatinine concentration. Method comparison analysis using D/S versus SPE demonstrated good agreement with all correlation coefficients (R2) > 0.95. Given the current analysis, we do not find sufficient benefit in adding SPE to our opiate testing.

Monday 2:00 PM
Poster #28 in Exhibit Hall
**High Throughput Measurement of Five Tobacco-specific Nitrosamines in Urine by Automation and Liquid Chromatography–mass Spectrometry**
*Baoyun Xia - Centers for Disease Control and Prevention (vvq2@cdc.gov)*

- A high throughput liquid chromatography tandem mass spectrometry (LC/MS/MS) method with robotic sample preparation and automatic data processing was developed and validated for the determination of five total tobacco-specific N-nitrosamines (TSNA), including both free and conjugated forms in urine. The sample size was decreased from previously reported 5 ml to 1.7 mL. The limit of detection for NNAL), NNN),NNK,NAT and NAB were 0.6, 1.8, 12.0, 2.0and 1.6 pg/mL respectively, with a linear calibration range of up to 20,000 pg/mL. The new robotic sample preparation method decreased the matrix effects and increased the throughput. The automatic data process by using Indigo Ascent™ combined peak integration and quality assurance, resulting in improved precision and reproducibility.

Monday 5:00 PM
Poster #29 in Exhibit Hall
**Highly Sensitive Screening for Antibiotic Resistance Using Parylene-matrix Chip**
*Jong-Min Park - Yonsei University (jmpark.nbsl@yonsei.ac.kr)*

- ß-lactamases (EC 3.5.2.6) are an important family of enzymes that confer resistance to ß-lactam antibiotics by catalyzing the hydrolysis of these antibiotics. Parylene-matrix chip and MALDI-TOF mass spectrometry are used to quantify ß-lactamase mediated hydrolysis of penicillin (m/z: [PEN+H]+=335.1 and [PEN+Na]+=357.8) into penicilloic acid (m/z: [PA+H]+=353.1 and [PA+Na]+=375.4) with minimal interference of low molecular weight noise peaks. The ß-lactamase assay was carried out with an antibiotic-resistant E.coli strain and an antibiotic-susceptible E.coli strain, revealing that the minimum number of E.coli cells required to screen for antibiotic resistance was 1000 cells for the MALDI-TOF mass spectrometry/parylene-matrix chip assay.
Monday 2:00 PM
Poster #30 in Exhibit Hall
Targeted Oxylipin Profiling for Clinical Diagnostic: A Novel Insight in Ventilator Associated Pneumonia

*Arnaud Wolfer* - Imperial College London (a.wolfer12@imperial.ac.uk) -- *Young Investigator Grantee*

- Ventilator associated pneumonia (VAP) is the most common infection in intensive care units (ICU), however robust diagnostic techniques for early detection are lacking. Blood samples of 58 brain injured patients were analysed through their ICU stay using a sensitive UPLC-MS profiling assay for the quantification of 48 inflammatory signalling molecules. Lipid mediators were able to differentiate patients with brain injuries or pneumonia and predictive modelling of VAP onset was performed. Serial oxylipins are of clinical merit in ICU, generating insight for potential disease biomarkers, stratifying critical patients based on their risk of adverse outcome and personalizing their therapeutic management.

Monday 5:00 PM
Poster #31 in Exhibit Hall
Small Molecule Analysis Using MALDI-TOF MS with Solid Nanostructure Matrices

*Jo-Il Kim* - Yonsei University (joiil@yonsei.ac.kr) -- *Young Investigator Grantee*

- TiO2 nanowire arrays and functional nanoweb matrices were synthesized and applied to MALDI-TOF mass spectrometry for the analysis of small molecules from human serum and milk samples qualitatively. TiO2 nanowires were synthesized by top-down hydrothermal process, and functional nanoweb matrices were synthesized by simultaneous process of electrospinning of nanoweb and electrospraying of TiO2 nanoparticles on the metal target plate. The feasibility of applying solid matrices to MALDI-TOF MS was demonstrated by the analysis of short peptides (leu-enkephalin) and amino acids. Amino acid in human sera and antibiotic drugs in milk were analyzed qualitatively and quantitatively using synthesized solid matrices.

Monday 2:00 PM
Poster #32 in Exhibit Hall
Is It Noroxymorphone or Nornaloxone, and Why Should You Care?

*Stephanie Marin* - ARUP Institute for Clinical and Experimental Pathology (stephanie.marin@aruplab.com)

- Noroxymorphine/nornaloxone positive results were evaluated to propose concentrations to determine the source (oxycodone, oxymorphone or naloxone) when detected alone. 16,273 positive results yielded 14,587 positive for oxycodone, oxymorphone, and/or noroxycodone. Presumed identity was normoxymorphone. Median concentration was 183ng/mL. 75% of results were >75ng/mL. 170 specimens with presumed identity of nornaloxone had compounds related to buprenorphine. 56 (33%) were positive for naloxone (median concentration 23ng/mL). Median concentration of nornaloxone was 35ng/mL. Our data suggest concentrations of noroxymorphone/nornaloxone <75ng/mL are from metabolism of naloxone. Ideally, urine testing should include precursor drugs, with results compared to patient prescriptions for accurate interpretation.

Monday 5:00 PM
Poster #33 in Exhibit Hall
UPLC-MS Analysis of Arginine Kinetics and Metabolism in Children with Severe Falciparum Malaria

*Haoyue Zhang* - Duke Medicine Biochemical Genetics Laboratory (zhang053@mc.duke.edu)

- We studied 10 healthy Tanzanian children (HC) and 10 with severe falciparum malaria (SM), using (13C6,15N4) labeled arginine tracer with measurement of isotopic enrichment in arginine and citrulline. These procedures allowed us to simultaneously determine the conversion of arginine to citrulline via the arginase-dependent (urea cycle) and nitric oxide synthase (NOS) pathways. Preliminary results show that arginine flux is higher in those with SM compared to HC children [median 108 (84-144) vs 85 (68-95) µmol/Kg/hr] and that citrulline production via the NOS pathway is reduced in malaria. These are the first direct measurements of arginine flux and metabolism in SM.
Monday 2:00 PM
Poster #34 in Exhibit Hall
Quantitative Analysis of IGF-1 Using Online Digestion Coupled to the Triple Quad LCMS-8050
David Colquhoun - Shimadzu Scientific Instruments (drcolquhoun@shimadzu.com)
• Automated digestion was used to accelerate sample analysis for a quantitative protein workflow analyzing the clinically important protein IGF-1. Digestion using a Perfinity Workstation was carried out in 4 minutes. The total run time (Workstation to Shimadzu LCMS-8050) was 20 minutes. Four peptides (10 transitions) were monitored. Digestion and LC-MS parameters were optimized using commercial standards, and the assay was evaluated using acetonitrile serum extracts spiked with IGF-1. IGF-1 was detected from 100 – 1000 ng/mL with r2 values >0.99. Accuracy was verified using a blinded “unknown” sample. This work demonstrates the feasibility of online digestion automation for IGF-1 analysis.

Monday 5:00 PM
Poster #35 in Exhibit Hall
Mass-Directed Isolation and Profiling of Small Molecule Analytes with SFC
Lu Dai - Theravance Biopharma US, Inc. (ldai@theravance.com)
• Electrospray ionization mass spectrometry (ESI-MS) coupled with supercritical fluid chromatography (SFC) is routinely being used in our laboratory for identification and isolation of small molecule analytes in supporting drug discovery efforts in PK analyses as well as other bioanalytical and clinical applications. This study demonstrates the utility of the preparative SFC-MS system to rapidly purify small molecule analytes for toxicology measurements and metabolite identification. The purification of one deuterated chiral isomer from its closely eluting impurity at the gram scale was accomplished within a day using 10 min mass-directed SFC methods. The limit of quantitation was reached below 1 ng.

Monday 2:00 PM
Poster #36 in Exhibit Hall
Development of a Rapid LCMS Method for Steroids in Plasma
Rachel Lieberman - Shimadzu Scientific Instruments (ralieberman@shimadzu.com)
• Steroids are important to the human body for normal biological activity. Steroid measurement is essential when evaluating disorders such as congenital adrenal hyperplasia, Cushing’s disease and polycystic ovarian disease. Traditional methods to measure steroids have been immunoassays, however, these assays lack specificity and could take up to a week to be completed. Liquid chromatography mass spectrometry (LC-MS/MS) has become the industry standard in evaluating steroids due to its specificity, precision and sensitivity. This presentation will focus on a rapid six minute method to analyze 19 steroids and internal standards in plasma at pg/mL levels using UHPLC-MS/MS detection.

Monday 5:00 PM
Poster #37 in Exhibit Hall
Use of 96-well Pipetting Workstation for Liquid-liquid Extraction of Adrenal Steroids
Geoffrey Rule - ARUP Laboratories (geoffrey.s.rule@aruplab.com)
• The compounds 17-hydroxyprogrenolone and pregnenolone are extracted with a 96-well pipetting workstation using methyl t-butyl ether. After derivatization the compounds, and isotopically labeled internal standards, are derivatized and quantified by HPLC tandem mass spectrometry. The method is rugged and reliable with precision and accuracy within acceptable limits. Studies to evaluate four collection tube types and special matrix types (icterus, hemolysis, and lipemia) are highlighted. Pooled human serum/plasma is used for preparation of control samples while 1%BSA solution is used for calibrator preparation.

Monday 2:00 PM
Poster #38 in Exhibit Hall
Comprehensive and Extended LC-MS Analysis of 166 Various Drugs and their Metabolites in Urine, Oral Fluid and Blood
Rory Doyle - Agilent Technologies, Inc (rory_doyle@agilent.com)
• Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. A sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of 166 drugs of the following drug classes: stimulants, benzodiazepines, antidepressants, opioids, muscle relaxants, hallucinogens, etc by QQQ. Using simple sample preparation techniques in urine and blood and chromatographic configurations achieves the required sensitivity and separation and is capable of quantitating the drugs over their relevant dynamic range. Excellent reproducibility was observed for all drugs (CV < 15%). All calibration curves displayed linearity with an R2 > 0.995.
Monday 5:00 PM  
Poster #39 in Exhibit Hall  
**LC-MS Quantitative Analysis of 25-Hydroxy-Vitamin D, 1, 25-Dihydroxy-Vitamin D, and their Isobars in Serum**  
*Jeff Keever - Agilent Technologies, Inc (jeff_keever@agilent.com)*  
- Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) are suited for rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of the 25-hydroxy-vitamin D and 1, 25-dihydroxy-vitamin D and their respective isobars. This method uses a simple offline sample preparation in serum. The described method achieves the required sensitivity and is capable of quantitating of these compounds over their relevant dynamic range. Excellent reproducibility was observed for all compounds (CV < 15%). All calibration curves displayed linearity with an R² > 0.995.

Monday 2:00 PM  
Poster #40 in Exhibit Hall  
**Innovations in Newborn Screening for Inborn Metabolic Disorders in Dried Blood Spots**  
*Zdenek Spacil - University of Washington (spacil@u.washington.edu) -- *Young Investigator Grantee*  
- Newborn screening of lysosomal storage disorders (LSDs) is being broadly implemented as new therapies became available and timely initiation of treatment improves outcome. A series of enzyme activity assays based on tandem mass spectrometry has been developed in our lab, greatly outperforming other available tests in terms of performance, simplicity and cost efficiency. We will demonstrate the potential of multiplexed enzyme activity assays coupled with analysis of metabolic markers to reliably screen for Pompe and Fabry diseases, sphingolipid disorders and mucopolysaccharidoses (I; II; IIIA; IVA; VI) in newborn dried blood spots. Several large scale pilot studies based on these assays have been completed or are underway.

Monday 5:00 PM  
Poster #41 in Exhibit Hall  
**Targeted Quantitation of 1-84 Parathyroid Hormone (PTH) by SID-MRM Mass Spectrometry**  
*Cheng Zhao - Abbott Laboratories (cheng.zhao@abbott.com)*  
- The measurement of PTH is used to assess hypocalcemia, hypercalcemia, metabolic bone disease, parathyroid gland tumors and intraoperative parathyroid hormone during parathyroidectomies. The bioactive amino acid sequence of PTH is 1-84. There are immunoassays specific for measuring PTH 1-84, however, these immunoassays can be susceptible to interference by cross-reacting PTH fragments. In this study, we developed a targeted quantitation method for PTH 1-84 using LC/MS/MS. The C-terminal peptide SLGEADKADVNVLTK was used as the surrogate peptide. PTH in normal patient samples was enriched by immunocapture, digested, and quantified by LC-MRM with a stable isotopically labeled IS.

Monday 2:00 PM  
Poster #42 in Exhibit Hall  
**Rapid, Simultaneous Analysis of Plasma Catecholamines and Metanephrines by Mixed-mode SPE and HILIC LC-MS/MS**  
*Sherri Naughton - Waters Corporation (sherri_naughton@waters.com)*  
- A single extraction and analysis method has been developed for plasma catecholamines and metanephrines for clinical research. Analytes were extracted using weak cation exchange mixed-mode SPE and analyzed using HILIC LC/MS/MS. Recoveries were good and matrix effects were minimal for most compounds. Linearity and QC results were excellent for all compounds down to 10 pg/mL, with % CV and bias values less than 10% at all QC points. This method enables rapid, simultaneous and accurate LC/MS/MS analysis of these challenging compounds without the challenges associated with traditional reversed-phase separation or ion-pairing techniques. For Research Use Only, not for use in diagnostic procedures.
Monday 5:00 PM
Poster #43 in Exhibit Hall
Value Assignment of Vitamin D Metabolites in Vitamin D Standardization Program (VDSP) Serum Samples
Karen Phinney - National Institute of Standards and Technology (karen.phinney@nist.gov)
- Assay variability has been cited as an obstacle to establishing optimal vitamin D exposure. As part of the Vitamin D Standardization Program (VDSP) effort to standardize the measurement of total 25(OH)D, value assignment of total 25(OH)D in 50 single donor serum samples was performed using two isotope-dilution LC-MS/MS methods. Both methods are recognized as reference measurement procedures (RMPs). These samples and their assigned values serve as the foundation for several aspects of the VDSP. To our knowledge, this is the first time that two RMPs have been used to assign values to such a large number of serum samples.

