The Association for

Mass Spectrometry: Applications to the Clinical Laboratory

4th Annual Conference & Exhibits

Final Program & Abstracts

Sheraton Hotel & Marina
San Diego, CA USA
January 14-18, 2012

Short Courses:	January 14-15
Conference:	January 15-18
Exhibition:	January 15-17

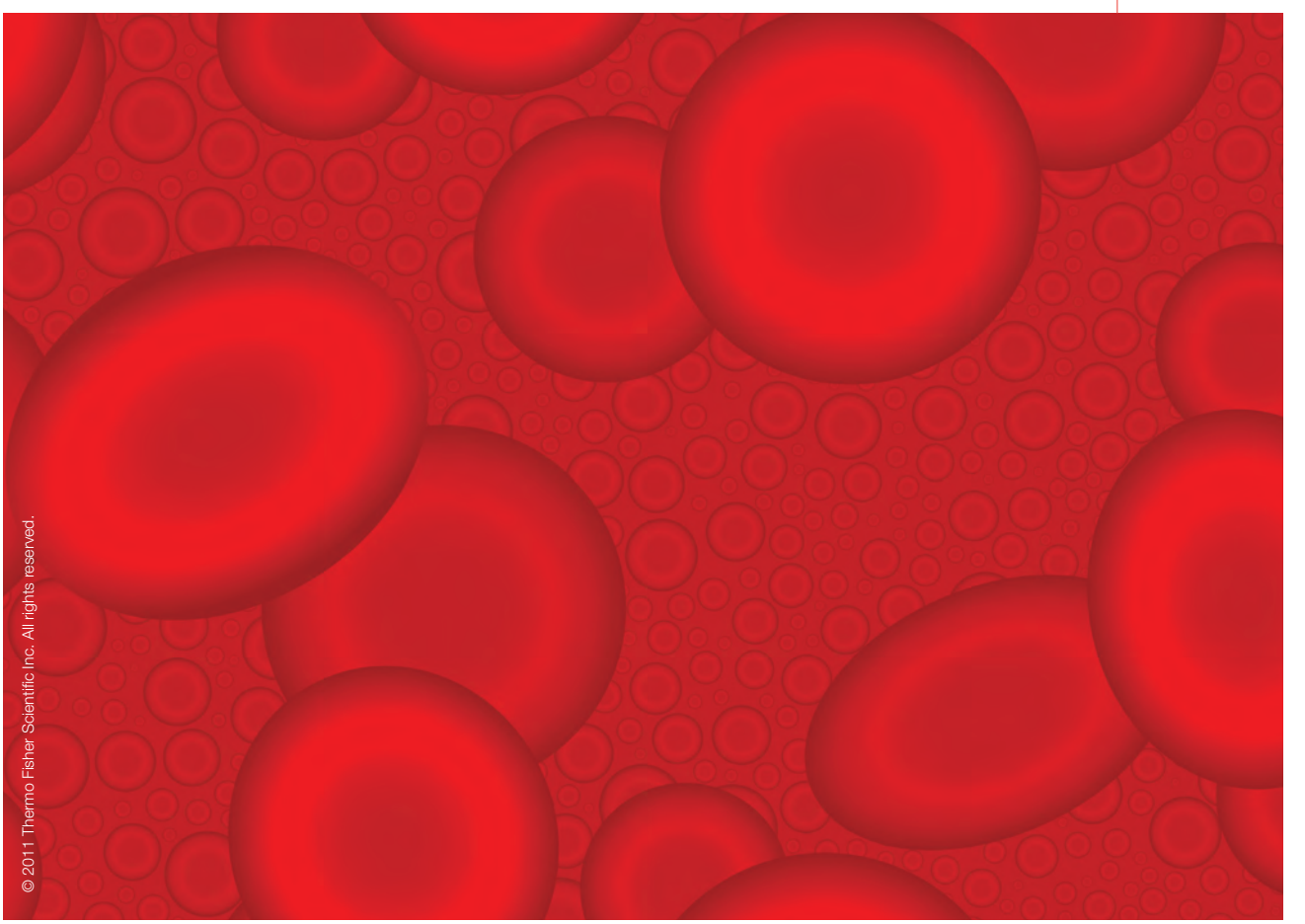
Thermo SCIENTIFIC

Innovation Applied

Connect. Collaborate. Solve. 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 need is to analyze small molecules or proteins. Whether your lab is large or small. We have the expertise and flexibility to supply the right answer.

to Clinical Research

- visit booth 7 or email **clinicallycurrent@thermo.com**



© 2011 Thermo Fisher Scientific Inc. All rights reserved.

MSACL 2012 Plenary Speaker Series

Plenary Speaker Overview

Day	Time	Speaker(s)
Sunday Evening	7:30 – 8:30 PM	Garth Ehrlich
Monday Morning	8:00 – 9:45 AM	Robin Patel and Andy Hoofnagle
Tuesday Morning	8:00 – 9:45 AM	Stanley Hazen and Ruedi Aebersold

SUNDAY EVENING PLENARY

Chair: David Herold

Sunday Evening January 15 from 7:30 – 8:30 PM
Location: Harbor Ballroom

Identification of Novel Microbial Pathogens in Clinical Specimens by Electrospray Ionization Mass Spectrometry

Sunday January 15 at 7:30 - 8:30 PM

Garth Ehrlich, PhD
Executive Director, Center for Genomic Sciences
Allegheny-Singer Research Institute
Professor of Microbiology and Immunology
Professor of Otolaryngology-Head and Neck Surgery
Drexel University College of Medicine

Microbial culture is inadequate for the identification of pathogens associated with chronic infections. We employed the Ibis MS-based technology to characterize persistent infections, providing an unbiased approach to pathogen identification. From multiple clinical trials we determined that culture detects < 20% of the pathogens present when compared to the Ibis. We have used deep 16S sequencing and 16S FISH as validation technologies, with both methods supporting the Ibis results. FISH not only provides species-specific validation, but also serves as proof-positive of the pathogenic role of unknown organisms when they are shown to be embedded within the diseased and damaged tissue.

MONDAY MORNING PLENARY

Chair: Russell Grant

Monday Morning January 16 from 8:00 – 9:45 AM
Location: Harbor Ballroom

Bacterial and Yeast Identification in the Clinical Microbiology Laboratory by Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry

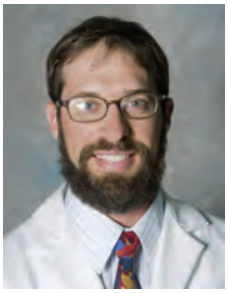
Monday January 16 at 8:10 - 9:00 AM

Robin Patel, MD(CM), FRCP(C), D(ABMM), FIDSA, FACP
Chair, Division of Clinical Microbiology
Consultant, Divisions of Clinical Microbiology and Infectious Diseases
Professor of Microbiology and Medicine
College of Medicine
Mayo Clinic

Matrix-assisted laser desorption ionization time of flight mass spectrometry can be used for routine identification of bacteria and yeast in the clinical microbiology laboratory. Numerous reports, including those from our group, have shown that matrix-assisted laser desorption ionization time of flight mass spectrometry accurately and reproducibly identifies bacteria and yeast from isolated colonies grown on culture plates in the clinical laboratory. Advantages include rapid turnaround-time and low cost.

Progress Toward the Quantification of Proteins in Clinical Samples by LC-MS

Monday January 16 at 9:00 - 9:45 AM



Andy Hoofnagle, MD, PhD
Assistant Professor, Lab Medicine
University of Washington

For decades the clinical laboratory has relied on immunoassays for the sensitive measurement of proteins in clinical samples. These assays have widely-recognized shortcomings that can lead to patient harm. Many laboratories have made progress toward quantitatively measuring proteins using mass spectrometry in clinical specimens. This presentation will review the motivation for using mass spectrometric approaches to measuring proteins, the success that our and other laboratories have had in measuring proteins with mass spectrometry, and some of the hurdles that remain before we can fully implement protein quantification by mass spectrometry in the clinical lab.

TUESDAY MORNING PLENARY

Chair: Gary Siuzdak

Tuesday Morning January 17 from 8:00 – 9:45 AM

Location: Harbor Ballroom

Development and Clinical Validation of Gut Flora Metabolites as Diagnostic Tests for Cardiometabolic Risk

Tuesday January 17 at 8:10 - 9:00 AM

Stanley Hazen, MD, PhD
Vice Chair, Translational Research,
Lerner Research Institute;
Section Head, Preventive Cardiology & Rehabilitation;
Director, Center for Cardiovascular Diagnostics & Prevention
Cleveland Clinic

Unbiased metabolomics studies hold promise for identifying novel metabolites and pathways linked to disease processes. Recent metabolomics studies in humans, and animal model studies from our group with germ free mice, indicate a role for gut flora in atherosclerosis (Wang et al, Nature 2011). We will review these findings, and new human clinical studies demonstrating the generation of pro-atherogenic metabolites by gut flora and their potential clinical prognostic significance for prediction of adverse cardiovascular event risk.

Mass Spectrometric Strategies for Protein Biomarker Discovery and Validation

Tuesday January 17 at 9:00 - 9:45 AM

Prof. Ruedi Aebersold, PhD
Institute of Molecular Systems Biology
ETH Zurich

A key barrier to the realization of personalized medicine for cancer is the identification of biomarkers. Of all types of biomarkers, plasma protein biomarkers are particularly attractive because they can be measured in easily accessible samples. Unfortunately, the search for plasma protein biomarkers has been highly challenging and met with surprisingly low level of success. Specifically, the comparison of plasma sample proteomes of control and disease affected individuals has to date not uncovered any new markers. On the backdrop of the emerging personal genome information and large scale cancer genome projects we have developed and applied a biomarker strategy that is driven by cancer genetic and genomic information. In a fist stage we use comparative genomic data to computationally predict which signaling systems might be perturbed in a particular type of cancer. We use targeted proteomic measurements on human tissue samples or tissue samples from suitable mouse models to experimentally validate these predictions, i.e. to determine which proteins are disregulated in the specific disease. We then use the validated perturbed molecular networks to select proteins that are likely to be secreted or otherwise released into plasma and quantify these proteins in sets of plasma samples by selected reaction monitoring, a highly sensitive targeted mass spectrometry technique. In this presentation we will discuss this novel biomarker strategy, its present status and expected directions. A case study on PTEN dependent prostate cancer will illustrate the concept.

ACHIEVE PEAK PERFORMANCE

Confidence means a proven path to adopting mass spectrometry in the clinical research lab. The move to mass spec is inevitable, but it comes with many implementation challenges. With Agilent you will have a proven partner at your side. Our leading mass spectrometry platforms combined with our comprehensive portfolio of sample preparation and chromatography technologies deliver peak performance to help you identify and quantify both endogenous and exogenous substances in complex biological matrices with the utmost sensitivity, accuracy, productivity and reliability. And our global support network can help you get your answer with minimal ramp-up and maximum productivity. Take your clinical research lab to a new level of performance with mass spectrometry from Agilent.
Visit **www.agilent.com/lifesciences/clinresMS**

The Measure of Confidence

© Agilent Technologies, Inc. 2011

Agilent Technologies

MSACL 2012

The Fourth Annual Meeting of The Association for Mass Spectrometry: Applications to the Clinical Lab, Inc.

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.

MSACL thanks the ***2012 Scientific Committee***:

David Herold, MD, PhD, Chair
UCSD/VA Medical Center-San Diego

Gary Siuzdak, PhD
The Scripps Research Institute

Andy Hoofnagle, MD, PhD
University of Washington

Russell Grant, PhD
Laboratory Corporation of America

Robert Kobelski, PhD
Centers for Disease Control

Donald Mason, Special Industry Advisor
Waters Corporation

PUSHING THE LIMITS IN CLINICAL RESEARCH

Visit us at booth #6

Everything depends on having a partner you can trust

RELY ON AB SCIEX FOR PROVEN LC/MS/MS SOLUTIONS

The limitations of conventional techniques are accelerating the adoption of mass spectrometry 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.

If you're ready to break through, look no further than the industry leader. With 25-years of experience delivering high-performance systems, software, and support, AB SCIEX is a partner you can trust to integrate easy-to-use mass spec technology into your lab's day-to-day research routine. Save time, cut costs, and solve analytical issues with greater confidence and better results. Isn't it time to break through the limits?

For more information, visit www.absciex.com/clinicalresearch

AB SCIEX

© 2011 AB SCIEX. For Research Use Only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of AB SCIEX Pte. Ltd. or their respective owners.

Table of Contents

Presenter Info and Guidelines

Podium/Talk

Location: *Harbor Ballroom*

- For 30 min Podium Presentations. 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.
- Speakers: Please make an effort to repeat any questions from the audience before answering in case audience members were unable to hear the question asked.
- Podium presentations are ~20-22 minutes with ~5 minutes for Q&A.
- PC Laptops running Windows 7 and Microsoft Office 2007 will be provided.
- Presenters should check-in 30 minutes prior to their Session with either the Session Chair or AV Support on-hand to set-up their computer or upload their presentation files to the primary presentation lap-top computer.
- It is ***highly recommended*** that presenters bring their presentations on thumb (USB) drives for placement on a single presentation computer from which all presenters will access their PowerPoint presentations. *Next year it will likely be obligatory.*
- Laser pointers will be provided.

Poster

Location: *Exhibit Hall in the Grande Ballroom*

Poster sessions are held on Sunday, Monday and Tuesday, January 15–17.

- Poster attendance (obligatory) is for 1 hour,
    - 2:00-3:00 PM (Odd numbered posters on Mon & Tue) or
    - 6:30-7:30 PM (All posters on Sunday and Even numbered posters on Mon & Tue).
- Posters should be placed on their numerically designated Poster Boards before 12:00 PM (for Mon & Tue posters) or 4:00 PM (for Sun posters) on the day of the presentation and remain in place until 7:30 PM of that day.
- Your Poster must be removed by 8:30 PM on the day of your presentation.
- Poster Board dimensions (for each presenter) are 8 wide x 4 ft high - providing about 7.5 x 3.5 ft to pin the poster.
- Poster Boards are Fabric.
- Poster Pins WILL BE provided.

General Information

Smoking
California law prohibits smoking in all public places.

Airport Shuttle
Travel Distance: Approximately 1.61 km/1.0 miles. Why walk when you can take the shuttle.

- Fee: Complimentary
- Hours: 24 hours;
- Complimentary airport shuttle runs every 20 minutes from the San Diego International Airport between the hours of 4.45 am and 12 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.

Conference Badges
Your MSACL2012 badge is your admission pass to the Conference, general receptions and the Exhibit Hall. Please display your badge prominently while attending the conference and all associated functions.

Parking
The visitor parking rate for the Sheraton is: $8 for Day Parking and $15 for Overnight Parking

Yoga
Yoga will be held every day of the conference from 5:45-6:30 AM in Spinnaker located just off the Harbor Island Foyer. MSACL will be providing a limited number of yoga mats, hopefully enough for all, but there are no guarantees. You may want to bring your own as insurance.

Breakfast
For **short course registrants** breakfast is served Saturday and Sunday, January 14-15 in the Harbor's Edge restaurant. Please show your conference badge to receive access to the breakfast buffet. This breakfast is ***restricted*** to short course registrants only.

For **conference registrants**, a light continental breakfast is served Monday - Wednesday, January 16 -18 in the Harbor Island Foyer.

Lunch
For **short course registrants** lunch is served in the Harbor's Edge Restaurant Saturday and Sunday at times that depend on the short course you are attending:

Russ Grant's Short Course: 11:30 – 12:30 PM
All Other Short Courses: 12:00 – 1:00 PM

For **conference registrants:**

Monday and Tuesday and Wednesday
Box lunches will be available for pickup in the Exhibit Hall (Grande Ballroom). Attendees may bring these lunches to the Corporate workshop of their choice in Harbor Ballrooms 1, 2, 3 or Marina 6.

Receptions
Held in the Exhibit Hall (Grande Ballroom)
Sun – Tue: 5:00 – 7:30 PM
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. This year, for the Sunday reception, the San Diego Youth Orchestra will be providing a selection of live classical music.

MSACL Discussion Groups
Rooms around the Harbor Island Foyer
Monday and Tuesday: 7:30 – 8:30 PM

The MSACL Discussion Groups are intended to provide a forum for focused discussion on the topics that matter to you most in clinical mass spectrometry.

The first round of Discussions will be held on Monday evening in rooms around the Harbor Island Foyer from 7:30 to 8:30 PM following the Reception in the Exhibit Hall. These discussions are intended to last about 1 hour, but they may go longer, and are meant to be "guided" by one or two individuals with interest and/or knowledge in the topic area. These are not intended to be presentations by those individuals, but are meant to engage the group for active discussion.

The Tuesday Evening Discussion Group topics will be dependent on the outcome of the discussions from the Monday group and may drill down further on topic concepts, continue the thread of the previous evening, transition to a different topic or end. The decision of the Group must be reported by the Group leader(s) to Chris Herold via email (chris.herold@msacl.org) or personal contact. The Tuesday Evening line-up will be posted by Tuesday morning and will be viewable on the web-based electronic program. Tuesday evening discussion groups may be completely different, possibly even having different leads.

Hospitality Receptions
Held at the ShoreLine Patio overlooking the San Diego Marina
Walk to the hotel lobby and then find the internet lounge. Walk left past the lounge to the door leading to the marina. Go out the door and follow the stairs down to the right. The ShoreLine Patio is just past the pool. There will be MSACL signs directing you to the location.
Sat – Tue: 8:30 to 10:30 PM.
Wed: 4:00 to 8:00 PM

Enjoy a winter's evening in San Diego while snacking on light appetizers.

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, committee meetings, in the Exhibit Hall, and during the plenary sessions.

Note: Throughout MSACL Conferences we will be videotaping and taking photographs to be used for future MSACL promotions. If you do not wish to appear on camera, please notify the videographer or photographer and your request will be honored.

Discussion Groups

Monday & Tuesday Evening
7:30-8:30 PM

The MSACL Discussion Groups are intended to provide a forum for focused discussion on the topics that matter to you most in clinical mass spectrometry. The first round of Discussions will be held on Monday evening from 7:30 to 8:30 PM following the Reception in the Exhibit Hall. These discussions are intended to last about 1 hour, but they may go longer, and are meant to be "guided" by one or two individuals with interest and/or knowledge in the topic area. These are not intended to be presentations by those individuals, but are meant to engage the group for active discussion.

The Tuesday Evening Discussion Group topics will be dependent on the outcome of the discussions from the Monday group and may drill down further on topic concepts, continue the thread of the previous evening, transition to a different topic or end. Tuesday Topic decisions should be reported by the Discussion leads to Chris Herold at the Hospitality event immediately following the Monday Evening Discussions or via email at chris.herold@msacl.org. The Tuesday Evening line-up will be posted by Tuesday morning and will be viewable on the web-based Conference Program at https://www.msacl.org/2012_program.

Monday Evening Discussion Groups

Topic	Lead(s)	Location
Metabolomics	Gary Patti	Harbor 1
Small Molecule Analysis/ Sample Prep	Russell Grant & Karl-Siegfried Boos	Harbor 2
Proteins, Proteomics and Disease Markers	Mike MacCoss & Nathan Yates	Harbor 3
Critical Method Assessment	Robert Kobelski & Jack Henion	Marina 3
Validation, Regulations & Standards	Julianne Bothelo	Marina 4
Proficiency Testing	Alan Rockwood & Walt Chandler	Marina 5
Microbiology	Nathan Ledeboer & George Goedesky	Marina 6
Toxicology	William Clarke	Seabreeze
MSACL 2012 Review and Recommendations Exhibitor and Vendor Feedback Focus Group	David Herold	Spinnaker

Tuesday Evening Discussion Groups

Topic	Lead(s)	Location
The Tuesday Evening Discussion Group topics will be dependent on the outcome of the discussions from the Monday group and may drill down further on topic concepts or continue the thread of the previous evening. Tuesday Topic decisions should be reported by the Discussion leads to Chris Herold.		
MSACL 2012 Review and Recommendations General feedback on how to improve MSACL.	Chris Herold	Spinnaker

SHIMADZU

You asked for faster speed, less cross-talk, easy operation and low cost ...

We Listened!

Shimadzu Introduces the LCMS-8030, the World's Fastest **Triple Quadrupole Mass Spectrometer**

Shimadzu's LCMS-8030 Triple Quad features:

- Fastest multiple reaction monitoring (MRM) transition times available today (dwell times of 1msec and pause times of 1msec) utilizing patented UFsweeper® technology
- High-speed multi-analyte detection with 500 MRM transitions in one second
- Unsurpassed polarity switching speed (15 msec)
- Synchronized survey scan technology, utilizing a high-speed scanning rate of 15,000 u/sec, allows full spectrum scans within a series of MRM measurements
- Easy system maintenance—enables greater uptime and usability
- Single-vendor solution (Nexera UHPLC/LCMS-8030) provides unmatched qualitative and quantitative analysis, increased productivity, and accelerated workflows for high-throughput analysis

Learn more about Shimadzu's LCMS-8030.
Call (800) 477-1227 or visit us online at
www.ssi.shimadzu.com/TripleQ

Order consumables and accessories on-line at http://store.shimadzu.com
Shimadzu Scientific Instruments Inc., 7102 Riverwood Dr., Columbia, MD 21046, USA
For research use only. Not for use in diagnostic procedures.

LCMS-8030

Triple Quadrupole Mass Spectrometer

Our Genius range has evolved.

Higher output and a small footprint make **Genius²** gas generators the perfect prodigy.

As your laboratory needs continually evolve so too does our Genius range of gas generators. With our customary touch of innovation we have developed the sleek new Genius^2 models and now lead the pack with exceptional performance and real value for money.

The transformation is remarkable. The new 3010, 3020 and 3030 Genius^2 generators have the unique benefit of double the gas flow, dual feeds and single control systems, all built into the one, space-saving casing.

Of course, we could go into all sorts of wonderfully technical detail on peak flow rates and testimonials about our world class service levels but we suggest you give us a call or better still visit us at **MSCAL, Booth #10** where we'll be exhibiting from the 14th - 18th January 2012.

Tel: **+1-866-647-1649**
Web: **www.peakscientific.com**
Email: **genius@peakscientific.com**

PEAK SCIENTIFIC. GO WITH THE FLOW.

PEAK® SCIENTIFIC

ACCENT® Continuing Education Credit

Short Courses

The American Association of Clinical Chemistry (AACC) designates the Saturday and Sunday Short Course for 6.0 ACCENT® credit hours each day for a maximum of 12.0 ACCENT® credit hours towards the AACC Clinical Chemist's Recognition Award.

General Scientific Sessions

The American Association of Clinical Chemistry (AACC) designates the General Scientific Sessions on Monday, Tuesday and Wednesday for 5.0 ACCENT® credit hours each day.

AACC is an approved provider of continuing education for clinical laboratory scientists in the states of California, Florida, Louisiana, Montana, Nevada, North Dakota, Rhode Island, and West Virginia.

Detailed instructions and relevant links for obtaining your certificate of credit will be available on the MSACL website under:

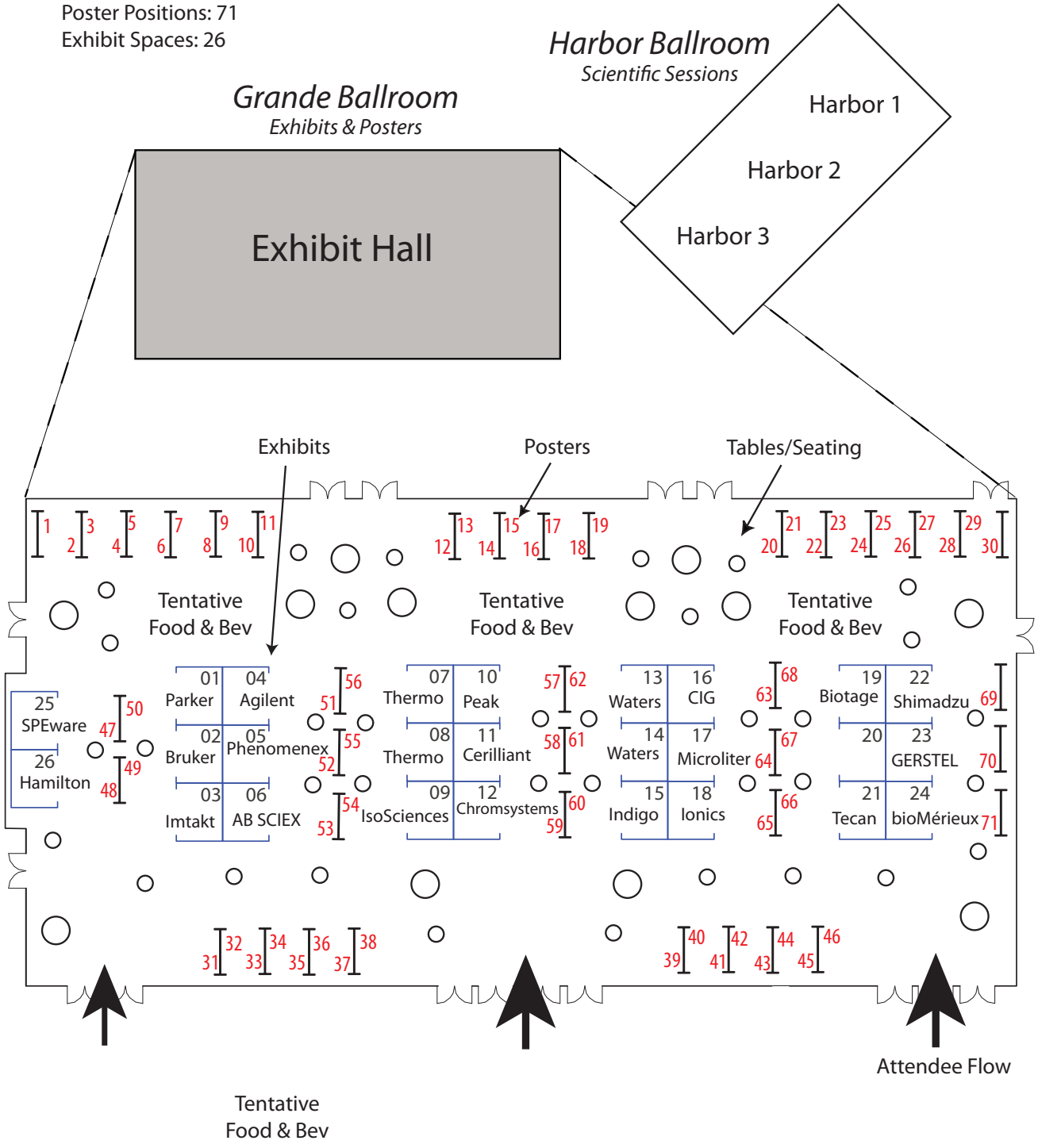
www.msacl.org > Conference 2012 > ACCENT® CE Credit

Hotel Map & Internet Access

Wireless Internet Access will be provided on the conference and exhibit floor. Wired internet access is provided free of charge in guest rooms booked within the MSACL block before January 10.

Wireless Network: **psav_network_solutions**
Password: **MSACL6064**

Exhibit Hall Map



Program-at-a-Glance

Saturday January 14, 2012

Saturday January 14, 2012	
9:00 am – 4:00 pm	***Short Courses (some may run until 5:00 pm, please confer with instructor)***
5:45 – 6:30 am	**Yoga** *Spinnaker*
7:00 – 8:45 am	**Breakfast** *Harbor's Edge Restaurant* – SHOW MSACL BADGE TO RECEIVE COMPED BREAKFAST BUFFET.
10:15 – 10:30 am	**AM Coffee Break** *Harbor Island Foyer*
11:30am – 12:30 pm	**Lunch** for ***Russ Grant*** **Short Course Group** *Harbor's Edge Restaurant* – SHOW MSACL BADGE TO RECEIVE COMPED LUNCH BUFFET.
12:00 – 1:00 pm	**Lunch** for ***All Other*** **Short Course Groups** *Harbor's Edge Restaurant* – SHOW MSACL BADGE TO RECEIVE COMPED LUNCH BUFFET.
2:15 – 2:30 pm	**PM Coffee Break** *Harbor Island Foyer*
5:00 – 8:30 pm	**Dinner** – On own with Discount Voucher: 25% off with $10 credit *Harbor's Edge Restaurant* – COLLECT **DISCOUNT VOUCHER** FROM MSACL REGISTRATION.
8:30 – 10:30 pm	**Hospitality Reception** *ShoreLine Patio*

Sunday January 15, 2012

Sunday January 15, 2012	
9:00 am – 4:00 pm	***Short Courses (some may run until 5:00 pm, please confer with instructor)***
5:45 – 6:30 am	**Yoga** *Spinnaker*
7:00 – 8:45 am	**Breakfast** *Harbor's Edge Restaurant* – SHOW MSACL BADGE TO RECEIVE COMPED BREAKFAST BUFFET.
10:15 – 10:30 am	**AM Coffee Break** *Harbor Island Foyer*
11:30am – 12:30 pm	**Lunch** for ***Russ Grant*** **Short Course Group** *Harbor's Edge Restaurant* – SHOW MSACL BADGE TO RECEIVE COMPED LUNCH BUFFET.
12:00 – 1:00 pm	**Lunch** for ***All Other*** **Short Course Groups** *Harbor's Edge Restaurant* – SHOW MSACL BADGE TO RECEIVE COMPED LUNCH BUFFET.
2:15 – 2:30 pm	**PM Coffee Break** *Harbor Island Foyer*
5:00 – 7:30 pm	**Reception -- EXHIBITS OPEN** *Exhibit Hall (Grande Ballroom)*
7:30 – 8:30 pm	**Opening Plenary Session** Introduction by *Chair:* ***David Herold***
	Identification of Novel Microbial Pathogens in Clinical Specimens by Electrospray Ionization Mass Spectrometry Garth Ehrlich *Allegheny Singer Research Institute*
8:30 – 10:30 pm	**Hospitality Reception** *ShoreLine Patio*

With Special Thanks to Our Sponsors, Who Have Made MSACL 2012 Possible.

Diamond Sponsor

MSACL thanks Thermo Scientific for leading the 2012 Trainee Travel Award program and their continued support.

Thermo SCIENTIFIC

Platinum Sponsor

MSACL thanks Agilent for supporting the 2012 Trainee Travel Award program and their continued support.