Monday 2:00 PM
Poster #44 in Exhibit Hall
Comparison of Tacrolimus Quantification Using the Waters MassTrak LC-MS/MS Assay with the Abbott Architect Immunoassay
Imir Metushi - University of California, San Diego - CALM (imetushi@mail.ucsd.edu)
- Immunoassays are the most common methodology utilized in the clinical laboratory for drug measurements. Although most immunoassays offer excellent sensitivity, cross reactivity of metabolites can result in decreased specificity as compared with mass spectrometry based assays. The Waters MassTrak assay is currently the only FDA cleared LC-MS/MS kit for quantification of tacrolimus in whole blood. Many clinical laboratories use the FDA cleared Abbott Architect Tacrolimus immunoassay because it is available on a reliable automated platform. This is the first comprehensive report comparing the Waters MassTrak LC-MS/MS assay with the Abbott immunoassay. Our results demonstrate that the immunoassay values range from 10 to 30% higher than values determined by isotope dilution (p < 0.0001; via two-tailed paired t-test).

Monday 5:00 PM
Poster #45 in Exhibit Hall
A Rapid and Accurate LC-MS/MS Method for the Analysis of Nicotine, Nicotine Metabolites, and Minor Tobacco Alkaloid in Urine
Shun-Hsin Liang - Restek Corporation (shun-hsinliang@restek.com)
- The analysis of nicotine metabolites has several aspects including monitoring public tobacco exposure, evaluation of nicotine replacement therapy, and drug therapy assessment. Most of the modern methods adapt the usage of high pH chromatography with relatively high concentration of additive reagents, which may not be applicable to all LC-MS instrumentation. An LC-MS/MS method was developed for urine test of nicotine, cotinine, trans-3'-hydroxycotinine, nornicotine, norcotinine, and anabasine. It was demonstrated that a fast and highly efficient analysis of these basic compounds can be achieved with the Raptor™ Biphenyl column using regular LC-MS solutions suitable for all LC-MS instrumentation.

Monday 2:00 PM
Poster #46 in Exhibit Hall
The Analysis of Synthetic Cannabinoids and their Metabolites in Human Urine by LC-MS/MS
Frances Carroll - Restek Corporation (frances.carroll@restek.com)
- The determination of cannabinoids and their metabolites, from a natural or synthetic source, has become routine in many forensic toxicology laboratories. The optimization of analysis time, resolution between metabolites, and method robustness is of ultimate importance when developing an efficient method for validation. The Raptor™ Biphenyl column combines the speed of superficially porous particles (SPP) with the resolution of highly selective USLC® technology to produce simple dilute and shoot methods with analysis times of less than 7 minutes for cannabinoids and their metabolites in urine.
Monday 5:00 PM  
Poster #47 in Exhibit Hall  
**Individualized Monitoring of Patients with Monoclonal Gammopathy: Optimizing Sample Preparation for Mass Spectrometry**  
*Patrick Vanderboom*  -  *Mayo Clinic*  
(vanderboom.patrick@mayo.edu)  
*‣* Plasma cell proliferative disorders are described by the expansion of a single clone of plasma cells. These cells commonly produce large amounts of monoclonal immunoglobulin. These monoclonal antibodies, clinically referred to as M-protein, are currently detected by agarose gel serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE). Recently, we have developed a top down mass spectrometry based method and coupled it to an optimized sample preparation procedure to yield a sensitive and high throughput platform capable of screening for monoclonal gammopathies and monitoring residual disease in myeloma patients.

Monday 2:00 PM  
Poster #48 in Exhibit Hall  
**Accuracy Evaluation of Three Routine 25-hydroxyvitamin D Assays by Comparing with LC-MS/MS**  
*Yeo-Min Yun*  -  *Konkuk University School of Medicine*  
(ymyun@kuh.ac.kr)  
*‣* Since the US Endocrine Society guideline defines vitamin D deficiency as 25(OH)D less than 20 ng/ml (50nmol/l), the accurate measurement of 25(OH)D in clinical laboratory is essential. We evaluated the accuracy and the effect of vitamin D binding protein (DBP) levels in three routine 25(OH)D immunoassays (Centaur, Elecsys, Architect) compared with LC-MS/MS using the samples of 48 healthy, 50 pregnant, and 50 ICU patients. All of three immunoassays showed significantly lower levels of 25(OH)D results than those of LC-MS/MS. Mean absolute biases [(routine assay) - (LC-MS/MS)] were -2.56 ng/mL (Centaur), -5.66 (Elecsys), and -6.21 (Architect). Although the mean value of DBP was significantly higher in pregnant women and lower in ICU patients than in healthy controls, the degree of biases in three immunoassays was not affected by the DBP levels.

Monday 5:00 PM  
Poster #49 in Exhibit Hall  
**Signal Enhancement in HPLC-ESI-MS/MS Analysis of Spironolactone Metabolites Using HFIP and NH4F as Eluent Additives**  
*Kalev Takkis*  -  *University of Tartu*  
(kalev.takkis@ut.ee)  --  *Young Investigator Grantee*  
*‣* We developed an HPLC-ESI-MS/MS method for simultaneous determination of spironolactone and its two metabolites, 7a-thiomethylspironolactone and canrenone from human blood plasma samples. Created as a part of a study evaluating the pharmacokinetic profile of spironolactone in children up to two years of age, the required sample volume was designed to be very small. That brought the question of ionization efficiency into our attentions and we investigated two signal boosting mobile phase additives, hexafluoroisopropanol and ammonium fluoride. Both were found to be beneficial and with the latter we observed more than 5 times increase in MS signal.

Monday 2:00 PM  
Poster #50 in Exhibit Hall  
**Using LC-MS/MS Urine Drug Testing to Identify Licit and Illicit Drug-Use in a Community-based Patient Population**  
*Adam Ptolemy*  -  *Gamma-Dynacare Medical Laboratories*  
(ptolemya@gamma-dynacare.com)  
*‣* Liquid chromatography tandem mass spectrometry-based (LC-MS/MS) urine drug testing (UDT) results from 165209 unique specimens were retrospectively reviewed to determine the number of positive results reported for each drug, metabolite and drug product tested. The relative frequencies of the detected drugs were: methadone > cannabinoids > opiates > oxycodone > cocaine > clonazepam > buprenorphine > diazepam > fentanyl > amphetamine > methylphenidate > heroin > alprazolam > nitrazepam > ecstasy > meperidine > flurazepam > flunitrazepam. The influences and considerations of drug metabolism on: observed positivity rates; monitoring patient compliance; and LC-MS/MS UDT method design will be presented.
Monday 5:00 PM
Poster #51 in Exhibit Hall
**Bioanalytical UPLC-MS/MS Method Development and Validation for Measuring Penicillins in Human Blood Plasma– Analyte Stability Issues**
**Karin Kipper - Institute for Infection and Immunity** (karin.kipper@gmail.com)
- Penicillins are the important group of antimicrobials widely used in children and adults for over 50 years. Penicillins are beta-lactam antimicrobials and therefore especially intolerant to the stress conditions since the degradation of penicillins occur in different ways in different conditions. UPLC-MS/MS method was developed and validated for determination of 5 in human blood plasma. The aim of developing and validating the bioanalytical method for measuring penicillins in blood plasma was to use it for the measurement of intensive care unit patients’ plasma samples, in order to use the data for the population pharmacokinetic modelling and dose optimization.

Monday 2:00 PM
Poster #52 in Exhibit Hall
**X13CMS and IsoMETLIN: Platforms for Isotope-Based Metabolomics**
**Kevin Cho - Washington University** (kevin.cho@wustl.edu) -- *Young Investigator Grantee*
- Mass spectrometry-based untargeted metabolomic technologies have been applied widely to compare the levels of hundreds to thousands of small molecules between sample groups unbiasedly, which has thereby enabled systems-level analyses. Recently, however, isotope-based metabolomics has been introduced to the standard metabolomic workflow, which a stable isotope is introduced into a biological system and metabolomic technologies are used to track its fate unbiasedly. Here we introduce X13CMS, an extension of the widely used mass spectrometry-based metabolomic software package XCMS, and isoMETLIN, an analog of the widely used METLIN database, that is designed to facilitate the identification of both isotopologues and isotopomers.

Monday 5:00 PM
Poster #53 in Exhibit Hall
**Usability Study of a New HPLC, a New Tandem MS and a New Data Processing Software**
**Jason Lai - Thermo Fisher Scientific** (jason.lai@thermofisher.com)
- Stability, robustness and time efficiency are three major challenges in laboratories where a large number of samples are analyzed routinely. Here we report results from a usability study of a new two channels HPLC, a new tandem mass spectrometer and a new data processing software to address these challenges. A total of 2000 crashed synthetic serum samples spiked with alprazolam and isotopically-labeled internal standard were analyzed continuously over 100 hours, with additional 44 QC samples inserted. On-line turboflow column helps removing sample matrix. Cross-channel RSD\% of retention time and concentration was observed at 0.85%, 1.49%, respectively. Instrument-to-instrument precision studies (3 units) are reported using two example compounds (testosterone, estradiol) with APCI probe in both positive and negative ionization modes. For in vitro diagnostic use.

Monday 2:00 PM
Poster #54 in Exhibit Hall
**Clinical Shotgun Proteomic Subtyping of Pituitary Adenomas**
**Surendra Dasari - Mayo Clinic** (Dasari.Surendra@mayo.edu) -- *Young Investigator Grantee*
- Accurate subtyping of pituitary adenomas is essential for prescribing therapy. A novel shotgun proteomics assay was developed for subtyping these tumors from formalin-fixed paraffin-embedded specimens. A cohort of 35 cases of five different subtypes was analyzed. Hormone profile of each case was correlated with patient’s clinical record, serum hormonal levels and electron microscopy. 100% of cases were in agreement with their clinical picture. Correlation of hormone profile with IHC battery revealed 89% agreement rate. Exceptions were gonadotrophs that had neither clinical symptoms nor serum elevations of FSH/LH. This assay is being validated for routine use in a CAP/CLIA clinical laboratory.
Evaluation of Bench-top Quadrupole Orbitrap Ultra High Resolution MS for Use in Clinical Research for Rapid Quantitative Analysis of Vitamin D in Human Plasma

Mindy Gao - ThermoFisher Scientific (mindy.gao@thermofisher.com)

- Clinical researchers commonly use a triple quadrupole mass spectrometer for analysis of 25-hydroxyvitamin D2 (25OHD2) and 25-hydroxyvitamin D3 (25OHD3) in plasma or serum. We evaluated Q Exactive Orbitrap mass spectrometer in fast, cost-efficient method collecting high resolution MS/MS spectra for improved method specificity. Protein precipitated plasma samples were analyzed with 3 min LC method, and high resolution MS/MS spectra were collected for each analyte. The most abundant fragment, besides water-loss, in each MS2 spectrum was selected for quantitative analysis. The linearity range was 1-100 ng/mL; precision and accuracy was with 15%; matrix effects and interferences were not observed.

Multi-channeling LC-MS/MS Forensic Methods for High-Throughput Urine Screening to Detect Buprenorphine and Ethanol Use

Joseph Di Bussolo - Thermo Fisher Scientific (joe.dibussolo@thermofisher.com)

- Two forensic LC-MS/MS methods to detect buprenorphine and/or ethanol uses were run on a multichannel UHPLC system utilizing positive-displacement pumps. A maximum throughput of 34 urine samples per hour was achieved. Desired quantitation ranges, accuracy and repeatability criteria were met for each application. Internal standard (IS) peak areas showed less than 25% coefficient of variation (CV) among calibrators, QCs and specimens (n = 20) on any of the four channels. Retention time variations throughout these batches were less than 3% CV. Results were within +/- 15% of those determined on a conventional multichannel system.

High Throughput Screening and Confirmation of 41 Pain Panel Drugs in Oral Fluid by an Integrated On-Line Extraction UHPLC-MS/MS System

Louis Maljers - Bruker (louis.maljers@bruker.com)

- A rapid and sensitive procedure for the quantification of Pain Panel Drugs (PPDs) in Synthetic Saliva was developed using Thomson filter vial for sample preparation and using an integrated On-Line Extraction (OLE)-UHPLC-MS/MS System. The lower limit of quantitation (LLOQ) was 0.2 ng/mL and upper level of quantitation (ULOQ) was 100 ng/mL. The linearity regression coefficient R2 was >0.99. The sub ng/mL level PPDs detection with about three orders of dynamic detection range will cover the clinical research needs. The method run cycle time was 8.5 minutes.

Differential Breast Cancer Glycosylation Detected by Gas Chromatography Nodal Glycan Analysis

Shayesteh R. Ferdosi - Arizona State Univerity (sroshdif@asu.edu) -- *Young Investigator Grantee*

- Aberrant Glycosylation is a hallmark of tumor cells that has been profoundly observed in breast cancer. Breast cancer as a heterogeneous disease has been classified based on the glycoprotein reseptors ER,PR and Her2. We propose differential glycosylation is associated with different breast cancer subtype. To implement this concept we measure glycan content of the enriched plasma membrane of the breast cancer cell lines from each subtype. Glycan permethylation and then semi purification and enrichment is followed by GC-MS to detect glycan "nodes" of N-, O-glycans pooled together.
Monday 5:00 PM
Poster #59 in Exhibit Hall
Optimization of LC-MS/MS Method for Thyroglobulin Quantitation in Human Serum
Lidong He - Department of Chemistry, University of Utah and ARUP Institute for Clinical and Experimental Pathology (cavalierhld@gmail.com) -- *Young Investigator Grantee*

- The incidence of thyroid cancer worldwide is estimated at 213,000 persons per year. Measurement of thyroglobulin (Tg) is used for monitoring of the thyroid cancer recurrence. After thyroidectomy, Tg concentrations drop to a very low or undetectable level, and elevated serum Tg concentration indicates cancer recurrence. However, the presence of endogenous thyroglobulin autoantibodies (Tg-AABs) can mask Tg epitopes and can lead to false-negative immunoassay results. We have established a LC-MS/MS method for Tg quantitation in human serum using a Tg-specific peptide FSPDDSAGASALLR as the surrogate peptide. Accurate concentration of thyroglobulin in serum can be measured without interference of Tg-AABs.

Monday 2:00 PM
Poster #60 in Exhibit Hall
Comprehensive Human Fecal Metabolome Analysis Using Chemical Isotope Labeling LC-MS
Nan Wang - Zhejiang University (nanwang@zju.edu.cn) -- *Young Investigator Grantee*

- Human fecal samples contain endogenous human metabolites, gut microbiota metabolites, and other components. We report a sensitive chemical isotope labeling LC-MS method for comprehensive and quantitative analysis of the amine-, phenol, and carboxylic acid-containing metabolites in fecal samples. A sequential water-acetonitrile extraction method is found to be optimal for extracting metabolites from fecal samples. Pre-analytical issues including sample storage conditions are investigated to study their effects on fecal metabolome profiling. The effect of diet on human fecal metabolome is studied and its implication on applying fecal metabolomics for disease biomarker discovery is discussed.

Monday 5:00 PM
Poster #61 in Exhibit Hall
Development and Validation of an Opioid LC-MS/MS Assay: Evaluation of Different β-glucuronidase Enzymes and Protein Precipitation Plates
He Yang - University of California, San Francisco/SF General Hospí (he.yang@ucsf.edu) -- *Young Investigator Grantee*

- We report the development and validation of an opioid confirmatory assay using enzyme hydrolysis and LC-MS/MS for the qualitative identification of thirteen opioids. We evaluated three different β-glucuronidase enzymes and three protein precipitation plates that are currently on the market. The combination of IMCSSme β-glucuronidase and Supleco protein precipitation plate provided an efficient and cost-effective hydrolysis and sample clean-up. LOD, Linearity, precision, matrix effect, recovery and carry-over of the final method were validated. This LC-MS/MS assay detected compounds missed by GC-MS due to interference or co-eluting peaks. This procedure is significantly faster and less expensive compared to the GC-MS method.

Monday 2:00 PM
Poster #62 in Exhibit Hall
A Novel Analytical Method to Analyze Phosphatidylcholine in Human Breath Using UHPLC-MS/MS
Shahid Ullah - Karolinska Institute (shahid.ullah@ki.se) -- *Young Investigator Grantee*

- Phosphatidylcholine (PC) is an important phospholipid of a lung surfactant. Dipalmitoylphosphatidylcholine (DPPC) is the most abundant form of PC, and is believed to be the most important pulmonary surfactant. Exhaled breath contains non-volatile substances including PC. Current analytical method do not account for analyzing the DPPC in human breath. This is the first time, we validates a LC-MS/MS method capable of analyzing DPPC (PC 32:0) and PC 16:0/18:1 in exhaled breath. The particles in exhaled breath were collected in a polymer filter and subsequently, 6.5 and 16 pg/filter of MLQs can be achieved for PC 32:0 and PC 16:0/18:1, respectively.
**Monday 5:00 PM**  
**Poster #63 in Exhibit Hall**  
**Development of a Fast Analytical Method for Separation of Urinary Arsenic Metabolites by Using HPLC-ICP-MS**  
*Ya-Ching Huang - Chang Gung Memorial Hospital, Linkou (hycymm@cgmh.org.tw)*  
- Identification of arsenic species in human urine has been used as a measure of exposure to inorganic arsenic. The purpose was to develop and validate a fast analytical method to quantify arsenic species. Urine samples were filtered, diluted with water and injected to HPLC-ICP-MS. The run-time was 5 minutes per injection with baseline resolved chromatographic separation. The analytical measurement range was 3.4 to 80 µg/L with r² values >0.999. Intra-assay imprecision (%CV) was less than 4.6% and inter-assay imprecision was less than 7.1%. The method demonstrated fast and acceptable performance for arsenic speciation in human urine.

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**Monday 2:00 PM**  
**Poster #64 in Exhibit Hall**  
**Using MALDI-TOF MS to Screen for Monoclonal Proteins in Serum**  
*Mindy Kohlhagen - Mayo Clinic (kohlhagen.mindy@mayo.edu)*  
- This study aims to evaluate MALDI-TOF MS as a clinical screening method for serum monoclonal proteins (M-proteins). A set of 556 serum samples previously tested by gel electrophoresis (PEL) and immunofixation (IFE) were analyzed by MALDI-TOF MS. The mass distributions of immunoglobulin light chains from each sample were compared to normal serum. Deviations from the normal distribution were considered positive. Upon analysis, 100% of PEL positive, 91% of IFE positive and 19% of PEL/IFE negative samples screened positive by MALDI-TOF MS. The results suggest that MALDI-TOF MS has near equivalent sensitivity compared to current methods and would be an effective screen for M-proteins.

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**Monday 5:00 PM**  
**Poster #65 in Exhibit Hall**  
**Accuracy Evaluation of High- and Low-density Lipoprotein Cholesterol Assays in Clinical Laboratories by Comparison with Isotope Dilution Mass Spectrometry**  
*Misuk Ji - Konkuk University School of Medicine (msji0402@gmail.com) -- *Young Investigator Grantee*  
- We evaluated the performance of high-density (HDL) and low-density lipoprotein (LDL) cholesterol assays commonly used in Korea. Five levels of commutable frozen serum pools were measured with five routine assays (Toshiba-Kyowa, Hitachi-Sekisui, Siemens, Roche, and Beckman Coulter). Target values were measured with CDC reference method by gas chromatography-isotope dilution mass spectrometry. The mean bias of HDL cholesterol was 1.5~3.5 mg/dL (3.2~8.0%). The LDL cholesterol values of one material with high triglyceride of 330 mg/dL were significantly overestimated (20.4 mg/dL) in all assays, at both refrigerated and frozen state, but the degree was quite different depending on the assays.

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**Monday 2:00 PM**  
**Poster #66 in Exhibit Hall**  
**A Fast, Sensitive, and High-throughput LC-MS/MS Assay for Benzodiazepines/Z-Drugs/Barbiturates**  
*Hui Qiao - IONICS Mass Spectrometry (huiq@ionics.ca)*  
- Benzodiazepines, Z-drugs, and barbiturates belong to a group of psychotropic drugs for the treatment of anxiety, depressant, and insomnia. However, these drugs have the potential for over dosage or abuse, which requires the development of fast and accurate methods for the screening and confirmation analysis. LC-MS/MS offers superior sensitivity, selectivity, and robustness for simultaneously detecting benzodiazepines and non-benzodiazepines in complex biological matrices. This work here presents a fast, reliable, and accurate LC-MS/MS method on an IONICS 3Q 120 triple quadrupole mass spectrometer with Restek Biphenyl column for the analysis of a total of 43 compounds, using fast polarity switching.
Monday 5:00 PM  
Poster #67 in Exhibit Hall  
**Value Assignment of Candidate Standard Reference Material® 3949 Folate Vitamers in Frozen Human Serum by Isotope-Dilution LC-MS/MS**  
**Johanna Camara** - National Institute of Standards and Technology (johanna.camara@nist.gov)  
‣ The National Institute of Standards and Technology (NIST) is developing a candidate Standard Reference Material (SRM) 3949 Folate Vitamers in Frozen Human Serum to replace SRM 1955 Homocysteine and Folate in Human Serum. Isotope dilution-liquid chromatography tandem mass spectrometry analysis at the Centers for Disease Control and Prevention and NIST will be utilized to assign values for folic acid and 5-methyltetrahydrofolate (5-mTHF), as well as several minor folates. Endogenous levels of folic acid and 5-mTHF in SRM 3949, enhanced folate stability via ascorbic acid addition, and additional minor folate values are improvements over SRM 1955 that should better serve customers.

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Monday 2:00 PM  
Poster #68 in Exhibit Hall  
**Development of Chemical Isotope Labeling LC-MS for Human Serum Metabolome Profiling from Dried Serum Spots**  
**Liyan Liu** - University of Alberta (liyan1@ualberta.ca)  
‣ Dried blood spots have been used as a means of sample collection and storage for diagnosis of inborn errors of metabolism and "omics" research with advantages such as convenience of storing and transporting samples. In this work, we describe a method for profiling the amine/phenol submetabolome of human serum from dried serum spots (DSS). Similar numbers of putative metabolites could be detected from DSS and serum matrix. Detailed workflow for DSS metabolomics will be presented. This method is shown to be useful to differentiate different groups of metabolomic samples with almost the same performance as using serum directly.

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Monday 5:00 PM  
Poster #69 in Exhibit Hall  
**A Comprehensive Study for Validation of a LC-MS/MS Method for the Simultaneous Determination of Four Immunosuppressive Drugs in Whole Blood**  
**Erdim Sertoglu** - Ankara Mevki Military Hospital, Anittepe Dispensary (erdimsertoglu@gmail.com)  
‣ In this study, we aimed to develop a rapid, sensitive, selective, and cost-effective LC-MS/MS method, to determine four immunosuppressive drugs in whole blood samples simultaneously. Non-isotopic internal standards ascomycin (tacrolimus, sirolimus, everolimus) and cyclosporine D (cyclosporine A) were obtained from ClinMass® while whole blood controls and calibrators were obtained from ClinChek® (lyophilised) and ClinCal® (lyophilised), respectively. Intra-day precisions were between 3.0-5.3%, 1.1-4.4%, 3.7-4.0, 3.1-6.3% and inter-day precisions were between 4.1-4.8%, 1.1-3.2%, 3.7-4.0%, 6.0-7.8 while the accuracy was varied from -6% to +10%, -7% to +4%, -6% to +5%, -4% to +11%, for Cyclosporine A, Tacrolimus, Sirolimus and Everolimus, respectively.

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Monday 2:00 PM  
Poster #70 in Exhibit Hall  
**A Fast Polarity Switching LC-MS/MS Analysis of Benzodiazepines and Barbiturates**  
**Joshua (Sha) Ye** - IONICS Mass Spectrometry (joshuay@ionics.ca)  
‣ ESI-LC-MS/MS has been widely used to monitor pain management drugs on a routine basis in many labs worldwide. Because some of these drugs ionized better in negative mode than that in positive electrospray ionization mode, the panel of interest usually is split into positive mode and negative mode panels. However, recent advancement of fast and robust polarity switching technology allows high throughput reliable implementation of combining all drugs into one panel disregard of positive and negative ionization modes. Current study demonstrates a fast, robust polarity switching ESI-LC-MS/MS method on an IONICS 3Q 120 triple quadrupole mass spectrometer.
Cortisol Measurement in Urine: LC-MS/MS Method Validation and Preliminary Clinical Application

Serkan Tapan - Gulhane School of Medicine (stapan@gata.edu.tr)

- In this study, we aimed to develop a rapid, sensitive and selective LC-MS/MS method for the determination of free cortisol levels in urine samples. Cortisol, methanol and ammonium formate were purchased from Sigma, and d4-cortisol was purchased from Cambridge Isotope Laboratories. Mobile phases were 5 mmol/L ammonium formate and methanol. An Agilent 6420 tandem mass spectrometer was used in the positive-ion mode with an APCI interface. Quantitative analysis was performed in the MRM mode. The intra-day and inter-day precisions were 1.9–4.5% and 2-5.4%, respectively while the accuracy was 103.4%. The method was linear from 2.0 to 500.0 ng/mL (r2 > 0.999). The limits of detection and quantification were 1 µg/L and 2 µg/L.

Extraction of Buprenorphine and Norbuprenorphine from Urine Samples Using New Nbe™ (Narrow Bore Extraction) Columns: Fully Automated Sample Preparation

Emmanuel Chanco - SPEware Corp. (luigi.chanco@speware.com)

- Sample preparation in clinical and toxicology laboratories has historically been very labor intensive. Individual portions of the analytical process, from sample accessioning to the instrumental analysis itself, have been streamlined and accelerated, but a comprehensive unattended process from sample aliquots to ready-to-analyze extracts has not been shown. Here, we present a process for robotic pipetting of urine samples, on-deck hydrolysis, automated extraction of the hydrolyzed samples, and subsequent analysis of the extracts for the presence of buprenorphine and norbuprenorphine. Analyses of the extracts were performed on an AB Sciex 5000 LCMS system with focus on linearity and LLOQ measurements.


Patrick Caron - CHU de Quebec Research Center (patrick.caron@crchul.ulaval.ca)

- We report a validated gas chromatography selected reaction monitoring – tandem mass spectrometry assay (GC-MS/MS) for the simultaneous quantification of progesterone, dehydroepiandrosterone, androstenediol, androstenedione, testosterone, dihydrotestosterone, androsterone, 5alpha-androstan-3beta-17beta-diol (3β-diol), estrone and estradiol. After addition of stable isotope internal standards to 250 µl of serum, the method involved a liquid-liquid extraction, derivatization and solid-phase extraction. Individual steroids were measured with high sensitivity, accuracy and reproducibility while its applicability was successful on serum from men, pre- and post-menopausal women. This method will allow measurement of ten steroids in clinical epidemiology and laboratory research while providing low quantification limits with a limited volume of sample.

Global Mapping of Nutrient Utilization by Untargeted Metabolomics

Liz Payne - Washington University in St. Louis (empayne@wustl.edu) -- *Young Investigator Grantee*

- Metabolomics has revealed that the complexity of cellular metabolism exceeds that expected from conventional textbooks. By integrating stable isotopes with untargeted metabolomic technologies, we can now identify novel pathway connections and nutrient fates. A limitation of isotope-based metabolomics applied to the clinic has been that each experiment required both a labeled cohort as well as an unlabeled control cohort. Here, we introduce a database called isoMETLIN that enables the distinction of labeled metabolites so that patients can be analyzed without unlabeled controls. This resource reduces the number of patients needed per experimental analysis and allows multiplexing with numerous isotopic labels.
1,25-di-hydroxy Vitamin D Analysis by LC-MS: Optimization for Sample Prep Automation and Medium Throughput Lab

**Dave van Staveren** - Tecan Schweiz AG (dave.vanstaveren@tecan.com)

- A manual RIA assay for 1,25di-OH Vitamin D (1,25DiOHVitD) from serum shall be replaced. Goal is to establish a LC-MS based workflow that significantly reduces hands on time (manual work), shortens the time to results and enables to analyze the samples on the existing LC-MS system. Extraction of 1,25DiOHVitD with the easy to automate AC Extraction Plate followed by derivatization with DAPTAD and analysis by LC-MS showed extraction efficiency of 84% at 100pg/mL and appeared to be very reproducible. The calibration curve for standard materials was linear for the concentration range of 5 to 160 pg/mL.

Rapid Quantification of Free and Glucuronidated THC-COOH in Human Urine Using Coated Well Plates and Column-switching LC-MS/MS

**Marianne Hädener** - University of Bern (marianne.haedener@irm.unibe.ch) -- *Young Investigator Grantee*

- Cannabis is the most commonly encountered illicit substance in workplace urine drug testing. Generally, cannabis consumption is detected by measuring the total concentration of the urinary THC-COOH metabolite obtained by hydrolyzing THC-COOH-glucuronide. To meet the workplace drug testing demands regarding rapid throughput, automation and low costs, we have developed a novel high-throughput method for the direct quantification of THC-COOH and THC-COOH-glucuronide in urine. Our method is based on minimal sample preparation employing Tecan AC Extraction Plates and subsequent quantification by two-dimensional chromatography and tandem mass spectrometry. The presented method was successfully validated and applied to several hundred authentic urine samples.

Rapid Mass Spectrometry Based Urine Drug Screening of 27 Antipsychotic and Antidepressant Medications

**Jeffrey Enders** - Ameritox, Ltd. (jeffrey.enders@ameritox.com)

- This poster will demonstrate figures of merit for screening 27 compounds using a two-minute dilute-and-shoot method on a SCIEX 4600 Triple TOF at a 10x dilution. Each compound’s positive identification relies upon it having significant abundance, acceptable retention time, exact parent mass, agreeable isotope ratio, and sometimes the appropriate fragmentation pattern. Figures of merit including limit of detection/limit of quantitation (LOD/LOQ), upper limit of linearity (ULOL), and possible inter-assay interferences will be discussed. Using patient samples, type I and type II errors will be assessed when compared with a traditional LC confirmation method.