Agilent Technologies

Gold Sponsors

MSACL thanks Waters, Bruker and AB SCIEX for supporting the 2012 Trainee Travel Award program and their continued support.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

BRUKER

AB SCIEX

Bronze Sponsors

CHROMSYSTEMS
DIAGNOSTICS BY HPLC & LC-MS/MS

phenomenex®
...breaking with tradition℠

SHIMADZU

Monday January 16, 2012

Monday January 16, 2012				
5:45 – 6:30 am	**Yoga** *Spinnaker*			
6:00 – 7:45 am	**Breakfast** *Harbor Island Foyer*			*Sponsored by:* Thermo SCIENTIFIC
8:00 – 9:45 am	**Plenary Session 2** Introduction by *Chair:* ***Russell Grant***			
	Bacterial and Yeast Identification in the Clinical Microbiology Laboratory by Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry Robin Patel *Mayo Clinic*			
	Progress Toward the Quantification of Proteins in Clinical Samples by LC-MS, Andy Hoofnagle *University of Washington*			
9:45 – 10:30 am	**AM Coffee Break** *Exhibit Hall (Grande Ballroom)*			
10:30 – 12:00 pm	**Scientific Session 1**			
	Track 1 **Targeted Metabolomics**	Track 2 **Small Molecule Analytes**	Track 3 **Tobacco**	
12:00 pm	**Lunch** *Pick up Lunch in Harbor Island Foyer; attend a Corporate Workshop*			*Sponsored by:* Thermo SCIENTIFIC
12:15 – 1:15 pm	**Corporate Workshops**			
	Harbor 1	***Harbor 2***	***Harbor 3***	***Marina 6***
	AB SCIEX	Waters THE SCIENCE OF WHAT'S POSSIBLE.™	CHROMSYSTEMS	BRUKER
2:00 – 3:00 pm	**Posters --** Odd numbered attended *Exhibit Hall (Grande Ballroom)*			
2:00 – 3:00 pm	**PM Coffee Break** in Exhibit Hall *Exhibit Hall (Grande Ballroom)*			*Sponsored by:* Thermo SCIENTIFIC
3:00 – 5:00 pm	**Scientific Session 2**			
	Track 1 **Discovery Metabolomics**	Track 2 **Small Molecule Analytes**	Track 3 **Microbiology**	
5:00 – 7:30 pm	**Reception** *Exhibit Hall (Grande Ballroom)*			
6:30 – 7:30 pm	**Posters** -- Even numbered attended *Exhibit Hall (Grande Ballroom)*			
7:30 – 8:30 pm	**Discussion Groups** *(See Page 13)* *Rooms around Harbor Island Foyer*			
8:30 – 10:30 pm	**Hospitality Reception** *ShoreLine Patio*			*Sponsored by:* McKinley Scientific

MassTRAK™ Solutions

Unmatched Sensitivity, Specificity and Flexibility

Stop by Booth # 13/14

Waters Corporate Workshops

Whatever the industry, whatever the challenges, Waters world-leading solutions will help lead you to the answer. Join us this week as we present our latest cutting-edge LC/MS work in clinical and proteomics applications.

Workshop 1

Monday, January 16th, 12:15–1:15
Location: Harbor Island Ballroom #2

Presentations Include:

- **Harmonization of LC-MS/MS Assays for Immunosuppressant Drug Monitoring**
  Donald Mason, Global Scientific Affairs Manager, Waters Corporation
- **Enhancing Laboratory Productivity through Automated Online SPE UPLC-MS/MS: A TDM Perspective**
  Martin Eastwood, PhD, Senior Clinical Application Scientist, Waters Corporation

Workshop 2

Wednesday, January 18th, 12:15–1:15
Location: Harbor Island Ballroom #2

Presentation Includes:

- **Application of Mass Spectrometry Based Proteomics in the Clinical Research Environment.**
  Johannes Vissers, Senior Market Development Manager Proteomics, Waters Corporation
- **Quantitation of Multiplex Cancer Pathway Proteins in FFPE Tissues Using Targeted Mass Spectrometry**
  Jon Burrows, Ph.D., Executive Vice President and Head of R&D, Expression Pathology Inc.

Xevo TQ-S

Working with clinical laboratories around the world, Waters has pioneered an integrated portfolio of MassTrak™ Solutions that include separations science, mass spectrometry, consumables, laboratory information management software, services and support. This comprehensive approach can help optimize your laboratory processes and minimize the risks associated with your assays.

MassTrak Solutions bring the power of Waters' medical devices in an easy-to-use, cost-effective package to address the needs of clinical diagnostic laboratories. Waters also provides general purpose laboratory research equipment for forensic toxicology and other clinical research applications.

Waters innovations and laboratory solutions allow you to deliver accurate, precise and high quality results using your assays.

Medical Devices

Waters Corporation manufactures medical devices that offer clinical diagnostic laboratories an alternative to general purpose laboratory research equipment. Unlike general purpose laboratory research equipment, medical devices are subject to the laws and regulations of the United States (US) Food and Drug Administration (FDA) and other regulatory agencies outside of the US. FDA regulations require medical devices to be designed and manufactured under a quality management system that complies with 21 CFR Part 820 (Quality System Regulation).

In Europe and Canada, a medical device manufacturer's quality management system is governed by ISO 13485:2003. ISO 13485:2003 is similar to FDA's Quality System Regulation. Waters quality management system complies with both the FDA's Quality System Regulation and ISO 13485:2003.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

©2011 Waters Corporation. UPLC, MassTrak, ACQUITY, Xevo, Waters, and The Science of What's Possible are trademarks of Waters Corporation.

Tuesday January 17, 2012

Tuesday January 17, 2012				
5:45 – 6:30 am	**Yoga** *Spinnaker*			
6:00 – 7:45 am	**Breakfast** *Harbor Island Foyer*			*Sponsored by:* Agilent Technologies
8:00 – 8:10 am	**Plenary Session 3** Introduction by *Chair:* ***Gary Siuzdak***			
8:10 – 9:00 am	**Metabolomics Studies Identify Gut Flora Metabolite as Predictor of Cardiovascular Risk** Stanley Hazen *Cleveland Clinic*			
9:00 – 9:45 am	**Mass Spectrometric Strategies for Protein Biomarker Discovery and Validation** Ruedi Aebersold *ETH Zurich and University of Zurich*			
9:45 – 10:30 am	**AM Coffee Break** *Exhibit Hall (Grande Ballroom)*			
10:30 – 12:00 pm	**Scientific Session 3**			
	Track 1 **Discovery Proteomics**	Track 2 **Emerging Toxicants**	Track 3 **Microbiology**	
12:00 pm	**Lunch** *Pick up Lunch in Harbor Island Foyer; attend a Corporate Workshop*			*Sponsored by:* Agilent Technologies
12:15 – 1:15 pm	**Corporate Workshops**			
	Harbor 1	***Harbor 2***	***Harbor 3***	***Marina 6***
	Agilent Technologies	Thermo Scientific	SHIMADZU	phenomenex
2:00 – 3:00 pm	**Posters** -- Odd numbered attended *Exhibit Hall (Grande Ballroom)*			
2:00 – 3:00 pm	**PM Coffee Break** *Exhibit Hall (Grande Ballroom)*			*Sponsored by:* Agilent Technologies
3:00 – 5:00 pm	**Scientific Session 4**			
	Track 1 **Targeted Proteomics**	Track 2 **Toxicology: K2/Spice**	Track 3 **InBorn Errors**	
5:00 – 7:30 pm	**Reception** *Exhibit Hall (Grande Ballroom)*			
6:30 – 7:30 pm	**Posters** -- Even numbered attended *Exhibit Hall (Grande Ballroom)*			
7:30 pm	**EXHIBITION CLOSED**			
7:30 – 8:30 pm	**Discussion Groups –** Continued from Monday *(See Page 13)* *Rooms around Harbor Island Foyer*			
8:30 – 10:30 pm	**Hospitality Reception** *ShoreLine Patio*			

Mass Spectrometry for Clinical Research

For research use only, not for use in diagnostic procedures.

Providing Separation and Mass Spectrometry based solutions for Clinical Research www.bdal.com/solutions.

Wednesday January 18, 2012

Wednesday January 18, 2012				
5:45 – 6:30 am	**Yoga** *Spinnaker*			
6:00 – 7:45 am	**Breakfast** *Harbor Island Foyer*			
8:00 – 10:00 am	**Scientific Session 5**			
	Track 1 **Disease Markers**	Track 2 **Toxicology**	Track 3 **Methods Validation**	
10:00 – 10:30 am	**AM Coffee Break** *Harbor Island Foyer*			
10:30 - 12:00 pm	**Scientific Session 6**			
	Track 1 **Regulations & Proficiency**	Track 2 **New Advances: Disease Markers**	Track 3 **Microbiology**	
12:00 pm	**Lunch** *Pick up Lunch in Harbor Island Foyer; attend a Corporate Workshop*			
12:15 – 1:15 pm	**Corporate Workshops**			
	Harbor 1	***Harbor 2***	***Harbor 3***	***Marina 6***
	AB SCIEX	Thermo SCIENTIFIC	Waters THE SCIENCE OF WHAT'S POSSIBLE.™	IONICS Mass Spectrometry Group Inc
1:15 – 2:00 pm	**PM Coffee Break** *Harbor Island Foyer*			
2:00 – 4:00 pm	**Scientific Session 7**			
	Track 1 **New Advances**	Track 2 **Sample Prep & Automation**	Track 3 **Microbiology**	
4:00 – 8:00 pm	**Hospitality/Closing Reception** *ShoreLine Patio*			
Conference Closed				

Your World, **Our Purpose**

Your Challenges, **Our Solutions**

Your Patients, **Our Commitment**

Your Future, **Our Vision**

Shaping Your World Together.

Visit bioMerieux booth #24 at the MSACL (Mass Spectrometry Application to the Clinical Lab) Meeting Jan. 14-18 in San Diego!

To learn more visit:
www.biomerieux-usa.com

BIOMÉRIEUX

System has not been cleared by the US Food and Drug Administration. Not for Clinical Use in the United States.

Young Investigator and Trainee Travel Awardees

MSACL 2012 Trainee Travel Awardees

Trainee Travel Awards are awarded to individuals directing clinical labs or who are in training for such a position and have not previously attended an MSACL conference. These individuals have had minimal exposure to mass spectrometry and are interested in gaining more understanding of its potential applications in the clinical lab. These awards cover hotel, conference and short course registration, and all or a portion of airfare.

Alaine Garrett *Nevada State Public Health Laboratory*
Alexandra Yates *University Hospital North Staffordshire*
Ami Grunbaum *McGill University*
Andre LeBlanc *University of Quebec in Montreal*
Anna K Fuezery *Johns Hopkins University*
Anthony N. Sireci *Columbia University*
Benjamin Mathis *University of Pennsylvania*
Brian Nicholas Kelly *University of Virginia*
Cheryl Rigg *Hull Royal Infirmary, NHS*
Chor Kwan Ching *Princess Margaret Hosp. Hong Kong*
Claudia L. Henemyre-Harris *Johns Hopkins University*
Edward KY Leung *University of Chicago*
Hans Frykman *University of British Columbia*
Heather Mack *University of Colorado, Denver*
Hemant K. Naikare *Texas Vet. Med. Diagnostic Lab*
Hongjie Chen *Mount Sinai School of Medicine*
Janetta Bryksin *Emory University*
Joe El-Khoury *Cleveland State University*
Joshua Warrick *Washington University*
Juli-Anne Gardner *Fletcher Allen Health Care*
Katherine Livingstone Devitt *University of Vermont*
Kitch Wilson *Stanford Medical Center*
Krystina M. Cocco *Virginia Commonwealth University*
Laura M. Bender *UNC at Chapel Hill*
Leslie J. Donato *Mayo Clinic*
Lindsay Bazydlo *University of Florida*
Mari DeMarco *Washington University*
Mark A. Marzinke *Johns Hopkins Medical Institutions*
Michael Angelo *UC San Francisco*
Nicole M. Green *LA County Public Health Laboratory*
Ramesh Saeedi *University of British Columbia*
Robert Benirschke *Mayo Clinic*
Sarah Shugarts *UC San Francisco*
Steven Cotten *UNC at Chapel Hill*
Thomas Kampfrath *University of Louisville*
Tiffany K Roberts-Wilson *Emory University*
Tom Gaulton *Health Protection Agency*
Vijay Bhoj *University of Pennsylvania*
Walter E. Kelley *NIH*
Yen-Michael S. Hsu *Washington University*
Yu Hou *California Department of Public Health*
Yungkang Lee *Cedars-Sinai Medical Center, Los Angeles*
Zhen Zhao *Washington University*

The 2012 Trainee Travel Awards are sponsored by:

MSACL 2012 Young Investigator Travel Awardees

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 hotel, conference and short course registration, and all or a portion of airfare.

Alexia Ortiz *Centre Nat'l de la Recherche Scientifique*
Alona Umali *TX Dept of State Health Services*
Andrea Bozovic *University of Toronto*
Andrei Drabovich *Mount Sinai Hospital & U. of Toronto*
Andrew VanSchoiack *University of Arizona*
Chengsi Huang *University of Arizona*
Chul Min Park *Konkuk University Medical Center*
Cody Goodwin *Vanderbilt University*
Deborah French *UC San Francisco*
Ethan den Boer *Erasmus Medical Center*
Gary Patti *Washington University*
Hari Nair *University of Washington*
He Wang *Purdue University*
Hee-Jung Chung *Kwandong University*
Jane Dickerson *University of Washington*
Jeanne M. Rhea *Emory University*
Julia Denes *Justus Liebig University*
Kara Lynch *UC San Francisco*
Lanette R. Hamilton *Washington University*
Laura Bechtel *Memorial Sloan Kettering Cancer Center*
Mark Fisher *University of Utah*
Matthew Petrie *UC San Francisco*
Matthew T. Olson *The Johns Hopkins Hospital*
Meiyao Wang *IBBR, University of Maryland*
Olgica Trenchevska *Sts.Cyril and Methodius University*
Phillip Bates *UNC at Chapel Hill*
Rebecca L. Edwards *University of Birmingham*
Roy Gerona *UC San Francisco*
Sean Hofherr *Mayo Clinic*
Simone Nicolardi *Leiden University Medical Center*
Steven J. Naleway *Virginia Commonwealth University*
Steven M. Truscott *University of Louisville*
Wei Wei *University of California, Davis*
Zdenek Spacil *University of Washington*

The 2012 Trainee Travel Awards are sponsored by:

MS ACL MSACL

Short Course Overview

The MSACL 2012 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.

One-Day Short Courses
SATURDAY, January 14; 9:00 am–4:30 pm

How To Implement A Business Strategy Tailored For Specific Inventions
Location: Spinnaker 2
Level 0
Anthony Craig, PhD, JD
Shaun Lonergan
A number of invention scenarios will be reviewed, the methods used to develop IP positions in terms of advantages and limitations and some real world examples will be discussed. Business strategy development for both start-up and established companies utilizing IP positions will also be explored. Exit strategies and the considerations given to IP during an acquisition or merger will be included in the discussion. This course will introduce the participant to a range of subjects some of which include investor types, terminal disclaimer, abandonment, request for continued examination, certificate of correction, ex parte reexamination, inter partes reexamination, broadening reissue, non-broadening reissue, patent troll, non-practicing entity, attorney types and practices, types of IP insurance.

Introduction to Mass Spectrometry
Location: Seabreeze 1
Level 1
Robert Kobelski, PhD
Kent Henry, PhD
This course will introduce the student to the type of information available in a mass spectrum and how to obtain the desire type of information from a mass spectrometry system. The principles of mass analysis for common mass spectrometers, single analyzer, tandem and hybrid, will be discussed along with commonly employed ionization techniques and ion sources. The types of data available from the various systems and the application of that data to the solution of analytical problems will be addressed.

One-Day Short Courses
SUNDAY, January 30; 9:00 am–4:30 pm

Preparing Manuscripts for Publication: Improving Your Chances for Success
Location: Spinnaker 2
Level 0
Thomas Annesley, PhD, Professor of Clinical Chemistry at the University of Michigan
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.

Chromatographic Principles for Mass Spectrometry Applications
Location: Seabreeze 1
Level 1
Robert Kobelski, PhD
Brian Crow, PhD
This 1-day course is designed for scientists with an interest in optimizing chromatographic separations prior to detection by a mass spectrometer. Discussions will start with understanding the basic contributions to chromatographic peak resolution in order to explore options for improving separations. Practical considerations for varying experimental parameters will be discussed and the benefits and trade-offs of each will be considered. Following a general discussion, specific considerations for both gas chromatography and liquid chromatography will be presented.

Metabolomics
Location: Marina 5
Level 3
Gary Siuzdak, PhD, Senior Director, Center for Mass Spectrometry, Associate Professor, Molecular Biology, TSRI
Gary Patti, PhD, Assistant Professor, Department of Chemistry, Washington University
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.

Two-Day Short Courses
SATURDAY AND SUNDAY
January 14 and 15, 9:00 am–4:30 pm

Understanding and Optimization of LC/MS/MS to Develop Successful Methods for Identification and Quantitation in Complex Matrices
Location: Marina 6
Level 2
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.

How to Develop Robust Assays Faster Using Free Data Analysis Tools
Location: Marina 4
Level 2
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 (as in “Free Beer”) 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.

New Developments in LC/MS and Mass Spectrometry
Location: Spinnaker 1
Level 3
Jack Henion, PhD, CSO, Advion BioSciences
This two-day, advanced course presents a systematic overview of key recent developments that are occurring in the exciting and rapidly developing field of mass spectrometry. The first lectures cover new ionization techniques which may be used with or without on-line separation science technology such as HPLC, UPLC or capillary electrophoresis (CE). This topic has evolved into so-called ambient ionization techniques such as DESI, DART, ASAP, etc. with examples and comparisons of the potential and pitfalls associated with these techniques. In addition the technique of MALDI and its exciting applications to tissue imaging as well as liquid extraction surface analysis (LESA) employing nano electrospray will be described as complimentary ionization techniques with representative key applications. Chip-based nanoscale HPLC separations coupled with the benefits of nanoelectrospray will also be covered with a focus on modern strategies to obtain lower detection limits with the benefits of reduced matrix suppression and more uniform analyte response.

Targeted Proteomics – **PLEASE NOTE: THIS COURSE STARTS AT 1:00 PM ON SATURDAY.
Location: Marina 3
Level 3
Andy Hoofnagle, MD, PhD, Assistant Professor, Lab Medicine University of Washington
Mike MacCoss, PhD, Associate Professor, Department of Genome Sciences, University of Washington
Nathan Yates, PhD, Visiting Associate Professor, University of Pittsburgh
This course will detail the development of targeted proteomics assays for use in complex matrices. We will discuss:

1. differential mass spectrometric assays and their application in the discovery of novel biomarkers of disease and therapy,
2. approaches to peptide and SRM/MRM transition selection and the software tools that have been developed to help facilitate this important step in a vendor neutral environment,
3. our experience with several different methods for the calibration of proteomic signals in human serum and plasma,
4. the different internal standards available and their use in targeted proteomics experiments,
5. approaches to the immunoaffinity enrichment of proteins and peptides in complex matrices There will be case studies used throughout to help illustrate the methods and the results of each of the possible approaches.

Manual Interpretation Of Electron Transfer Dissociation (ETD) Mass Spectra Of Peptides
Location: Marina 5
Level 3
Donald F. Hunt, PhD, University Professor of Chemistry and Pathology, University of Virginia
Electron transfer dissociation mass spectrometry is a break-though technology for sequencing post-translationally modified peptides. The purpose of the workshop is to provide instruction on how to manually interpret peptide ETD mass spectra. Following a lengthy tutorial about ion structures, fragmentation pathways, predictable changes in fragment ion isotope patterns, etc., we will outline a general approach for the manual interpretation of peptide ETD spectra, solve the sequence of several post-translationally modified peptides, assign homework spectra, and reconvene on the second day of the workshop to go over the homework problems and to explore additional applications of this powerful technology. A packet of lecture notes and handouts will be provided. Attendees should bring an inexpensive calculator, pad of paper, and a small ruler.

Development and Validation of Quantitative LC-MS/MS Assays for Use in Clinical Diagnostics
Location: Harbor Ballroom 1
Level 3
Russell Grant, PhD, VP R&D, Lab Corp.
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.

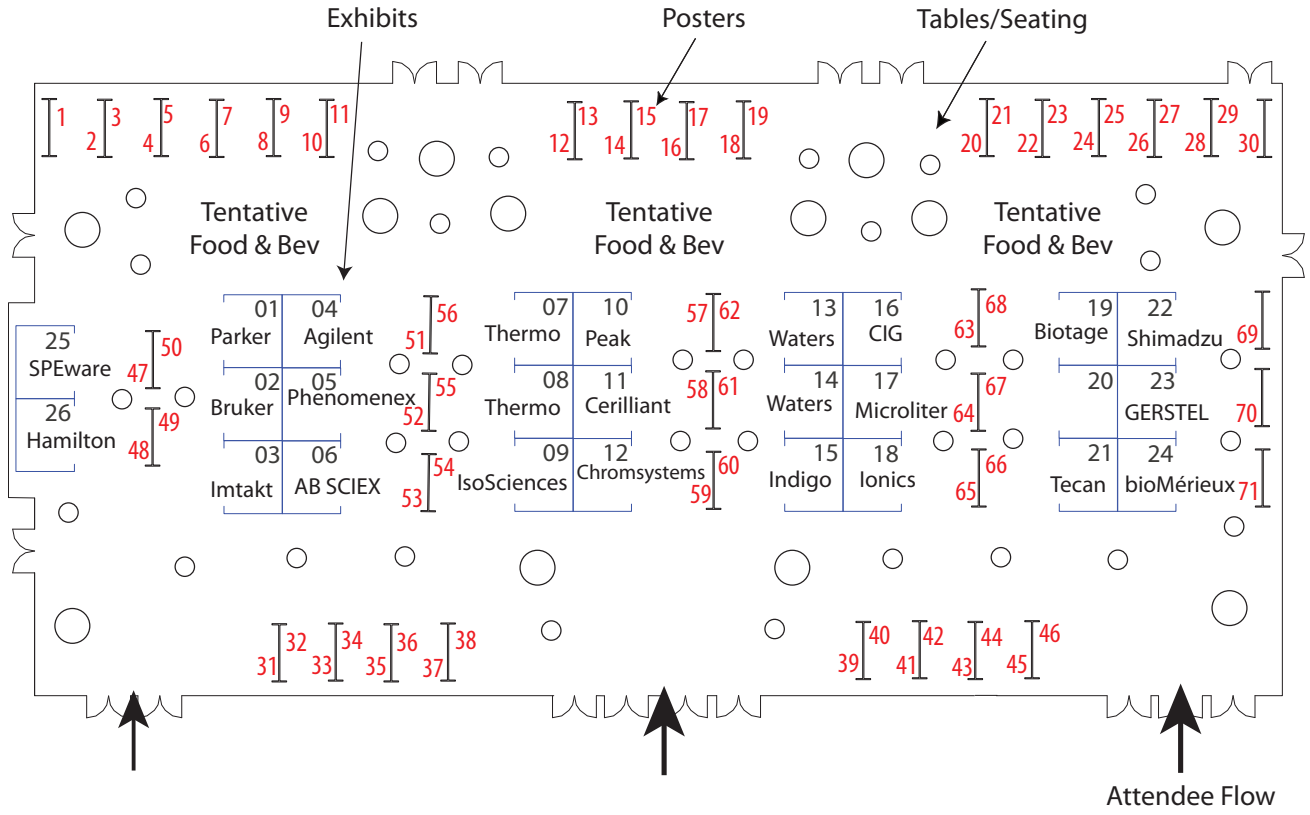
Exhibitor Summary

The Exhibits officially open at 5:00 PM on Sunday with the Opening Reception in the Grande Ballroom. Exhibits are open for viewing from 9:45 AM to 7:30 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 are listed below.

Sunday January 15, 2012	
9:00 am – 5:00 pm	Exhibitor Set-Up (EXHIBITS CLOSED)
5:00 – 7:30 pm	**Opening Reception** in Exhibit Hall
Monday January 16, 2012	
9:45 – 10:30 am	**AM Coffee Break** in Exhibit Hall
12:00 – 3:00 pm	**Lunch** provided in the Exhibit Hall. Attendees likely to visit Workshops from 12:15-1:15 PM.
2:00 – 3:00 pm	**Posters** in Exhibit Hall
2:00 – 3:00 pm	**PM Coffee Break** in Exhibit Hall
5:00 – 7:30 pm	**Reception** in Exhibit Hall
Tuesday January 17, 2012	
9:45 – 10:30 am	**AM Coffee Break** in Exhibit Hall
12:00 – 3:00 pm	**Lunch** provided in the Exhibit Hall. Attendees likely to visit Workshops from 12:15-1:15 PM.
2:00 – 3:00 pm	**Posters** in Exhibit Hall
2:00 – 3:00 pm	**PM Coffee Break** in Exhibit Hall
5:00 – 7:30 pm	**Reception** in Exhibit Hall
7:30 pm	**END OF EXHIBITS**
Midnight	**Deadline for removal of Exhibits from Exhibit Hall**

The Exhibit Hall is located in the ***Grande Ballroom***.



Exhibitors

AB SCIEX Booth #6
Gold-Level Corporate Sponsor
http://www.absciex.com/
AB SCIEX helps to improve the world we live in. AB 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. AB 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 AB SCIEX systems, including the AB 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.absciex.com/clinicalresearch

---

Agilent Technologies Booth #4
Platinum-Level Corporate Sponsor
http://www.agilent.com/chem
Agilent Technologies delivers premiere analytical tools for clinical research ensuring your success from sample prep to final answer. These include a comprehensive portfolio of innovative LC/MS and GC/MS solutions which enables the identification and quantification of both endogenous and exogenous substances in complex biological matrices with the utmost accuracy, productivity and reliability.

---

bioMérieux Booth #24
http://www.biomerieux.com
bioMérieux 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 #19
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 #2
Gold-Level Corporate Sponsor
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 Labs Booth #16
http://www.isotope.com
Cambridge Isotope Laboratories is the world leader in the manufacture and separation of stable isotopes and stable isotope labeled compounds. CIL offers a diverse array of highly-pure compounds that are uniformly or selectively enriched in 13C, 15N, D or 18O, 17O. Our inventory of stable isotope labeled reagents are used across many scientific fields ranging from proteomics, metabolic and environmental applications for quantitative mass spectrometry. CIL's full suite of products for these applications include SILAC protein quantitation kits, 99% enriched amino acids, MouseExpress™ labeled mouse feed and mouse tissue, growth media for protein expression (bacterial, mammalian, insect and yeast), cell free protein synthesis products, environmental contaminants standards for ultra trace environmental analysis, steroids, acyl carnitines, drug metabolites, nucleic acids, lipids, and carbohydrates.

Cerilliant Booth #11
http://www.cerilliant.com
Analytical Reference Standards & Certified Spiking Solutions®-Cerilliant offers 2800+ catalog standards (labeled & unlabeled) including Drugs (pharmaceutical, OTC, & TDM such as hormones, steroids and immunosuppressants), 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 also incorporates cGMP and GLP. A COA is provided with every product. Call 512-238-9974 or visit www.cerillant.com.

Chromsystems Booth #12
****Bronze-Level Corporate Sponsor***
http://www.chromsystems.com
CHROMSYSTEMS
DIAGNOSTICS BY HPLC & LC-MS/MS
Chromsystems is one of the worldwide leading designers and suppliers of analytic techniques. For more than 20 years our products link high technology with the needs of clinical diagnostic laboratories. Chromsystems combines technical advantages of LC-MS/MS and HPLC with the smooth workflow of reagent kits, quality controls, and calibration standards. Our products add value to laboratories, providing results with less effort, more reliability and fulfilling regulatory rules with ease. The product range covers a large spectrum of diagnostic analyses such as therapeutic drug monitoring, newborn screening, vitamin profiling, biogenic amines, vitamin D and many more. Diagnostic kits are complete, ready-to-use and comprehensively validated. Several products are FDA-listed. Additionally, an individual customer service is integral part of Chromsystems. For more information please visit www.chromsystems.com

---

GERSTEL Booth #23
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.

Hamilton Booth #26
http://www.hamiltoncompany.com
Summary not provided.

Imtakt USA Booth #3
http://www.imtaktusa.com
Imtakt designs, manufactures, and sells HPLC columns. Our columns offer chromatographers: 1) Novel Chemistry, 2) High Resolution, and 3) Faster Throughput. We have over 20 complimentary phases, which allows end-users to find the right selectivity for their specific application. Scherzo Family: Our Scherzo family enables a multi-separation mode in LC-MS compatible conditions. Retain polar compounds, without the use of ion-pairing additives in the mobile phase. Elute strong ionic compounds and basic compounds. Retain weak ionic compounds, such as zwitterions. Faster Throughput with our HT and UP: these 3um columns that can be used at high pressure (up to 15,000 psi) for ultra fast seperations. Clinical end users have gotten run times as short as 10-20 seconds.

Indigo Biosystems Booth #15
http://indigobiosciences.com/
The scientists and engineers at Indigo BioSystems imagined a solution for obtaining patient results from LC-MS simply and easily using patented algorithms and informatics technology. ASCENT™ is the intelligent system for production chromatography quantitative analysis and quality review. ASCENT™ provides expert MS quantitation that is easy, flexible, and automated. With ASCENT™, dramatically increase your lab throughput, decrease cost and reduce turn around time all while improving quality through consistent and objective application of your QC rules. ASCENT™ is in production at world leading clinical diagnostic, reference and pain medication monitoring laboratories. Come talk to Indigo BioSystems about how we can help enable auto verification of your chromatographic test.

Ionics Mass Spectrometry Group Booth #18
http://www.ionics.ca
IONICS Mass Spectrometry Group has designed a next generation triple quadrupole mass spectrometer to address the critical requirements of sensitivity, uptime, throughput and day after day after day reliability. For more than ten years we have been creating and developing advanced solutions to improve sensitivity, robustness and throughput for triple quads. In 2009/2010 we launched the 3Q Molecular Analyzer, a true next generation triple. Our 3Q's are specifically designed for the clinical research environment, and engineered to minimize complexity of running and owning a sophisticated instrument while providing the highest levels of sensitivity and throughput, often achieving low femtogram detection levels. To learn more about us, join us at our workshop at noon Wednesday Jan 18th in Marina 6, or stop by booth 18.

IsoSciences Booth #9
http://www.isosciences.com/
IsoSciences (www.isosciences.com) is a world leader in the synthesis of stable isotope labeled mass spec standards including vitamins, steroids, drug substances, metabolites and other compounds of interest. IsoSciences 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 also specializes in the custom synthesis of any compound or metabolite, labeled or unlabeled, that you require.

Microliter Analytical Supplies Booth #17
http://www.microliter.com
MicroLiter Analytical Supplies, Inc. specializes in consumable products for Sample Handlers for Chomatography. MicroLiter 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. MicroLiter offers a variety of methods to test for Pain Management, Vitamin D, Immunosuppressant drugs, and other important compounds. MicroLiter also offers a complete line of vials, closures with septa, inserts and 96-well microplates for chromatography. Stop by MicroLiter's booth to review the posters and find out how to manually try ITSP in advance of an instrument purchase.