Enhancing Ion Abundances and Spatial Homogeneity of Glycans by Regulating the Substrate Temperature in MALDI MS: A Physical Chemistry Perspective

**Yin-Hung Lai** - Genomics Research Center, Academia Sinica (yhlai@gate.sinica.edu.tw) -- *Young Investigator Grantee*

- Identifying glycans provides critical insights into the pathogenesis of a cancer. Mass spectrometric analyses, especially matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS), are the major tools for the identification of glycans. However, owing to the thermally labile nature, ionization efficiency, and heterogeneity of ion population, the identification of glycans in MALDI MS is still less effective. A laboratory-built sample preparation compartment was built to facilitate a rapid regulation of the substrate temperature. This preparation method successfully enhanced the abundances and eliminated the spatial heterogeneity of ions. A theoretical simulation was conducted to discuss the homogeneity of ion populations.
Monday 5:00 PM
Poster #79 in Exhibit Hall
**RapidFire-Based Screening of THCA: Comparison with Established Methods**  
*Jennifer Hitchcock - Ameritox, Ltd.* (jennifer.hitchcock@ameritox.com)
- A rapid mass spectrometry-based screening method for THCA has been recently developed and implemented in our pain management laboratory. Following implementation, it was observed that the false positive rate in patient samples increased when compared to the previously used EIA-based method. Data from both EIA and RF methods will be summarized and a discussion of false positive rates observed in authentic patient samples via both screening methods will be presented.

Monday 2:00 PM
Poster #80 in Exhibit Hall
**Investigation of Arachidonic Acid and Its Metabolites as Biomarkers for Potential Efficacy Endpoints for Monoacylglycerol Lipase Inhibition**  
*Kimberly Navetta - Pfizer Global Research and Development* (kimberly.a.navetta@pfizer.com)
- Brain inflammation can occur with infection, traumatic brain injury or disease and arachidonic acid (AA) and metabolites are increased. Monoacylglycerol lipase (MAGL) is an enzyme that controls brain levels of AA and pro-inflammatory metabolites. To further develop MAGL inhibition as a target for diseases with neuroinflammation, efficacy biomarkers are needed in investigative studies. A quantitative assay was developed using liquid chromatography coupled with mass spectrometry to measure AA and metabolites in human cerebral spinal fluid. AA and metabolites were measured from control patients and patients with brain inflammation and the biggest change was observed in patients with intra-cerebral hemorrhage.

Monday 5:00 PM
Poster #81 in Exhibit Hall
**Detection and Quantitation of Exemestane, Letrozole and Anastrozole in Human Serum by LC-MS/MS and Atmospheric Pressure Chemical Ionization**  
*Julia Addiss - Quest Diagnostics, Inc.* (julia.d.addiss@questdiagnostics.com)
- Carcinomas expressing estrogen receptors are sensitive to the proliferation of estrogens. An effective therapeutic option for the treatment of these estrogen-responsive cancers in postmenopausal women involves the use of aromatase inhibitors including exemestane, letrozole and anastrozole. These selective drugs interfere in the synthesis of estrogen in peripheral tissues by inactivating aromatase. The resulting decrease in circulating estrogen levels can slow, and even stop, the growth of breast cancer cells. Monitoring the serum concentrations of exemestane, letrozole and anastrozole will help determine the patient’s adherence to the drugs, as well as provide useful information regarding concentration-dependent side effects and therapeutic efficacy.

Monday 2:00 PM
Poster #82 in Exhibit Hall
**Identifying the Proteome of Different Mycobacterial Species Using Orbitrap™ Mass Spectrometry**  
*Suraj Saraswat - ARUP Lab* (suraj.saraswat@aruplab.com)
- Advances in next generation sequencing, of whole bacterial genomes, have led to a plethora of useful genetic data. However, these data often lack correlation with expressed protein profiles. It has been well demonstrated that even very closely related genomes express drastically different phenotypes which often have major roles in pathogenicity. Therefore, it is just as important to have a method for examining the proteome of a bacterium. We setout to develop a method for the identification and characterization of the mycobacterial proteome using reversed-phase liquid chromatography and a Q Exactive™ Plus Orbitrap™ MS with both Top-down and Bottom-up approaches.
**Posters by Day: TUESDAY**

**Tuesday 5:00 PM**
Poster #1 in Exhibit Hall

**HPLC-UHPLC Hybrid 2D Platform for LC-MS Analysis of Biological Samples. Back to the Future**

*Eduard Rogatsky - Albert Einstein College of Medicine (eduard.rogatsky@einstein.yu.edu)*

- We developed a cost-efficient hybrid LC platform that's ideal for biological sample analysis by LC/MS. This platform is based on Agilent 1100 and Agilent 1290 series LC devices. The first dimension of the analytical system used a standard pressure binary HPLC pump, where the sample was injected using a standard analytical autosampler into a pre-analytical column. The fraction containing analytes of interest was transferred through a UHPLC valve to a fused core column. We successfully used this platform for LC/MS analysis. Instead of retiring an entire functioning Agilent 1100 LC system, we just added one UHPLC pump to achieve much greater overall performance, functionality and lower cost, compared to a single pump UHPLC system purchase.

**Tuesday 2:00 PM**
Poster #2 in Exhibit Hall

**Standardized and Quantitative Metabolic Phenotyping of Bile Acids in Blood - An International Inter-laboratory Ring Trial Test**

*Ralf Bogumil - BIOCRATES Life Sciences AG (ralf.bogumil@biocrates.com)*

- Bile acids are relevant endogenous markers for liver cell functions, inflammation, apoptosis, gut microbiome but also are signalling molecules regulating triglycerides, cholesterol and glucose metabolism. We have developed the worldwide first kit including analytical reagents to harmonize the analysis of individual bile acids from only 10 µL of either human plasma or human serum (16 bile acids) or mouse plasma samples (19 bile acids) for UHPLC-MS/MS analysis. A ring trial test with 14 participants has been successfully performed. Bile acid profile of mice is quite different from that of human; relevant aspects for translational medicine.

**Tuesday 5:00 PM**
Poster #3 in Exhibit Hall

**Optimization and Validation of Online SPE-UHPLC-MS/MS for Trace Level Quantitation of Bisphenol A Analogues in Human Urine**

*Wei Zou - EHLB, California Department of Public Health (wei.zou@cdph.ca.gov)*

- California enacted legislation banning the use of BPA in baby bottles. Manufacturers are considering BPA analogs or derivatives as replacements for BPA in various applications. In the present study, mass spectrometry parameters were optimized to achieve the lowest possible instrument limit of detection. In addition, on-line solid phase extraction protocol was optimized for maximal absolute recovery rates and high throughput. Initial demonstration of capability and control charts were established.

**Tuesday 2:00 PM**
Poster #4 in Exhibit Hall

**Validation of a Reliable LC-MS/MS Method for Analysis of Five Steroids Simultaneously in Clinical Laboratory**

*Atecla Alves - Universidade de São Paulo/HCFMUSP (atecla@terra.com.br)*

- Our aim was to developed a LC-MS/MS method for 17-hydroxyprogesterone, androstenedione, 11-desoxycortisol, 21-desoxycortisol and cortisol quantification in serum using protein precipitation, isotopic internal standards and two dimensional liquid chromatography consisting of trapping column and reverse-phase C18 analytical column following atmospheric pressure chemical ionization and mass spectrometry detection. Functional sensitivity was less than 0.5 ng/mL, precision was less than 15%, recovery ranged from 93% to 120%, linearity ranged from 89% to 111%, accuracy was considered adequate for all compounds tested. In conclusion, we developed a suitable method for routine measurement of 17-hydroxyprogesterone, androstenedione, 11-desoxycortisol, 21-desoxycortisol and cortisol in serum.
Tuesday 5:00 PM
Poster #5 in Exhibit Hall
Measurement of Human Urinary Organophosphate Pesticide Metabolites in a Clinical Setting
Stephen Donovan - NMS Labs (stephen.donovan@nmslabs.com)
- Organophosphorus (OP) pesticides are among the most widely used and toxic pesticides used in the United States. Human exposure to these compounds can come from consumption of fruits and vegetables, applying the pesticides to crops, exposure to insecticides used inside homes, and even from broadcast spraying for mosquitoes. There are many compounds used in this class, and not everyone is exposed to all of them, but an analysis of urine for the six downstream OP metabolites is an effective way to assess the overall exposure to this class. In the case of agricultural workers, an analysis of their urine can assess the effectiveness of chemical hygiene procedures used during crop protection applications of these toxic compounds. A number of published approaches were examined and we also explored recent novel techniques to enhance the response of anions in the positive mode in LC/MS/MS.

Tuesday 2:00 PM
Poster #6 in Exhibit Hall
Multimodal Imaging Mass Spectrometry for Probing Aβ-Plaque Pathology
Jörg Hanrieder - Gothenburg University (jorg.hanrieder@chalmers.se) -- *Young Investigator Grantee*
- Alzheimer's disease is the most common neurodegenerative disorder affecting 12% over 65. The exact mechanisms underlying AD remain unknown but cognitive decline has been linked to formation of β-amyloid (Aβ) deposits as senile plaques. Changes in peptide truncation and plaque associated neuronal lipid species have been implicated with proteopathic mechanisms in AD. The aim of this study was therefore to employ SIMS and MALDI based imaging mass spectrometry (IMS) to probe Aβ plaque pathology in tgARCSWE mice with particular focus on associated neuronal lipid species and Aβ truncations. SIMS revealed plaque associated lipid species including sulfatides, triglycerides and cholesterol. MALDI IMS in turn revealed AD implicated Aβ peptide truncation (e.g. pyro-Glu). In conclusion, multimodal IMS is a powerful approach to interrogate chemical plaque pathology in AD.

Tuesday 5:00 PM
Poster #7 in Exhibit Hall
Rapid Detection of Microbial Resistance to Lactam Antibiotics by LC-MS/MS
Michael Jarvis - SCIEX (michael.jarvis@sciex.com)
- Rapid Detection of Microbial Resistance to Lactam Antibiotics by LC-MS/MS Michael Jarvis, SCIEX, 71 Four Valley Dr., Vaughan Ontario L4K 4V8 Current methods of assessing antibiotic resistance using turbidometric or disk diffusion methods to assess bacterial growth require prolonged incubation periods of up to 24 hours or more. A rapid detection strategy using multiplexed antibiotic mixtures incubated with bacteria for periods of 1 hour followed by antibiotic extraction and detection by electrospray LC-MS/MS yields comparable information in time periods as short as 90 minutes. The assay strategy includes quantitation of parent antibiotic levels and detection of metabolites produced by beta lactamases. Antibiotics screened in this manner include penicillin, ampicillin, amoxicillin, cloxacillin, piperacillin and cefotaxime in the presence and absence of tazobactam.

Tuesday 2:00 PM
Poster #8 in Exhibit Hall
Altered Adrenal and Gonadal Steroids Biosynthesis in Patients with Burn Injury
Maria Bergquist - Uppsala University (maria.bergquist@medsci.uu.se) -- *Young Investigator Grantee*
- Previous studies have reported gender differences in outcome following burn injury, suggesting that steroids may play a role. For this single-center, retrospective descriptive study, we used high-sensitivity liquid chromatography tandem mass spectrometry (LC-MS/MS) based steroid quantification to determine endogenous steroid concentrations in plasma samples of male patients with burn injury. Our data indicate that burn injury alters endogenous steroids biosynthesis, with decreased testosterone concentrations and elevated estrone concentrations during the first 21 days after the injury. Several of the measured steroids were found to correlate positively with the area of the burn injury. Further studies are needed to delineate the underlying mechanisms behind alterations in steroid biosynthesis after burn injury.
Separation and Quantification of Serum L- and D-2-hydroxyglutarate Enantiomers by LC-MS/MS Following Derivatisation
Laura Bernstone - University Hospital of South Manchester (laura.bernstone@nhs.net) -- *Young Investigator Grantee*

- The metabolite 2-hydroxyglutarate (2-HG) is normally present at low levels within cells and body fluids. It exists as two stereoisomers (L-2-HG and D-2-HG) due to an asymmetric carbon atom in the carbon backbone. Elevated 2-HG is found in inherited metabolic disorders and in malignancies such as acute myeloid leukaemia due to enzyme mutation, and it is important to differentiate between the stereoisomers in these cases. We have used the chiral derivatisation reagent DATAN (diacetyl-L-tartaric anhydride) to form diasteroisomers of L-2-HG and D-2-HG which we were then able to separate and quantify using an LC-MS/MS method.

LC-MS Analysis of Nicotine and Its Metabolites in Urine, Oral Fluid and Blood
Julie Cichelli - Agilent Technologies, Inc (julie_cichelli@agilent.com)

- Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. A sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of nicotine and its metabolites by QQQ. Using simple sample preparation techniques in urine, oral fluid and blood, and chromatographic configurations achieves the required sensitivity and separation and is capable of quantitating the drugs over their relevant dynamic range. Excellent reproducibility was observed for all drugs (CV < 15%). All calibration curves displayed linearity with an R2 > 0.995.

Converting a Liquid-liquid Extraction Method for Vitamin D to a 96-well Plate Supported-liquid Extraction Format: A Case Study with Real Patient Plasma Samples
Katerina Sadilkova - Seattle Children’s Hospital (katerina.sadilkova@seattlechildrens.org)

- The objective of this study was to transfer a validated liquid-liquid extraction method to a supported liquid extraction method to leverage advantages in reduced workflow and support a throughput of ~550 samples/month. During this evaluation, it was determined that sample processing time was reduced by ~50% for a de-identified patient set (n=30). To optimize the method to maximize analytes sensitivity, pH adjustment in the sample pre-treatment, elution volumes for extraction, and MRM transitions for analytes detection were evaluated. Once optimized and validated, method was applied to real patient sample sets. The samples were split and processed by each method for verification.

Sample Preparation of Three Steroids for Quantitative Determination by LC-MS/MS – Comparison of Two Extraction Procedures
Dave van Staveren - Tecan Schweiz AG (dave.vanstaveren@tecan.com)

- Two sample preparation procedures for the steroids testosterone, androstendione and 17a-hydroxyprogesterone using commercially available extraction plates were automated on a Liquid Handling System. The performance between a SLE-plate for supported liquid extraction and the AC Extraction Plate™, a deep well plate having wells coated with an absorptive material acting as the extraction phase, was compared. Plasma calibrators and controls as well as human plasma samples were used to determine linearity, accuracy, precision and LOD. The overall handling of both procedures on a Tecan Freedom EVO® liquid handling system was evaluated.
**Analysis of Sex Steroids in Urine by LC-MS/MS**  
*Maria Ospina - Centers of Disease Control and Prevention (mospina@cdc.gov)*

- Measurements of steroid panels in urine can provide information about steroid status and metabolism. We developed an automated isotope dilution LC-MS/MS method using solid phase extraction on a polymeric, reverse-phase sorbent to quantitate 12 steroids which includes progestagens, estrogens and androgens as well as some of their conjugates in human urine without derivatization. Steroids are separated on a C18 column and analyzed separately by electrospray ionization in the positive and negative ion mode for maximum sensitivity. The measurement range covers four orders of magnitude. The method is used to measure steroids in urine from men, women and children.

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**Comparison of 25-hydroxy Vitamin D Extraction Using Supported Liquid Extraction and Phospholipid Depletion Plate Technology Prior to LC-MS/MS Analysis**  
*Lee Williams - Biotage GB Limited (lee.williams@biotage.com)*

- Vitamin D analysis has extremely important clinical relevance. Many sample preparation approaches to the extraction of 25-hydroxy vitamin D have been employed prior to LC-MS/MS analysis. This poster compares the use of supported liquid extraction and a novel protein and phospholipid depletion plate, for the extraction of 25-hydroxy-vitamin D from serum. Optimum protocols were compared for recoveries, extract cleanliness, calibration line performance using PBS/BSA, calibrated serum samples and DEQAS external quality control samples. The extraction protocol was ultimately transferred to an SPE automation platform and method performance versus manual processing compared.

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**Triplex Tandem Mass Spectrometry Assays for Screening of 3 Lysosomal Storage Disorders in a Korean Population**  
*Sung Eun Cho - LabGenomics Clinical Laboratories (secho0824@gmail.com)*

- We evaluated the performance of triplex tandem mass spectrometry assays for screening of Pompe, Fabry, and Gaucher diseases with Acquity UPLC CSH C18 column (Waters, USA) and TQD triple quadrupole mass spectrometer (Waters, USA) in the multiple-reaction-monitoring mode. We evaluated the precisions, linearity, limit of detection, recovery, carryover, and ion suppression with 3 enzyme activities in 376 anonymous newborn dried blood spots. Intra- and inter-assay precisions were between 0% and 18.9%. The linearity of each enzyme activity was good (R2=0.9952, 0.9982, 0.9974, respectively). The lower limit of detection was 0.22 umol/h/L, 0.39 umol/h/L, 0.79 umol/h/L, respectively. The recovery and the carryover was good. There was no ion suppression. The performance for screening of 3 lysosomal storage disorders was acceptable in a Korean population.

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**Multiple Reaction Monitoring Assay for Identification of Borrelia burgdorferi**  
*Crystal Cheung - Institute for Bioscience and Biotechnology Research (cheung96@ibbr.umd.edu) -- *Young Investigator Grantee*|

- Lyme disease is caused by Borrelia burgdorferi. Currently, Lyme infection is identified by the presence of skin lesion called erythema migrans, and is later confirmed by immunological assays. However, such an approach has a low therapeutic value because delayed diagnosis may lead to serious complications. Herein we developed a mass spectrometric method to detect the presence of Borrelia burgdorferi using multiple reaction monitoring with a quantification concatamer as an internal standard. Two quantification concatamers containing signature peptides from the six most abundant Borrelia burgdorferi proteins have been constructed and will be used to improve the detection limit of Lyme infection.
Validation of an Automated Method to Remove β-Glucuronidase from Hydrolyzed Pain Management Urine Samples

Shahana Huq - Phenomenex (shahanaH@phenomenex.com)

- The escalating abuse of pain medicines has mandated the health care providers and practitioners to routinely perform urine drug testing. Most laboratories opt for a dilute-and-shoot approach, along with enzymatic hydrolysis that requires no sample clean up. The addition of extra protein to the urine can result in fouling of the LC column and loss of productivity. In this work, we present an automated method that uses an Impact Protein Precipitation plate to remove majority of the proteins from hydrolyzed urine.

HDL and Total Cholesterol Analysis by LDTD-MS/MS Analysis in 7 Seconds Per Sample with Cross-validation Data from a Clinical Laboratory Using Real Patient Serum Samples

Alex Birsan - Phytronix Technologies, Inc (a.birsan@phytronix.com)

- A fast and quantitative method for the measurement of Total and HDL cholesterol in human serum sample using the LDTD-MS/MS system. A liquid-liquid extraction is performed for total cholesterol and HDL isolated fraction. Organic upper layer is directly applied to the LazWell plate and dried prior to analysis. Standard method validation criteria are evaluated. All analyses were cross-validated and compared with an enzymatic coloration technique from an external clinical laboratory. The cross-validation results show low percentage differences between the two methods. Samples were analyzed in 7 seconds per sample using LDTD-MS/MS.

Preliminary Experience with the Waters Unispray™ Source

Brian Keevil - University Hospital of South Manchester (brian.keevil@uhsm.nhs.uk)

- We have tested the Waters Unispray™ source in a routine clinical laboratory for a number of applications including steroids, therapeutic drugs and plasma metanephrines. In the Unispray™ source the gas flow and droplets from a grounded nebuliser strike a rod target held at approximately 1kV upstream from the sample cone. The source has proved to be robust and we have seen a three fold increase in sensitivity for plasma metanephrines and sirolimus (pos). Gains in sensitivity are not seen for all compounds and we have seen no real improvement over the electrospray technique for testosterone, androstenedione or aldosterone.

Development of a Rapid Mass Spectrometry Based Screen of Tricyclic Antidepressants as an Alternative to Immunoassay Screening

Erin C. Strickland - Ameritox, Ltd. (erin.strickland@ameritox.com)

- While immunoassay is a prevalent screening technique it is also prone to issues such as high false positive rates due to lack of analyte specificity. Mass spectrometry was therefore investigated as an alternative screening technique for the ability to improve analyte specificity on a comparable time scale. In this study, a Rapid Online Sample Preparation and Injection method was developed using a commercially available guard cartridge on a conventional LC-MS/MS system. Using a two-point calibration curve to provide semi-quantitation, a robust method was developed and validated that improved upon the high false positive rate observed in immunoassay screening.

Analysis of Aldosterone in Plasma for Clinical Research Using Automated Extraction

Heather Brown - Waters Corporation (heather_a_brown@waters.com)

- Here we evaluate a newly developed UPLC-MS/MS method for the measurement of plasma aldosterone for clinical research purposes. An analytically sensitive method was developed using a mixed-mode Solid Phase Extraction (SPE) sorbent in 96-well plate format. Automated extraction was employed, enabling high throughput of samples. Analysis was performed using an ACQUITY UPLC® I-Class system, with samples injected onto a 2.1 x 100mm Waters CORTECS UPLC C18 column, separated using a water/methanol gradient and quantified with a WatersXevo® TQ-S mass spectrometer to obtain quantitative measurement of aldosterone at high sensitivity. For Research Use Only, Not for use in diagnostic procedures.
Tuesday 2:00 PM
Poster #22 in Exhibit Hall
**Improved Method for the Analysis of 31 Drugs of Abuse/Pain Management Panel in Oral Fluid Samples Using the Thomson EXTreme® Filter Vials by LC-MS/MS**
*Nadine Koenig - Health Networks (nadine.koenig@healthnetworklabs.com)*

- The goal of this study was to improve the sample preparation for the analysis of drugs of abuse in oral fluids. Oral fluid samples were collected with Intercept® i2he™ Oral Fluid Collection Devices. The samples were prepared using eXtreme|FV®, followed by LC/MS/MS analysis. The most critical aspects of reliable analysis are the reduction of interferences from the sample matrix and analyte recovery. Traditionally, SPE, SLE and centrifugation have been used to reduce matrix interference. These techniques are time consuming, adversely impact recovery, use large amounts of solvent and are expensive. The improved method allows for the analysis of 31 drugs.

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Tuesday 5:00 PM
Poster #23 in Exhibit Hall
**Multi-mode Desorption Electrospray Ionization (DESI) Mass Spectrometry Imaging at Different Pixel Resolutions for Human Tissue Imaging**
*Khalid Khan - Waters Corporation (khalid_khan@waters.com)*

- DESI a surface analysis technique using an electrospray probe, can be used as a spatially resolved imaging technique. The droplets impact upon the surface, with ionization occurring due to the charge imparted onto the droplets and requires no modifications to the sample by matrix addition. The profile of the spray on the surface affects the spatial resolution and modification can be achieved using different gas & solvent flow rates, affecting the spatial resolution. DESI does not destruct the tissue surface and the tissue section can be re-analyzed under different conditions or techniques. Here we describe the workflow that allows one tissue image profile to be rapidly acquired at low spatial resolution, followed by the analysis of a region of interest at a higher spatial resolution and finally the same tissue section being H&E stained. Example of human tissue sections will be presented.

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Tuesday 2:00 PM
Poster #24 in Exhibit Hall
**High Throughput Pulse-chase Analysis of Metabolite Turnover in Microorganisms Followed by LAESI Mass Spectrometry**
*Sylwia Stopka - The George Washington University (stopka@gwmail.gwu.edu) -- *Young Investigator Grantee*

- Metabolic fluxes and the pools of the associated chemical species are closely linked to their turnover in a living organism. Pulse-chase analysis, using stable isotope labeling, has been applied to monitor dynamic changes in metabolite buildup and decay. The lipid metabolism of Chlamydomonas reinhardtii has been extensively studied using conventional extraction and separation techniques. Here, we introduce a high-throughput method for pulse-chase analysis based on laser ablation electrospray ionization (LAESI) mass spectrometry (MS) with ion mobility separation (IMS). Simultaneous detection, identification, and quantitation of some molecular constituents, e.g., metabolites, lipids, and peptides, in a microorganism can be achieved within minutes. Pulse-chase analysis by LAESI-IMS-MS can be applied to follow changes in metabolic fluxes during disease.

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Tuesday 5:00 PM
Poster #25 in Exhibit Hall
**A Data Independent LC-MS Based Method for a Multi-omic Approach to Investigate Obesity Treatment Within a Mouse Model**
*Giuseppe Astarita - Waters Corporation (giuseppe.astarita@waters.com)*

- Obesity is one of the risk-factors associated with metabolic syndrome. Pharmacological manipulation of glycosphingolipid metabolism has been shown to improve the symptoms of metabolic syndrome. A global view of the alterations in the proteome, metabolome and lipidome during development is still to be fully explored. The work presented here is to provide a multi-omic analysis of protein and lipid liver extracts from control and obese mouse models undergoing treatment to prevent or revert obesity. Integrated pathway analysis was used to review the complimentary datasets and provide an understanding of the underlying biology of differentially expressed proteins and lipids.
Tuesday 2:00 PM
Poster #26 in Exhibit Hall
Desorption Electrospray Ionization (DESI) for Tissue Imaging on a Time-Of-Flight (TOF) Mass Spectrometer
Darwin Asa - Waters Corporation (darwin_asa@waters.com)

DESI mass spectrometry imaging, when used with a TOF mass spectrometer, is a very effective technique that determines the spatial localization and molecular distribution of molecules within a variety of samples. It can be applied to animal and human thin tissue sections and be utilized to differentiate tissue types or study the distribution of molecules within tissues. In this study, good sensitivity for a wide range of lipid and small molecule metabolites from tissues was achieved in positive and negative mode, allowing a more comprehensive understanding of the sample by imaging the sample using a TOF mass spectrometer equipped with DESI.

Tuesday 5:00 PM
Poster #27 in Exhibit Hall
A UPLC-MS/MS Method for the Analysis of Plasma Mycophenolic Acid for Clinical Research
Michelle Wills - Waters Corporation (michelle_wills@waters.com)

Here we present a UPLC-MS/MS method to measure mycophenolic acid in human plasma for clinical research purposes. An analytically sensitive method across the range 0.1 – 20 µg/mL was developed using protein precipitation extraction (PPE), providing a fast and cost effective technique. The method demonstrates good linearity, precision (< 10% CV) and accuracy (≤ 5.1% bias). For Research Use Only, not for use in diagnostic procedures.

Tuesday 2:00 PM
Poster #28 in Exhibit Hall
A High Throughput LC-MS/MS Method for the Analysis of Multiple Vitamin D Analytes in Serum and Placenta Using Supportive Liquid-liquid Extraction
Carl Jenkinson - University of Birmingham (C.Jenkinson@Bham.ac.uk) -- *Young Investigator Grantee*

Serum analysis of 25OHD3 is routinely used to define patient vitamin D status. Quantification of multiple vitamin D metabolites may provide more accurate definitions of vitamin D status for specific patients and disease scenarios. The aim of this study was to develop an LC-MS/MS method for multiple Vit-D metabolites in serum and placenta. This method ensures accurate 25OHD3 measurements, separating inactive C-3 epimers and isobar interferences. LC-MS/MS analysis was carried out using a Waters AQUITY UPLC coupled to a XEVO TQ-S detector. Separation of multiple vitamin D analytes was achieved in a 9 minute run time and optimised sample preparation.

Tuesday 5:00 PM
Poster #29 in Exhibit Hall
Development of a Consolidated LC-MS/MS Assay for Quantification of Voriconazole, Posaconazole and Teriflunomide
Pratistha Ranjitkar - University of Washington (prati@uw.edu) -- *Young Investigator Grantee*

Clinical laboratories are increasingly challenged with decreasing test reimbursements and the concomitant need to generate high quality patient results at lower cost. The consolidation of multiple tests onto a single platform or workflow can increase productivity and lead to reduced expense. The aim of this work is to develop a single LC-MS/MS method to quantify voriconazole and posaconazole, anti-fungal agents commonly used in immunocompromised patients, as well as teriflunomide, a drug with both immunosuppressive and anti-viral properties used in renal transplant patients. We present the data for voriconazole and are working toward adding posaconazole and teriflunomide.
Tuesday 2:00 PM
Poster #30 in Exhibit Hall
High-throughput Targeted Screening and Definitive Method for Barbiturate Drugs in Urine Using LDTD-MS/MS with Ultra-fast Analysis at 9 Seconds Sample to Sample
Pierre Picard - Phytronix Technologies, Inc (p.picard@phytronix.com)

- An ultra-fast quantitative targeted screening and a definitive confirmation method for the detection of Barbiturates drugs in urine have been developed for the LDTD-MS/MS system. Extraction is a liquid-liquid procedure at acidic pH. Organic upper layer is applied to the LazWell plate and dried prior to analysis. Mass spectrometer with ion mobility system is used for adequate isobaric drug resolution (amobarbital and pentobarbital) as confirmation method. Standard method validation was performed. All analyses were cross-validated with a LC-MS/MS method. Cross-validation results show low percentage differences between the two methods. All samples on LDTD-MS/MS were analyzed at 9 seconds per sample.