Parker Hannifin Filtration & Separation Division, Balston Operation Booth #1
http://www.labgasgenerators.com
Parker Balston Gas Generators for analytical instruments eliminate the expense and danger associated with high pressure compressed gas cylinders. The inconvenience of changing cylinders and supply interruptions will no longer be a concern. A Parker Balston Gas Generator offers long term price stability and eliminates long-term commitments, contract negotiations and tank rental fees. A continuous supply of consistent purity is available 24/7 without the need for operator attention. Parker Balston offers Gas Generators for a variety of analytical applications including LCMS, GC, FTIR, and NMR. Parker offers global distribution and support.

Peak Scientific Booth #10
http://www.peakscientific.com/
Peak Scientific Instruments Ltd are a manufacturer of laboratory Gas Generators including nitrogen, hydrogen and zero air suitable to operate most laboratory analytical applications such as LCMS (liquid chromatography mass spectroscopy) and GC (Gas chromatography). With varying flow rates, purities & pressures of gas generators, available with or without internal air compressors, Peak are confident to offer the complete solution. We are delighted to announce the launch of the NEW Genius2 range. The range consists of three cutting edge Nitrogen Generators. The 3010, 3020 & 3030 All the Generators in the Genius2 are range are designed with the same concept offering a mobile, quiet, convenient and simple plug and play system that is more cost effective than any other gas supply method.

Phenomenex Booth #5
Bronze-Level Corporate Sponsor
http://www.phenomenex.com
phenomenex
...breaking with tradition℠
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.

Shimadzu Booth #22
Bronze-Level Corporate Sponsor
http://www.SHIMADZU.com
SHIMADZU
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 in science and industry, including life sciences, pharmaceuticals, food safety, environmental, chemicals, and forensics. Instruments include chromatographs (HPLC/UFLC/UHPLC, GC); mass spectrometers (GCMS, LCMS, LC-MS/MS. MALDI); spectrophotometers (FT-IR, Fluorescence, UV-VIS-NIR); atomic spectrometers (AA, ICP); X-ray spectrometers (EDX, XRD, XRF); thermal & particle size analyzers; Total Organic Carbon analyzers; data systems/software; balances; protein sequencers and materials testers. International sales, service, and technical support facilities are located in Germany, Singapore, Australia, China, Brazil, and Turkey, with numerous regional offices and distributors worldwide.

SPEware Booth #25
http://www.speware.com
SPEware Corporation is a manufacturer of unique and innovative Solid Phase Extraction Columns & Products. Our Cerex Product Line provides solutions to many extraction problems that require a high degree of purification and efficient processing

---

Tecan Booth #21
http://www.tecan.com
Tecan (www.tecan.com) is a leading global provider of laboratory instruments and solutions in biopharmaceuticals, forensics, and clinical diagnostics. The company specializes in the development, production and distribution of instruments and automated workflow solutions for laboratories in the life sciences sector. Its clients include pharmaceutical and biotechnology companies, university research departments, forensic and diagnostic laboratories. As an original equipment manufacturer, Tecan is also a leader in developing and manufacturing OEM instruments and components that are then distributed by partner companies. Founded in Switzerland in 1980, the company has manufacturing, research and development sites in both Europe and North America and maintains a sales and service network in 52 countries.

Thermo Scientific Booth #7-8
****Diamond-Level Corporate Sponsor***
http://www.thermoscientific.com
Innovation applied to clinical research. 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. www.thermoscientific.com

---

Waters Booth #13-14
****Gold-Level Corporate Sponsor***
http://www.waters.com
Waters Corporation, the premium brand in the analytical instruments industry, creates business advantages for laboratory-dependent organizations by delivering practical and sustainable scientific innovation to enable significant advancements in healthcare delivery, environmental management, food safety, and water quality worldwide. Bringing keen understanding and deep experience to those responsible for laboratory infrastructure and performance, Waters helps customers make profound discoveries, optimize laboratory operations, deliver product performance, and ensure regulatory compliance. Pioneering a connected portfolio of separations and analytical science, laboratory informatics, mass spectrometry, as well as thermal analysis, Waters' technology breakthroughs and laboratory solutions provide an enduring platform for customer success.

Corporate Workshops

Mon Tue & Wed

January 14, 15 & 16, 2012

12:15-1:15 PM

As part of your registration you may attend, at no charge, any Corporate Workshop.

Workshops are held during lunch on Monday and Tuesday from 12:15 - 1:15 PM
Lunch break: Monday and Tuesday 12:30 - 3:00 PM.
Lunch break: Wednesday 12:30 - 3:00 PM.

Some sponsoring vendors may request that attendees register, but it is ***not*** required.

Vendors may, however, provide priority seating to pre-registered workshop attendee if there are space limitation issues.

Box lunches will be availableat 12:00 in the Exhibit Hall on Monday and Tuesday, and in the Harbor Island Foyer on Wednesday so that you may pick up your lunch and bring it into the Workshop.

You will have time after the workshop to:

1. Attend the exhibits,
2. Enjoy the afternoon coffee break (2:00 – 3:00 in Exhibit Hall),
3. View the posters (2:00 – 3:00 in Exhibit Hall),
4. Enjoy a walk outside along the bay.

AB SCIEX - Harbor Ballroom 1

Recent Developments in LC-MS/MS Applications for the Clinical Research Laboratory
Michael Jarvis, Technical Marketing Manager, Clinical Research, AB SCIEX

In this workshop attendees will learn about recent developments in the application of LC-MS/MS technology in the clinical research laboratory, with a focus on the analysis of steroid biomarkers. The revolutionary new SelexION™ ion mobility technology will be presented, which introduces a new dimension of selectivity for LC-MS/MS analyses for clinical research. Examples will be discussed to illustrate how this technology enables the separation of isomers that cannot be resolved by high-resolution mass spectrometry, and furthermore enables improved limits of detection by removing background interferences. In addition, a guest speaker from Phytronix Technologies will discuss how the SelexION™ technology can be combined with the ultra-fast LDTD ionization source to achieve unprecedented throughput and selectivity for MS/MS measurement of analytes such as testosterone.

Waters - Harbor Ballroom 2

Harmonization of LC-MS/MS Assays for Immunosuppressant Drug Monitoring and Enhancing Laboratory Productivity through Automated Online SPE UPLC-MS/MS:A TDM Perspective
Don Mason, Global Scientific Affairs Manager, Waters Corporation and Martin Eastwood, PhD, Senior Clinical Application Scientist, Waters Corporation

Recent data from proficiency testing schemes indicate that up to fifty percent of participating laboratories are now using LC-MS for immunosuppressant TDM. The majority of these centers are using laboratory developed tests that require in-house method development and validation, and post implementation surveillance by proficiency testing. While improvement in inter-laboratory agreement has been observed in proficiency testing schemes over the years, there is still an acute need for harmonization/standardization of LC-MS based testing in the clinical laboratory.1,2 This workshop will feature some recent data demonstrating that harmonization of immunosuppressant TDM using LC-MS is possible, and that the technique can meet or exceed all other analytical criteria offered by immunoassay techniques.

Chromsystems - Harbor Ballroom 3

Diagnostic kits for high throughput analysis by LC-MS/MS
Dr. Claudia Halter

TDM refers to the determination of the concentration of drugs in a biological matrix, usually blood, with the aim of optimizing a patient's treatment regimen. TDM has been proven to be beneficial in many areas in which it is performed as a routine procedure. Chromsystems exclusively offers for MassTox® Series A – beside established parameter sets- new parameter sets in the same reagent modular system for 20 benzodiazepines, 13 tricyclic antidepressants and more than 25 antiepileptic drugs.

Bruker Daltonics - Marina 6

Bruker MALDI Biotyper: Tools for Advanced Analysis for Next Generation Applications in Microbiology
Markus Kostrzewa, Ph. D.

In this workshop both existing tools and some under development for next generation applications of MALDI-TOF technology in microbiology using the Bruker MALDI Biotyper will be discussed. Among these will be workflows, statistical tools and software modules for: ID direct from positive blood culture bottles; detection and identification of multiple species in mixed cultures; differentiation of closely related species and strain differentiation; determination of some types of antibiotic resistance using mass spectrometry. Come to this workshop to understand the exciting future of mass spectrometry in the microbiology laboratory and why mass spectrometry will soon be found in most microbiology labs.

Corporate Workshops - Tuesday

Tuesday January 17, 2012
12:15-1:15 PM

Agilent - Harbor Ballroom 1

Innovations and Analytical Breakthroughs: High-Throughput Quantitation of Protein Biomarkers by SISCAPA and Underivatized Estradiol Analysis by LC/MS/MS
Leigh Anderson PhD , SISCAPA Assay Technologies, Andre Szczesniewski, Agilent Technologies

Quantitation of proteotypic peptides in digests of plasma by SRM-MS allows specific, internally-standardized measurement of protein biomarkers and can achieve sub-nanogram/ml detection levels when specific anti-peptide antibodies are used to enrich target peptides from the plasma digests (SISCAPA). Results will be presented demonstrating the performance of the workflow for high-throughput quantitation of protein biomarkers. In addition, underivatized 17ß-estradiol can now be accurately and rapidly quantified in serum using a simple sample prep and LC-MS/MS approach. Traditionally done also by GC/MS, the quantitative performance by this LC-MS/MS method is comparable in robustness, accuracy and precision. A derivatized LC-MS/MS method will also be presented in comparison.

Shimadzu - Harbor Ballroom 2

Prepare Your Lab for the Future ... From Automated Sample Prep to Ultra Fast Mass Spectrometry
Jeff Dahl, Shimadzu and Kevin Meyer, Perfinity Biosciences

This workshop will demonstrate how ultra fast LC and mass spectrometry technologies increase the speed and scope of clinical assays. Exceptional triple quadrupole scan speeds of 15,000 u/sec and the ability to perform 500 MRM measurements per second enable researchers to fully utilize the capabilities of UHPLC to develop faster assays. Applications of ultra fast MS to drugs of abuse, forensics and clinical toxicology will be presented. This workshop will also show how manual sample preparation steps used in protein/peptide analyses can be replace with a faster, more reproducible, fully-automated sample prep platform. Incorporating the Perfinity Workstation dramatically increases assay reproducibility while enabling complete digestion of plasma/sera samples in less than six minutes. For Research Use Only. Not For Use in Diagnostic Procedures.

Thermo Scientific - Harbor Ballroom 3

Innovation Applied to Clinical Research
Dr. Nigel Clarke, Quest Diagnostics and Dr. Matthew Petrie, San Francisco General Hospital

In this workshop our speakers will present two comprehensive mass spectrometry workflows, LC-MS/MS for peptide quantitation and high-resolution, accurate mass for analysis of designer drugs and unknowns.

Phenomenex - Marina 6

Practical LC/MS/MS Method Development for Clinical Assays and Overcoming the Challenges Encountered During Validation
Seyed Sadjadi[1], Jeff Layne[1], and Carrie Haglock[2]
[1]Phenomenex, Torrance, CA; [2]ARUP Laboratories, Salt Lake City, UT

There are many unique challenges associated with the analysis of a biological specimen that must be considered when developing and validating a new assay. Utilizing the separation power of the HPLC combined with the selectivity of the mass spectrometer can help overcome some of these. In this session we will outline the steps involved in a new method development and optimization of clinical LC/MS/MS assays. We will also discuss challenges that arise and how to overcome them. Specific examples will be given for both a nicotine/cotinine and an aldosterone assay.

Corporate Workshops- Wednesday

Wednesday January 18, 2012
12:15-1:15 PM

AB SCIEX - Harbor Ballroom 1

Advances in LC-MS/MS Hardware and Software Solutions for the Toxicology Lab
Adrian Taylor, Technical Marketing Manager, Forensic Toxicology, AB SCIEX

In this workshop attendees will learn about AB SCIEX's comprehensive portfolio of innovative LC-MS/MS solutions for qualitative and quantitative analysis on a single platform. Using unique QTRAP® and TripleTOF™ technologies, rapid targeted and non-targeted screening methods have been developed to identify a wide variety of analytes, using automatically triggered acquisition of full-scan MS/MS to confirm compound identifications based on comparisons to a comprehensive spectral library. The novel SelexION™ ion mobility technology will be discussed, with the aim of demonstrating how this highly selective device can be used to improve the quality of MS/MS spectra acquired in dirty matrix samples. In addition, Dr. Randall K. Julian of Indigo Biosystems will discuss the use of Ascent software to simplify and automate the processing of LC-MS/MS data.

Waters - Harbor Ballroom 2

Application of Mass Spectrometry Based Proteomics in the Clinical Research Environment and Quantitation of Multiplex Cancer Pathway Proteins in FFPE Tissues Using Targeted Mass Spectrometry
Johannes Vissers, Senior Market Development Manager Proteomics , Waters Corporation and Jon Burrows, Ph.D., Executive Vice President and Head of R&D, Expression Pathology Inc.

Join Waters Corporation as leading experts discuss the application of mass spectrometry based proteomics in a clinical research environment. Tandem quadrupole mass spectrometers are in routine use in clinical laboratories for diagnostic applications such as Therapeutic Drug Monitoring, Metabolic Diagnostics and Toxicology. Liquid Tissue-Selected Reaction Monitoring (SRM) assays allow highly multiplexed protein quantification using mass spectrometry from small amounts of laser micro-dissected and dissolved formalin-fixed paraffin-embedded tissue. SRM protein identification relies on the ability to detect target peptide fragments of proteins whose sequence and mass-to-charge are unique to the targeted protein or specific to a variant of that protein. The clinical validation of the performance of these assays to measure protein expression in relevant human clinical trial cohorts will be demonstrated.

Thermo Scientific - Harbor Ballroom 3

Innovation Applied to Clinical Research
TBD and Dr. Chao Yuan, Cleveland Clinic

In this workshop our speakers will present two comprehensive mass spectrometry workflows, immunoaffinity LC-MS/MS for parathyroid hormone (PTH) analysis and automated online sample preparation with TurboFlow technology for pain management drug analysis.

Ionics Mass Spectrometry Group - Marina 6

Breaking Through Productivity Barriers for High Sensitivity Assays
Dr. Lisa Cousins, VP Research and Development

Clinical Research has embraced mass spectrometry as the instrument of choice when sensitivity and quantitation are key drivers. Adoption has been limited by the feasibility of putting research grade instruments into an industrial setting. The IONICS 3Q Molecular Analyzer has the sensitivity and the robustness to stand up to any setting. With low femtogram detection levels and robustness to run day after day the IONICS mass spec platform continues to gain recognition as the next standard in clinical research.

General Scientific Session

Monday AM Session 1: Track 1: *Targeted Metabolomics* (Chair: Gary Patti)

Monday 10:30 AM Session 1: Track 1: *Targeted Metabolomics* – Podium

Clinical Implications of Metabolomics for Elucidating Novel Biochemical Mechanisms of Disease
Gary Patti (gjpattij@wustl.edu) -- *Young Investigator Awardee*
Washington University School of Medicine
Neuropathic pain is a debilitating condition that develops after injury to the nervous system. Recently, untargeted metabolomics identified a previously uncharacterized sphingomyelin metabolite dysregulated in the disease. However, the physiological mechanism underlying its etiological role had remained unclear. Here we provide a robust characterization of the metabolite, examine its effects on cell cultures and known lipid-signaling cascades, and describe a new pathway in which methylation is critically linked to disease pathogenesis. By using stable-isotope labeling metabolomics, we provide evidence supporting a mechanism where methylation of the metabolized sphingoid base prevents termination of neurotransmission and discuss implications for new pain therapeutics.

Monday 11:00 AM Session 1: Track 1: *Targeted Metabolomics* – Podium

High resolution mass spectrometry and post-acquisition data mining for thorough metabolite screening
Andre LeBlanc (leblanc.andre.3@courrier.uqam.ca) -- *Young Investigator Awardee*
University of Quebec in Montreal (UQAM)
The use of untargeted high resolution mass spectrometric detection in combination with generic acquisition and separation methods is an accessible and time-saving approach for performing metabolite screening and is applicable to a wide range of xenobiotics from a variety of biological matrices. Relying solely on post-acquisition processing for compound-specific screening, such a method can provide useful information for subsequent metabolism related studies due to its sensitivity and specificity. Using this new approach, we analyzed in vitro metabolites formed in human liver microsomes from atrazine. Not only have we detected all its previously-known biotransformations, but also identified several new metabolites.

Monday 11:30 AM Session 1: Track 1: *Targeted Metabolomics* – Podium

Massive Multiplexing of Tandem Mass Spectrometry Assays for the Early Detection of Lysosomal Storage Disorders by Newborn Screening
Frantisek Turecek (turecek@chem.washington.edu)
University of Washington
Enzyme assays in dried blood spots (DBS) based on tandem mass spectrometry (MS/MS) have been developed for eleven lysosomal storage diseases. The assays use synthetic substrates and monitor product formation upon incubation in DBS. The enzymatic products have orthogonal molecular masses to allow simultaneous monitoring by MS/MS. We now report a new approach to multiplex monitoring of nine diseases by fast LC/MS/MS using two incubation buffers and a single injection into the mass spectrometer. The system uses dual short UPLC columns and achieves complete separation and MS/MS analysis of nine enzyme products and internal standards in less than 2 minutes.

Monday AM Session 1: Track 2: *Small Molecule Analytes* (Chair: Deborah French)

Monday 10:30 AM Session 1: Track 2: *Small Molecule Analytes* – Podium

Improving the Selectivity of Endogenous Steroid Analysis Using Differential Mobility Spectrometry
Deborah French (deborah.french@ucsf.edu) -- *Young Investigator Awardee*
University of California San Francisco
Background: Quantification of testosterone by LC-MS/MS can be subject to many interferences. Objective: Determine if using differential mobility spectrometry reduces these interferences. Methods: An ABSCIEX 5500 QTRAP® in ESI positive mode was utilized coupled to a Shimadzu Prominence UPLC. Pediatric patient samples extracted by liquid-liquid extraction or protein precipitation were run with and without using differential ion mobility. Results: Differential ion mobility demonstrated significant removal of interferences from the different serum matrices and obtained more accurate ion ratios and concentration determination. Conclusions: A reliable and robust testosterone LC-MS/MS method with differential ion mobility that reduced the potential interferences was developed.

Monday 11:00 AM Session 1: Track 2: *Small Molecule Analytes* – Podium

Moving a Primary Aldosteronism Screening program from RIA to LC-MS/MS
Daniel Holmes (dtholmes@mail.ubc.ca)
University of British Columbia Department of Pathology and Laboratory Medicine
We review the challenges and benefits of moving our primary aldosteronism (PA) screening program from RIA to LC-MS/MS. Liquid-liquid extraction and supported liquid extraction methods using the ABSCIEX API-5000 LC-MS/MS system for the duplex analysis aldosterone and cortisol will be discussed in application to screening, confirmation and tumor localization for PA. Elimination of large interferences seen in RIA for chronic kidney disease patients have been realized, vastily-improved analysis of adrenal vein samples, and superior turnaround for neonates. Fortuitous diagnoses in adults and children will be demonstrated. A plasma renin activity assay by LC-MS/MS using immunoprotection of angiotensin-I will be discussed.

Monday 11:30 AM Session 1: Track 2: *Small Molecule Analytes* – Podium

Direct measurement of Free Estradiol in Human Serum by Equilibrium Dialysis-LC-MS/MS and Reference Intervals of Free Estradiol in Women
Julie Ray (julie.ray@aruplab.com)
ARUP Laboratories
Over 90% of circulating estradiol is bound to proteins. Measuring the free form of the hormone provides better representation of its bioactive form. Free estradiol is typically measured using indirect methods. We have developed a direct method of measuring the free hormone by equilibrium dialysis-LC-MS/MS using the AB SCIEX TripleQuadTM 5500 mass spectrometer. The limit of quantification was 0.5 pg/mL. Total imprecision was <10% at 1.3, 6.0 and 24.9 pg/mL. When compared with an indirect tracer dialysis technique we found good correlation, while observing a proportional bias of 2.70. Reference interval ranges in premenopausal women have been established.

Monday AM Session 1: Track 3: *Tobacco* (Chair: Ben Blount)

Monday 10:30 AM Session 1: Track 3: *Tobacco* – Podium

Tobacco and tobacco smoke constituents: from products to people.
Clifford Watson (cow1@cdc.gov)
Centers for Disease Control and Prevention
Tobacco use remains a leading cause of preventable disease and death. Cigarette design and behavior influence exposure to toxic constituents. Emission testing using standard cigarette machines does not adequately reflect smokers' intake. Measuring exposure bio-markers provides a definitive means for exposure assessment, but is often invasive, require special handling, and utilize advanced analytical instrumentation. Less invasive techniques show promise for estimating smoke uptake. For example, cigarette filter analysis has shown utility in examining total intake and smoker individualistic variations. A compressive tobacco approach is important to help reduce addiction, exposure, and ultimately the morbidity and mortality associated with tobacco use.

Monday 11:00 AM Session 1: Track 3: *Tobacco* – Podium

Tobacco Exposure Biomarkers of Adverse Health Effects
Lanqing Wang (lfw3@cdc.gov)
CDC
The use of tobacco remains the leading preventable cause of morbidity and mortality in the United States. The measurement in the U.S. population of biomarkers associated with tobacco use and exposure that may have direct links to adverse health effects is an important consideration. Division of Laboratory Sciences at CDC has developed precise and accurate analytical methods to measure tobacco exposure biomarkers: serum/saliva cotinine, urinary NNAL, urinary nicotine metabolites and aromatic amines, by using LC/MS/MS and GC/MS. Serum cotinine analyses have been performed on all NHANES samples since 1988. Urinary NNAL has been measured in NHANES participants aged 6 and above since 2007.

Monday 11:30 AM Session 1: Track 3: *Tobacco* – Podium

Direct, Quantitative Analysis of Nicotine and Its Metabolites in Biofluid Samples Using Paper Spray Mass Spectrometry
He Wang (wang41@purdue.edu) -- *Young Investigator Awardee*
Purdue University
We have developed paper spray for direct analysis of biological samples without sample preparation and chromatographic separation. In this study, we applied paper spray mass spectrometry analysis for direct, quantitative analysis of nicotine and its metabolites from biofluids including whole blood, urine and saliva. Methods have been developed for analysis of dried as well as fresh wet samples and LOQs below 5 ng/mL for blood samples were obtained. Preliminary data show potential of this technique for practical use in smoking test and smoking related disease diagnosis.

Monday PM Session 2: Track 1: *Discovery Metabolomics* (Chair: Thomas Hankemeier)

Monday 3:00 PM Session 2: Track 1: *Discovery Metabolomics* – Podium

Development of a Platform for Online Analysis of the Metabolic Footprint
Jeffrey Enders (jeffrey.r.enders@Vanderbilt.Edu)
Vanderbilt University
Metabolomics and metabolic profiling is said to provide an instantaneous snapshot of cell physiology. While considerable effort has been applied to interpreting metabolic profiles to assess phenotype, there is substantial room for improving current sampling methodologies. It seems a waste of valuable information to take these instantaneous metabolite targets and average their contributions over the course of an entire experiment. This work delineates the progress towards an online platform capable of performing truly integrated systems biology research using a combination of microfluidics, online desalting techniques, and ion mobility-mass spectrometry detection capabilities to provide dynamic exometabolomic sampling of biological systems.

Monday 3:30 PM Session 2: Track 1: *Discovery Metabolomics* – Podium

A diagnostic metabolomic platform designed for use in the clinical laboratory by non-metabolomic specialist
Gary Siuzdak (siuzdak@scripps.edu)
The Scripps Research Institute
Although the success of metabolite analysis has already been demonstrated in the area of clinical diagnostics, a major limitation in further extending the application of metabolomics to the clinic has been in data processing. Data analysis has required installation that varied depending on computer specifications, operating systems, and the vendor of the instrument. Consequently data analysis has typically required familiarity with software settings and command-line driven commands, limiting the number of investigators using the approach. Here, we introduce the first web-based software for the non-metabolomic specialist that minimizes many of these technical issues. The software will be described and demonstrated for the general user.

Monday 4:00 PM Session 2: Track 1: *Discovery Metabolomics* – Podium

Metabolomics: new opportunities for personalized medicine
Thomas Hankemeier (hankemeier@lacdr.leidenuniv.nl)
Netherlands Metabolomics Centre
Metabolic processes are the core of physiology, and consequently, metabolomics is ideally positioned to distinguish between health and disease, and to predict the efficiency of interventions. Several metabolomics platforms have been developed such as global profiling of lipids, central metabolism and targeted platforms such as for oxylipins. Recent developments for the lipid profiling platforms will be discussed such as the identification of position of the double bond in lipids and tracer studies to zoom into biochemical mechanisms. Several applications to (pre)clinical studies are discussed, also showing that these approach is well suited to detect individual differences between patients.

Monday 4:30 PM Session 2: Track 1: *Discovery Metabolomics* – Podium

Detection of Reactive Metabolites by Glutathione Trapping and UHPLC-MS-MS with Fast Precursor Ion and Neutral Loss Scanning
Richard van Breemen (breemen@uic.edu)
University of Illinois College of Pharmacy
Toxicity is the primary cause of lead compounds failing during drug development. Glutathione trapping and UHPLC-MS-MS is underutilized for the detection of reactive metabolites forming during incubation of lead compounds with metabolic enzymes. Since slow scanning and polarity switching of many mass spectrometers limit the usefulness of this approach for rapid screening, we developed an improved GSH screening assay using a fast triple quadrupole mass spectrometer that enables screening on a UHPLC timescale. This method was validated using standards and then applied to an investigation of the mechanism-based inhibition of the metabolic enzyme CYP3A4 by a compound in licorice.

Monday PM Session 2: Track 2: *Small Molecule Analytes* (Chair: Brian Rappold)

Monday 3:00 PM Session 2: Track 2: *Small Molecule Analytes* – Podium

Conventional Wisdom and Truth in Development of Quantitative HILIC-MS/MS Assays.
Brian Rappold (rappolb@labcorp.com)
Laboratory Corporation of America
Hydrophilic interaction liquid chromatography is a powerful separation technique applicable to the quantitation of biomarkers. In research and development of assays, a wealth of literature yields a number of misconceptions regarding HILIC separations, including reconditioning time, solvent amenability and cycle time. This work demonstrates the fallacy of a number of HILIC doctrines when applied to targeted metabolite analysis and addresses throughput, selectivity, sensitivity, and associated components. Examples of observational experimental results to elucidate the steps to successful high thtoughput HILIC-MS/MS assay will also be presented.

Monday 3:30 PM Session 2: Track 2: *Small Molecule Analytes* – Podium

Rapid Analysis of Carnitine and Acylcarnitines by UHPLC-MS/MS
Paul Minkler (paul.minkler@case.edu)
Case Western Reserve University School of Medicine
We developed and validated a rigorously quantitative, accurate, and precise HPLC-MS/MS method for carnitine and acylcarnitine analysis in plasma, urine, and tissue samples. We investigate moving that method to a new UHPLC-MS/MS platform and report a reduction in analysis time from 6 min/sample to 2 min/sample for carnitine and from 21 min/sample to 6 min/sample for acylcarnitines. Chromatographically, both carnitine and acylcarnitines were successfully separated using this new UHPLC-MS/MS platform. Quantification using the rapid carnitine method was successful; acylcarnitine quantification on the new platform is in development.

Monday 4:00 PM Session 2: Track 2: *Small Molecule Analytes* – Podium

A new stable isotope dilution LC-ESI-MS/MS method for the quantification of methotrexate polyglutamates in red blood cells.
Ethan den Boer (e.denboer@erasmusmc.nl) -- *Young Investigator Awardee*
Department of Clinical Chemistry, Erasmus Medical Centre
The folate antagonist methotrexate (MTX) is the anchor drug in the treatment of rheumatoid arthritis. The therapeutic effects of MTX are attributed to the intracellular levels of MTX, present in the cell as polyglutamates (MTXPGn). We developed an UPLC-ESI-MS/MS based method for the separate analysis of MTXPG1-5 in red blood cells using stable isotope labelled internal standards for each MTXPGn. Separation was performed during a 7 minute run time per sample. Intra- and inter-day precision were <4% and <15% for all concentrations and MTXPGn. The method was linear from 0-250 nmol/L (r2>0,99). LLOQ was determined at 1 nmol/L.

Monday 4:30 PM Session 2: Track 2: *Small Molecule Analytes* – Podium

Challenges and Successes in developing a LC-MS/MS Method for the measurement of Aldosterone, Cortisol, 11-Deoxycorticosterone, Corticosterone, 18-Hydroxycorticosterone and 18-Hydroxy-11-Deoxycorticosterone in Human Serum.
Grace van der Gugten (GvanderGugten@providencehealth.bc.ca)
University of British Columbia
The measurement of aldosterone, cortisol, 11-deoxycorticosterone, corticosterone, 18(OH)-11-deoxycorticosterone, and 18-hydroxycorticosterone in a multiplex fashion would be beneficial in the investigation of hypertension caused by mineralocorticoid excess. Developing a LC-MS/MS method to measure these 6 closely related compounds proved challenging. Specific examples of challenges faced with interferences and chromatographic separation will be discussed and the process by which barriers were overcome will be explained. Application to routine clinical specimens and to adrenal vein samples (both successfully and unsuccessfully cannulated) will be shown. Other fortuitous diagnoses we have encountered through multiplex analysis will be highlighted.

Monday PM Session 2: Track 3: *Microbiology* (Chair: Steve Hofstadler)

Monday 3:00 PM Session 2: Track 3: *Microbiology* - Podium
The future of MALDI-TOF mass spectrometry in microbiology – new clinical applications on the horizon

Markus Kostrzewa (km@bdal.de)
Bruker Daltonik GmbH
Recently, MALDI-TOF MS fingerprinting for microbial identification appeared as a powerful alternative to biochemical methods and DNA sequencing. Improved accuracy, reduced costs, and significantly shortened time-to-result have been demonstrated. New application areas are currently investigated where the technology could further improve and speed up diagnostic in clinical microbiology. These are including databases for particular difficult microorganisms, dedicated protocols to identify microorganisms directly from body fluids or enrichment broths, subtyping, e.g. for virulence-prediction, or in epidemiology, and resistance detection. The talk will give an overview about such new approaches and their future perspectives.

Monday 3:30 PM Session 2: Track 3: *Microbiology* - Podium
Further Development of a Mass Coding Based Multiplex-PCR Pathogen Detection Application for Clinical Research
Peter Sheffield (peter_sheffield@agilent.com)
Agilent Technologies
We recently presented a MassCode multiplex PCR research solution for the analysis of samples for 10-30 nucleic acid targets simultaneously. This solution includes nucleic acid extraction, cDNA synthesis, multiplex PCR involving mass-tagged primers, amplicon purification, and tag analysis by LC/MS. Here we report efforts to streamline the present solution. Combination of reagents for cDNA synthesis and PCR into a single master mix represents a valuable improvement for RNA-target panels, decreasing contamination risk and eliminating workflow steps for overall reduced turnaround and hands-on time. Automation of amplicon purification through use of chromatographic methods is another valuable step to free operator time.