Tuesday 5:00 PM
Poster #31 in Exhibit Hall
Rapid and Robust Plasma Proteomics Platform for Clinical Settings
Philipp Emanuel Geyer - Max Planck Institute of Biochemistry (geyer@biochem.mpg.de)

- Mass spectrometry (MS)-based proteomics should in principle be an ideal technology to discover disease indicators in the blood plasma proteome. Recent developments in our group such as the ‘in StageTip’ (iST) sample-preparation workflow and optimized high-throughput LC-MS measurement technologies enable fast and multiplexed sample processing with very high reproducibility (R² ≥ 0.99) and low coefficients of variation (CV < 20 %) for the majority (81 %) of the top 200 quantified proteins. We anticipate that these developments will facilitate MS-based blood plasma proteomics for routine, quantitative analysis of patient samples in clinical settings.

Tuesday 2:00 PM
Poster #32 in Exhibit Hall
Comparison of Different Liquid-liquid Sample Preparations for LC-MS/MS Assays of Total Serum Testosterone Measurements
Yuyong Ke - EndoCeutics, Inc (yuyong.ke@endoceutics.com)

- The removal of protein binding and phospholipids is usually carried out in two steps in the reference method of total testosterone measurements. In the present study, a simpler approach of one-step liquid-liquid extraction is examined against the reference procedure. QCIs and unknown samples were compared in terms of interference, accuracy, recovery and lipid removal. The difference of accuracies of QCs and values of unknown samples between the simple procedure and the reference one is less than 5%. This simple procedure could replace the reference procedure for the accurate measurement of testosterone in human serum.

Tuesday 5:00 PM
Poster #33 in Exhibit Hall
A Hybrid Approach to Proteomic Sequencing of Immunoglobulins
Natalie Castellana - Digital Proteomics LLC (natalie@digitalproteomics.com)

- Primary sequence determination of an antibody is a precursor to accurate characterization. When the source cell is unavailable, direct proteomic sequencing is the only option. Unlike other proteomic applications, no comprehensive database of antibody sequences exists. We utilize a hybrid approach that combines the strengths of database-mediated peptide identification and de novo protein sequencing in order to sequence an antibody and determine sites of common post-translational modifications. In this study we demonstrate how new acquisition modes that combine CID, ETD, and HCD fragmentation can improve sequencing accuracy. In addition, we expand on our algorithm to sequence a mixture of a heavy and light chain as well as a mixture of three monoclonal antibodies.
Comparison of Non-derivatization and Derivatization Tandem Mass Spectrometry Methods for Analysis of Amino Acids and Acylcarnitines in Dried Blood Spot

Xiaolei Xie - Thermo Fisher Scientific (xiaolei.xie@thermofisher.com)

- Flow injection tandem mass spectrometry has been frequently used to analyze amino acids (AA), acylcarnitines (AC), and succinylacetone (SUAC) in dried blood spots. While butyl esterification method is routine, it is possible to detect AAs, ACs, and SUAC as their native free acids. Using quality control DBS samples, we conducted a comprehensive study to evaluate and compare non-derivatization and derivatization methods on a triple quadrupole mass spectrometer. 12 AAs, 18 ACs, and SUAC were detected and quantified. Massive data analysis involving 26,000 calculations were performed using a streamlined meta-calculation software. Both methods had excellent analytical precision (within-run: <10%; run-to-run, <15%). Minor differences (<15%) between quantitative values resulting from derivatization and non-derivatization methods were observed for the majority of the analytes.

Paper Spray Ionization - Tandem Mass Spectrometry for Quantification of Prescription Drugs in Oral Fluid

Karen Cesafsky - Purdue University (kcesafsk@purdue.edu)

- Paper Spray (PS) ionization is an ambient technique which creates ions in a mechanism similar to electrospray ionization. When coupled with a triple quadrupole mass spectrometer, quantification of analytes in a complex matrix can be achieved with no time consuming sample preparation or chromatography. This automated 3 minute multiple reaction monitoring method was used to quantitatively measure opioids, benzodiazepines, and tranquilizers at ng/mL levels in <10µL saliva. This PS method may be adapted for other biological matrices and the speed and simplicity of the technique is ideal for point of care diagnostics, roadside drug testing and many more clinical applications.

Swab Touch Spray - Mass Spectrometry for Direct Analysis of Bacteria and Drugs in Oral Fluid

Karen Cesafsky - Purdue University (kcesafsk@purdue.edu)

- Touch spray mass spectrometry (TS-MS) is an ambient ionization technique in which a sample is transferred to a probe where ionization occurs via electrospray-like mechanisms. The probe can be metallic or a medical swab, the latter having potentials for direct and in vivo oral fluid analysis. Here we present oral fluid testing by TS-MS to detect Streptococcus pyogenes, the leading cause of strep throat, and drugs of abuse, for clinical-toxicological applications. The approach outlined is intended for point-of-care testing using oral fluid in clinical-toxicological applications. Proof-of-concept results presented will require extension and refinement in analytical procedures to meet clinical/legal requirements.

Determination of Plasma Catecholamines by LC-MS/MS for Clinical Research

Linda Cote - Agilent Technologies (linda_cote@agilent.com)

- Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is ideally suited for the rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS method has been developed for the quantitation of catecholamines (dopamine, epinephrine and norepinephrine). An efficient sample preparation procedure by filtration on Captiva NDLipids and by solid phase extraction (SPE) allows simultaneous extraction of all analytes in plasma. The described method achieves the required functional sensitivity and is capable of quantitating analytes over a wide dynamic range with a single injection. Excellent reproducibility was observed (CV < 5%). All calibration curves displayed linearity with an R² > 0.9997.
**Tuesday 2:00 PM**

**Poster #38 in Exhibit Hall**

**Improved Detection of 17ß-Estradiol and Estrone in Serum Through Derivatization with Dansyl Chloride Utilizing LC-MS/MS Technology**

*Andre Szczesniewski - Agilent Technologies (andre_szczesniewski@agilent.com)*

- Sub-picogram levels for determination of 17ß-estradiol and estrone presents several challenges for traditional analysis of the molecule. Molecular similarities leave some methods susceptible to cross-reactivity with other steroids, leading to poor analytical accuracy. A lack of highly ionisable functional groups also poses a challenge for analysis by mass spectrometry. Through the use of high-end triple detection and derivatization, detection limit was improved to the point that 17ß-estradiol and estrone can be quickly and accurately quantified down to 0.5 pg/mL using an LC/MS/MS approach.

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**Tuesday 5:00 PM**

**Poster #39 in Exhibit Hall**

**An Improved Platform for the Recovery and Analysis of Cannabinoids from Dried Blood Samples**

*James Hill - Spot On Sciences (jameshill@spotonsciences.com)*

- In order to quantitate three cannabinoids; delta-9 tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) from dried blood spots (DBS), we examined the recoveries from four platforms routinely used in dried blood spot collections; 903™ Card, DMS™ Card, HemaSpot-HF™ and HemaSpot-SE™. While recovery was poor (40-60%) from cotton fiber based absorbents, 80% could be recovered from the glass fiber membrane-based HemaSpot-SE™, which separates blood cells from plasma. Cannabinoid levels from wet plasma were equal to levels found from the dried plasma portion of the HemaSpot-SE™ (99% correlation).

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**Tuesday 2:00 PM**

**Poster #40 in Exhibit Hall**

**Determination of Plasma Renin Activity by LC-MS/MS for Clinical Research**

*Linda Cote - Agilent Technologies (linda_cote@agilent.com)*

- A highly sensitive and specific LC/MS/MS method has been developed for the determination of plasma renin activity for clinical research. Plasma samples are incubated for 3 hours-37oC. A sample preparation procedure by solid phase extraction (SPE) allows efficient extraction of Angiotensin I in plasma. Plasma renin activity is calculated by subtracting Angiotensin I concentration in a blank plate. The described method achieves the required functional sensitivity and is capable of quantitating Angiotensin I over a wide dynamic range with a single injection. Excellent reproducibility was observed (CV < 6%). All calibration curves displayed linearity with an R2 > 0.9996.

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**Tuesday 5:00 PM**

**Poster #41 in Exhibit Hall**

**Effect of Sodium and Carbon on Analytical Determination of Ultratrace Metals in Bio Specimens by ICP-MS**

*Key-Young Choe - California Department of Public Health (Key-Young.Choe@cdph.ca.gov)*

- Disparity between composition of external calibration standards and bio specimens, like blood or human plasma, is a main impediment in accurate determination of metals at ultratrace level by ICP-MS. We have overcome the problem by addition of 1% of NaCl to the intermediate calibration standards and use of at least 3% n-butanol in the diluent as a source of carbon. The addition of carbon is necessary to maximize elemental signal enhancement due to charge transfer from C+ ions to the analytes and internal standards, while Na+ provides a matrix-match effect with the inorganic easy ionized elements present in the bio specimens.
Tuesday 2:00 PM
Poster #42 in Exhibit Hall
Measurement of Urinary Serotonin for Clinical Research, Using Mixed Mode SPE and a High-strength Silica PFP Column
Sherri Naughton - Waters Corporation (sherri_naughton@waters.com)
‣ A single extraction and analysis method has been developed for urinary serotonin for clinical research. Serotonin was extracted using weak cation exchange mixed-mode SPE and analyzed by LC/MS/MS. Recoveries were good and matrix effects were minimal for most compounds. Linearity and QC results were excellent, with % CV and bias values less than 3% at all QC points. This method enables rapid, simultaneous and accurate LC/MS/MS analysis of these challenging compounds without the challenges associated with traditional reversed-phase separation or ion-pairing techniques. For Research Use Only, not for use in diagnostic procedures.

Tuesday 5:00 PM
Poster #43 in Exhibit Hall
The Analysis of Fentanyl and Its Analogues in Human Urine by LC-MS/MS
Landon Wiest - Restek Corporation (Landon.Wiest@restek.com)
‣ Synthetic opioid drugs, such as fentanyl and sufentanil, have very high analgesic potency. Abuse of these prescription opioids and their illicit analogue, acetyl fentanyl, is a growing public health problem. In this study, a simple dilute and shoot method was developed with an analysis time of less than 3.5 minutes for fentanyl, norfentanyl, acetyl fentanyl, and sufentanil in human urine by LC-MS/MS using the Raptor™ Biphenyl column.

Tuesday 2:00 PM
Poster #44 in Exhibit Hall
Detection of Ethyl Glucuronide and Ethyl Sulfate in Urine by Hydrophilic Interaction Liquid Chromatography (HILIC)-MS/MS
Maricar Dube - EMD Millipore (maricar.dube@emdmillipore.com)
‣ Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are biomarkers of alcohol abuse. LC-MS/MS methods that detect EtG and EtS usually employ reversed phase columns. But EtG and EtS are very polar metabolites, making retention in reversed phase challenging. Polar hydrophilic compounds that are difficult to separate in reversed phase columns are separated on HILIC columns. This paper demonstrates that a zwitterionic HILIC column can be used for EtG and EtS detection in urine (ESI-ion trap detection). The method developed produced sharp peaks and achieved baseline separation in less than 7 min. A lower limit of quantitation 1 lg/L was achieved.

Tuesday 5:00 PM
Poster #45 in Exhibit Hall
A Rapid and Sensitive LC-MS/MS Method for the Analysis of Free Thyroid Hormones
Shun-Hsin Liang - Restek Corporation (shun-hsinliang@restek.com)
‣ The unbound or “free” thyroxin (T4) and tri-iodothyronine (T3) are the active forms of thyroid hormones which only exist at low levels (pg/mL) in the circulation. The measurement of these free hormones is necessary to assess thyroid function for both veterinary and human clinicians. A fast, accurate, and sensitive method was developed for the analysis of thyroid hormones at the free form levels using the highly efficient and selective Raptor™ Biphenyl column. The clinical applicability of the method was demonstrated by analyzing the fortified thyroid hormone in phosphate buffer saline containing 4% human albumin.

Tuesday 2:00 PM
Poster #46 in Exhibit Hall
Metformin Treated Wistar Rats Demonstrate Remarkable Alterations in Lipid and Bile Acid Plasma Levels
Panagiotis Vorkas - Imperial College London (p.vorkas09@imperial.ac.uk) -- *Young Investigator Grantee*
‣ Type 2 diabetes is one of the greatest threats of public health. The beneficial effects of metformin towards mitigating disease manifestation have been connected to gut microbiota and inflammation onset. In total, 12 animals were treated with Metformin for 5 weeks with 12 used as controls. Using UPLC-TOF-MS methodologies, highly significant alterations were detected for the levels of pro- and anti-inflammatory lipid precursors in plasma samples, and of sulfated and glucosylated bile acids in plasma and liver tissue. This study may provide insights of the pathways able to function beneficially for diabetic patients, as well as of blood markers reflecting modifications in gut microbiota.
**Tuesday 5:00 PM**
**Poster #47 in Exhibit Hall**
**LC-MS/MS Measurement of Urinary 2,3-dinor-11β-Prostaglandin F2α in Patients with Systemic Mastocytosis: Improved Diagnostic Accuracy Compared to ELISA**

*Alan Lueke* - *Mayo Clinic Rochester* (lueke.alan@mayo.edu)

- Systemic mastocytosis (SM) results in the accumulation of mast cells in tissues. Currently, urine concentrations of 11-β-prostaglandin F2α (BPG) are measured by ELISA and used for screening and monitoring of SM. Comparison with a novel LC-MS/MS method found no measurable BPG in urine specimens despite measureable immunoassay values. However, ELISA BPG concentrations did correlate with the LC-MS/MS measured 2,3-dinor isoform of BPG. In a clinical study of 203 patients LC-MS/MS analysis of 2,3-BPG improved clinical sensitivity and specificity for SM detection over the immunoassay both alone, and when combined with other urinary markers of SM.

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**Tuesday 2:00 PM**
**Poster #48 in Exhibit Hall**
**Rapid Discovery of Differentially Expressed Proteins in T2D Plasma Samples Using Improved UHPLC Chromatography and PSMART Data Acquisition**

*Scott Peterman* - *Thermo Fisher Scientific BRIMS* (scott.peterman@thermofisher.com)

- Translational clinical proteomics links global protein discovery to targeted quantification with the ultimate goal of identifying and verifying clinically relevant disease biomarkers. Studies incorporating LC-MS can identify thousands of proteins and peptides per study requiring automated data processing to increase data interrogation. To better facilitate commercially available software, higher flow rates utilizing UHPLC pumps significantly increases the LC reproducibility. Incorporation of pSMART data acquisition results in significant increase in detection/sequencing reproducibility and better relative quantitation. We applied these new workflows to investigate protein differences associated with type 2 diabetes (T2D).