Monday 4:00 PM Session 2: Track 3: *Microbiology* - Podium
MALDI TOF - Expanding to New Horizons for Microbial Identification and Beyond
Nedal Safwat (nedal.safwat@biomerieux.com)
bioMerieux Inc.
MALDI TOF technology provides a new horizon for the microbiology lab. Different approaches have been used to develop unique fingerprints for species of organisms, but most significantly a method called SuperSpectra that relies on identifying specific proteins related to a specific species and even to a strain level. The ability to test whole cells showing patterns of mass signals that were reproducible and specific up to strain levels made the technology attractive for potential routine clinical microbiology lab testing. Recent publications show improved identification specificity to a species level with even the ability to identify from blood culture samples.

Monday 4:30 PM Session 2: Track 3: *Microbiology* - Podium
THE ABBOTT PLEX-ID: A NOVEL PLATFORM FOR PATHOGEN DETECTION AND CHARACTERIZATION VIA HIGH THROUGHPUT ESI-TOF MS ANALYSIS OF AMPLIFIED NUCLEIC ACIDS
Steven Hofstadler (shofstad@ibisbio.com)
Ibis Biosciences, An Abbott Company
High throughput electrospray ionization time-of-flight (ESI-TOF) mass spectrometric analysis of polymerase chain reaction (PCR) amplicons represents a novel and universal strategy for the detection and characterization of microorganisms associated with emerging infectious diseases. The process uses mass spectrometry, signal processing, and base composition analysis of PCR amplification products from biologically conserved regions of microbial genomes to simultaneously identify the organisms present in a sample without the need for culture. This strategy distinguishes this approach from other detection/identification strategies in that it requires little or no prior knowledge about an organism in order to identify it in a sample.

Tuesday AM Session 3: Track 1: *Discovery Proteomics* (Chair: Randy Nelson)

Tuesday 10:30 AM Session 3: Track 1: *Discovery Proteomics* – Podium

Apolipoprotein A-I and Apolipoprotein E Mass Spectrometric Immunoassays
Randall Nelson (randal.nelson@asu.edu)
Arizona State University
Molecular heterogeneity within apolipoproteins A-I (apoAI) and E (apoE) can affect their capacity to remove cholesterol and triglycerides from circulation and can result in cardiovascular disease. Current clinical assays quantify apoAI and apoE but are unable to determine the extent to which the proteins exist as a variety of unique molecular forms. Mass spectrometric immunoassay (MSIA) was employed to survey the molecular heterogeneity and quantify the relative abundance of apoAI and apoE variants in plasma samples from donors with varying degrees of cardiovascular disease. Results demonstrate the unique ability of MSIA to measure apolipoprotein variants relevant to heart disease.

Tuesday 11:00 AM Session 3: Track 1: *Discovery Proteomics* – Podium

Proteomics and transfusion medicine: pathogen inactivation using methylene blue and UV-light and its impact on fibrinogen, the key protein in clotting process
Alexia Ortiz (alexia.ortiz@ed.univ-lille1.fr) -- *Young Investigator Awardee*
USR 3290 Laboratoire MSAP (Miniaturisation pour la Synthèse, l
One of the objectives of the French Blood Agency is to preserve the transfusion quality of plasma. The most used inactivation technique is the irradiation of plasma by UV light using methylene blue (MB). This treatment is known to affect the biological activity of fibrinogen, the main protein involved in the clotting process. This study aims at characterizing the impact of BM-treatment on this clotting factor. Based on high resolution mass spectrometry analysis, we identified specific modifications. We correlated them with the decrease in fibrinogen clottability. The present results also demonstrate that proteomics has a potential role in transfusion medicine.

Tuesday 11:30 AM Session 3: Track 1: *Discovery Proteomics* – Podium

Verification of Protein Biomarkers with Mass Spectrometry-Based Selected Reaction Monitoring Assays
Andrei Drabovich (adrabovich@gmail.com) -- *Young Investigator Awardee*
Samuel Lunenfeld Research Institute, Mount Sinai Hospital
Translation of proteomics discovery data from research into clinical practice is constrained due to the lack of efficient biomarker verification and validation methods. Here, we present a biomarker verification workflow based on mass spectrometry and selected reaction monitoring (SRM) assays that provide high selectivity of analysis and superior multiplexing potential. A robust sample preparation protocol for quantification of proteins in various biological fluids will be presented. Specific examples will include verification of protein biomarkers for differential diagnosis of male infertility. Such verification allowed us to propose a panel of biomarkers for differential diagnosis with near absolute specificity and sensitivity.

Tuesday AM Session 3: Track 2: *Toxicology: Emerging Toxicants* (Chair: Matthew McMullin)

Tuesday 10:30 AM Session 3: Track 2: *Toxicology: Emerging Toxicants* – Podium

Development of LC-TOF/MS and LC-MS/MS Methods for the Investigation of Drug Adulteration Trends and Clinical Cases Involving Prevalent Adulterants

Kara Lynch (kara.lynch@ucsf.edu) -- *Young Investigator Awardee*
University of California San Francisco

Illicit drugs often contain substances that pose serious health risks, however these adulterants and contaminants are often not tested for in clinical laboratories. These adulterants can pose challenges for clinicians treating patients with a mixed clinical presentation after exposure to an illicit drug. The objective of this study was to develop an untargeted LC-MS/TOF method to identify cocaine adulterants in clinical samples and targeted LC-MS/MS methods to further investigate 1) adulteration trends and 2) clinical cases involving the identified adulterants. The prevalence of adulterants in cocaine positive samples will be presented along with the investigation of the most common adulterant in clinical cases.

Tuesday 11:00 AM Session 3: Track 2: *Toxicology: Emerging Toxicants* – Podium

Quantitation of Synthetic Cannabinoid Metabolites in Urine Utilizing High Resolution Accurate Mass (HRAM) Orbitrap Mass Spectrometer

Kristine Van Natta (kristine.vannatta@thermofisher.com)
Thermo Fisher Scientific

An LC-HRAM-MS method has been developed for quantitative determination of alky hydroxylated and alkyl carboxylated metabolites of JWH-018 and JWH-073 in urine. The utilization of HRAM enables the separation of compounds with the same nominal mass, but different exact masses by the mass spectrometer rather than by chromatography. This increased mass resolution yields shorter runs times. Different hydrolysis methods and conditions were tested on human samples. Sample processing was dilute and shoot, consisting of enzymatic hydrolysis followed by quenching, centrifugation and dilution of supernatant. Calibration curves were generated from 2 to 1000 ng/mL of urine. No matrix effects were observed.

Tuesday 11:30 AM Session 3: Track 2: *Toxicology: Emerging Toxicants* – Podium

Excretion profiles for 11 designer synthetic cathinones in human urine. Interpretive value of reduced beta-hydroxy metabolites.

Victor Uralets (vuralets@redwoodtoxicology.com)
Redwood Toxicology Laboratory

Designer stimulants - synthetic derivatives of cathinone (“bath salts”) and their metabolites excreted free in human urine were studies in over 8000 routine samples obtained from Redwood Toxicology Laboratory clients. Mephedrone, buphedrone, flephedrone, 4-methylethcathinone, 3,4-dimethylmethcathinone and pentedrone largely convert in the body to a variety of respective substituted ephedrines by reduction of beta-keto- into hydroxy- group. A substantial number of specimens in this group contained reduced metabolites, but not the parent drug. Methylone, ethylone and butylone on the contrary are excreted mostly as such. For MDPV reduced metabolites were not detected at all.

Tuesday AM Session 3: Track 3: *Microbiology* (Chair: Nathan Ledeboer)

Tuesday 10:30 AM Session 3: Track 3: *Microbiology* – Podium

Development of a Mass Spectrometry-Based Diagnostic for Coccidioidomycosis
Andrew VanSchoiack (vansca@email.arizona.edu) -- *Young Investigator Awardee*
University of Arizona
Valley Fever, or Coccidioidomycosis is a disease caused by the fungus Coccidioides spp. (Cocci) It presents with a variety of symptoms, however a false negative diagnosis can occur in up to 50% of infections. Described here is a mass spectrometry based diagnostic technique. Using three previously identified target proteins and MRM transitions for each, a method using an LTQ Orbitrap Velos is under development. Fragmenting only the parent ions of interest and performing full MS/MS product ion scans, the transitions will be established after data collection. This technique will be applied to Cocci positive mouse and de-identified human samples.

Tuesday 11:00 AM Session 3: Track 3: *Microbiology* – Podium

Rapid detection of multidrug resistant bacteria by Mass Spectrometry
Michael Hodsdon (michael.hodsdon@yale.edu)
Yale University
Multi-drug resistant gram negative rods have limited diagnostic and treatment options. Carbapenemases, enzymes that hydrolyze the carbapenem class of beta lactam antibiotics, are associated with resistance to most modern antibiotics. We have developed a novel, rapid, and scalable mass spectrometric (MS) assay that detects carbapenemase expression within 30 minutes from complex biological matrices. Carbapenemase activity leads to the addition of H2O to the parent drug, and we use UPLC/MS/MS to monitor for the appearance of the 18 Da increase in molecular weight associated with enzyme activity. We are able to accurately differentiate among related resistance mechanisms and detect enzyme activity from positive blood cultures.

Tuesday 11:30 AM Session 3: Track 3: *Microbiology* – Podium

Features of the Proteome of E coli 0104:H4 though 1D-LC-MS/MS; Model of an Emerging Mosaic Clone
Raju Misra (raju.misra@hpa.org.uk)
Health Protection Agency
In late May 2011 over 4000 people were infected by a atypical Shiga-toxin producing strain of E. coli that was multi-drug resistant prompting a worldwide effort to characterise this new pathogen. Within days several genomes were sequenced and using crowd-sourcing of data its genome was assembled. However, the genome blue print alone was insufficient to provide information on disease severity. To decipher the expressed virulence determinants we utilised an established method of cell lysis, followed by separation on SDS-PAGE tracks and analysed the tryptic peptides by (LC-MS/MS) using two different LTQ Orbitrap instruments to reveal a plethora of virulence determinants.

Tuesday PM Session 4: Track 1: *Targeted Proteomics* (Chair: Christoph Borchers)

Tuesday 3:00 PM Session 4: Track 1: *Targeted Proteomics* – Podium

Development of Automated SISCAPA Assays for High-Throughput Quantitation of Protein Biomarkers
Leigh Anderson (leighanderson@siscapa.com)
SISCAPA Assay Technologies
Quantitation of proteotypic peptides in digests of plasma by SRM-MS allows specific, internally-standardized measurement of protein biomarkers and can achieve sub-nanogram/ml detection levels when specific anti-peptide antibodies are used to enrich target peptides from the plasma digests (SISCAPA). We have developed an automated protocol for implementing this immunoaffinity enrichment of biomarker peptides, allowing processing of 96 samples in less than 30 minutes. For an 11-plex assay, this automated sample preparation has been combined with a rapid, conventional flow LC/MS analysis (3 min cycle time). Results will be presented demonstrating the performance of the workflow for high-throughput quantitation of protein biomarkers.

Tuesday 3:30 PM Session 4: Track 1: *Targeted Proteomics* – Podium

Assessment of Multiple Myeloma Patients with Quantitative Mass Spectrometry
John Koomen (john.koomen@moffitt.org)
Moffitt Cancer Center
Quantitative mass spectrometry assays for measurement of immunoglobulins in multiple myeloma (MM) patients provide another approach for diagnosis and monitoring tumor burden. Liquid chromatography-multiple reaction monitoring mass spectrometry (LC-MRM) assays were optimized prior to assessment of serum from 83 patients. LC-MRM can routinely quantify expression of the immunoglobulins and their isoforms, based on application to patient samples and comparisons with existing clinical methods. Peptide-based LC-MRM assays for diagnosis and prognosis of MM determine the type and isoform of the involved immunoglobulin and quantify its expression in a single test, further illustrating the clinical utility of LC-MRM for measuring protein biomarkers.

Tuesday 4:00 PM Session 4: Track 1: *Targeted Proteomics* – Podium

Novel Quantification Method Based on High-Resolution Mass measurements Combined with Polypeptides Internal Standards
Bruno Domon (bdomon@crp-sante.lu)
Luxembourg Clinical Proteomics Center
A quadrupole / orbitrap mass spectrometer with high resolution and high mass accuracy capabilities was used to analyze clinical samples and to reliably discriminate the targeted peptides from background interferences. This technique was applied to quantify bladder and lung cancer biomarker candidates in urine and plasma samples, respectively. Analyses were performed in selected ion monitoring mode and in MS/MS modes. Quantification was achieved using novel concatenated polypeptides comprising three proteotypic sequences representing the protein of interest and one reporter peptide, which enables precise determination of the internal standard concentration. In addition, all analyses were replicated on a triple quadrupole instrument.

Tuesday 4:30 PM Session 4: Track 1: *Targeted Proteomics* – Podium

An iMALDI-based Assay for Measuring Plasma Renin Activity for the Diagnosis of Secondary Hypertension
Christoph Borchers (christoph@proteincentre.com)
University of Victoria
Plasma renin activity (PRA) is an essential analytical tool for screening/diagnosis of secondary hypertension. Typically, PRA is measured by competitive radioimmunassay (RIA), but there are significant drawbacks, including the requirement for use of radioisotopes, non-specificity, and long-analysis times. We have developed a PRA assay using iMALDI, a method combining selective immunocapture with mass-spectrometry-based detection, which overcomes these drawbacks. Our iMALDI assay showed a linear correlation to RIA used with a high correlation coefficient (R2=0.94, 42 samples). The short time-to-result and simple experimental set-up for iMALDI, combined with its improved specificity, make this method very promising as a clinical diagnostics tool.

Tuesday PM Session 4: Track 2: *Toxicology: K2/Spice* (Chair: Jeffrey Moran)

Tuesday 3:00 PM Session 4: Track 2: *Toxicology: K2/Spice* – Podium

A State Response to 'Legal Highs': Forensics to the Clinic
Jeffery Moran (jeffery.moran@arkansas.gov)
Arkansas Department of Health
This presentation briefly reviews the history of synthetic cannabinoids, illustrates why these new designer drugs pose significant public health threats, and reviews several analytical challenges that had to be met to provide Arkansas with the analytical capacity necessary to support proposed legislation and protect public health. A particular focus is placed on new GC-MS forensic and LC-MS/MS toxicological testing platforms developed through private, academic, and governmental partnerships. This capacity forms the basis for understanding the toxicity of these drugs and enacting appropriate statewide regulations.

Tuesday 3:30 PM Session 4: Track 2: *Toxicology: K2/Spice* – Podium

The Designer Drug Phenomenon from Fake Pot to Bath Salts: Commercial Toxicology Laboratory's Challenges, Partnerships and Solutions
Matthew McMullin (matthew.mcmullin@nmslabs.com)
NMS Labs
The rapidly changing landscape of designer drugs has pushed commercial labs outside of the normal comfort zone and in-house expertise. Collaborations are needed to quickly obtain the expertise required to determine the analytes and develop the appropriate analytical procedures to provide testing services in a production lab environment. These "legal highs" can be divided into two groups: 1) synthetic cannabinoids and, 2) bath salts. An overview and analytical approaches will be presented for both the synthetic cannabinoids and the bath salts.

Tuesday 4:00 PM Session 4: Track 2: *Toxicology: K2/Spice* – Podium

Quality Assessment of Clinical Laboratory Services for the Analysis of Synthetic Cannabinoids
Richard Jenny (rwj03@health.state.ny.us)
New York State Dept of Health
We surveyed in August 2011 the fifty-six clinical and forensic laboratories around the country that provide toxicology services to healthcare providers in NYS to identify the laboratories that are testing biological specimens for synthetic cannabinoids. Twelve laboratories responded that testing is directly provided, primarily for JWH-018 and JWH-073 in urine. No two laboratories tested for the same panel of metabolites, and the limits of quantification varied more than 100-fold. We have submitted urine-based performance testing specimens for analysis. The clinical validity of metabolite panels selected by laboratories and the accuracy of metabolite quantification are being assessed and will be presented.

Tuesday 4:30 PM Session 4: Track 2: *Toxicology: K2/Spice* – Podium

Challenges and Opportunities in the Manufacture of Analytical Standards for Emerging Drugs of Abuse
Greg Endres (gendres@caymanchem.com)
Cayman Chemical
This presentation will discuss some of the challanges faced in the production of analytical standards for emerging drugs of abuse. The focus will be on synthetic cannabinoids and cathone-based compounds and describe the evolution of these classes of designer drugs.

Tuesday PM Session 4: Track 3: *InBorn Errors* (Chair: Charles Hoppel)

Tuesday 3:00 PM Session 4: Track 3: *InBorn Errors* – Podium

Detailed Analysis of Acylcarnitines in Metabolic Disease Research
Charles Hoppel (charles.hoppel@case.edu)
Case Western Reserve University School of Medicine
Detailed analysis of acylcarnitines is used for: 1) Follow-up to positive tandem MS / newborn screening (TMS/NBS) results to eliminate false positives and resolve isomeric acylcarnitines, 2) Monitoring with accurate and precise values (as opposed to a semi/pseudo quantitative screen), and 3) In support of clinical research. We developed and validated a rigorously quantitative, accurate, and precise HPLC-MS/MS method for acylcarnitine analysis in plasma, urine, and tissue samples. Acylcarnitine species were resolved and quantified regardless of sample type, acyl chain length, or isomeric configuration. Chromatographic separation of isomeric acylcarnitines allowed for detailed analysis of patient samples not possible by TMS/NBS.

Tuesday 3:30 PM Session 4: Track 3: *InBorn Errors* – Podium

Application of High Resolution Mass Spectrometry in Newborn Screening
Julia Denes (Julia.Denes@anorg.Chemie.uni-giessen.de) -- *Young Investigator Awardee*
Justus Liebig University, Institute for Inorganic and Analytical Chemistry
Effective combination of single-stage high resolution mass spectrometry and nanoelectrospray was applied for determination of metabolites from dried blood spot samples. The method was tested on samples taken from healthy newborns and patients with different metabolic diseases and the diagnostic results were in agreement with the diagnosis. Since the entire mass spectral pattern is specific to a given condition, linear statistical pattern recognition methods were also successfully used for data analysis, besides quantitative determination of known biomarkers. Due to the extended range of detected metabolites and the complex data analysis, reduction of the false-positive rate of newborn screening is possible.

Tuesday 4:00 PM Session 4: Track 3: *InBorn Errors* – Podium

Analysis of Glycosaminoglycans in Urine using Tandem Mass Spectrometry: Potential for Therapeutic Monitoring of Patients with Mucopolysaccharidoses
Haoyue Zhang (haoyue.zhang@duke.edu)
Duke University Biochemical Genetics Laboratory
Mucopolysaccharidoses (MPSs) are complex storage disorders that result in the accumulation of glycosaminoglycans (GAGs) in urine, blood, brain and other tissues. Enzyme replacement (ERT) and hematopoietic stem cell (HSCT) therapies are increasingly used to treat several MPSs. We developed a stable isotope dilution UPLC-ESI-MS/MS method to quantify the GAGs, chondroitin sulfate (CS), dermatan sulfate (DS) and heparan sulfate (HS), in urine. The method is diagnostically valuable, having the required sensitivity and specificity to discriminate patients with MPS III and MPS VI from MPS I, MPS II and from normal controls, and is useful in monitoring patients following therapeutic intervention.

Tuesday 4:30 PM Session 4: Track 3: *InBorn Errors* – Podium

A Multiplexed Enzyme Assay Utilizing Tandem Mass Spectrometry for the Diagnosis of the Oligosaccharidoses
Sean Hofherr (hofherr.sean@mayo.edu) -- *Young Investigator Awardee*
Mayo Clinic
Oligosaccharidoses are a rare group of lysosomal storage disorders. Current clinical testing for the oligosaccharidoses is performed in biochemical genetics laboratories and involves thin layer chromatography (TLC) screen lacking sensitivity and specificity. Positive TLC screen is followed by individual fluorometric (4MU-substrate) enzyme assays in WBCs or fibroblasts, which although specific are ordered and performed piecemeal. This translates into increased cost and time from clinic visit to diagnosis. We addressed this widespread deficiency by developing a multiplexed enzyme assay for all oligosaccharidoses utilizing tandem mass spectrometry, which in preliminary experiments on patient cell lines, was able to detect individual enzyme deficiencies.

Wednesday Early AM Session 5: Track 1: *Disease Markers* (Chair: Dan Raftery)

Wednesday 8:00 AM Session 5: Track 1: *Disease Markers* – Podium

Development and Initial Validation of a Metabolite Profile for the Early Detection of Breast Cancer Recurrence
Daniel Raftery (raftery@purdue.edu)
Purdue University
The detection of recurrent breast cancer is limited by poorly performing CA tests that are both insensitive and late markers. Because of their sensitivity to biological status, metabolite markers may provide better diagnostic performance and earlier detection. Combining MS and NMR methods improves global metabolomics, resulting in a serum based metabolite profile that is twice as sensitive as the CA 27.29 assay, and detects recurrence 12 months earlier. The profile has been ported to a single MS platform and validated using an independent set of ~100 patient samples. Assay performance, and an outlook for the approach will be discussed.

Wednesday 8:30 AM Session 5: Track 1: *Disease Markers* – Podium

Discovery and Measurement of Immunogenic Gluten Peptides
Robert Voyksner (robert_voyksner@lcmslimited.com)
LCMS Limited
Gluten is one of eight allergens that FDA has identified in the consumer and food protection act. Dietary gluten (a class of proteins present in wheat, barley and rye) detection and quantification is important because some gluten proteins are implicated in a variety of immune diseases, food allergies and intolerances. This presentation will discuss the development of an analytical LC-MS based methodology for the identification and quantification of physiologically relevant gluten marker peptides that can serve as markers for gluten in food and consumer products. This approach utilized LC/ion trap, LC/QQQ and LC/Q-TOF accurate mass analysis.

Wednesday 9:00 AM Session 5: Track 1: *Disease Markers* – Podium

Determination of α–amanitin in human urine by negative ion MRM and LC/MS/MS
Christopher Pittman (ctpittman@cdc.gov)
Centers for Disease Control and Prevention
In response to numerous reported poisonings annually from α-amanitin, a fungal toxin found in mushrooms including the Death Cap and Destroying Angel species, an LC/MS/MS analytical method has been developed to quantitate human exposure by laboratory analysis of urine. Symptoms of α-amanitin poisoning can be delayed up to 24 hours and may be initially dismissed as food poisoning. An early definitive test for α-amanitin could lead to more rapid diagnoses and treatment of poisoning cases. To address the need for a rapid, sensitive and robust analytical test, we have developed a novel, high throughput LC/MS/MS urinary assay.

Wednesday 9:30 AM Session 5: Track 1: *Disease Markers* – Podium

A Quantitative Proteomic Workflow for Characterization of Frozen and Archival FFPE Clinical Biopsies: Laser Capture Microdissection Coupled with Label-Free Mass Spectrometry
Michael Freitas (freitas.5@osu.edu)
The Ohio State University
This abstract describes a highly efficient, robust proteomic workflow for routine quantitative proteomic analysis of Laser Microdissected tissues. Quantitative proteomics was performed with as few as 500 cells from frozen or archival FFPE biopsies. The approach wa compatible with spectral count quantitation. Comparative proteomic analysis of microdissected 1) glomeruli from frozen and FFPE needle biopsies from patients with glomerular nephritis and 2) tumor microenvironment (tumor vs. stroma) from murine breast cancer models. Validation of differentially expressed proteins by immunohistochemistry will be presented. These examples illustrate the capabilities of tissue proteomics for biomarker discovery from limited human tissues.

Wednesday Early AM Session 5: Track 2: *Toxicology* (Chair: Jane Dickerson)

Wednesday 8:00 AM Session 5: Track 2: *Toxicology* – Podium

Open-Access Liquid Chromatography Tandem Mass Spectrometry for the Analysis of Drugs of Abuse and Therapeutic Monitoring in Quantitative Clinical Toxicology.
Matthew Crawford (crawfm1@labcorp.com)
Laboratory Corporation of America
The evolution of drug screen/confirmation assays has been driven by the replacement of low-throughput technologies (LC-UV, GC-MS) with open-access multiplexed TFC-LC-MS/MS and HILIC-MS/MS in our laboratory. Enhancements in multiplexing technology, mass spectrometric sensitivity and chromatographic system design have been the primary drivers for such advancements. The combination of analytical cassetting, generic automated sample preparation and tuned methodologies using generic LC formats have allowed for the analysis of greater than 2000 samples per day in a walk-up platform for a high throughput clinical diagnostic laboratory. This paper describes the systems and methodologies applied in the quantification of targeted clinical toxicology analytes.

Wednesday 8:30 AM Session 5: Track 2: *Toxicology* – Podium

Finding the Cheats: Measurement of 14 Opioids and 6 Opioid Glucuronide Metabolites in Urine by UPLC-MS/MS
Jane Dickerson (janed4@uw.edu) -- *Young Investigator Awardee*
University of Washington
When chronic pain patients are suspected of being non-compliant, their therapy can be withdrawn. Therefore, sensitive and specific confirmatory testing is important in identifying diversion and adherence. In this work, we aimed to develop a liquid chromatography tandem mass spectrometry method to detect 14 opioids and 6 opioid glucuronide metabolites. Detecting glucuronides increases the detection window and adds confidence. Internal standards were not available for every analyte to critically evaluate for ion suppression. Instead, we designed a novel approach to achieve the most rigorous quality control possible in which we evaluated the recovery of each analyte in each negative sample.

Wednesday 9:00 AM Session 5: Track 2: *Toxicology* – Podium

Analytical assessment of the suitability of Liquid Chromatography- Time-of-Flight Mass Spectrometry (LC-TOF/MS) as a platform for a comprehensive serum drug of abuse panel
Alan Wu (Alan.Wu@ucsf.edu)
San Francisco General Hospital/ UC San Francisco
LC-MS/MS has emerged as the gold standard for confirmatory and quantitative drug analysis. More recently, the high resolution and mass accuracy of LC- TOF/MS has raised the possibility of having a good alternative to LC-MS/MS in targeted drug screening and confirmation. Coupled with retention time matching, the high mass accuracy of LC-TOF/MS allows targeted drug identification without the need for parent compound fragmentation. We have assessed and demonstrated the suitability of a comprehensive LC-TOF/MS serum drug of abuse panel to screen and deliver semi-quantitative data for 214 drugs that may cause intoxication in cases seen at the emergency department.

Wednesday 9:30 AM Session 5: Track 2: *Toxicology* – Podium

Rapid Analysis of Small Molecule Analytes in Plasma Using Ultra-Fast Online SPE/MS/MS
Vaughn Miller (vaughn.miller@agilent.com)
Agilent Technologies
We evaluated the ability of an ultra-fast SPE/MS/MS system to analyze small molecule analytes in plasma with much faster sample cycle times and similar analytical results compared to LC/MS/MS methods. Critical bioanalytical parameters were systematically investigated using small molecule analytes (i.e. hydroxymidazolam) spiked into plasma. Comparable accuracy, precision, linearity, and sensitivity were achieved at rates 20-30 fold faster than LC/MS/MS. SPE/MS/MS may be useful for the fast and efficient analysis of similar analytes in biological matrices.

Wednesday Early AM Session 5: Track 3: *Methods Validation* (Chair: Bob Kobelski)

Wednesday 8:00 AM Session 5: Track 3: *Methods Validation* – Podium

Validation of Efficient LC-MS/MS Calibration Strategies
Matthew Olson (molson8@jhmi.edu) -- *Young Investigator Awardee*
The Johns Hopkins Hospital
The measurement of the standard curve for every batch of clinical samples comprises a major source of labor and materials costs for LC-MS/MS quantitative analysis. This work focused on the use of more efficient calibration strategies and their effects, if any, on assay quality of clinical nortriptyline serum concentration measurements. The strategies included: the use of the so-called “one-point” and “internal” calibration strategies. Retrospective (n=212 patients) and prospective (n=190 patients) analyses demonstrated excellent agreement with method versus method slopes of .97 to 1.03 ($R > 0.99$) for this patient cohort. Larger validation sets are in progress.

Wednesday 8:30 AM Session 5: Track 3: *Methods Validation* – Podium

Method Validation in the Clinical Laboratory: Mass Spectrometric Methods and the College of American Patholgists Checklists
Michael Peat (michael.a.peat@questdiagnostics.com)
Quest Diagnostics
Mass spectrometry is increasing used in the clinical laboratory, primarily for toxicology and endocrinology testing. Validation can be considered as a series of analytical and statistical procedures designed to objectively demonstrate the testing procedures applicability for the intended purpose. Validation of GC-MS methods is included in a number of the CAP Checklists, particularly for toxicology. These validations have included both qualitative and quantitative procedures, for example specificity and sensitivity (limit of detection). Specific checklist questions will be used to frame this discussion. The second part of the discussion will focus on the newer areas of mass spectrometry.

Wednesday 9:00 AM Session 5: Track 3: *Methods Validation* – Podium

FDA-Approved LC-MS Test Procedure for Clinical Use: Method Validation Support by Independent Laboratories
Kimberly Napoli Eaton (kimberly.n.eaton@gmail.com)
Andor Laboratories, LLC
The benefit of LC-MS technology for clinical assays is now recognized, but laboratory-developed tests require rigorous validation. FDA-approved reagent kits can ease this burden. One such kit for determination of tacrolimus has achieved FDA approval following performance evaluations by the manufacturer and an industry guideline-based validation performed by independent clinical labs. The validation experiments demonstrated that, by analyzing actual clinical specimens with the kit, predetermined goals for linearity, limits of detection/quantitation, precision, and accuracy as well as comparison to established LC-MS procedures were achieved. The ability to dilute and freeze/thaw specimens was demonstrated as were sample stability, and freedom from interference.

Wednesday 9:30 AM Session 5: Track 3: *Methods Validation* – Podium

Method Validation Practices in Forensic Toxicology
Jarrad Wagner (jarrad.wagner@okstate.edu)
Oklahoma State University-Ctr for Health Sciences
Proper method validation is required in forensic toxicology in order to ensure that results are correct, and that they will stand up under the scrutiny that may be encountered in a court of law. Method validation requirements depend on the type of method being performed. Qualitative or screening methods may require only the determination of limit of detection, selectivity, processed sample stability, and matrix effects. Quantitative methods may require further validation of accuracy, precision, calibration model, carryover, limit of quantitation, and recovery.

Wednesday AM Session 6: Track 1: *Regulations & Proficiency* (Chair: Donald Mason)

Wednesday 10:30 AM Session 6: Track 1: *Regulations & Proficiency* – Podium

Development and Evaluation of an Isotope-Dilution Liquid Chromatography-Mass Spectrometry Method for the Determination of Creatinine in a Novel Urine-based Standard Reference Material
Johanna Camara (johanna.camara@nist.gov)
National Institute of Standards and Technology
An isotope dilution liquid chromatography-mass spectrometry (ID-LC-MS) method was developed and evaluated for the value assignment of creatinine in a novel urine-based Standard Reference Material® (SRM). Spiking urine with d3-creatinine followed by dilution in acidic solution and ID-LC-MS analysis resulted in appropriate accuracy (recovery=104 %) and precision (<=2 %). ID-LC-MS response was linear over a wide range (0.05 mg/dL-1.0 mg/dL) and separation of creatinine from creatine was verified by LC-MS. This method will be employed in the value assignment of urine creatinine in the newly developed SRM and may be utilized to add creatinine values to other clinical urine SRMs.

Wednesday 11:00 AM Session 6: Track 1: *Regulations & Proficiency* – Podium

The CDC Hormone Standardization (HoSt) Program: Update and New Criteria
Julianne Botelho (gur5@cdc.gov)
CDC
The CDC Hormone Standardization (HoSt) Program is working toward providing accurate and reliable results through harmonizing laboratories measurements of steroid hormones regardless of the method, the measurement procedure, and the laboratory where the analyses are carried out. The HoSt Program was established in 2009 and currently has 18 active participants. To continue to improve measurements, the acceptable criteria for testosterone are currently being reviewed and will be adjusted in 2012 to further improve research translation and patient care. An update on these changes will be provided as well as information on the addition of estradiol to the HoSt Program.

Wednesday 11:30 AM Session 6: Track 1: *Regulations & Proficiency* – Podium

Results from the Initial Exercises of the NIST/NIH Vitamin D Metabolites Quality Assurance Program
Mary Bedner (mary.bedner@nist.gov)
National Institute of Standards and Technology
There have been four completed exercises of the NIST/NIH Vitamin D Metabolites Quality Assurance Program (VitDQAP). For each exercise, program participants used immunoassay and/or LC methods to determine 25-hydroxyvitamin D in human serum or plasma study materials that were selected to represent different clinical concentration ranges. NIST used LC-MSn methods and quantitation with isotopically labeled standards to evaluate the materials. The community consensus statistics for the participant results for each study material were determined by NIST. A summary of the participant and NIST results for selected study materials from the first four exercises will be presented and discussed.

Wednesday AM Session 6: Track 2: *New Advances* (Chair: Al Yergey)

Wednesday 10:30 AM Session 6: Track 2: *New Advances* – Podium

SimulTOFTM Mass Spectrometry for High Performance MS and MS-MS
Marvin Vestal (Marvin.Vestal@virgininstruments.com)
VIC Systems
A new principle for time-of-flight (TOF) mass spectrometry is described together with applications to analysis of small molecules, peptides, and intact proteins by MALDI. This advance allows simultaneous space and velocity focusing and provides both high resolving power and high sensitivity using a relatively simple analyzer. Existing TOF instruments employ "space focusing" and "delayed extraction" to reduce effects of initial position and initial velocity, but it is impossible to simultaneously achieve both space focusing and velocity focusing in these instruments. In recent work we have developed an alternative approach to focusing in TOF that overcomes this limitation. Applications to tissue imaging and MSIA are presented.

Wednesday 11:00 AM Session 6: Track 2: *New Advances* – Podium

SelexION™ Ion Mobility Technology: Introducing a New Dimension of Selectivity for LC-MS/MS Analyses
Michael Jarvis (michael.jarvis@absciex.com)
AB SCIEX
For challenging LC-MS/MS analyses an orthogonal separation technique, such as differential ion mobility spectrometry, may be used to resolve isobaric species that cannot be separated by tandem mass spectrometry alone. In the work presented here the new SelexION™ ion mobility technology has been utilized to differentiate between isobaric analytes, and to improve limits of quantitation by reducing chemical background for analytes such as steroids and prostaglandins. The ability to completely resolve isobaric species provides analysts with the opportunity to dramatically decrease chromatographic run-times in LC-MS/MS methods, since complete chromatographic separation of interfering compounds is no longer a requirement.

Wednesday 11:30 AM Session 6: Track 2: *New Advances* – Podium

Direct and Rapid Analysis of Biological Samples by Paper Spray-MS: Toward Point of Care Mass Spectrometry
Nicholas Manicke (nmanicke@purdue.edu)
Purdue University
Paper Spray is a simple and low cost method for the direct analysis of dried matrix spots by MS. Analysis is performed by depositing the sample on a porous substrate, such as a dried blood card, that has been cut to a sharp point. Target compounds are simultaneously extracted and sprayed into the MS by wetting the substrate with a solvent and applying a high voltage. Quantitative accuracy and precision over a range of 1 to 10,000 ng/mL or more from dried blood are routinely obtainable. In addition to assay validation data, a cross-validation study with LC-MS will be presented.

Wednesday AM Session 6: Track 3: *Microbiology* (Chair: James Stephenson)

Wednesday 10:30 AM Session 6: Track 3: *Microbiology* – Podium

Identification of bacteria from positive blood cultures using MALDI-TOF
Nathan Ledeboer (nledeboe@mcw.edu)
Medical College of Wisconsin
We evaluated the performance of MALDI-TOF to identify bacteria directly from positive blood cultures. Of the cultures analyzed, 81 contained gram-positive bacteria (GPB), 31 contained gram-negative bacteria (GNB), 5 contained yeast, and 12 were polymicrobial. Initial interrogation with MALDI produced "acceptable" identifications (score >1.7) for 79.0% GPB cultures. Narrowing the mass range increased acceptable identifications to 92% for GPB. MALDI produced "acceptable" identifications for 100% of GNB cultures. Time from culture positivity to identification using routine methods ranged from 19.2 hrs to 128.5 hrs. Time to identification for any isolate using the Biotyper/Sepsityper kit was 30 min

---

Wednesday 11:00 AM Session 6: Track 3: *Microbiology* – Podium

Identifying Pathogens in "Culture Negative" Infections: a Case Series exploring PCR and Electrospray Ionization Mass Spectrometry for Microbial Identification
Raymond Ranken (rranken@ibisbio.com)
Ibis Biosciences, An Abbott Company
PCR/ESI-MS is a novel diagnostic method that combines two highly sensitive techniques. We show that this approach may prove to be useful in clinical situations when a pathogen is difficult to detect or culture methods are inadequate. These characteristics can assist in the clinical evaluation, direct specific therapy, minimize unnecessary antibiotic use, and target appropriate ancillary management. Some of the limitations of culture are averted by PCR/ESI-MS, including dependence on maintaining viability of organisms, and delay between culture inoculation and organism identification of anywhere from one to two days or, in the case of fungi, one to two weeks.

Wednesday 11:30 AM Session 6: Track 3: *Microbiology* – Podium

Detection and Quantitation of human JC polyomavirus and Host Response in Formalin fixed Paraffin Embedded Human Brain Tissue by Mass Spectrometry
Jing Wei (jwei@jadebio.com)
Jadebio Inc.
PML is a destructive JCV infection of CNS white matter. PML is receiving increased attention due to its occurrence in a rare subset of patients on a variety of selective immunomodulatory agents. Fundamental aspects of JCV biology and PML pathology remain mysterious. We analyzed a case of histologically-confirmed PML. Brain tissues were analyzed by global quantitative proteome profiling and MRM absolute quantitation. The result were correlated with immunohistochemical findings as well as qPCR. These studies serve as a foundation to better understand JCV biology and PML pathology, with the goal of improving risk management in patients on selective immunomodulatory therapies.

Wednesday PM Session 7: Track 1: *New Advances* (Chair: Michael Hodsdon)

Wednesday 2:00 PM Session 7: Track 1: *New Advances* – Podium

" Find the needle in the Haystack" with on-line pI selection of proteins in complex samples with a new HX-IA Instrument™ coupled to HR-API-Mass Spectrometry
Thorleif Lavold (tl@biomotif.com)
Biomotf AB
The new unique micro-fluidic H/DX technology will enable researchers to study structure, conformation, dynamics and molecular interactions in the liquid phase without tethering. The use of our latest" Find the needle in the Haystack" invention coupled to API-MS as a novel analytical tool for on-line pI protein separation, digestion and quantitative analysis of peptides will be discussed. The HX-IA Instrument™ (prototype) could potentially become a unique tool for diagnosing patients with Alzheimers, Parkinsons and other neurodegenerative diseases in the early stage.

Wednesday 2:30 PM Session 7: Track 1: *New Advances* – Podium

Shooting 100% of nanoliter volumes of liquid samples, cells and more into Mass Spectrometers.
Drew Sauter (adsauterjr@gmail.com)
nanoLiter LLC
In this paper, we present a new way to dispense 100% (!!!) of liquid samples and liquid sample contents into mass spectrometers including: drugs of abuse; metals; peptides; proteins and cell or cell fragments using an induction base fluidic dispenser. We discuss and we show the technology that can also provide excellent MALDI depositions for bacteria characterizations, along with the specific physics of discrete droplet production and ion generation. We speculate as to why the integral of the 100% liquid sample introduction IBF approach could become the most efficient way to find biomarkers and how it might replace ESI.

Wednesday 3:00 PM Session 7: Track 1: *New Advances* – Podium

Electron Transfer Dissociation of proteins in clinical labs
Alexander Scherl (alexander.scherl@unige.ch)
University of Geneva
Diagnosis of hemoglobin disorders such as sickle cell disease is complex, time consuming and requires multiple laboratory equipment. We developed a mass spectrometry-based assay in an ion trap instrument combining the specificity of selected reaction monitoring and the protein activation capabilities of electron-transfer dissociation. We showed that this method allows the unambiguous detection of hemoglobin S and C only hours after blood collection, including confirmation of the precise point-mutation sites. The presented ETD-based assay shows capabilities to be automated with GxP-compatible software. We conclude that the assay can be used in a clinical environment for the diagnosis of hemoglobinopathies.

Wednesday 3:30 PM Session 7: Track 1: *New Advances* – Podium

Quantiative Analysis of Insulin by Liquid Chromatography-Tandem Mass Spectrometry
Zhaohui Chen (zhaohui.x.chen@questdiagnostics.com)
Quest Diagnostics Nichols Institute
Insulin measurement is primarily used by immunoassays in the clinical laboratory for the diagnosis and management of glycemic disorders and insulin resistant syndromes. However, differences in results between antibody-based platforms are well known. We have successfully developed a quantitative LC/MS/MS method to analyze the insulin concentrations. After off-line extraction, the serum sample underwent a reduction to free the insulin B chain, and then followed by online extraction and analytical separation before triple quadrupole mass spectrometry. Internal standards were applied and the validation has been completed in a high throughput clinical laboratory.

Wednesday PM Session 7: Track 2: *Sample Prep & Automation* (Chair: Karl-Siegfried Boos)

Wednesday 2:00 PM Session 7: Track 2: *Sample Prep & Automation* – Podium

Sample Preparation using Supported Liquid Extraction for Clinical Applications prior to LC-MS/MS Analysis
Lee Williams (Lee.Williams@Biotage.com)
Biotage Employee, R&D
This presentation is designed to give an introduction to the use of supported liquid extraction in a clinical environment prior to LC-MS/MS analysis. Extract cleanliness is a major factor when investigating sample preparation strategies. Data will be presented with respect to serum/plasma in terms of endogenous matrix components; specifically proteins and phospholipids. pH control and extraction solvent selection can significantly influence extract cleanliness. We will then briefly discuss three applications where extraction optimization has been performed; corticosteroids, vitamin D and steroid hormones.

Wednesday 2:30 PM Session 7: Track 2: *Sample Prep & Automation* – Podium

Automated, Rapid Identification of Single Amino Acid Polymorphisms Directly from Complex Biological Fluids
Nicholas Herold (nherold@perfinity.com)
Perfinity Biosciences, Inc.
For a given protein it is common for a single amino acid variation to change biological activity. Differentiating between isoforms is an essential part of quantification and establishing structure-function relationships. Mass spectrometers are capable of resolving these differences. Through use of a Perfinity Workstation, it is possible to couple extraction, buffer exchange, digestion, desalting and RPC such that proteins are ready for MS based detection in approximately 10 minutes. Preparation and analysis of samples containing hemoglobin variants was used to demonstrate this method. This workflow couples automated sample preparation to MS detection allowing for highly reproducible results (CV's < 10%).

Wednesday 3:00 PM Session 7: Track 2: *Sample Prep & Automation* – Podium

Haemoglobin Variant Analysis by Liquid Extraction Surface Sampling Mass Spectrometry of Neonatal Dried Blood Spots
Rebecca Edwards (RLE975@bham.ac.uk) -- *Young Investigator Awardee*
School of Biosciences, University of Birmingham
Established methods for screening haemoglobin variants, such as sickle cell disease, in newborn screening programmes are laborious: They involve the resolubilisation of the dried blood spot (DBS) followed by analysis using high pressure liquid chromatography and/or isoelectric focusing, with the results being presumptive rather than absolute. Direct sampling of DBS by liquid extraction surface sampling eliminates the need for any sample preparation and the subsequent analysis by high resolution tandem mass spectrometry rapidly and unequivocally determines haemoglobin variants, including variants with mass shifts of <-1 Da (such as HbC and HbD) and those uncharacterised by standard clinical laboratory screening methods.

Wednesday 3:30 PM Session 7: Track 2: *Sample Prep & Automation* – Podium

A fully automated platform for in-line pretreatment and on-line clean-up of whole blood samples prior to LC-MS/MS analysis
Karl-Siegfried Boos (boos@med.uni-muenchen.de)
Laboratory of BioSeparation, Institute of Clinical Chemistry, Medical Center of the University
Towards a total automation of LC-MS/MS analysis of whole blood samples we developed two simple and reliable procedures which convert whole blood into a novel blood matrix, i.e. cell-disintegrated blood (CDB). For production of CDB a blood sample first is spiked with an Internal Standard and thereafter perfused through a heated (LC)-capillary or aspirated into a syringe-needle and snap-frozen in liquid nitrogen . Both processes are fully automated, highly efficient and highly reproducible using a dedicated instrument. After in-line generation of CDB by heat-shock or cryogenic treatment CDB can be further subjected to on-line SPE prior to LC-MS/MS analysis.

Wednesday PM Session 7: Track 3: *Microbiology* (Chair: Yi-Wei Tang)

Wednesday 2:00 PM Session 7: Track 3: *Microbiology* – Podium

Analysis of the "10 percent rule" in bacterial identification by MALDI-TOF MS
Mark Fisher (mark.fisher@path.utah.edu) -- *Young Investigator Awardee*
University of Utah and ARUP Laboratories
Spectra from multiple species may yield high-scoring matches for a single isolate in the Bruker MALDI Biotyper. Some laboratories resolve this issue with relative score cutoff such as the “10% rule,” which excludes species scoring >10% below the top match. We analyzed relative cutoffs ranging from 0-35% on a diverse set of isolates (170 species) to identify optimal values for excluding additional species. We concluded there is no universal cutoff, and further showed that “consistency categories” in the newest Biotyper software may obviate the need for arbitrary cutoffs. However, some consistency calls may be too conservative and require critical evaluation.

Wednesday 2:30 PM Session 7: Track 3: *Microbiology* - Podium
Use of MALDI-TOF for Bacterial Identification in a Large Reference Laboratory

Kenneth Van Horn (kvanhorn@focusdx.com)
Focus Diagnostics
Rapid identification of bacteria in cultures can aid in therapeutic decision-making. In this study, the Bruker Biotyper MALDI-TOF was used at 2 Infectious Disease laboratories at Quest Diagnostics to identify various bacterial isolates whose identifications were obtained using standard laboratory methods. Overall, 374 of 450 total isolates (83.1%) were correctly identified to the genus and species level, and 40 isolates (8.9%) were correctly identified to the genus level only. Using MALDI-TOF, 27 (6.0%) isolates were not identified and only 9 isolates (2.0%) were mis-identified with 16S rRNA sequencing to resolve discrepancies. This MALDI-TOF assay thus accurately identifies bacterial isolates.

Wednesday 3:00 PM Session 7: Track 3: *Microbiology* – Podium

Mass Spectrometry Biotyper System Identifies Bacterial Pathogens Associated with Urinary Tract Infections
Yi-Wei Tang (tangy@mskcc.org)
Memorial Sloan-Kettering Cancer Center
We evaluated a MALDi-TOF MS-based Biotyper system for identifying the bacterial pathogens in comparison a Phoenix system. A total of 1,024 urine bacterial isolates were identified and the Biotyper and Phoenix correctly identified 99.9% and 99.5% to the genus level ($P>0.05$) and 99.1% and 98.5% to the species level ($P>0.05$), respectively. A protein extraction processing was required for 85 (8.3%) isolates by Biotyper in which 28.1% were Gram-positive cocci and 2.8% were Gram-negative bacilli ($P<0.0001$). Both systems provide reliable identification while the Biotyper system offers an evolutionarily rapid tool for bacterial pathogen identification in urine.

Wednesday 3:30 PM Session 7: Track 3: *Microbiology* – Podium

Finding Protein Biomarkers in Mouse Plasma as Diagnostic Targets for Invasive Aspergillosis
Chengsi Huang (cshuang@email.arizona.edu) -- *Young Investigator Awardee*
University of Arizona
The goal of this study is to identify Aspergillus fumigatus proteins that are specifically present in patients with invasive aspergillosis, a potentially fatal pulmonary infection caused by A. fumigatus. Mice were used as the model organism and were divided into three groups: naïve control, asthma-model and IA-model. Plasma samples were analyzed via a bottom-up proteomics approach on an LTQ Orbitrap Velos. We identified one potential protein target that is exclusive to IA, and the expressed recombinant protein is used as a standard for MRM-type analysis. Several IA-exclusive pan-aspergillus and pan-fungal proteins were also identified and can be used as potential targets.

Sunday 6:30 - 7:30 PM
Poster #1 in the Exhibit Hall
Aryl Piperazine Motifs Can Cause False Positive Amphetamine Screens: Utilizing High Resolution Mass Spectrometry to Identify Cross-reacting Substances
Matthew Petrie (matthew.petrie@ucsf.edu) -- *Young Investigator Awardee*
University of California-San Francisco
Among screening immunoassays, those designed to detect amphetamines have suffered from a particularly high rate of false positives. We present two cases of suspected designer amphetamine drug abuse which ultimately allow us to determine that drugs such as trazodone and aripiprazole, which contain aryl piperazine motifs, can cause false positive amphetamine screens. The cross-reactivity is not due to the parent drugs, but their primary aryl piprerazine metabolites after delkylation in the liver. Overall, we show drugs that contain aryl piperazine motifs can cause false positive amphetamine screened and laboratories should be aware of the limitations of their specific amphetamine assays.

Sunday 6:30 - 7:30 PM
Poster #3 in the Exhibit Hall
Development and comparison of two LC-MS/MS assays for the fast and simple quantification of serum testosterone
Jeanne Rhea (jmrhea@emory.edu) -- *Young Investigator Awardee*
Emory University
While both immunoassays and liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays are used clinically, the latter is considered the “gold standard” for measuring total testosterone at low concentrations (i.e. in women, children, and hypogonadal men) due to the specificity and sensitivity of MS. We compared the sensitivity of an in-house LC-MS/MS testosterone assay to a LC-MS/MS assay using a novel derivatization reagent, with the goal of developing a method to rapidly and accurately measure both free and total serum testosterone in clinical samples from both sexes and across all ages.

Sunday 6:30 - 7:30 PM
Poster #5 in the Exhibit Hall
Comparison of three available deuterated testosterone internal standards in an LC-MS/MS assay for quantification of testosterone in patient samples
Jeanne Rhea (jmrhea@emory.edu) -- *Young Investigator Awardee*
Emory University
Isotopically labeled internal standards (ISs) are critical components of clinical mass spectrometry (MS) assays, and are used to control for extraction, HPLC injection, and ionization variability. We evaluated the usefulness of three deuterated testosterone internal standards in our LC-MS/MS assay for testosterone quantification, and found that a naturally occurring isotope of testosterone may interfere with the measurement of D2 as an IS. The problem of using D2 as an IS is further confounded by our finding that unlabeled transitions are present at significant levels when analyzing D2 alone, and that measurement of these transitions significantly affects testosterone quantification in patient samples.

Sunday 6:30 - 7:30 PM
Poster #7 in the Exhibit Hall
Assessing Effects of CYP2C19 Inhibitors on Carisoprodol Metabolism in Urinary Excretion Data in Patients with Pain Using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)
Stephanie Tse (s1tse@ucsd.edu)
UC San Diego Skaggs School of Pharmacy & Pharmaceutical Sciences
Carisoprodol is a skeletal muscle relaxant metabolized by CYP2C19. Using LC-MS/MS, the purpose is to evaluate the effects of CYP2C19 inhibitors on urinary excretion data in patients prescribed carisoprodol. This retrospective analysis of de-identified urinary excretion data was quantitated by LC-MS/MS at Millennium Laboratories. The metabolic ratio (MR) was assessed and calculated as meprobamate/carisoprodol. The mean MRs were: control, 74.0; esomeprazole, 50.5; fluoxetine, 55.8; omeprazole, 73.8; and lansoprazole, 77.6. Esomeprazole and fluoxetine use was associated with a smaller MR compared to control ($p<0.05$), suggesting these drugs are strong inhibitors. Further elucidation of inhibitor effects may be important for clinical applications.

Sunday 6:30 - 7:30 PM
Poster #9 in the Exhibit Hall

Diazepam Metabolism Shifts with Cytochrome P450 (CYP) Inhibitors in Patients with Chronic Pain as Identified by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS): A Urinary Approach

Samantha Luk (sluk@ucsd.edu)
UCSD Skaggs School of Pharmacy and Pharmaceutical Sciences

Diazepam is metabolized by cytochrome P450 (CYP) 2C19 into nordiazepam and CYP 3A4 into temazepam. Both are further metabolized to oxazepam. The purpose of this retrospective data analysis was to determine if CYP inhibitors affect diazepam pharmacokinetics using urinary excretion data from Millennium Laboratories tested by LC-MS/MS between 2008 – 2011. De-identified urine specimens from subjects on diazepam and CYP 3A4 inhibitors had a 17.7% decrease in the temazepam fraction and a 9.5% increase in the oxazepam fraction. Those on diazepam, CYP 3A4 inhibitors, and CYP 2C19 inhibitors had a 25.4% decrease in the temazepam fraction and a 13.3% increase in the oxazepam fraction.

Sunday 6:30 - 7:30 PM
Poster #11 in the Exhibit Hall

Rapid Purification of Glycosaminoglycans from Human Serum for Disease Diagnosis and Prognosis

Wei WEI (viwei@ucdavis.edu)
University of California, Davis

Heparin/heparan sulfate glycosaminoglycans (HSGAGs) are widely involved in many diseases such as rheumatoid arthritis. Because of their complex structures, HSGAGs are one of the most informative biopolymers in nature and have diverse biological functions. Evaluating the alternations (i.e. presence and quantity) of HSGAGs in serum or plasma at the disaccharide level could provide information on their heterogeneity, which has a great potential for disease diagnostics and prognosis. Herein, we describe a rapid method for the in-depth compositional analysis of 12 heparin/HS-derived disaccharides from human serum using fractionation and enrichment techniques for enhanced detection prior to LC-MS/MS analysis.

Sunday 6:30 - 7:30 PM
Poster #13 in the Exhibit Hall

The Analysis of Aromatic Amine Urinary Adducts in Smokers and Non-Smoker Urine by Gas Chromatography Tandem Mass Spectrometry

Elizabeth Cowan (ecowan@cdc.gov)
Centers for Disease Control

Aromatic amines such as ortho-toluidine (o-Tol), 2-aminonapthalene (2-AMN), 3- and 4-aminobiphenyl (3-and 4-ABP) are known carcinogens found in tobacco smoke. Most GC-MS methods currently used to measure aromatic amines use negative ion chemical ionization (NICI). Here we report the validation of a precise, sensitive method for the quantification of o-Tol, 2-AMN, and 4-ABP urinary adducts in smoker and non-smokers. Unlike most methods currently in literature, this method uses a lower sample volume (2mL urine) and GC-MS/MS positive ion electron impact ionization.

Sunday 6:30 - 7:30 PM
Poster #15 in the Exhibit Hall

Impact of CYP2D6 and CYP3A4 Inducers, Inhibitors, and Substrates on Urinary Hydrocodone and Metabolite in Patients with Pain as Identified by LC-MS/MS

Neveen Barakat (nhbarakat@gmail.com)
University of California San Diego, Skaggs School of Pharmacy and Pharmaceutical Sciences

Hydrocodone is metabolized by cytochrome P450 (CYP) 2D6 into hydromorphone and by CYP3A4 into norhydrocodone. This retrospective analysis evaluated the effect of CYP2D6 and CYP3A4 inducers, inhibitors, and substrates on hydrocodone metabolism. Urinary excretion data from de-identified specimens collected from patients with pain during routine office visits were analyzed at Millennium Laboratories by LC-MS/MS between January and August 2011. The mole fraction of the metabolites hydromorphone and norhydrocodone increased in the presence of CYP 2D6 and/or CYP 3A4 inducers, and decreased with enzyme inhibitors. The presence of CYP2D6 substrate increased the mole fraction of hydromorphone suggesting pathway saturation.

Sunday 6:30 - 7:30 PM
Poster #17 in the Exhibit Hall
Determination of argininosuccinic acid and its anhydride forms by LC-MS/MS
Zhen Zhao (zhaoz@path.wustl.edu) -- *Young Investigator Awardee*
Washington University in St. Louis
Argininosuccinic acid (ASA) lyase deficiency is a urea cycle defect characterized by accumulation of ASA in blood and urine. In MS procedures employing butylation, at least twenty different derivatives of ASA may be formed, complicating quantitation. We describe an LC-MS/MS method for simultaneous assessment of butylated derivatives of ASA including the tricarboxylate form, anhydrides, and deaminated anhydrides. Using homoarginine as an internal standard, the ten most abundant ASA derivatives were used to assess linearity and recovery. Quantitation was linear to at least 500 μM, and ASA recovery in plasma was 80%-100% compared to 30-40% using a previously published method.

Sunday 6:30 - 7:30 PM
Poster #19 in the Exhibit Hall
Development and Clinical Validation of Alpha-Synuclein Peptides as Parkinson's Disease Diagnostic Biomarkers
Faith Hays (fahays@shimadzu.com)
Shimadzu
Currently there is no clinical biomarker available for the diagnosis of Parkinson's Disease (PD) or PD-related cognitive impairment. Using a triple quadrupole mass spectrometer, we have developed a highly sensitive selected reaction monitoring (SRM) assay for quantitating six phosphorylated and two nitrated alpha-synuclein peptides and their corresponding tryptic peptides. Using this protocol, we will screen cerebrospinal fluid from patients with PD-related cognitive impairment, un-impaired PD patients, and healthy controls to determine if modified alpha-synuclein contributes to the pathophysiology of PD and if any of the selected alpha-synuclein peptides themselves may serve as a clinical diagnostic.

Sunday 6:30 - 7:30 PM
Poster #21 in the Exhibit Hall
Determination of the Correlation between Whole Blood Lead and Salivary Lead Levels
Erica Guice (erica@westernslopelabs.com)
Western Slope Laboratory, LLC
Lead, even at low levels, can be very detrimental to human beings. As such, lead concentrations are monitored and action levels are set by the CDC. Currently, body lead levels are screened using whole blood levels. This testing requires invasive and painful blood draws that necessitate special handling and specifically trained staff. This study focused on validating a new test protocol that utilizes LC/ICP-MS to test oral fluid for inorganic lead and compared it to whole blood lead levels. This testing will allow for a wide spread utilization of lead screening in communities that cannot afford whole blood screening.

Sunday 6:30 - 7:30 PM
Poster #23 in the Exhibit Hall
Development and implementation of an LC-MSMS method for pyrolytic and metabolic markers of cocaine
Cheryl Rigg (cheryl.rigg@hey.nhs.uk) -- *Young Investigator Awardee*
Hull Royal Infirmary, NHS
Anhydroecgonine and anhydroecgonine methyl ester are produced when cocaine is heated, referred to as pyrolytic products. They are detected specifically in the urine of crack cocaine users. Identifying the route of administration of cocaine can be beneficial in the addiction management of patients and in forensic studies. A method has not previously been established which can identify both pyrolytic products of cocaine. This study has developed an LC-MSMS method for the analysis of cocaine, its metabolites and pyrolytic products, suitable for use in the clinical laboratory.

Sunday 6:30 - 7:30 PM
Poster #25 in the Exhibit Hall

Simultaneous Detection of Plasma Methylmalonic acid, Total homocysteine, Methionine and 2-Methylcitric acid Using Liquid Chromatography and Mass Spectrometry (LC/MS/MS)

Xiaowei Fu (xfu@chla.usc.edu)
Department of Pathology and Lab Medicine

A very simple, fast and sensitive LC/MS/MS method was developed for simultaneous detection of plasma total homocysteine, methylmalonic acid, methionine and 2-methylcitric acid. Only 100uL of plasma or serum is needed. 10uL was injected after a very simple extraction without derivatization. Instrument run time was 6 minutes. tHcy and Met were quantified in positive ion mode. MMA and 2MCA were quantified in negative ion mode. Linearity, accuracy, precision and interference studies were carried out. Reference intervals were established. A total fifteen patients with variable disorders were successfully diagnosed.

Sunday 6:30 - 7:30 PM
Poster #27 in the Exhibit Hall

Production and Application of Quality Control and Proficiency Testing samples in California Genetic Disease Screening Program

Yu Hou (yhou@cdph.ca.gov) -- *Young Investigator Awardee*
Genetic Disease Laboratory, California Depatment of Public Health

The Genetic Disease Screening Program (GDSP) has been recognized as an international leader in genetic disease screening. On an annual basis, approximately one million pregnant women and newborns in California are screened for nearly 80 disorders, among which 47 disorders are detected via tandem mass spectrometry (MS/MS) screening. Testing is conducted from the Genetic Disease Laboratory (GDL) and seven contract laboratories over California. The GDL is responsible for maintaining consistent performance of analytical instruments at the contract laboratories by exporting quality control and proficiency testing samples and evaluating test results to determine whether any remedial action is required.

Sunday 6:30 - 7:30 PM
Poster #29 in the Exhibit Hall

Simultaneous detection of nine opiates, including buprenorphine and norburprenophine, in urine using UPLC/MS/MS

Phillip Bates (pbates@unch.unc.edu) -- *Young Investigator Awardee*
UNC Healthcare

Urine opiate confirmations are currently run by LC/MS/MS for seven opiates including codeine, morphine, oxycodone, oxymorphone, hydrocodone, hydromorphone, and 6-monoacetylmorphine with a runtime of 13 minutes. Buprenorphine and its metabolite norbuprenophine are also run using LC/MS/MS with a runtime of 7 minutes. The goal of the study was to validate a new assay using UPLC/MS/MS and combining all nine opiates into one run with a faster runtime. The final assay using UPLC/MS/MS has a runtime of 6 minutes with an average coefficient of variation of 5.7 % for the low control and 7.6 % for the high control.