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**Tuesday 5:00 PM**
**Poster #49 in Exhibit Hall**
**A Rapid Sample Preparation Method for Quantitative Analysis of Cortisol in Saliva and Urine by LC-MS/MS**

*Daniel Zhou* - *Stanford Health Care* (DaZhou@stanfordhealthcare.org)

- Late night saliva and 24-hour urine cortisol are among the initial screening tests for cortisol excess [i.e. Cushing’s syndrome or disease]. Early morning saliva cortisol may be used to assess and rule out adrenal insufficiency [i.e. Addison’s syndrome]. Since conventional LC-MS/MS approach requires lengthy manual sample preparation by liquid-liquid extraction [LLE] or solid phase extraction [SPE], we investigated commercially available dispersive pipette extraction tips [DPX tips] to shorten this preparation time. Results were comparable with a LC-MS/MS method in clinical use and may reduce sample preparation by 80%, making it much more suitable for a clinical diagnostic lab.

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**Tuesday 2:00 PM**
**Poster #50 in Exhibit Hall**
**Proteomic and Metabolic Changes in Malnutrition**

*Evelyn Gitau* - *Kemri Wellcome Trust Research Programme* (egitau@kemri-wellcome.org) -- *Young Investigator Grantee*

- Evidence suggests that the risk of death from infections in children with severe acute malnutrition may be sustained after apparent medical stabilization and nutritional rehabilitation. Biomarkers may allow identification of children at highest risk as well as identify physiological processes involved, thereby guiding development of new intervention strategies to reduce mortality in malnourished children. Using mass spectrometry we assessed the relationship between plasma protein and metabolite levels and subsequent survival of severely malnourished Kenyan children enrolled in a clinical trial of daily antimicrobial prophylaxis in severe malnutrition after apparent medical stabilization. Preliminary results will be presented.
High Throughput Determination of Multiple Drugs in Plasma and in Blood Using Solid Phase Microextraction
Nathaly Reyes-Garces - University of Waterloo (nreyesga@uwaterloo.ca) -- *Young Investigator Grantee*

- Solid phase microextraction in high throughput thin film format (TF-SPME) has demonstrated its potential and usefulness as sample preparation tool in multiple areas of application, including the bioanalytical field. In this study, an analytical protocol for the determination of multiple prohibited drugs in plasma samples using TF-SPME and liquid chromatography-tandem mass spectrometry (LC-MS/MS) is presented. Additionally, SPME devices prepared on an alternative material as a support are introduced and evaluated by extracting selected drugs from whole blood. Overall, results herein presented demonstrate the great convenience of SPME as sample preparation tool in the clinical lab.

Comparison of SDVB-Monolithic & Bead-based Columns Used in Nanoflow LC-MS for Proteomic Study
Sung-Fang Chen - National Taiwan Normal University (sfchen@ntnu.edu.tw)

- NanoLC-ESI-MS/MS is a powerful tool in proteomic analysis. The preparation conditions including the choice of monomer, inside diameter of capillary, and porogen is optimized. The performance of polymeric column was also compared and evaluated with micro particle-filled capillary columns, including a totally porous silica C18 column and a HALO® fused core C18 column. With all optimized conditions, the monolithic capillary column was prepared by in-situ polymerization of styrene and divinylbenzene (SDVB) inside a 4 meter-long, 50 µm i.d. fused silica capillary. This continuous unitary porous structure has more robust and high separation efficiency when comparing with the bead-based columns. The characterization of this novel monolithic media will be a promising addition to the stationary phase used in capillary column for proteome research.

Strategies for the Direct Coupling of Solid Phase Microextraction (SPME) to Mass Spectrometry: Applications in the Clinical Lab
Germán Augusto Gómez-ri os - University of Waterloo (gagomezr@uwaterloo.ca) -- *Young Investigator Grantee*

- In this work we introduce two novel strategies for the direct coupling of SPME devices to MS for trace analysis of target compounds: coated blade spray (CBS) and solid phase microextraction-transmission mode (SPME-TM). Contrary to the general believes that sample prep is slow, analyte-enrichment and sample-clean-up is performed in 1 minute, and the total analysis time does not exceed 3 minutes in both approaches. Detection limits at the low ppt levels are feasible in complex matrices of clinical relevance. Given the structural configuration of the apparatus, sample prep can be used to perform extractions independently of the sample complexity or its dimensions.

Evaluation of a Method for Forensic Quantitative Screening of Over 120 Drugs of Abuse on a Triple Quadrupole Mass Spectrometer
Kristine Van Natta - Thermo Fisher Scientific (kristine.vannatta@thermofisher.com)

- A liquid-liquid extraction scheme (LLE) was developed for analyzing a wide range of compounds in human urine in a forensic toxicology setting. Samples preparation was enzymatic hydrolysis followed by LLE using commercially available extraction tubes. All samples were analyzed on a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer. The method was tested with over 100 compounds from a wide chemical space including polar and non-polar compounds as well as positively and negatively ionizing compounds. Limits of quantitation (LOQ), LLE recoveries, precision, and matrix effects were determined. LOQs met forensic toxicology requirements for 95% of the compounds tested.
Simple Sample Preparation for Measuring Methylmalonic Acid in Blood Serum by LC-MS

**Joseph Di Bussolo** - Thermo Fisher Scientific (joe.dibussolo@thermofisher.com)

Many researchers need to measure methylmalonic acid (MMA) in blood serum to assess vitamin B12 sufficiency. Most perform sample preparations that involve laborious and expensive solid-phase or liquid extraction followed by derivatization to form the butyl ester. Butyl-MMA can then be measured by LC-MS or GC-MS. We developed a simpler approach involving protein precipitation with acetonitrile followed by evaporation of the supernatant. After the residue was butylated and the remaining butylation reagent was evaporated, the residue was reconstituted with a water/methanol diluent and subjected to a five-minute LC-APCI-MS/MS method reliably achieving a quantitative range of 50 to 1000 nM.

Personalized Chemotherapy Through the Combination of Microdosing and Accelerator Mass Spectrometry

**Maike Zimmermann** - University of California, Davis (maike.zimmermann@ucdmc.ucdavis.edu) -- *Young Investigator Grantee*

Platinum-based chemotherapy is the primary therapeutic intervention for over 300,000 cancer patients per year in the US. Although some patients are cured by this type of chemotherapy, 50-70% of patients show no benefit and only suffer the side effects. Currently, there is no diagnostic test that predicts therapy response towards these therapeutics before the treatment is started. These compounds are DNA alkylating agents that kill cancer cells through formation of DNA-adducts. We are developing a predictive test for cancer patient’s response to platinum-based chemotherapy drugs. The technology utilizes the ultra-sensitive accelerator mass spectrometry method to measure drug-DNA adduct frequency.

Online Analysis of Immunosuppressants in Whole Blood with the Evoq Triple Quad

**Rafaela Martin** - Bruker Daltonik GmbH (rafaela.martin@bruker.com)

A robust and reliable research method to quantitate the immunosuppressants cyclosporine A, tacrolimus, sirolimus and everolimus in whole blood samples using the Bruker Advance UHPLC with OLE coupled to the EvoQ Elite triple quad is demonstrated. The integrated online extraction option of the UHPLC together with the ClinMass® LC-MS/MS complete kit (MS11000) provides fast and easy sample cleanup. Interlacing the online extraction and chromatographic separation reduces the overall run time to 3 minutes per sample. The calibrations showed excellent linearity (r²≥0.997) and the assay had a very good precision with RSD <9% as well as high accuracy with bias <±6.5%.

Fast and Confident Identification of Drugs and their Metabolites Using Ion Trap LC-MSn Analysis and a Library of >4,500 Compounds

**Rafaela Martin** - Bruker Daltonik (rafaela.martin@bruker.com)

Comprehensive screening of urine samples in forensic toxicology and clinical research is focused on the unambiguous identification of parent drugs and their corresponding metabolites. Here, we present the evaluation of a comprehensive and robust forensic toxicology ion trap based LC-MSn spectral library screening to detect and confirm both parent drugs and metabolites in urine as alternative or complementary technology to GC-MS. Acquired data (full scan MS, MS2 and MS3) were searched against the Toxtyper library (900 compounds) and the recently published Maurer/Wisenbach/Weber (MWW, Wiley-VCH, Weinheim, Germany, 2014) LC-MSn library which contains > 4500 compound entries including 3000 metabolites.
Tuesday 5:00 PM
Poster #59 in Exhibit Hall

A Novel High Resolution Metabolite MS/MS Spectral Library for Accurate Metabolite Identification in Human Biofluids

**Annette Michalski** - *Bruker Daltonics* (annette.michalski@bdal.de)

- Identification of metabolites in human biofluids remains a major analytical challenge that we here address in a library-based approach. We describe a workflow for rapid and accurate metabolite profiling based on the use of a high resolution spectral MS/MS library of about 800 metabolites selected from the Human Metabolome Database (http://www.hmdb.ca/).

Tuesday 2:00 PM
Poster #60 in Exhibit Hall

Phosphoproteomic Analysis by HAMMOC Enrichment and LC-MS/MS

**Ting-Yu Wei** - *National Taiwan Normal University* (a67586danielwei@gmail.com)

- The combination of HAMMOC enrichment and LC-MS/MS analysis were employed for the phosphoproteome analysis in this study. A total of 2953 phosphopeptides and 1231 phosphoproteins were found in milligram proteins of RAW264.7 cell lysate by combining two desalting methods coupled with HAMMOC-sIEF approach. The use of centrifugal filter and HAMMOC coupled with three various fractionation strategies, including sIEF;BSCX and HILIC, had shown good complementarity (86.8% more phosphopeptide identifications than SCX only). Finally, 114 phosphoproteins were found differentially expressed between control and lipopolysaccharide-treated RAW264.7 from 200 microgram proteins by using label-free comparative approach after HAMMOC and LC-MS/MS. Results indicate that HAMMOC followed by LC-MS/MS analysis is suited for the in-depth analysis of qualitative and quantitative phosphoproteome profiling.

Tuesday 5:00 PM
Poster #61 in Exhibit Hall

An Illustrative Example of the Need for Ongoing Clinical Microbiology Competency in the Era of MALDI-TOF MS Microorganism Identification: Neisseria spp.

**Neil Anderson** - *Washington University School of Medicine* (NAnderson@path.wustl.edu) -- *Young Investigator Grantee*

- We report the case of a penile lesion culture containing Neisseria cinerea misidentified as Neisseria meningitidis by MALDI-TOF MS. To further investigate this phenomenon, four characterized isolates, including the patient isolate of N. cinerea, a laboratory isolate of Neisseria mucosa, and ATCC strains of Neisseria lactamica, and Neisseria sicca were analyzed at various days growth by the Bruker MALDI Biotyper (Bruker, Billerica, MA) and the VITEK MS (bioMérieux, Marcy l’Etoile, France). N. meningitidis was the first or second match for three isolates during at least one day tested by the Bruker Biotyper. These isolates were correctly identified by VITEK MS. N. sicca failed identification on both platforms, and on Day 5 was misidentified as N. mucosa by VITEK MS. These findings suggest MALDI-TOF MS may not be an accurate stand-alone identification method for Neisseria species.

Tuesday 2:00 PM
Poster #62 in Exhibit Hall

Differential Proteomic Analysis of PLC/PRF/5 Cell Lines Treated with Various Anti-cancer Drugs by iTRAQ Labeling and Mass Spectrometry

**Shih-Hua Huang** - *National Taiwan Normal University* (happykim056@gmail.com)

- Sorafenib, sunitinib and tivozanib are oral small molecular VEGF receptor tyrosine kinase (RTK)-targeted drugs, and two of them are approved to treat anti-angiogenesis. The iTRAQ technology was used to investigate the protein profiles in PLC/PRF/5 cell lines treated with these three anti-cancer drugs. A total of 11233 unique peptides were identified which were associated with 2010 proteins in two biological replicate experiments. Among them, 187, 112 and 128 differential expressed proteins were selected for Metacore analysis. They are found to be associated with oxidative phosphorylation, cytoskeleton remodeling and transcription role of AP-1 in regulation of cellular metabolism.
Tuesday 2:00 PM  
Poster #63 in Exhibit Hall  

**Catecholamine Analysis: Evaluation of Method Optimization to Improve Sensitivity and Reduce Limits of Quantitation Using LC-MS/MS**  
*Adam Senior* - *Biotage GB Limited* (adam.senior@biotage.com)  
- Catecholamines are classic biomarkers for the detection of diseases like hypertension, pheochromocytoma and neuroblastoma. The main target analytes are epinephrine, norepinephrine and dopamine and are traditionally analyzed using liquid chromatography with electrochemical detection. This poster investigates various parts of the method development process to evaluate the sensitivity of LC-MS/MS analysis. A highly sensitive LC/MS system, a Shimadzu Nexera UHPLC coupled to an SCIEX 5500 triple quadrupole MS was used for analysis. Method sensitivity in terms of pre-cursor ion selection and MRM transitions, chromatography and solid phase extraction protocols were all evaluated for increased sensitivity.

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Tuesday 2:00 PM  
Poster #64 in Exhibit Hall  

**Analysis of COL6A Proteins as a Potential Therapeutic Marker for Ullrich Muscular Dystrophy and Bethlem Myopathies**  
*Sunhee Jung* - *Seattle Children's Hospital Research Institute* (sunhee@uw.edu)  
- Abnormalities in the extracellular matrix are common in human muscular dystrophies (MD). Mutations in COL6A are prevalent causes of congenital MD such as Ullrich MD, Bethlem myopathies and yet poorly defined intermediate form. Most of the COL6A mutations lead to altered extracellular deposition of COL6A; thus COL6A proteins have enormous potential in monitoring and evaluating efficacy of new therapeutic trials for COL6A-related MD. Approaches to accurately access the level of COL6A in cell-based assays are critically needed. Here, we propose that a LC-SRM-MS assay can detect the cellular level and extracellular release of COL6A in human fibroblasts and cultured media.

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Tuesday 5:00 PM  
Poster #65 in Exhibit Hall  

**Clinical and Forensic Monitoring of Zopiclone Through the Use of a Degradation Product**  
*Anna Miller* - *MedTox Laboratories, LabCorp* (milla20@labcorp.com)  
- The clinical and forensic monitoring of zopiclone in biological samples is severely limited by poor ex vivo stability of both the parent drug and the primary metabolites zopiclone-N-oxide and N-desmethylzopiclone. All three rapidly degrade to 2-amino-5-chloropyridine (ACP) under ambient and refrigerated conditions, with limited degradation also occurring in frozen samples. An acidic stabilization process is effective, but impractical for remote and poorly controlled sample collections. Alternatively, we are implementing a process in which all zopiclone and primary metabolites are chemically converted into ACP prior to analysis. Measured ACP levels are then utilized as a surrogate analyte reflecting zopiclone concentrations.