Sunday 6:30 - 7:30 PM
Poster #31 in the Exhibit Hall

Streamlined Identification of Clinically Relevant Yeast Species Using a Modified Direct-Smear, Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometery (MALDI-TOF MS) Approach

Lanette Hamilton (lhamilton@path.wustl.edu) -- *Young Investigator Awardee*
Department of Pathology and Immunology

Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was used to identify 130 yeast isolates of which 8 were ATCC strains, including Candida albicans (n=34), Candida krusei (n=2), Candida glabrata (n=21), Candida parapsilosis (n=18), Candida tropicalis (n=13), Cryptococcus neoformans (n=8), and other less frequently isolated yeast species (n=34). In this study, a traditional centrifugation-based formic acid extraction procedure (FA) was compared to the direct smear method with a 1 uL formic acid overlay (DS). Using the DS method, 77(80%) of the yeast were identified to Genus and species level, respectively, compared to 81 (84%) using the FA procedure

---

Sunday 6:30 - 7:30 PM
Poster #33 in the Exhibit Hall

Simultaneous quantification of 19 drugs/metabolites in urine important for pain management by liquid chromatography-tandem mass spectrometry

Chao Yuan (yuanc@ccf.org)
Cleveland Clinic

Monitoring pain management drugs and frequently abused drugs is important for physicians to assess patient compliance. In this report, a novel liquid chromatography-tandem mass spectrometry method for simultaneously monitoring 19 drugs/metabolites in urine important for pain management was developed and validated. Sample preparation included hydrolysis, dilution, and turbulent flow online extraction. Lower limits of quantification ranged from 5 to 25 ng/mL. Within the linear range, analytical recovery was between 85.8% and 119.4%. Intra-assay and total coefficient of variations were between 0.2% and 12.7%. This method showed excellent performance in comparison with mass spectrometry methods offered by other reference laboratories.

Sunday 6:30 - 7:30 PM
Poster #35 in the Exhibit Hall

Lipidomic Screening and Biomarker Discovery using UPLC/Ion Mobility/MSE

Giuseppe Astarita (giuseppe_astarita@waters.com)
Waters Corporation

Lipids play essential roles in health and disease. The discovery of novel alterations in lipid levels related to various human diseases could lead to the development of novel biomarkers and future diagnostic testing. The challenge with global lipid analysis is the chemical complexity and the large range of concentrations of thousands of lipid species in biological samples. In this study, we developed a robust workflow for global lipid profiling, which employs UPLC®/ion mobility/time-of-flight (TOF) MSE (HDMSE) for the high throughput and automated identification of lipids in complex biological matrices.

Sunday 6:30 - 7:30 PM
Poster #37 in the Exhibit Hall

Simultaneous and Comprehensive Analysis of the Metabolism of Multiple Drugs in Liver Microsomes Using Ultra High resolution time of flight mass spectrometry and UHPLC

Jeffrey Patrick (jeff_patrick@leco.com)
LECO Corporation

The analysis of pharmaceutical metabolites is important in testing drug candidates. One model system used is the liver microsome system. This system is used to monitor a metabolic time-course for a suite of ten pharmaceuticals metabolized simultaneously in a mixture to investigate multiplex capabilities. Time points were then analyzed in an untargeted fashion using UHPLC and mass spectrometric analysis on a TOF-MS with a Folded-Flight-Path(TM) and with a total analysis time of 2 minutes. High performance TOFMS with structural confirmation by ion fragmentation demonstrates the capability to perform this multiplex analysis. Simulatneous analysis of clearance and metabolites is readily achieved.

Sunday 6:30 - 7:30 PM
Poster #39 in the Exhibit Hall

Analysis of Substances of Abuse in Urine Using High resolution TOFMS by direct analysis and UHPLC – Enhanced Detection Limits using a novel parent-fragment signal amplification

Jeffrey Patrick (jeff_patrick@leco.com)
LECO Corporation

Chemicals are used to enhance the performance of athletes in competition. The challenges to analysts aresensitivity, matrix complexity, structural confirmation, and a comprehensiveness. High performance TOFMS with a Folded Flight Path (FFP™) is applied to the detection of steroids in urine after enrichment using solid-phase extraction. Analyses are performed by UHPLC and plug injection. Data demonstrate the utility of high mass accuracy in identification and high resolving power in selectivity. The high resolving power and mass accuracy are extended to fragment ions to provide novel post-acquisition signal amplification with significantly enhanced S/N.

Sunday 6:30 - 7:30 PM
Poster #41 in the Exhibit Hall

Fully Automated Extraction and Analysis of Immunosupressents in whole blood using the Gerstel MPS Workstation with 2DLC-MS/MS

Adrian Taylor (adrian.taylor@absciex.com)
AB SCIEX

As with many HPLC-MS/MS applications, offline sample preparation is needed to extract immunosuppressant drugs from whole blood. Online 2-dimensional sample cleanup can be used to minimize the amount of offline sample processing required, however some manual preparation of samples is still needed. We present here a solution using the Gerstel MPS sample processing workstation, coupled to a 2-dimensional HPLC-MS/MS system, to provide a fully automated extraction and analysis approach for this application. In addition, intelligent softwares and automated reporting systems are exploited to minimize the automated sample preparation time and dramatically improve throughput, giving reduced time from sample to result.

Monday 2:00 - 3:00 PM
Poster #1 in the Exhibit Hall

Quantitative measurement of plasma free metanephrines by ion-pairing solid phase extraction and liquid chromatography-tandem mass spectrometry with porous graphitic carbon column

Xiang He (kevin.he@thermofisher.com)
Thermo Scientific

Plasma free metanephrine and normetanephrine (Pmets) are the best biomarkers for pheochromocytoma. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become the preferred technology to measure Pmets. In this study, a solid phase extraction (SPE) method using ion-pairing reagent and C18 stationary phase was used for sample preparation. Porous graphitic carbon column was tested for chromatographic separation. This method was linear from 7.2 to 486.8 pg/mL for metanephrine and 18.0 to 989.1 pg/mL for normetanephrine, respectively. Inter-assay and intra-assay CV for metanephrine and normetanephrine ranged from 2.0% to 10.9%. In conclusion, this LC-MS/MS method for Pmets is simple and sensitive.

Monday 6:30 - 7:30 PM
Poster #2 in the Exhibit Hall

A Comparative Study of Common Urine Sample Preparation Techniques for LC-MS/MS Analysis of a Comprehensive Panel of Pain Management Drugs

Peter Stone (kevin_mccann@agilent.com)
Agilent Technologies

Determining the most effective and efficient manner to pre-treat urine samples for the LC/MS analysis of large panels of pain medications and illicit drugs is a challenge. Two of the most common techniques – ‘dilute and shoot’ (D&S) and solid phase extraction (SPE) – are widely used due to cost-effectiveness and speed, but each has advantages and disadvantages. This research compares and contrasts sample preparation approaches using a comprehensive and rapid targeted LC/MS analysis method of a panel of over 68 compounds extracted from urine. The recovery results from several batches are reported.

Monday 2:00 - 3:00 PM
Poster #3 in the Exhibit Hall

Evaluation of LCMSMS Deuterium Scrambling in Clinically Significant Small Molecules

Joshua Cooper (Josh_Cooper@cerilliant.com)
Cerilliant Corporation

LCMSMS in clinical applications is a powerful tool that provides significant benefits but also presents unique challenges including matrix effects. Deuterium-labeled internal standards are frequently used to compensate for matrix effects. Some deuterium labeled compounds exhibit hydrogen-deuterium scrambling. This was investigated in numerous small molecules of clinical importance: hydroxyvitamin D, testosterone, immunosuppressants, bath salts, and spice cannabinoids. Results show that scrambling clearly does occur, but can be mitigated. The key parameter is selection of transition, as other parameters (including changing instrument types and vendors, matrix, and concentration) do not appear to impact the extent of scrambling.

Monday 6:30 - 7:30 PM
Poster #4 in the Exhibit Hall

Modern Liquid Chromatography Joins Tandem Mass Spectrometry for Multiplex Newborn Screening of Lysosomal Storage Disorders

Zdenek Spacil (spacil@u.washington.edu) -- *Young Investigator Awardee*
Department of Chemistry, University of Washington

The increasingly promising potential to treat lysosomal storage disorders with enzyme replacement therapy and hematopoietic stem cell transplantation results in wide implementation of LSD newborn screening. Previously, the MS/MS enzyme assays were incubated separately and analyzed by direct injection into mass spectrometer. However, multiplexing of both incubation and MS/MS analysis substantially saves time, labor and material. Recently, we have shown advantages of on-line liquid chromatography in multiplexing of individual enzyme assays. A yet higher level of multiplexing will be demonstrated, combining nine enzyme specific substrates into the two incubation buffers. The multiplexed samples were analyzed by modern chromatographic methods.

Monday 2:00 - 3:00 PM
Poster #5 in the Exhibit Hall

Preliminary Evaluation of Calibration, Specimen Type and Binding for an LC-MS/MS Method for Testosterone Measurements in Females and Children

Krystina Cocco (coccokm@vcu.edu) -- *Young Investigator Awardee*
Virginia Commonwealth University

A LC-MS/MS method for low testosterone concentrations was performed to develop the calibration curve and to determine acceptable sample collection tubes. Testosterone binding to glass test tubes was investigated. The calibration curve equation was obtained. Linearity resulted in r2 of 0.9984. Commercial control within-run CVs were 5.6% and 18%. Female serum sample within-run CVs were 3.3% and 1.6%. An interfering peak was present in SST samples. Testosterone was not significantly different in Red-top vs. EDTA tubes (p=0.98, 0.65). A (-)25% bias was observed for SST (p=0.009, <0.001). No significant reduction of testosterone was observed due to glass test tube binding.

Monday 6:30 - 7:30 PM
Poster #6 in the Exhibit Hall

Validation of Alpha-1-Antitrypsin S and Z phenotyping by LC-Mass Spectrometry

Robin Karras (karras.robin@mayo.edu)
Mayo Clinic

Alpha-1-antitrypsin (A1A) is a liver-synthesized protein that inhibits the enzyme neutrophil elastase. Individuals deficient in A1A are susceptible to early onset emphysema and liver disease. Isoelectric focusing electrophoresis (IEF) is the primary method for characterizing allelic variants. A PCR-based assay is also available to detect the deficient S and Z alleles in A1A-SERPINA1 gene. Clinical validation of these two alleles using an LC-MS/MS-phenotype was undertaken in our lab. This method compared favorably with IFE and genotyping with 100 percent concordance. LC-MS/MS-phenotype was also found to have increased sensitivity for detection of the two deficient alleles in comparison to IFE.

Monday 2:00 - 3:00 PM
Poster #7 in the Exhibit Hall

±-threo-Ritalinic acid-D10 Hydrochloride, an internal standard for quantitation of ritalinic acid: Synthesis and determination of isotopic distribution by qNMR and LCMS

Elizabeth Marek (beth_marek@cerilliant.com)
Cerilliant

Ritalinic acid is a major metabolite of and synthetic precursor to methylphenidate (Ritalin®). ±-threo-Ritalinic acid-D10 HCl was synthesized with a purity of 99% and an isotopic purity ratio of D0/D10 = 0%. The presence of D9-D7 isomers, by LCMS, prompted extensive structure elucidation by NMR. qNMR, was used to determine the percentage of hydrogen, and therefore deuterium, present on each carbon. LCMSMS studies indicated that the compound was suitable for use as an internal standard for quantitation of methylphenidate as ritalinic acid. This study highlights chemical interactions that factor into the design of stable labeled internal standards for LCMS applications.

Monday 6:30 - 7:30 PM
Poster #8 in the Exhibit Hall

Measurement of Diabetes-Predictive Amino Acids from a Dried Blood Spot

Jeanette Hill (jeanetterhill@spotonsciences.com)
Spot On Sciences

Elevated plasma levels of five amino acids were recently shown to be predictive of diabetes development (Wang, Nature Medicine, 2011) up to 12 years prior to disease onset. To show that amino acids could be accurately quantitated from dried blood, we compared levels in plasma and dried plasma, whole and dried blood from venipuncture and dried blood from finger stick. Concentrations of 45 amino acids were measured by LC-MS/MS in a single injection. A high correlation was observed for amino acid concentrations between the sample types demonstrating the feasibility to screen for diabetes from a dried blood spot from a finger stick.

Monday 2:00 - 3:00 PM
Poster #9 in the Exhibit Hall

Determination of Trace Elements in Hair Using Inductively Coupled Plasma-Mass Spectrometry : Experience of the Eone Reference Laboratory

Sung Eun Cho (secho@eonelab.co.kr)
Eone reference laboratory

We evaluated trace elements in hair by inductively coupled plasma-mass spectrometry (ICP-MS). We evaluated the precision, accuracy, lower limit of quantification (LLOQ) and linearity using ICP-MS (Agilent 7700: Agilent Technologices, Tokyo, Japan). Both intraday and interday precision CVs were within 10% for 26 concentrations. The concentrations of the trace elements in sub-samples of the certified reference material were all within acceptable certificated ranges. Accuracy was 80-120% for all concentrations. The ICP-MS method demonstrated good linearity with R2 > 0.99. The LLOQ ranged from 0.1 ng/g to 0.198 µg/g. This study demonstrated excellent results for the determination of trace elements in hair.

Monday 6:30 - 7:30 PM
Poster #10 in the Exhibit Hall

Rapid Quantitative Analysis of Immunosuppressant Drug Panels in Blood and Plasma by LC-MS/MS

Linda Cote (linda_cote@agilent.com)
Agilent Technologies

Highly sensitive and specific methods have been developed for the quantitation of a panel of up to five common immunosuppressant drugs – Cyclosporine A, Everolimus, Mycophenolic Acid (MPA), Sirolimus and Tacrolimus. The first is a rapid, 2-minute method suitable for the reliable quantification of Cyclosporine A, Everolimus, Sirolimus and Tacrolimus. The second method contains a longer gradient, critical to the analysis of Mycophenolic Acid (MPA). Excellent reproducibility was observed for all compounds (LLOQ CV <20%; all other CV < 10%). All calibration curves displayed excellent linearity with an R2 > 0.998.

Monday 2:00 - 3:00 PM
Poster #11 in the Exhibit Hall

A Quantitative HPLC-MS/MS Method for Therapeutic Drug Monitoring of Docetaxel

Autumn Breaud (abreaud1@jhmi.edu)
The Johns Hopkins University

Here we describe the development and validation of an automated, robust and reproducible LC-MS/MS method for therapeutic drug monitoring of the anti-neoplastic agent docetaxel. The method requires only 100uL of serum for analysis and is linear from 8.1-1978.6 ng/ml. Recovery was observed to be within 5% of expected concentration at eight points across the linear range. The lower limit of quantitation is 8 ng/mL and we established that matrix effects account for <14% of signal enhancement or suppression. The within-run, between-run and between-day precision (coefficients of variation, %CV)s were all less than 6.2%.

Monday 6:30 - 7:30 PM
Poster #12 in the Exhibit Hall

Performance Characteristics of Liquid Chromatography-Tandem Mass Spectrometry as Confirmatory Test for Drugs of Abuse from Urine Samples

Sun-Hee Jun (sunnyeyo@snubh.org)
Seoul National University Bundang Hospital

We evaluated the performance characteristics of the LC/MS/MS method by multiplexing 8 major drugs and their metabolites. Imprecision, limit of quantification, linearity, and correlation with external quality control materials were analyzed. Multiplex test for DOA from urine specimen by LC/MS/MS showed acceptable performance characteristics. This method is simple, efficient and accurate enough to be used as confirmatory tests for DOA and may be an alternative to the GC/MS method.

Monday 2:00 - 3:00 PM
Poster #13 in the Exhibit Hall
Sensitive and Accurate Measurement of Serum Total and Free Prostate-Specific Antigen Using Immunoaffinity Depletion Coupled to Selected Reaction Monitoring: Correlation with Clinical Immunoassay Tests
Tao Liu (tao.liu@pnnl.gov)
Pacific Northwest National Laboratory
We developed for the first time immunoaffinity depletion based workflows and selected reaction monitoring mass spectrometry (SRM-MS) assays that enable sensitive and accurate quantification of total and free prostate-specific antigen (PSA) in serum without the requirement for specific PSA antibodies. Low ng/mL level quantification of both total and free PSA was consistently achieved; the SRM-MS assay and conventional immunoassay results showed very good correlation in several independent clinical sample sets. Simultaneous measurement of the free and bound forms of PSA, as well as other biomarkers that have similar complexation/binding characteristics, can be performed in a single multiplexed SRM-MS analysis.

Monday 6:30 - 7:30 PM
Poster #14 in the Exhibit Hall
Analysis of Pain Management Drugs using Automated Disposable Pipette Extraction and LC/MS/MS
Oscar G. Cabrices (ogcabrices@gerstelus.com)
Gerstel Inc.
This presentation demonstrates the use of disposable pipette extraction (DPX) for rapid and automated sample preparation of biological matrices for comprehensive LC/MS/MS screening. A GERSTEL MPS 2 with a DPX option coupled to an Agilent 6460 LC/MS/MS instrument was used for the extraction of over 40 pain management drugs and metabolites in a single urine specimen. The automated DPX-LC/MS/MS solution provided rapid, just-in-time sample preparation for high throughput analysis. Linearity was achieved between 0.1-1000 ng/mL for most analytes, recoveries and %RSDs for over 40 drugs are shown to be greater than 70% and less than 10%, respectively, in most cases.

Monday 2:00 - 3:00 PM
Poster #15 in the Exhibit Hall
Analyses of Rat and Rat Leukocyte Metabolomic Response to Cocaine Stimulation using Multi-dimensional Separations Methods
Cody Goodwin (cody.r.goodwin@vanderbilt.edu) -- *Young Investigator Awardee*
Vanderbilt University
Nanoflow rate-ultra performance liquid chromatography-ion mobility-mass spectrometry (nUPLC-IM-MS) was used to analyze effects of cocaine on the metabolome and exometabolome of rat leukocytes. Leukocytes originated from unexposed rats and rats that had self-administered cocaine premortum. Self-administering rats were further classified as “addicted” or “recreational users” based on behavioral criteria. This research seeks to differentiate the metabolic response of leukocytes from each class to cocaine stimulus applied in vitro. Multivariate statistical analyses were used for the determination of significant metabolites specific to prior exposure. Preliminary data suggest leukocyte metabolic response to cocaine stimulation can be used to indicate prior abuse.

Monday 6:30 - 7:30 PM
Poster #16 in the Exhibit Hall
MEA Chips for High-throughput Mass Spectrometry
Daojing Wang (djwang@lbl.gov)
Lawrence Berkeley National Laboratory
Mass spectrometry (MS) is the enabling technology for proteomics and metabolomics. There is still a tremendous push for higher sensitivity and throughput in MS, for a variety of applications ranging from single cell analysis to clinical proteomics. Taking advantage of the robust and scalable silicon microfabrication technologies, and using a top-down approach, we have demonstrated for the first time the monolithic integration of multinozzle electrospray emitters with a microfluidic channel, M3 emitters. More recently, we have further developed the silicon-based monolithic multinozzle emitter array (MEA) chip and demonstrated its proof-of-principle applications in high-sensitivity and high-throughput nanoelectrospray mass spectrometry.

Monday 2:00 - 3:00 PM
Poster #17 in the Exhibit Hall

Development and Validation of a New High Throughput LC/MS/MS Assay for Nicotine, Metabolites, and Anabasine for Determination of Tobacco Exposure

Carrie Haglock (carrie.haglock@aruplab.com)
ARUP Laboratories

The use of tobacco products, and particularly smoking, is the leading preventable cause of death and disability in the United States, and is a worldwide problem. The current work describes the development and validation of a LC/MS/MS assay for the accurate identification and quantification of the presence of nicotine (NIC) and nicotine metabolites: cotinine (COT), trans-3-hydroxy cotinine (3HCOT), nornicotine (NRNC). The presence of Anabasine (ANAB), an alkaloid present in tobacco plants, in urine samples was used to differentiate persons using a tobacco product from those who were on nicotine replacement therapy (NRT).

Monday 6:30 - 7:30 PM
Poster #18 in the Exhibit Hall

Visualizing Large Data Sets – Applications to Steroid LC-MS and 2D Gels

Alfred Yergey (aly@helix.nih.gov)
NICHD, NIH

Current research and clinical mass spectrometry experiments generate large (>100 MB) data sets. While manufacturer's software-based analysis is useful for extracting information from the data, delving into more subtle features might be done more effectively by viewing the data set in its entirety. We have devised a tool that enables one to look at a complete data set independently of any manufacturer's software and applied it to the development of an assay for profiling known and unknown urinary steroids.

Monday 2:00 - 3:00 PM
Poster #19 in the Exhibit Hall

Analysis of Anions in Positive Electrospray Ionization Mode Using Di- and Tri- Cationic Ion-Pair Reagents

Rick Link (rick.link@sial.com)
Supelco/Sigma-Aldrich

Analysis of anions is important in a number of diverse fields. Most commonly anions are determined by ion chromatography, ion selective electrodes or flow injection analysis. These methods may lack sensitivity or specificity. In this work, a low concentration of ion pairing reagent is added to the mobile phase. For a monovalent anion, the dication pairs with the singly charged anion resulting in a complex with a plus one charge. Detection by mass spectrometry can then be in the positive ion mode. Anions studied with the dication reagent were perfluorooctanate, nitrate, thiocyanate, arsenate and perchlorate. Detection limits were in the low nanogram range.

Monday 6:30 - 7:30 PM
Poster #20 in the Exhibit Hall

CHARACTERIZATION OF NOVEL O-GLYCANS ISOLATED FROM TEAR-SALIVA: IMPLICATIONS FOR DISEASE MARKER DISCOVERY

Sureyya Ozcan (sozcan@ucdavis.edu)
UC Davis Chemistry

Glycomics is emerging as a simple yet highly sensitive diagnostic tool for disease onset and progression. Human fluids are the most relevant biological source for monitoring actual body status from a glycomics perspective.In this research,O-glycans from tear and saliva samples were profiled and their structures were elucidated using mass spectrometry-based technique.As a case study the O-glycan profile found in control tear and saliva was compared to that of patients suffering from ocular rosacea.These comparisons showed that there is a significant alteration on acidic O-glycans in saliva and tear.The findings demonstrate that patient samples were readily differentiated by a group of O-glycans,suggesting their potential as a biomarker for ocular rosacea.

Monday 2:00 - 3:00 PM
Poster #21 in the Exhibit Hall

Steroid Profiling by Liquid Chromatography-Tandem Mass Spectrometry as a Second Tier Test in Newborn Screening for Congenital Adrenal Hyperplasia

Hyung-Doo Park (nayadoo@hanmail.net)
Departments of Laboratory Medicine & Genetics, Samsung Medical Center

Newborn screening for CAH, which is based on measuring 17-hydroxyprogesterone (17-OHP) levels by fluorescent immunoassay, generates false-positive results. To solve this problem, we simultaneously determined the levels of 17-OHP, androstenedione, and cortisol by liquid chromatography-tandem mass spectrometry (LC-MS/MS). We analyzed 298 samples of dried blood spot (DBS) of newborns (218 positive and 80 negative samples). Steroid profiling with the cutoff value of 3.75 for the ratio of the sum of 17-OHP and androstenedione concentrations divided by cortisol level confirmed 2 true positive cases from false positive cases. Steroid profiling of DBS by LC-MS/MS appeared to be a reliable and convenient tool.

Monday 6:30 - 7:30 PM
Poster #22 in the Exhibit Hall

A Fast and Simple Assay for Direct Argatroban Quantitation in Hospitalized Patient Plasma Samples by Liquid Chromatography-Tandem Mass Spectrometry

Jeanne Rhea (jmrhea@emory.edu) -- *Young Investigator Awardee*
Emory University

A method for the direct measurement of argatroban in human plasma was developed and compared with a commercially available indirect activity-based assay. Direct analyses were performed by ultra performance liquid chromatography with tandem mass spectrometry detection (UPLC-MS/MS). Correlation was demonstrated between direct determination by UPLC-MS/MS and the indirect activity-based plasma argatroban assay using patient samples. The present UPLC-MS/MS method provides a relatively simple and sensitive assay with a short turn-around-time. It has successfully been applied to assess plasma argatroban concentrations in hospitalized patients and may provide a more accurate determination of argatroban concentrations in certain clinical conditions.

Monday 2:00 - 3:00 PM
Poster #23 in the Exhibit Hall

ANALYSIS of SERUM METHYLMALONIC ACID by LC-MS/MS in LOW VOLUME SAMPLES.

Ekaterina Paliakov (epaliakov@cdc.gov)
CDC

The National Health and Nutrition Examination Survey (NHANES) has measured methylmalonic acid (MMA) concentrations, a functional biomarker of vitamin B-12 insufficiency, in the U.S. population from 1999-2004 using a lengthy GC/MS procedure. No data are available for 2005-2010. A 2010 expert roundtable discussing “NHANES Monitoring of Biomarkers of Folate and Vitamin B-12 Status” recommended that serum MMA measurements be reinstated in future NHANES in conjunction with serum vitamin B12 measurements. We have adapted and validated an isotope dilution LC-MS/MS method for population monitoring of serum MMA and compared this simpler procedure to the previous GC/MS procedure.

Monday 6:30 - 7:30 PM
Poster #24 in the Exhibit Hall

Development and Applications of Automated Benchtop Dynamic Pressure Generators For Biological Sample Preparation and High Pressure Perturbation of Protein Structure.

Alexander Lazarev (alazarev@pressurebiosciences.com)
Pressure BioSciences, inc.

We report the development of the two modular computer-controlled appliances offering hydrostatic pressure control within the range of 20 to 1,380 Bar or 50 to 4,000 bar, respectively. The new instruments process samples is either of the two pressure vessels, accommodating 12 and 48 samples, respectively or in automated on-line mode, using a flow-through high pressure module. The new instruments are intended for automated optimization of sample preparation methods for biological mass spectrometry. This work presents several applications, such as extraction of hydrophobic proteins from tissue samples, pressure-enhanced in-solution and in-gel tryptic digestion, and enzyme kinetics.

Monday 2:00 - 3:00 PM
Poster #25 in the Exhibit Hall

Molecular Imaging Studies of Drugs and Metabolites in Tissue using Desorption Electrospray Ionization (DESI) coupled to Mass Spectrometer/Comparison to MALDI-MS

John Chakel (chakel@prosolia.com)
Prosolia, Inc.

In DESI, a charged droplet beam produced by electrospray is pneumatically directed towards a surface resulting in production of molecular ions sampled by MS. It is a new method for visualizing tissue distributions of drugs and metabolites, simultaneously and directly from intact sections. DESI does not require any matrix and is an ambient technique. Scanning the charged-droplet beam across a tissue surface produces molecular images. DESI-MS images are shown for the endogenous lipids in rat brain; several antipsychotic and anti cancer drugs where both MALDI-MS and DESI-MS were performed on the same tissue samples; and antibiotics in rabbit ocular tissue.

Monday 6:30 - 7:30 PM
Poster #26 in the Exhibit Hall

Detecting New Drugs of Abuse using LC-MS-MS with Fast Precursor Ion and Neutral Loss Scanning

Jeff Dahl (jhdahl@shimadzu.com)
Shimadzu

Forensics and anti-doping labs rely on LC-MS-MS for detection of controlled and banned substances. However designer drugs may not be detected by traditional MRM-based methods since their transitions are not always known in advance. Precursor ion and neutral loss scanning can be used to detect designer drugs which share similar functional groups. We developed LC-MS methods that utilize extremely fast precursor ion and neutral loss scanning for detection of designer cannabinoids, amphetamines, barbiturates, and their metabolites. Data-dependent MS-MS using fast product ion scanning was used to characterize unknown designer drugs in real time.

Monday 2:00 - 3:00 PM
Poster #27 in the Exhibit Hall

Quality Control Measures for Routine, High-Throughput Targeted Protein Quantitation Using Tandem Capillary Column Separation

Scott Peterman (scott.peterman@thermofisher.com)
Thermo Fisher Scientific

Mass spectrometry-based targeted protein quantitation experiments presents many challenges to high-throughput assay demands. Our approach to accommodate a longer list of targets is to incorporate tandem capillary column separation with high resolution accurate mass analysis. Tandem capillary columns enable mass spectral acquisition to be performed only over the target peptide elution profile. A set of well-characterize trainer peptides is spiked into each sample enabling quality control for LC and MS performance for each injection. A set of 424 peptides were targeted from urine bladder cancer.

Monday 6:30 - 7:30 PM
Poster #28 in the Exhibit Hall

Correlating Glycan Expression with Gene Expression in Milk Glycoproteins

Jincui Huang (hjchuang@ucdavis.edu)
University of California Davis

Lactoferrin (LF), an iron-binding glycoprotein with multiple physiological functions (antimicrobial activities, bacterial pathogenesis, gene regulation, and immune modulation), is one of the most important proteins present in mammalian milk.Glycans are an integral part of glycoproteins and affect their overall conformation, and thus, may affect functions as well. Human milk LF has been characterized with three potential N-glycosylation sites. In order to investigate N-glycosylation of LF during lactation, a systematic method was applied to purify LF from milk. The variations of glycosylation were studiedby both (MALDI FT-ICR) MS and HPLC-Chip/TOF MS. The results were supported by glycosylation-related gene expression studies.

Monday 2:00 - 3:00 PM
Poster #29 in the Exhibit Hall
Urine Barbiturate Analysis Using an Improved SPE Protocol and LC/MS/MS; Achieving Chromatographic Resolution of Isobaric Amobarbital & Pentobarbital
Michael Rummel (michaelr@phenomenex.com)
Phenomenex
Barbiturates are a notoriously difficult class of compounds to analyze in drugs of abuse testing panels. Additionally, two of the commonly screened barbiturates, pentobarbital and amobarbital share the same mass transitions meaning full chromatographic resolution is necessary for LC/MS/MS analysis. Therefore, the goal of this work was to develop a streamlined SPE extraction for barbiturates in urine that removed potential matrix contaminants and a LC/MS/MS method that resolved pentobarbital amobarbital, butalbital, secobarbital, and phenobarbital. Much emphasis was placed on the sensitivity and reproducibility of the method over a linear range from 40-125% of the cutoff concentration set at 300 ng/mL.

Monday 6:30 - 7:30 PM
Poster #30 in the Exhibit Hall
Quantitation of Haloperidol and Reduced Haloperidol in Serum by LC-MS/MS
Paula Ladwig (ladwig.paula@mayo.edu)
Mayo Clinic
This assay gives accurate, robust identification and quantitation of haloperidol and reduced haloperidol in serum. Haloperidol is a typical antipsychotic used in the treatment of schizophrenia. Unfortunately, the use of haloperidol is associated with significant toxic side effects. It is extremely important to have available a quantitative assay for haloperidol and reduced haloperidol for monitoring of clinical response and possible toxicity, optimizing drug dosage, and assessing compliance. Haloperidol and reduced haloperidol are extracted from serum by SPE. The extraction containing deuterated internal standards is analyzed by LC-MS/MS giving quantitative results for haloperidol and reduced haloperidol.

Monday 2:00 - 3:00 PM
Poster #31 in the Exhibit Hall
A sparkplug for clinical assays: progressing towards autonomous global spectral repositories
Barbara Frewen (barbara.frewen@gmail.com)
University of Washington
Targeted protein quantitation using mass spectrometry has emerged as a viable alternative for affinity-based quantitation. Spectral libraries provide a repository of peptide information which can be used to validate peptide identifications or to provide a starting point for developing targeted assays. Existing libraries are designed with identification in mind. We present a spectral library especially suited for developing targeted assays which characterizes the variation in observed data and normalizes retention times and abundances to an internal standard. Data from synthetic or recombinant peptides are treated separately as “reference” data to be compared with the variation seen from endogenous sources.

Monday 6:30 - 7:30 PM
Poster #32 in the Exhibit Hall
XCMS Online & METLIN: Resources for web-based metabolomics data processing
Kevin Cho (kevincho@scripps.edu)
The Scripps Research Institute
Metabolomics provides a tool to study cellular metabolism and has particular power for investigating biochemical applications for the clinical laboratories. One of the major challenges in global metabolomics, however, is the complexity of the datasets and the significant number of unknown metabolites. Metabolomics data analysis is challenging giving the large number differentially regulated metabolites. To facilitate this effort, we have developed a new web-based platform for untargeted metabolomics data, called XCMS Online. Furthermore, a new scoring system has been integrated that allows the matching of user MS/MS spectra against the METLIN MS/MS database for rapid metabolite identification.

Monday 2:00 - 3:00 PM
Poster #33 in the Exhibit Hall

Chemical imaging of germ-free and gnotabiotic mice with MALDI mass spectrometry—towards characterizing the metabolic interactions that define the healthy microbiome

Christopher Rath (crath@ucsd.edu)
University of California at San Diego, Skaggs School of Pharmacy and Pharmaceutical Sciences

Microbial communities in the gut are thought to play key roles in the health of the host organism. We hypothesize that inter- and intra- species interactions in the microbiome are mediated by diffusible metabolic exchange factors. Herein, we investigate the distribution of these compounds, both host and microbiome-derived, through chemical imaging of germ-free and gnotabiotic mice with MALDI-TOF mass spectrometry. The application of an imaging mass spectrometry platform for exploring metabolic exchange has the potential to revolutionize our understanding of the gut microbiome—presenting the possibility of identifying new strategies for treating disease in the host.

Monday 6:30 - 7:30 PM
Poster #34 in the Exhibit Hall

Rapid Identification of Disseminated Aspergillus terreus Infection by PCR and Electrospray Ionization Mass Spectrometry

Christian Massire (cmassire@ibisbio.com)
Ibis Biosciences, An Abbott Company

A. terreus infection occurs most commonly in patients with leukemia. Even with timely initiation of antifungal therapy, disseminated A. terreus is almost certainly fatal. Inherent resistance of A. terreus to amphotericin B complicates management. , Diagnosis by conventional methods has low sensitivity, and may be complicated by morphologic variations among isolates of A. terreus. One impediment to timely diagnosis is the low yield of fungal cultures from respiratory specimens. We describe a fatal case of disseminated A. terreus infection that was readily diagnosed by PCR/ESI-MS. Ultimately, the diagnosis was confirmed by more invasive methods before the patient expired.

Monday 2:00 - 3:00 PM
Poster #35 in the Exhibit Hall

Urine Profiles of Oxycodone, Noroxycodone, Oxymorphone, and Noroxymorphone

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

Monitoring chronic pain patient compliance is necessary for prevention of prescribed medication diversion and/or concurrent use of unauthorized/illicit drugs. Metabolite detection is crucial, but result interpretation is complicated by the availability of several opioid metabolites as prescription drugs. Our current opioid confirmation assay was expanded to include noroxycodone and noroxymorphone. Previous findings that the inclusion of normetabolites can reduce false negatives for parent opioids use were investigated for oxycodone, oxymorphone, noroxycodone and noroxymorphone and were confirmed. Five distinct patterns for fraction of the total, calculated as analyte concentration/sum of concentrations for all investigated opioids, were identified using clustering analysis.

Monday 6:30 - 7:30 PM
Poster #36 in the Exhibit Hall

Alpha-lytic protease: orthogonal specificity complementary to trypsin for proteomics

Elizabeth Komives (ekomives@ucsd.edu)
UC San Diego

To increase PTM and sequence coverage, proteases of alternative specificity to trypsin are needed. Alpha-lytic protease (aLP) is exceptionally stable, and highly active in various denaturants. The average peptide length produced by aLP digestion was similar to those produced by trypsin. Digestion of the S. pombe proteome with aLP results in identification of >1000 proteins with at least one peptide below a 1% FDR when CID/ETD fragmentation pairs are searched with MSGFDB. The combination of aLP and trypsin doubles the number of proteins showing significant iTRAQ quantifiable changes, showing the significant advantage of using a protease with “orthogonal” specificity.

Monday 2:00 - 3:00 PM
Poster #37 in the Exhibit Hall

The Measurement of Nucleoside Reverse Transcriptase Inhibitor (NRTI) Antiretroviral Drugs by LC-MS/MS

Michael Jarvis (michael.jarvis@absciex.com)
AB SCIEX

The nucleoside reverse transcriptase inhibitors (NRTI) are an important class of antiretroviral drugs, which inhibit growth of the viral DNA strand by causing chain termination. In this work, an LC-MS/MS method has been developed for the quantitative analysis of Abacavir, Didanosine, Emtricitabine, Lamivudine, Stavudine, and Zidovudine, using a hybrid triple quadrupole / linear ion trap mass spectrometer. The method employs the Multiple Reaction Monitoring (MRM) scan mode to perform quantitation, while also utilizing the linear ion trap to simultaneously perform the Information-Dependent Acquisition (IDA) of MS/MS spectra to confirm the identity of the detected antiretroviral drugs.

Monday 6:30 - 7:30 PM
Poster #38 in the Exhibit Hall

Simplified LC-MS/MS Workflow for the Parallel Measurement of Methotrexate and Other Common Immunosuppressants

Hari Nair (nairh@uw.edu -- *Young Investigator Awardee*)
Department of Laboratory Medicine, University of Washington, Seattle.

A combined LC-MS/MS work flow for highly specific therapeutic monitoring of methotrexate, in parallel with other common immunosuppressant drugs tacrolimus, sirolimus, cyclosporine, and everolimus has been developed and validated for clinical application. The workflow was designed by modifying an existing LC-MS/MS assay used for the routine measurement of common immunosuppressant drugs. The assays were successfully validated for methotrexate and other immunosuppressant drugs and a favorable correlation with a previously validated immunoassay was demonstrated. Such an approach for parallel analysis of methotrexate and common immunosuppressant drugs can be adopted in clinical laboratories to extend the utility of the existing mass spectrometric instrumentation.

Monday 2:00 - 3:00 PM
Poster #39 in the Exhibit Hall

Establishing Analytical Run Acceptability Criteria for a 25-OH Vitamin D LC-MS/MS Assay for Use in a Clinical Laboratory Setting

Steven Naleway (snaleway@mcvh-vcu.edu) -- *Young Investigator Awardee*
Virginia Commonweatlh University

There is little information regarding development of LC-MS/MS quality assurance (QA) parameters. During preliminary evaluation for 25-OHD, max between-run calibrator ISrec difference was < 30%. Within-run sample-specific ISrec difference was < 20% of average calibrator ISrec. During production, failed criteria for wtnr ISrec indicated instrument contamination. Failure of btwr ISrec in 4/35 runs indicated instrument problems. 3 runs failed calibration slope/intercept criteria, but QC or ISrec was unacceptable. 5.3% of patient samples failed sample-specific ISrec and 4.6% of those failed repeat criteria. Btwr calibrator ISrec and wtnr sample-specific ISrec are useful QA parameters that detect LC-MS/MS system or sample problems.

Monday 6:30 - 7:30 PM
Poster #40 in the Exhibit Hall

Coupling Nanoparticle-Assisted Discovery with Immuno-MS Validation to Improve Clinical Diagnostics

Brian Feild (bjfeild@shimadzu.com)
Shimadzu Scientific Instruments

Development of clinical diagnostic tests requires identification and measurement of low- level, disease-specific biomarkers directly from complex biological fluids. This presentation focuses on a nanoparticle-assisted biomarker candidate discovery approach and an automated, high-throughput Immuno-MS validation strategy. Serum samples from a muscular dystrophy study were used to illustrate the ability to rapidly select and optimize disease biomarker candidates. The differentially expressed proteins from nanoparticle enrichment were identified using MALDI-TOF MS profiling followed by in gel tryptic digestion and LC-MS/MS. Work is underway to use a microfluidics based sample preparation workstation to refine and validate CXCL7 as a candidate biomarker with Immuno-MS.

Monday 2:00 - 3:00 PM
Poster #41 in the Exhibit Hall

LC-ICP-MS for Stability Assessment of Lanthanide Complexes used for Implementation of Elemental Labeling Strategies in Bio-analytical Assays.

Daniela Kretschy (daniela.kretschy@boku.ac.at)
Division of Analytical Chemistry, Department of Chemistry, University of Natural Resources and Life Sciences, BOKU Vienna

Labeling strategies offer enhanced sensitivity, selectivity and robustness for analysis of bio-molecules in difficult matrices. Our work deals with the stability assessment of elemental labels at realistic conditions for the intended use in bio-assays. Previously, we investigated complex stabilities of non-functionalized chelating moieties combined with lanthanides and this continuative study will address complex stability of the same chelators functionalized with linkers for bio-molecule derivatization, i.e maleimide, isothiocyanato, benzyle and iodoacetamide groups. For this purpose we have developed a LC based separation method combined with ICP-MS detection for simultaneous, quantification of the free and complexed fractions of the employed lanthanides.

Monday 6:30 - 7:30 PM
Poster #42 in the Exhibit Hall

Converting Proteomic Based Discovery into a Validative Assay for Cardiac Ischemic Biomarkers

Faith Hays (fahays@shimadzu.com)
Shimadzu

Using a triple quadrapole mass spectrometer, we have developed a Selected Reaction Monitoring assay to quantify Albumin PTMs (phosphorylations and cysteinylations) implicated in cardiac ischemia. Unlike most SRM-MS assays that require multiple, independent sample preparation steps, our sample preparation occurs within a Perfinity Workstation. By replacing bench-top preparative steps with column-mediated digestion, desalting, and Reverse Phase HPLC separation performed in-line, the reproducibility and throughput of our developed SRM-assay has been improved significantly. Selecting the Shimadzu Perfinity Workstation as the front-end of a validation pipeline has ensured that an SRM-MS assay could be used as an emergency department diagnostic for cardiac ischemia.

Monday 2:00 - 3:00 PM
Poster #43 in the Exhibit Hall

A Simple and Fast Liquid Chromatography-Tandem Mass Spectrometry Method for the Quantitation of Iothalamate in Serum and Urine.

Joe El-Khoury (joe.eldouss@gmail.com) -- *Young Investigator Awardee*
Cleveland State University

Radioactive iothalamate clearance is routinely used for the determination of glomerular filtration rate (GFR). To avoid exposure to radioactive materials, methods that measure non-radioactive iothalamate have been developed. However, these methods suffer from long chromatography and/or tedious sample preparation, or are susceptible to interferences. We have developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to measure iothalamate in serum and urine. Sample preparation for both matrices is protein precipitation then dilution followed by a 2.1 min LC-MS/MS run time. Assay precision is less than 8.8%, accuracy is 87-110% and assay is free from ion suppression, interference and carryover.

Monday 6:30 - 7:30 PM
Poster #44 in the Exhibit Hall

Quantitative Analysis of Human C-peptide by LC-MS Isotope-Dilution Assay and Microheterogeneity of Internal Standard

Alexander Stoyanov (stoyanova@health.missouri.edu)
University of Missouri, School of Medicine

Any quantitation based on IDA is connected with additional errors due to heterogeneity of the labeled analogue. The existence of multiple isoforms of labeled peptide which possess comparable abundance can result in highly overestimated C-peptide concentration in biological fluids when the concentration of labeled (internal) standard is determined by nitrogen, amino acid analysis, or by radioimmunoassay. In order to provide an accurate C-peptide measurement, heterogeneity of the internal standard must be taken into account.

Monday 2:00 - 3:00 PM
Poster #45 in the Exhibit Hall

Comparison of Automated SPE/HPLC/MS/MS Methods to Traditional Immunoassay with MS Confirmation of Driving Under the Influence Samples

Ken Lewis (klewis@opans.com)
OpAns

Immunoassay for screening followed by solid phase extraction (SPE) coupled with GC/MS or LC/MS/MS is well established for identification and confirmation/quantification of drugs and/or poisons from complex biological matrices submitted to forensic laboratories. However, reduced budgets and staffing necessitate improved operational efficiency. This poster details our initial comparison of operational efficiency using in-line automated SPE HPLC/MS/MS, versus traditional methods, for the analysis of urine samples submitted in Driving Under the Influence of Drugs (DUI-D) cases.

Monday 6:30 - 7:30 PM
Poster #46 in the Exhibit Hall

High Sensitivity Analysis of Testosterone from Dried Blood Spots

Michal Weinstock (michal.weinstock@absciex.com)
AB SCIEX

Herein presented an ultra -high sensitivity LC/MS/MS method to analyze Testosterone (Te) in human Dried Blood Spots (DBS) using ESI-LC/MS/MS and a novel derivatization chemistry. The workflow is simple and quick: DBS are extracted with Hexane/ethyl acetate prior to derivatization and the quantitation of Te is enabled by adding a known concentration of isotopically enriched Te as Internal Standard (IS). LLOQ after derivatization is <50pg/mL. Linearity is demonstrated ($R2 \geq 0.999$) over the range of 50-10000 pg/mL, with 11% CV at LLOQ (n=20). The above method enabled Te measurement from pediatric and female DBS which was not possible without derivatization.

Monday 2:00 - 3:00 PM
Poster #47 in the Exhibit Hall

INCREASED URINARY 4-AMINOBIPHENYL IN NONSMOKERS EXPOSED TO SIDESTREAM CIGARETTE SMOKE UNDER CONTROLLED CONDITIONS

Tiffany Seyler (tvh2@cdc.gov)
CDC

Exposure of non-smokers to second hand smoke (SHS) remains a significant public health concern, and surveys of nonsmokers' exposure to SHS based on serum cotinine have shown certain groups such as males and non-Hispanic Blacks having relatively higher exposure. To better understand the differences, we enrolled 40 nonsmokers who were exposed to cigarette smoke under controlled conditions and measured change in total urinary 4-aminobiphenyl as a tobacco exposure biomarker. Under controlled exposure conditions, level of 4-abp was significantly increased after exposure in all participants ($p < 0.027$). Our results suggest that total urinary 4-ABP is an effective biomarker for short term tobacco exposure in non-smokers.

Monday 6:30 - 7:30 PM
Poster #48 in the Exhibit Hall

Proteomic Biomarkers for the Early Detection of Ovarian Cancer Progression

Mark Marzinke (mmarzin1@jhmi.edu) -- *Young Investigator Awardee*
Johns Hopkins Medical Institutions

Ovarian carcinoma is a highly metastatic disease rarely detected during the early stages of cancer progression, resulting in decreased survival. A cascade of molecular and morphological events results in uncontrolled cancer growth and ultimate metastasis. These events change over time, and thus can be temporally monitored. The purpose of this work is to analyze these temporal changes in order to identify biomarkers that may be useful for the early detection of ovarian cancer. Using the adenocarcinoma OVCAR-3 cell line as a model system, molecular and mass spectrometry-driven proteomic analyses were performed to identify candidate biomarkers differentially regulated during cellular hyperproliferation.

Monday 2:00 - 3:00 PM
Poster #49 in the Exhibit Hall

Selectivity Enhancement in High Throughput Analysis of Testosterone using Differential Ion Mobility Coupled to LDTD MS/MS

Pierre Picard (p.picard@phytronix.com)
Phytronix Technologies inc

The Laser Diode Thermal Desorption™ (LDTD) ionization source has been coupled to a mass spectrometer equipped with the SelexION™ differential ion mobility cell, enabling a high throughput capacity for the analysis of testosterone in biological matrix, with sample-to-sample analysis time of 7 seconds. Sample preparation consisted of a liquid-liquid extraction of plasma with ethyl acetate. The lower limit of quantitation for the LDTD analysis of testosterone was limited by the blank interference from the presence of isobaric analytes, however the use of the orthogonal differential mobility spectrometry device allowed separation of those interferences, yielding an LOQ of 0.1 ng/ml testosterone.

Monday 6:30 - 7:30 PM
Poster #50 in the Exhibit Hall

Quantification of 8-isoprostaglandin F2α in Urine using Microfluidic Chip-Based LC-MS/MS

Shan-An Chan (jimmy_chan@agilent.com)
Agilent Technologies

In this study, we exploited a µ-fluidic chip-based LC-MS/MS system for the determination of (8-isoPGF2α) in urine. Solid-phase extraction (SPE) method was employed as a sample pre-treatment procedure to extract the analytical component from urine. A µ-fluidic chip consisting of C18 columns and a QQQ Mass spectrometer were was used for LC separation and determination. Gradient elution was employed for LC separation. Multiple reaction monitoring (MRM) was utilized for 8-isoPGF2α (m/z 353→193) quantitative determination. Good linearity (0.005 to 10 ng/ml) and detection limit (~ 0.001 ng/ml) were obtained. Reproducibility, precision and accuracy of this method have been evaluated.

Monday 2:00 - 3:00 PM
Poster #51 in the Exhibit Hall

Identification and Quantitation of Toxins from the Common Oleander Plant (Nerium oleander) and the Yellow Oleander (Thevetia peruviana) in Clinical Samples.

Alaine Garrett (amgarrett@medicine.nevada.edu) -- *Young Investigator Awardee*
Nevada State Public Health Laboratory

The seeds from the yellow oleander (Thevetia pruviana) have been used as a weight loss aid under the name Alamendra Quema Grassa. Recently two patients have been admitted to hospitals in Nevada due to ingestion of these seeds. Treatment of acute poisoning with digoxin-specific Fab antibody fragments has been show as a successful treatment but efficient treatment requires quantitation of toxic compounds in the body. This poster will demonstrate a quantitative LC/MS/MS method for clinical samples for the major toxins found in the common oleander (Nerium oleander) in clinical samples.

Monday 6:30 - 7:30 PM
Poster #52 in the Exhibit Hall

200 µm ID chip based columns for increased throughput peptide quantitation with nanoLC/MS

Remco van Soest (rvansoest@eksigent.com)
Eksigent, part of AB SCIEX

NanoLC coupled with mass spectrometry is the method of choice for sensitive peptide quantitation. In this presentation we will report on the use of 200 µm ID chip columns to address the typically observed delays in nanoLC using 75 µm ID columns caused by gradient delay in the nanoLC system itself, delay in the autosampler and sample loop, and the delay caused by connecting tubing. The increased sample throughput obtained using these 200 µm ID chip columns can be especially beneficiary for samples with reduced complexity, such as digests from protein enriched samples or peptide enriched samples (i.e. SISCAPA workflow).

Monday 2:00 - 3:00 PM
Poster #53 in the Exhibit Hall

Apple may offset oxidative stress and inflammation known to occur in inflammatory bowel diseases: Identification of polyphenols in the dried apple peel using LC-MSD Tof.

Marie-Claude Denis (marie-claude.denis.1@umontreal.ca)
CHU Sainte-Justine/University of Montreal

Polyphenols are known antioxidants which maintain natural defenses against various diseases. We hypothesize that polyphenols extracted from apple peels (DAPP) may offset oxidative stress and inflammation in intestine. An LC-MS method has been developed to separate and identify masses and chemical structures of polyphenols in DAPP. Flavonols were identified in crude extracts and flavanols in purified fractions. Intestinal Caco-2/15 cells were incubated with iron-ascorbate and lipopolysaccharide to evaluate the lipid peroxidation and inflammation, respectively. Exposure to DAPP extracts prevented lipid peroxidation and reduced inflammation in Caco-2/15 cells. Our findings provide evidence of polyphenols capacity to reduce oxidative stress and inflammation in intestine.

Monday 6:30 - 7:30 PM
Poster #54 in the Exhibit Hall

Comprehensive Targeted Quantitative Proteomics – Taking Multiplexed Assays to a New Level

Christie Hunter (christie.hunter@absciex.com)
AB SCIEX

The extreme complexity and dynamic range of proteins in typical proteomic samples challenges traditional data dependent workflows by requiring very high speed MS/MS acquisition to reproducibly and deeply interrogate the sample. The application of data independent acquisition strategies to increase the reproducibility and comprehensiveness of data collection has been limited by the speed of current mass spectrometers. Recent QqTOF innovations providing high speed acquisition of high resolution MS/MS spectra have enabled a new data independent acquisition strategy, called MS/MSALL with SWATH™ Acquisition. The utility of this workflow for highly multiplexed targeted quantification in plasma samples will be discussed.

Monday 2:00 - 3:00 PM
Poster #55 in the Exhibit Hall

Quantification of 25OH-Vitamin D by LC-MS/MS – is there a 'right' ionisation mode and MRM-transition

Roland Geyer (roland.geyer@tecan.com)
Tecan Trading AG

LC-MS/MS becomes the preferred method for assessing Vitamin D status of humans as it specifically detects the 25OH-VitD3/D2 metabolites utilizing either Atmospheric Pressure Chemical Ionisation (APCI) or Electrospray Ionisation (ESI). While the more gentle ESI preserves intact molecular ions to yield [M+H]+ in APCI hydroxylated analytes easily undergo loss of water to yield [M-H2O+H]+ resulting in differing MRM-transitions. On the other hand APCI is less prone to matrix effects and often displays lower noise levels due to thermal decomposition of clusters and background molecules. A comparative survey on both ionisation methods for 25OH-VitD analysis will be presented.

Monday 6:30 - 7:30 PM
Poster #56 in the Exhibit Hall

Analysis of Pain Management Compounds and Drugs of Abuse by LC/MS using a Perfluorophenyl Stationary Phase

Peter Simms
(psimms@kozmary.com)
Kozmary Center for Pain Management

Amphetamine, methamphetamine, MDA, MDMA, MDEA, cocaine, benzoylcegonine, trazodone and tapentadol were analyzed by LC/MS using a perfluorophenyl stationary phase. The separation of all eleven compounds could be achieved in approximately seven minutes using a water/50:50 acetonitrile/methanol/0.1% formic acid mobile phase. The separation was affected by varying the percentage of methanol and acetonitrile in the organic modifier. The chromatographic conditions allowed for baseline resolution of methamphetamine and phentermine, which shared MRM transitions. This decreased the rate of false positives for methamphetamine in our patients.

Monday 2:00 - 3:00 PM
Poster #57 in the Exhibit Hall
Accurate mass analysis of background contaminants in cotton cellulose paper
Jonathan Wilson (jonathan.wilson@perkinelmer.com)
PerkinElmer
An accurate mass MS study of dried bloods spots used a flow-through Spark Holland prototype DBS clamping device. Contaminants desorbed from the blank paper could suppress ionization thus reducing sensitivity and linearity in quantitative clinical screening assays. The elemental formulae of the major contaminants were determined from the accurate mass and isotopic pattern of the peaks. One component was identified as an inert component in pesticides used on cotton plants and confirmed by MS and HPLC of standards. Other components were also investigated. Brands of DBS paper were compared, and contaminant levels were determined by comparison with spiked paper calibrators.

Monday 6:30 - 7:30 PM
Poster #58 in the Exhibit Hall
Colorectal Cancer Screening based on Serum Peptide Precision Profiles
Simone Nicolardi (s.nicolardi@lumc.nl) -- *Young Investigator Awardee*
Leiden University Medical Center (LUMC)
We report the proteomics results of a biomarker discovery study for colorectal cancer obtained from mass spectrometry (MS)-based precision profiles. Discriminating profiles were found in a discovery set that consisted of more than 200 CRC patients and 400 healthy controls. This profiling study was performed by matrix-assisted laser desorption ionization (MALDI) coupled with time-of-flight MS. In addition, the profiles were obtained on a MALDI Fourier Transformation Ion Cyclotron Resonance MS. Finally, we will present initial data from a screening study that involves more than 1200 human serum samples. This cohort is embedded in the Dutch Center for Translational Molecular Medicine.

Monday 2:00 - 3:00 PM
Poster #59 in the Exhibit Hall
High Throughput Liquid Chromatography-Tandem Mass Spectrometry Method for Serum 25-hydroxyvitamin D using a TurboFlow Online Extraction Technology
Andrea Bozovic (Andrea.Bozovic@uhn.on.ca) -- *Young Investigator Awardee*
University Health Network
Increased knowledge about vitamin D benefits has improved our understanding of the implications of vitamin D deficiency. Now, vitamin D analysis seems to be one of the most commonly performed tests in clinical labs. To improve the throughput of LC–MS/MS analysis, we developed a quick and sensitive quantitative method for measuring clinically-relevant metabolites of vitamin D: 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 in serum, using a turbulent-flow technology. Automatic online extraction based on this technology efficiently removed large matrix components while extracting analytes of interest. We achieved more than two-fold increase in throughput by the multiplexing capability of TLX-2 instrument.

Monday 6:30 - 7:30 PM
Poster #60 in the Exhibit Hall
Semi-Automated Direct Elution of Dried Blood Spots for the Quantitative Determination of Guanfacine in Human Blood
Jack Henion (henionj@advion.com)
Advion Bioanalytical Labs
Direct analysis of dried blood spot (DBS) samples was investigated using a semi-automated robotic device that allows for the direct elution of sample spots from a paper substrate DBS card to an in-line solid-phase extraction (SPE) cartridge. A high-throughput and very sensitive method based on this flow-through concept was developed for the quantitative determination of guanfacine in fortified human whole blood DBS samples using on-line SPE followed by analysis with high performance liquid chromatography coupled with a tandem mass spectrometer system (LC-MS/MS) operated in the selected reaction monitoring (SRM) mode.

Monday 2:00 - 3:00 PM
Poster #61 in the Exhibit Hall

Extraction of Methylmalonic Acid (MMA) from Human Serum Using Supported Liquid Extraction (ISOLUTE® SLE+) in 96-Well Plate and Column Formats Prior to LC-MS-MS Analysis

Victor Vandell (Victor.Vandell@Biotage.com)
Biotage

The screening for elevated levels of Methylmalonic Acid (MMA) in serum is commonly used as a clinical diagnostic indicator of Cobalamin (Vitamin B12) deficiency in mammals. While commonly done, the detection and quantification of MMA can be problematic due to the abundant presence of the endogenous isobaric Succinic Acid (SA). The development of reliable and automation-compatible sample cleanup methods is necessary for the accurate detection and quantification of MMA using mass spectrometry. This poster demonstrates a rapid and reliable Supported Liquid Extraction assay for Methylmalonic Acid from serum at microgram per milliliter concentration levels prior to LC-MS/MS analysis.

Monday 6:30 - 7:30 PM
Poster #62 in the Exhibit Hall

Improved LC/MS/MS Method for Analysis of Ethyl-Glucuronide and Ethyl-Sulfate in Urine

Tania Sasaki (tsasaki@avee.com)
Avee Laboratories

An improved LC/MS/MS method for analysis of EtG and EtS in urine has been developed. Sample preparation was simple dilution and separation utilized dihexyl ammonium acetate as an ion pair reagent, optimizing retention of both compounds on a short 2.1 mm x 50 mm column and eliminating the necessity for post-column infusion of organic solvent. EtG and EtS were also resolved from known matrix interferences, minimizing ion suppression. Run time was less than seven minutes and multiplexing the assay reduced analysis times to less than four minutes per sample, making the method useful for high throughput sample analysis.

Monday 2:00 - 3:00 PM
Poster #63 in the Exhibit Hall

Immuno-MS: Increased Dimensionality Over Immunoassays

Rachel Lieberman (ralieberman@shimadzu.com)
Shimadzu Scientific Instruments

The benefits of immunoassay, namely speed, low cost and high efficiency, can be coupled with the exceptional resolving power of mass spectrometric detection, delivering improved detection and quantitation capabilities. Multiple Reaction Monitoring (MRM) MS has recently expanded its use as a powerful method for the quantitative analysis of proteins. MS based methods are more likely to be complementary to immunoassays, especially when protein isoforms or PTMs add diagnostic value. This presentation will review the advantages and disadvantages of various immune-MS strategies and describe the development of a fully automated workstation capable of performing a proteomic workflow prior to MS detection.

Monday 6:30 - 7:30 PM
Poster #64 in the Exhibit Hall

New advance in measuring 1,25-dihydroxyvitamin D by LC-MS/MS for the clinical laboratory

Chao Yuan (yuanc@ccf.org)
Cleveland Clinic

Measurement of 1,25-dihydroxyvitamin D by LC-MS/MS is challenging due to its low level in blood and high fragmentation rate in the MS ion source. Derivatization or adduct formation has been used to improve sensitivity. However eliminating interference is difficult even after tedious sample preparation. Recently we have developed a novel LC-MS/MS method combining immunoaffinity extraction and lithium adduct formation to achieve clean chromatography with high sensitivity and precision. Important features of this method along with full validation data will be discussed in this presentation.

Monday 2:00 - 3:00 PM
Poster #65 in the Exhibit Hall

Rapid Analysis of Drug Analytes in Urine for Forensic Toxicology Using Ultra-Fast Online SPE/MS/MS

Vaughn Miller (vaughn.miller@agilent.com)
Agilent Technologies

The ability of an ultra-fast SPE/MS/MS system to analyze metabolites of drugs such as benzoylecgonine and THCCOOH in urine with much faster sample cycle times and similar analytical results compared to GC/MS assays was evaluated in the present study. Excellent linearity, precision, accuracy, and signal-to-noise ratios were determined for both analytes. Results were comparable to GC/MS but with much faster analysis times. This SPE/MS/MS system may be useful for fast and efficient detection of similar small molecule analytes in urine.

Monday 6:30 - 7:30 PM
Poster #66 in the Exhibit Hall

A Novel Solid-Phase Extraction Method for Paralytic Shellfish Marine Toxins Saxitoxin and Neosaxitoxin from Human Urine

Justin Jacob (JJacob@cdc.gov)
Centers for Disease Control and Prevention

Saxitoxin (STX) and neosaxitoxin (NEO), marine toxins produced by red tide algae, can contaminate shellfish and cause paralytic shellfish poisoning (PSP) when consumed. A novel, high-throughput, solid-phase extraction method with LC/MS/MS detection has been developed for clinical analysis of urine samples, improving upon an existing published clinical method by decreasing the limit of detection. Additionally, the use of a multi-mode solid phase sorbent enables the extraction of several other paralytic shellfish toxins such as tetrodotoxin and the gonyautoxins, which are not included in the currently-used clinical method and for which no urinary extraction method currently exists.