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Tuesday 2:00 PM  
Poster #66 in Exhibit Hall  

**Imaging Analysis of Metals, Lipids, and Proteins in Biological Tissues via Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry**  
*Christopher Shiea* - *Kaohsiung Medical University* (shinzxtian@gmail.com) -- *Young Investigator Grantee*  
- The relationships between metals, lipids, and proteins in biological tissues are complex and are linked to disease. A technique that can explore the correlations between the different analytes and their normal and abnormal distributions has exciting potential for clinical diagnosis. In this study, analyses of metal, lipid, and protein distributions in biological tissues were studied using MALDI-TOF imaging mass spectrometry. Current studies have focused on the metal, lipid, and protein profiles for oyster tissues, in which future studies will involve the application of the technique to other biological and clinical tissues.
Tuesday 5:00 PM
Poster #67 in Exhibit Hall

**Discrimination of Human Brain Tumors by Desorption Electrospray Ionization - Mass Spectrometry Imaging**

*Alan Jarmusch - Purdue University (ajarmusc@purdue.edu) -- Young Investigator Grantee*

- We aim to acquire diagnostic information directly from human brain tissue using desorption electrospray ionization - mass spectrometry (DESI-MS) for intraoperative diagnosis and tumor margin assessment. We have expanded the types of human brain tissue (i.e. normal tissue and pituitary tumors). Preliminary results suggest that normal, meningioma, low-grade glioma, high-grade glioma, and pituitary tumors have characteristic lipid profiles that allow for discrimination. Further, small metabolites (m/z <200) detected in the negative mode were found to differ between the classes – an analogous metabolite profile. One promising metabolite, N-acetyl-aspartic acid (NAA), was detected and appears to decrease in neoplastic areas.

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Tuesday 2:00 PM
Poster #68 in Exhibit Hall

**Mapping c-Src Phosphorylation Sites as Potential Disease Biomarkers**

*Kunhong Xiao - Duke University Medical Center (xiao0003@duke.edu)*

- Protein post-translational modifications (PTMs) are essential in regulation of many physiological processes and dysregulation of these modifications is usually indicative of disease states. Therefore, mapping PTMs in signaling proteins has become a powerful tool to discover biomarkers for disease diagnosis and progression. Here, we mapped the phosphorylation sites on c-Src using LC/MS/MS and will further use the identified sites to develop MRM-based approaches to monitor the phosphorylation status of c-Src in different cancer biopsies for biomarker discovery. These MRM methods are readily transferable to other tissues and across instrument platforms and are scalable to search for novel biomarkers.

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Tuesday 5:00 PM
Poster #69 in Exhibit Hall

**Quantification of Drugs for Drug-Facilitated Crimes in Human Urine by LC-MS/MS**

*Claudio De Nardi - Thermo Fisher Scientific (claudio.denardi@thermofisher.com)*

- An analytical method based on liquid chromatography tandem mass spectrometry for the quantification of drugs for drug-facilitated crime in human urine is described. The method includes ketamine, its metabolites norketamine and dehydronorketamine, phencyclidine and gamma-butyrolactone (GBL); the method is also suitable to detect gamma-hydroxybutyric acid (GHB) at physiological levels. Sample preparation was based on extraction using methanol containing 0.1% formic acid. Mass spectrometric detection was performed by single reaction monitoring (SRM) on a Thermo Scientific TSQ Quantum Access MAX triple quadrupole using heated electrospray ionization. Linearity of the method was evaluated on duplicate curves for each analyte.

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Tuesday 2:00 PM
Poster #70 in Exhibit Hall

**Combine HDX-MS and NMR for Polycystin-2 C-terminal Tail Structural Characterization**

*Yifei Yang - Yale University (yifei.yang@yale.edu) -- Young Investigator Grantee*

- Polycystin-2 (PC2) is a calcium-regulated calcium-channel. The C-terminal tail of human PC2 is crucial for channel assembly and function. We have combined biophysical and structural approaches to study the calcium-dependent molecular mechanism within the C-terminal tail. The goal of our study is to define the oligomeric states of PC2 C-terminal tail in solution, to characterize its calcium-binding property, and to map its calcium-responding elements. This study provides a structural basis for regulation of the PC2 channel by its cytosolic C-terminal domain, with an improved understanding of the functional role of PC2 in regulating intracellular calcium signaling.
A Practical and Sensitive Plasma Catecholamines Measurement via LC-MS/MS After an Integrated Solid Phase Extraction (SPE) and Derivatization Sample Preparation

Qi Huang - Quantalytical Labs (qih@unionbiopharma.com)

- Clinical measurement of plasma catecholamines are a challenge due to the low abundance and chemical instability of the endogenous analytes. We have developed a robust and sensitive plasma catecholamines measurement process which provides linear responses at 6-200pg/mL for dopamine, epinephrine and norepinephrine. This process integrated a solid phase extraction (SPE) sample preparation with an elution, derivatization, and direct shoot (EDDS) procedure. Finally all three analytes were separated and quantified via LC-MSMS. The assay took 200uL of plasma sample volume, the sample preparation was done on an automated liquid handler, and the quantification was carried out on an AB Scieix API 5000 Tandem Mass Spectroscopy coupled with Shimadzu LC-20AD LC pumps.

Quantitative Analysis of Precursor Prostate Specific Antigen Isoforms to Improve Prostate Cancer Detection by Immunoprecipitation and Mass Spectrometry

I-An Wei - National Taiwan Normal University (anne81721@hotmail.com)

- Prostate specific antigen (PSA) is a widely used serum marker for prostate cancer (PCa). More recently, promising data is emerging regarding one precursor form of free PSA, proPSA is associated with PCa. ProPSA is comprised of native proPSA as well as truncated proPSA forms, [-2] proPSA, [-5] proPSA, and [-7] proPSA, which have been shown to be more cancer-associated than native proPSA. In this study, we developed and optimized a method for an immunoprecipitation-based platform and MRM-MS assay capable of sensitive and accurate quantification of proPSA in serum.

Simultaneous Quantification of 17-β-oestradiol and Oestrone in Human Plasma by LC-MS/MS

Sherry Gregory - Thermo Fisher Scientific (sherry.gregory@thermofisher.com)

- A simple, rapid and sensitive procedure for the simultaneous quantification of 17-β-oestradiol and oestrone in serum and human plasma using a combination of liquid-liquid extraction and liquid chromatography-tandem mass spectrometry (LLE-LC/MS/MS) has been developed and validated. Employing standards and quality control samples prepared in a matrix of human plasma and charcoal stripped serum, the analytes were isolated and subsequently chromatographically separated on a 150 x 2.1 mm column under reversed-phase conditions. The LLE-LC/APCI/MS/MS procedure possesses good accuracy, specificity, sensitivity and precision, and may be adopted as a convincing alternative to immunological approaches for the measurement of oestrogens in routine investigations.

Quantitative Omics Strategies for Investigiting the Oral Microbiome in Dental and Systemic Diseases

Anna Merrill - University of Wisconsin (alarson@chem.wisc.edu) -- *Young Investigator Grantee*

- Microbes play an integral role in many human biochemical and metabolic processes. Perturbations of the normal microbial community have been linked to deleterious health outcomes. The oral microbiome is of particular interest, as the mouth is a primary point of entry into the body. The switch from a positive/symbiotic host-oral microbiome relationship to a negative/pathogenic disease state is likely mediated by proteins and/or small molecules. Yet large-scale surveys of the proteome and metabolome of oral microbiota, and the connection of these to disease states, have been lacking. Recent advances in quantitative, mass spectrometry-based technologies have made such investigations feasible. We present here a beginning analysis of the oral microbiome proteome and metabolome isolated from dental plaque samples collected in collaboration with the Marshfield Clinic Research Foundation.
Complete Annotation of the Untargeted, LC-MS Based Metabolomic Analysis of Escherichia coli
Nathaniel Mahieu - Washington University, St. Louis (nathaniel.mahieu@wustl.edu)

- The large number of signals commonly detected in LC/MS based metabolomics has led to widely varying estimates of the number of metabolites being assayed in these experiments. Understanding the composition of these datasets is critical to making informed decisions during experimental design and data interpretation. In this work we thoroughly annotate the peaks detected in an LC/MS based untargeted metabolomic analysis of E. coli metabolic extract. MS/MS analysis of peaks is used to empirically annotate fragment peaks. Further, an estimate of known and unknown compounds is made by matching fragmentation spectra to metabolite databases. This information is critical to the planning and interpretation of metabolomic experiments.

A Simplified, Rapid LC-MS/MS Assay for Serum and Salivary Creatinine
Laura Bernstone - University Hospital of South Manchester (laura.bernstone@nhs.net) -- *Young Investigator Grantee*

- In routine clinical laboratories serum creatinine is usually measured by colourimetric or enzymatic assays using automated analysers, however these methods are prone to interferences. We have developed a straightforward and rapid LC-MS/MS assay for serum creatinine using methanol extraction, and separation with a strong cation exchange column. With a short run time of 1.1 minutes and imprecision of 1.1-1.4% at the concentrations tested, this assay provides an alternative for patient samples where interference is likely in routine creatinine methods. In addition salivary creatinine can be measured and we have found that concentrations are on average 15% of serum levels.

Versatile Platform for Fully Automated Sample Preparation of Forensic Whole Blood for LC-MS Analysis
Brian Rasmussen - University of Copenhagen (brian.rasmussen@sund.ku.dk)

- A robotic setup for efficient and fully automated sample preparation of forensic whole blood for LC-MS analysis was developed based on a Tecan Freedom EVO liquid handler. The platform was extended with devices such as a balance, centrifuge and evaporator and is capable of processing whole blood from 96 primary tubes into injection ready extracts in a couple of hours. Automated steps include weighing of aliquots of whole blood for each sample, addition of internal standard, pretreatment by centrifugation, solid phase extraction, evaporation of eluate and finally reconstitution. Results from validated methods in routine production for toxicological screening and quantification of drugs of abuse in whole blood will be presented.

A User’s Perspective on UPLC-Q-ExactiveTM High Resolution Mass Spectrometry: Application to Comprehensive Drug Screening in Clinical Laboratory
Cristiana Stefan - Centre for Addiction and Mental Health (cristiana.stefan@camh.ca)

- The availability of comprehensive urine drug screening is crucial for patient care in many clinical settings. This presentation introduces the audience to a complex, powerful, yet user-friendly and cost-effective high-resolution mass spectrometry methodology for comprehensive drug screening in matrices of interest at the Centre for Addiction and Mental Health in Toronto (urine, swabs and/or herbal products). Using an UPLC system coupled to the Q-ExactiveTM mass spectrometer (Quadrupole-Orbitrap) we established an in-house full scan/ddMS2 protocol based on monoisotopic mass, retention time and MS2 databases/libraries comprising of multiple drug classes and metabolites (cannabinoids, cocaine, opiates/opioids, benzodiazepines, antipsychotics, antidepressants, synthetic cannabinoids, bath salts, other designer drugs). Case discussions are included.
Fast, Simple Method for the Analysis of Benzodiazepines in Meconium and an Interlaboratory Method Comparison

William Brewer - University of South Carolina (brewer@sc.edu)

- A novel method for the quantitation of 10 commonly prescribed benzodiazepines and/or their metabolites, 7-aminoclonazepam, clonazepam, α-hydroxyalprazolam, alprazolam, nordiazepam, diazepam, midazolam, oxazepam, lorazepam, and temazepam, in meconium was developed using enzymatic hydrolysis, WAX-S dispersive pipette extraction (DPX) tips, and LC-MS-MS analysis. The method was linear over a range of 5 ng/g to 1000 ng/g with all correlation coefficients above 0.99. Within-run, between-run, and total imprecision were evaluated; all fell below 13% CV. A blind study with a corresponding laboratory of 35 positive patient samples resulted in approximately 92% correlation, with 100% accuracy in qualitative results.

Development of a High-throughput UPLC-MS/MS Method for Medroxyprogesterone Acetate (MP 17 Acetate) Quantification in Human Plasma

Pamela Hummert - Johns Hopkins University School of Medicine (phummer1@jhmi.edu) -- *Young Investigator Grantee*

- Medroxyprogesterone acetate (MPA), a synthetic analog of the hormone progesterone, has been commonly employed as a contraceptive agent. MPA quantification can be useful in determining potential drug-drug interactions, and as a result, robust analytical methods are required for drug quantification. While current LC-MS/MS methods are laborious and require high sample volumes, we have developed a rugged method for MPA determination with a sample volume of 0.6 mL of plasma and a lower limit of quantification of 0.2 ng/mL. The method was validated according to the recommendations of the FDA, Guidance for Industry: Bioanalytical Method Validation document.

Biomarker Candidates Discovery of Myocarditis Using MALDI Imaging Mass Spectrometry

Jungju Seo - Korea Basic Science Institute (jjseo@kbsi.re.kr)

- Spatial distribution of inflammation relative metabolites from myocarditis heart tissues was visualized by imaging mass spectrometer. MALDI-TOF/TOF, UPLC-Q-TOF M), and high resolution magic angle spinning (HR-MAS) nuclear magnetic resonance were employed to identify metabolites. Decrease level of creatine and NADH in myocarditis heart tissues were analyzed by imaging analysis. In addition, increase of lipids involved choline, especially sphingomyelins and lysophosphatidylcholines that has associated in inflammatory processes were detected. Increase level of choline and glycerol-3-phosphocholine were proved that involved in phosphatidylcholines and SMs synthesis and carnitine and acyl-carnitine that transported acyl-CoA was derived lipids levels are reduced in myocarditis heart tissue.

LC-MS/MS Method for the Measurement of Free 25-OH Vitamin D3

Steven Soldin - National Institutes of Health (steven.soldin@nih.gov)

- The measurement of total 25-OH Vitamin D3 is suboptimal with serum concentrations correlating poorly with PTH. For this reason we developed a method to quantify the free fraction employing ultrafiltration at 37°C and LC-MS/MS. The range of results from 34 healthy volunteers was 1.5 to 17.9 pg/mL. This cohort was supplemented with 8 patients with elevated parathyroid hormone (PTH). The free 25-OH Vitamin D3 concentration correlates excellently with the concentration of PTH and poorly with the total 25-OH Vitamin D3 concentration. A poor correlation was observed between total 25-OH Vitamin D3 and PTH. We can now evaluate the role of free 25-OH Vitamin D3 in patients with bone and/or a variety of malignant diseases.
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