Monday 2:00 - 3:00 PM
Poster #67 in the Exhibit Hall

Heavy Isotope-Labeled Parathyroid Hormone as an Immunocapture and Digestion Efficiency Internal Standard for Mass Spectrometry (MS)-based Immunoassays

John Rogers (john.rogers@thermofisher.com)
Thermo Fisher Scientific

Parathyroid hormone (PTH) assays for monitoring PTH and PTH variants are becoming important for the accurate diagnosis of endocrine and osteological diseases. We describe the production, characterization and use of heavy isotope-labeled PTH to quantitatively assess sample preparation for selected reaction monitoring (SRM)-based immunoassays. Normal PTH and two heavy forms of PTH were translated in vitro using a human cell-free extract system and characterized by MS. The proteins were then used as internal standards to assess immune-capture and trypsin digestion efficiency for the detection of PTH variants, including aa7–84 and aa34–84.

Monday 6:30 - 7:30 PM
Poster #68 in the Exhibit Hall

Determination of Heavy Metals in Blood by ICP-MS with Calibration in Synthetic Matrix

Josephine Alvaran (Josephine.Alvaran@cdph.ca.gov)
California Department of Public Health

The effects of matrix matching between blood samples and calibration standards using a synthetic matrix (SM) for intermediate calibration standard preparation are presented. When using SM for calibration standard preparation, an excellent agreement was observed between the Pb results and reference values for all four levels of NIST reference material, and results were within uncertainty of the mean for Cd and Hg results. The calibration standard preparation using SM has become an approved method for the determination of heavy metals in blood for the California Biomonitoring program, and is used for routine blood lead analysis for patients being treated for lead poisoning.

Monday 2:00 - 3:00 PM
Poster #69 in the Exhibit Hall

Comparing Large and Small Molecule LC/MS Differential Analyses of the Effects of Hypoxia on BeWo Cells

Michael Athana (athanas@vastscientific.com)
VAST SCIENTIFIC

Preeclampsia is thought in many cases to be caused by a shallowly implanted placenta which becomes hypoxic, leading to an immune reaction characterized by secretion of up-regulated inflammatory mediators from the placenta. In this study, we modeled the effects of hypoxia and explore secreted compounds using placental-derived BeWo cell culture under normal and hypoxic conditions. We present perspectives from various pathway analysis tools such as Ingenuity Pathway Analysis to elucidate and contrast differential expression patterns in both the small and large molecule analyses.

Monday 6:30 - 7:30 PM
Poster #70 in the Exhibit Hall

Rapid separation of 25-OH-vitamin D3 and 3-epi-25-OH-vitamin D3 in human serum under RP-LC conditions and tandem mass spectrometry detection

Jeff Layne (JeffL@phenomenex.com)
Phenomenex

The monohydroxy vitamin D and its metabolically inactive isomer, C3-epi, cannot be distinguished by tandem mass spectrometry. This inactive metabolite is present in children less than one year old and can interfere with accurate quantitaion of the active metabolite. A chromatographic method is described here that fully resolve the isomeric species by a reversed phase LC column within a short run time.

Monday 2:00 - 3:00 PM
Poster #71 in the Exhibit Hall

Determination of the Amniotic Fluid Lecithin/Sphingomyelin Ratio Using Cation-Exchange Resin and Liquid Chromatography-Quadrupole Mass spectrometry

Hee-Jung Chung (vivid.hee@gmail.com) -- *Young Investigator Awardee*
Department of Laboratory Medicine, Cheil General Hospital & Women

We developed a novel method for determination of the amniotic fluid lecithin/sphingomyelin ratio using a pipette tip containing cation-exchange resin and MS. Choline-containing phospholipids were purified by passage through the resin tip. Purified samples could be directly analyzed by LC-MS/MS and MALDI-TOF. The L/S ratio is determined by MS and other fetal lung maturity test showed a positive correlation between each measurement and gestational age. The L/S ratio by our method was less subject to interference by blood or meconium contamination than other methods. Our method was simpler, quicker, and more efficacious than conventional TLC, more accurate than TDx-FLMII result.

POSTERS: Tuesday

Tuesday 2:00 - 3:00 PM
Poster #1 in the Exhibit Hall
Probing Mass Spectrometric Strategies for High Sensitivity Quantification of Bioactive Peptides
Christie Hunter (christie.hunter@absciex.com)
AB SCIEX
The increased throughput needed for protein biomarker validation often requires reduced sample preparation and / or accelerated chromatography which increases the chance of interferences that could confound robust quantification. The purpose of this study is to explore a range of new MS analysis methodologies that enable higher selectivity quantification. In this study, we compare the quantification of the bioactive peptide BNP using various strategies including combinations of sample preparation and mass spectrometric methodologies on different mass spectrometric platforms. MRM, MRM3 and differential mobility separation will be the technologies explored.

Tuesday 6:30 - 7:30 PM
Poster #2 in the Exhibit Hall
UPLC-MS/MS Procedure Detecting the Use of the Psychoactive Plant Kratom
Gregory Janis (gjanis@medtox.com)
MEDTOX Laboratories
The psychoactive plant kratom is abused to obtain both stimulating and opiate-like effects. As kratom abuse increases, efficient procedures to detect its use are needed. We have developed a mixed quantitative / qualitative LC/MS/MS procedure detecting kratom use in urine; the procedure utilizes both MRM and enhanced product ion analyses on an AB Sciex 5500 QTrap. The primary kratom alkaloid, mitragynine, is quantified from 1 to 500 ng/mL. Simultaneously, the presence of two metabolites and the related, active alkaloid 7-hydroxymitragynine are qualitatively monitored as confirming evidence of use. The fully validated method has been successfully employed for clinical toxicology analyses.

Tuesday 2:00 - 3:00 PM
Poster #3 in the Exhibit Hall
Proteomic Identification of Red Blood Cell Membrane Proteins in Hereditary Spherocytosis by Nano-Ultra Performance Liquid Chromatography-Quadrupole-Time of Flight Tandem Mass Spectrometry
Chul Min Park (i004@snu.ac.kr) -- *Young Investigator Awardee*
Konkuk University Medical Center
Hereditary spherocytosis (HS) is characterized by red blood cell (RBC) membrane protein defects. Sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is used for HS diagnosis, despite its labor-intensive procedures. We performed RBC membrane protein analysis using quadrupole-time of flight (Q-TOF) tandem mass spectrometry (MS/MS). Five HS patients/three controls were studied. Both SDS-PAGE and proteomic analysis were performed. Tryptic peptides were analyzed with liquid chromatography/Q-TOF MS/MS. The intensity for each protein fraction was compared, and there were acceptable correlations between methods. This method will be helpful in the diagnosis of HS and could replace the current labor-intensive electrophoretic method if well adjusted.

Tuesday 6:30 - 7:30 PM
Poster #4 in the Exhibit Hall
Clinical Evaluation of Chromium, Cobalt, and Titanium by ICP-MS for Metal on Metal Implants
Anna Miller (amiller@medtox.com)
Medtox Scientific
The potential for bioaccumulation of chromium, cobalt, and titanium from metal-o- metal joint replacements is an increasing medical concern for patients, physicians and device manufacturers. We developed and validated ICP-MS based bioanalytical methods to quantify these metals in blood and plasma samples for use in monitoring studies. Circulating levels of chromium and cobalt in patients with metal-o- metal hip implants commonly exceed levels found in normal populations. Specimen analysis was performed by acidic dilution and ICP-MS utilizing helium gas octopole reaction system to reduce matrix based interferences. Rapid analysis is achieved utilizing 96-well plates and integrated sample introduction systems.

Tuesday 2:00 - 3:00 PM
Poster #5 in the Exhibit Hall

Confirmational Analysis of Urinary Synthetic Cannabinoids using Rapid Solid Phase Extraction and Dual Polarity Electrospray LC/MS/MS

Michael Rummel (michaelr@phenomenex.com)
Phenomenex

Synthetic cannabinoids are chemical compounds that bind to the same receptors as the pharmacologically active component of marijuana, THC. The products are reported to have a much stronger effect than THC, producing reported signs of vomiting, increased addictive properties, loss of consciousness, psychosis and seizures. As such, drug screening and testing programs are readily adopting procedures to test for these substances in routine drug panels. In this study, the goal was to provide a rapid and reproducible confirmational test for the synthetic cannabinoids JWH-018, JWH-073, JWH-122, JWH-200, CP47,497, and CP47,497-C8 (Cannabicyclohexanol) in urine using SPE followed by LC/MS/MS analysis.

Tuesday 6:30 - 7:30 PM
Poster #6 in the Exhibit Hall

PBDE analysis in Human serum by Twister© Stirbar Sorptive Extraction and GC/ MSD-ECNI-SIM

Bonita Taffe (taffeb@michigan.gov)
Michigan Department of Community Health Bureau of Laboratories

Polybrominated diphenyl ethers (PBDEs), widely used fire retardants, are currently under investigation as agents interfering with immunological, embryonic development and endocrine functions. Low population background levels have favored analysis by high resolution mass spectrometry (HRMS), limiting this analysis to highly specialized laboratories. This presentation describes a rapid solvent free extraction of PBDEs from a small serum sample (0.25 ml), with processing time of 2-3 hours using Twister® stirbar sorptive extraction. Gas chromatographic separation with electron capture negative ion mass selective detection (GC/ MSD-ECNI-SIM) produces quantitative results with detection limits fit for purpose using instrumentation readily available in most analytical laboratories.

Tuesday 2:00 - 3:00 PM
Poster #7 in the Exhibit Hall

ABSOLUTE QUANTIFICATION of CHOLESTEROL HYDROXYLASES in ALZHEIMER'S DISEASE USING 15N-LABELED INTERNAL STANDARDS and MRM MS

Meiyao Wang (meiyaow@umd.edu) -- *Young Investigator Awardee*
IBBR, University of Maryland

We have developed a sample preparation method suitable for quantification of membrane proteins using MRM MS and successfully quantified cholesterol hydroxylases CYPs 27A1 and 46A1 in the temporal lobe of Alzheimer’s disease (AD). By comparing the group of control (Braak II/III) with the groups of patients (Braak IV-VI), elevation of protein CYP27A1 level at different AD stages was observed, supporting altered cholesterol metabolism in AD. No protein level change was observed for CYP46A1. Validation of the observation with a larger sample size and further investigation of post translational modification will be performed.

Tuesday 2:00 - 3:00 PM
Poster #9 in the Exhibit Hall

MALDI-TOF MS for functional detection of ß-lactam resistance of bacteria from positive blood cultures

Markus Kostrzewa (km@bdal.de)
Bruker Daltonik GmbH

Recently, a simple and rapid MALDI-TOF MS based assay for detection of ß-lactam resistance of bacteria was described. In this assay the hydrolysis of antibiotics by ß-lactamases is monitored by mass spectrometry. Here we report its application to bacteria obtained by a rapid isolation procedure from positive blood cultures. The method enables the detection of ß-lactamase activity of bacteria within one to three hours from a positive blood culture and can be performed with standard MALDI-TOF mass spectrometers as they are already used in several hundred microbiology laboratories worldwide, thereby enabling resistance testing as well as ESBL/KPC monitoring.

Tuesday 6:30 - 7:30 PM
Poster #10 in the Exhibit Hall

Sensitive Quantification of Digoxin by LC-MS/MS as Groundwork for the Development of a Clinical Test for an Endogenous ROR-gamma-t Ligand

Steven Truscott (smtrus02@louisville.edu) -- *Young Investigator Awardee*
University of Louisville School of Medicine

Recent publications indicate that digoxin binds to and antagonizes the transcriptional activity of ROR-gamma-t, known to drive development of pathogenic interleukin-17-producing T helper cells (Th17 cells), which induce autoimmune disease. Sensitive and specific quantification of digoxin and endogenous digoxin-like ligands of ROR-gamma-t will likely be critical. Here we report an LC-MS/MS method with increased sensitivity (LLOQ 0.03 ng/mL digoxin in methanol) relative to our current clinical immunoassay (LLOQ 0.3 ng/mL digoxin in serum/plasma). This project is groundbreaking for future efforts to quantify endogenous digoxin-like mammalian cardenolides that are likely physiological ROR-gamma-t ligands that control the development of pathogenic T cells.

Tuesday 2:00 - 3:00 PM
Poster #11 in the Exhibit Hall

MEASUREMENT of ARYLSULFATASE (ASA) ACTIVITY USING NATURAL SUBSTRATE by UPLC-MS/MS in LEUKOCYTES and DRIED BLOOD SPOTS

Junghan Song (songjhcp@snu.ac.kr)
Seoul National University Bundang Hospital

We evaluated the feasibility of UPLC-MS/MS for ASA assay using natural substrate (N-hexadecanoyl-sulfatide) and adopted this revised method for the newborn screening for MLD using DBS specimens. We measured enzyme reaction product (N-hexadecanoyl-galactopsychosine) and its IS (N-hexadecanoyl-D3-glucopsychosine) using UPLC-MS/MS and evaluated the performance of revised methods Substrates, products and IS were easily separated and detected in positive ion mode by the UPLC-MS/MS system with an injection cycle time of 3 min. We found the performance of the revised method to be generally acceptable. This method could be directly applied to DBSs and allows an effective newborn screening test for MLD.

Tuesday 2:00 - 3:00 PM
Poster #13 in the Exhibit Hall

Extraction of Testosterone and Other Endogenous Steroid Hormones from Plasma using Supported Liquid Extraction (SLE) prior to UPLC-MS/MS Analysis.

Adam Senior (adam.senior@biotage.com)
Biotage GB ltd

Steroid hormones are important physiological compounds controlling: inflammation, immune functions, salt and water balance, and development of sexual characteristics. Steroid hormone testing, especially for testosterone, is becoming more common to screen for a variety of clinical conditions, as well as athlete doping. Here we demonstrate a rapid and reliable 96 well Supported Liquid Extraction assay for the extraction of endogenous steroids from human biological fluids. A variety of pH and extraction solvent combinations were investigated. Final extraction conditions were selected based on compound recoveries and extract cleanliness. Full results, discussion and conclusions will be shown in the final poster.

Tuesday 6:30 - 7:30 PM
Poster #14 in the Exhibit Hall

An insight into the frequency and concentration of protein variants in general population

Olgica Trenchevska (olja.trencevska@gmail.com) -- *Young Investigator Awardee*
Institute of Chemistry, Sts.Cyril and Methodius University

Quantification of the individual protein variants is critical to better understanding of their role in pathological processes. Quantitative mass spectrometry immunoassay was used to quantify variants of beta-2-microglobulin, cystatin C, transthyretin and rethinol-binding protein in 500 human plasma samples. A longitudinal protein variants study was also executed on samples from 4 individuals collected on a weekly basis, during a period of 6 months. The results obtained provide a unique insight into the quantitative distribution of protein variants within a population and over time.

Tuesday 2:00 - 3:00 PM
Poster #15 in the Exhibit Hall

Multiplexed LC-MS/MS SRM assay for parathyroid hormone (PTH) and variants: Correlation with current clinical immunoassay methods.

Bryan Krastins (bryan.krastins@thermofisher.com)
Thermo Fisher Scientific - BRIMS Center

The heterogeneity of PTH has traditionally been an impediment to the development of assays that distinguish full length PTH (PTH1-84) from N-terminally truncated PTH. To date, most immunoassays used to monitor PTH levels are based on traditional sandwich ELISA methods and cannot accurately discriminate intact from truncated PTH. We developed multiplexed SRM assays for PTH that allow quantification of four fully-tryptic monitoring peptides and two semi-tryptic variant specific peptides. Comparison of the MSIA–SRM assay with the commercial ELSA assays demonstrated good correlation. However, the data also showed that the commercial immunoassays overestimated the amount of intact PTH as compared with the MSIA-SRM.

Tuesday 2:00 - 3:00 PM
Poster #17 in the Exhibit Hall

A Novel Method for the Extraction of 25-Hydroxy-vitamin D and Analysis using UPLC-MS/MS.

Lee Williams (lee.williams@biotage.com)
Biotage GB limited

For a variety of reasons vitamin D analysis has extremely important clinical relevance. This poster presents a novel high throughput method for the extraction of 25-hydroxy-vitamin D. The method was developed from spiked plasma and serum samples at various levels and subjected to partial validation, investigating calibrated sample performance. Overall method performance demonstrated high analyte recoveries and low ion suppression, allowing limits of quantitation at low ng/mL levels. The analysis of calibrator samples showed good linearity and coefficients of determination and DEQAS supplied samples demonstrated excellent correlation to previously documented values.

Tuesday 6:30 - 7:30 PM
Poster #18 in the Exhibit Hall

Urinary VOC metabolites and blood cyanide in smoke inhalation patients

Alona Umali (alona.umali@dshs.state.tx.us) -- *Young Investigator Awardee*
APHL-CDC/NCEH and Texas Dept of State Health

Smoke inhalation injury, especially when associated with cutaneous burns, causes higher death rates than burn injury alone in fire accident victims. The pathophysiology of smoke inhalation injury has been studied extensively. However, smoke toxins that may exacerbate injury have not been identified. Herein, we explore by LC-MS/MS various VOC metabolites in urine produced from smoke inhalation, to determine which metabolites could potentially indicate morbidity in smoke inhalation injury patients. Results from urinary VOC metabolites analysis and blood cyanide analysis obtained by GC-MS were analyzed by principal component analysis (PCA). Elemental analysis by ICP-MS of urine samples was also done.

Tuesday 2:00 - 3:00 PM
Poster #19 in the Exhibit Hall

Quantitation of Cystine and Identification of Related Metabolites in White Blood Cells Using a High Resolution Accurate Mass LC/MS Approach

Na Pi Parra (na_pi@agilent.com)
Agilent Technologies

High resolution accurate mass (HRAM) LC/MS approach was demonstrated for quantitation and profiling of small molecule metabolites in complex biological samples. Excellent assay performance was achieved in the quantitation of cystine in white blood cells (WBCs) using the ultra-high resolving power and mass accuracy of an accurate-mass Q-TOF LC/MS System. Further, related metabolites were successfully identified and quantitatively profiled. The HRAM LC/MS data acquired in this study can be retrospectively analyzed to search for more metabolites and biomarkers without sample re-injection.

Tuesday 2:00 - 3:00 PM
Poster #21 in the Exhibit Hall

The use of Nitric Oxide as a Radiosensitiser of Hypoxic Prostate Cancer Characterized by Data Independent Label-free Ion Mobility LC-MS

Hans Vissers (hans_vissers@waters.com)
Waters Corporation

Radical prostatectomy and radiotherapy are curative treatment methods for localized prostate cancer whilst the cancer is hormone sensitive. Radiotherapy as treatment of prostate cancer is common but it is also used in combination with hormonal manipulation for advanced cancer. Hypoxia occurs in most solid tumors and an independent prognostic indicator of poor clinical outcome. Hypoxia is known to cause resistance to radiotherapy. NO-NSAID compounds can abrogate hypoxia response and radio-sensitize prostate cancer cells in vitro. The aim of this study was to evaluate protein changes in PC-3 prostate cancer cells following treatment with hypoxia, NO-NSAIDs and radiation.

Tuesday 6:30 - 7:30 PM
Poster #22 in the Exhibit Hall

Low pg/ml Detection of Underivatized 17ß-Estradiol in Serum through Increased Ion Sampling Efficiency Using LC-MS/MS

Andre Szczesniewski (andre_szczesniewski@agilent.com)
Agilent Technologies

Low-level determination of 17ß-estradiol presents several challenges for traditional analysis of the molecule. Due to a lack of highly ionizable functional groups, mass spectrometric approaches have relied on laborious derivatization methods to achieve a sufficient LLOQ. Through the use of dual ion funnel technology, ion sampling efficiency has been improved to the point that underivatized 17ß-estradiol can be quickly and accurately quantified down to 1 pg/ml using an LC/MS approach. Quantitative performance of the presented LC-MS/MS method is comparable to GC-MS/MS which has been recognized as the only methodology capable to achieve these LLOQs with robustness, accuracy and precision.

Tuesday 2:00 - 3:00 PM
Poster #23 in the Exhibit Hall

Method Comparison of Underivatized 1,25-Dihydroxyvitamin D2 and D3 Quantitative Analysis in Serum by LC-MS/MS Utilizing Ion Funnel Technology

Peter Christensen (peter.christensen@agilent.com)
Agilent Technologies

1,25-dihydroxyvitamin D has proven to be a challenging compound to analyze due to the low pg/ml range relevant to clinical research. In order to address this challenge, two sensitive methods have been developed for the quantitation of underivatized 1,25-dihydroxyvitamin D2 and D3 in serum. The first method utilizes lithium adducts for quantitation while the second uses ammonium adducts. Preliminary data shows a greater response and S/N using the lithium adducts, but long-term robustness will be an important factor in the determination of the optimal method.

Tuesday 2:00 - 3:00 PM
Poster #25 in the Exhibit Hall

Extraction of Sub-Nanogram Levels of Catecholamines from Human Plasma Using EVOLUTE® WCX Mixed-Mode Cation Exchange Resin Prior to LC-MS-MS Analysis

Victor Vandell (Victor.Vandell@Biotage.com)
Biotage

Epinephrine, Dopamine and Norepinephrine are typically found in biological matrices at trace levels <50 pg/ml. The extraction and LC-MS analysis of these catecholamines from plasma, serum and urine can be problematic due to analyte instability and matrix interferences. Reliable and automation-compatible sample preparation methodologies are needed to effectively minimize unwanted ionization effects and to concentrate the target analytes prior to LC-MS analysis. This poster demonstrates a rapid and reliable mixed-mode weak cation exchange (WCX) SPE assay for the extraction of Epinephrine, Dopamine and Norepinephrine from plasma at subnanogram per milliliter levels prior to LC-MS/MS analysis.

Tuesday 6:30 - 7:30 PM
Poster #26 in the Exhibit Hall
Serum estrone and estradiol quantitation with a liquid chromatography-tandem mass spectrometry method and automated online sample preparation\r\n\r\n
Xiang He (kevin.he@thermofisher.com)
Thermo Scientific
Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been widely used in clinical research. This study aimed to develop a fast and sensitive LC-MS/MS method to simultaneously measure estrone and estradiol in serum with turbulent flow on line extraction. This method was linear from 3.8 to 1000.9 pg/mL for estrone and 3.7 to 993.1 pg/mL for estradiol. Inter-assay and intra-assay precision at two different concentrations in both spiked charcoal stripped serum and pooled human plasma ranged from 3.5% to 18.0%. In conclusion, this method issimple, sensitive and suitable for serum estrone and estradiol quantitation in clinical research.

Tuesday 2:00 - 3:00 PM
Poster #27 in the Exhibit Hall
The Impact of Preanalytical Variables on Biomarker Research
David Craft (David_Craft@BD.com)
BD Diagnostics
The scientific efforts on biomarker discovery research in the past five years have resulted in numerous potential biomarker candidates. These biomarkers, however, require further investigation by verification and validation in the clinical setting prior to specific application. One major hurdle in the transition from the research lab to the clinical lab is preanalytical variability, most notably, time and temperature, which have significant impact on analyte stability. This poster will present the potential impact of sample handling on protein and peptide stability and how this variability can be controlled through the use of protease inhibitors.

Tuesday 2:00 - 3:00 PM
Poster #29 in the Exhibit Hall
Initial Development of a Method to Measure VX in Human Serum, as either Adducted to Butyrylcholinesterase or as Free Agent
Carter Abney (carter.abney@cdc.hhs.gov)
Centers for Disease Control and Prevention
An existing method for measuring exposure to nerve agents was modified to measure VX in human serum, as either adducted to butyrylcholinesterase or free agent. Antibutyrylcholinesterase monoclonal antibodies were conjugated to protein-G ferromagnetic particles for measuring BuChE in serum. For live agent measurements, these beads were further conjugated with BuChE from unexposed human serum. Aliquots from VX-spiked serum were taken at different time points, digested, and analyzed by LC/MS/MS. Adduction of VX to BuChE was observed to be very rapid, with most occurring within 60 minutes, though unadducted VX was detectable more than 48 hours after spiking.

Tuesday 6:30 - 7:30 PM
Poster #30 in the Exhibit Hall
Comparative Analysis of Serum Samples from Cervical Cancer Patients for Biomarker Discovery using iTRAQ labeling
Natalia Govorukhina (N.Govorukhina@rug.nl)
RUG
We performed a quantitative study of serum samples from 2 cervical cancer patient sets related to the early and late stage of disease versus 2 healthy control groups using chemical stable isotope labeling (iTRAQ® 4-plex). In total approximately 250 proteins were identified according to HUPO criteria that overlapped between all samples. Analytical variability was assessed in replicate analyses per iTRAQ kit. Statistical analysis of analytical and biological variability showed that data from both iTRAQ labeling experiments can be combined.

Tuesday 2:00 - 3:00 PM
Poster #31 in the Exhibit Hall

Advantages of a Two-Pass Workflow for Biomarker Discovery in Plasma or Serum Samples for Clinical Research.

Maryann Vogelsang (maryann.vogelsang@thermofisher.com)
Thermo Fisher Scientific

Sample preparations relying on fractionation to simplify the complexity and large dynamic range of plasma or serum samples do so at a cost that can result in inaccurate or unreliable abundance measurements. Here we describe a simplified approach to biomarker discovery using a Two-Pass workflow that reduces the need for physical sample fractionation. The workflow covers robust, reproducible sample preparation, chromatography and strong informatics-driven data analysis. Our workflow reduces the number of replicates needed. In our hands, the Two-Pass Workflow also provides 20-50% more quantitatively-associated protein identifications than a single pass experiment and at shorter times (2-5X or greater).

Tuesday 2:00 - 3:00 PM
Poster #33 in the Exhibit Hall

Ultra-fast Analysis of Small Molecule Analytes in Urine and Blood-based Matrices Using an SPE/MS/MS System

Vaughn Miller (vaughn.miller@agilent.com)
Agilent Technologies

We evaluated the ability of an ultra-fast SPE/MS/MS to analyze small molecule analytes in human urine or blood-based matrices with much faster sample cycle times and similar analytical results compared to LC/MS/MS assays. Several analyte/matrix pairs were assessed including: benzodiazipines, marijuana metabolite, cocaine metabolite, tacrolimus, levetiracetam and hydroxymidazolam in urine, serum, plasma or whole blood. The SPE/MS/MS methods had comparable linearity, carry-over, accuracy and precision to LC/MS/MS methods. However, the analysis cycle time for SPE/MS/MS was always approximately 10 times faster (9-12 seconds/sample) providing a significant improvement in laboratory throughput and speed to results.

Tuesday 6:30 - 7:30 PM
Poster #34 in the Exhibit Hall

Quantitative Analysis of Free and Total Thyroid Hormones in Serum With and Without Online Sample Cleanup using LC-MS/MS

Rory Doyle (rory_doyle@agilent.com)
Agilent Technologies

Thyroid hormones can be challenging compounds to analyze due to the low levels relevant to clinical research. In order to address this challenge, sensitive liquid chromatography-tandem mass spectrometry methods for the simultaneous analysis of Thyroxine (T4), 3,3’,5-Triiodothyronine (T3) and 3,3’,5’-Triiodothyronine (rT3) in serum samples were developed. Optimal quantitation was achieved using positive electrospray ionization (ESI). The LC system was configured for on-line sample cleanup and results were compared to a standard LC configuration. Excellent linearity and reproducibility was achieved across the entire range of analysis.

Tuesday 2:00 - 3:00 PM
Poster #35 in the Exhibit Hall

Validation of an LC-MS/MS assay for verification of midazolam in pharmacy solutions.

Eric Korman (korman.eric@mayo.edu)
Mayo Clinic

Drug diversion of prescription drugs such as midazolam by hospital staff puts patients at risk and reflects poorly on healthcare institutions. We validated a precise LC-MS/MS assay for verifying pharmacy solution concentrations for routine surveillance and suspected cases of drug diversion. We used serial dilutions of pharmacy solutions into the appropriate linear dynamic range of an Agilent 6410 LC-MS/MS. The assay was linear from 100 to 10000 ng/ml and had inter-day (n = 10) and intra-day (n = 20) CV’s of less than 2%.

Tuesday 2:00 - 3:00 PM
Poster #37 in the Exhibit Hall

Determination of lysophosphatidic acid/lysophosphatidylcholine ratio by LC-MS/MS: potential biomarker for ovarian cancer

Won-Ki Min (heeya1205@paran.com)
Department of Laboratory Medicine, University of Ulsan College of Medicine & Asan Medical Center

Lysophosphatidic acid (LPA) levels in plasma have been identified as potential biomarkers for human diseases such as ovarian cancer, but the results are still controversial. We established a new method that spontaneously measured lysophosphatidylcholine (LPC) and LPA in negative precursor ion scan. Our results revealed that LPA/LPC ratio may represent potential biomarker for ovarian cancer. These are preliminary and are based on a limited study population. Further studies will be required to prove more useful than other biomarker (CA-125).

Tuesday 6:30 - 7:30 PM
Poster #38 in the Exhibit Hall

Characterization of Influenza Vaccine Preparations by Mass Spectrometry: Analysis of Post-Translational Modifications

Jonathan Bundy (jbundy@cdc.gov)
Centers for Disease Control

Influenza hemagglutinin (HA) is the primary regulated antigen used to induce immunity in commercial vaccines. Post-translational modifications of HA (N-linked glycosylation) can affect virulence by interfering with the cell recognition or by masking antigenic regions. Characterization of glycosylation sites and differential glycosylation patterns between strains is useful determining the efficacy of novel virus expression platforms as well as to determine the virulence of particular influenza strains. This presentation will discuss the application of LC-MS/MS approaches for determining glycosylation site occupation and investigating the glycans attached to those sites.

Tuesday 2:00 - 3:00 PM
Poster #39 in the Exhibit Hall

LC-MS/MS validated method for the quantitation of ribavirin in serum.

Darlington Danso (danso.darlington@mayo.edu)
Mayo Clinic, Rochester.

A LC-MS/MS method has been developed and validated for the quantitation of ribavirin in serum. Ribavirin is a nucleoside analog used for the treatment of chronic hepatitis C.This method utilizes 13C5- ribavirin as an internal standard, protein precipitation with acetonitrile and an analytical column Hypercarb designed for retention of polar analytes. The assay was linear from 50 -5000 ng/mL with a correlation coefficient of 0.9998. The intra-day (n=20) and inter-day (n=12) imprecision studies showed CVs of <5% at 200, 750, 2000 and 4000 ng/mL. Isobaric interferences were chromatographically separated from the analyte of interest in both human and bovine serum, and were identified as endogenous nucleosides.

Tuesday 2:00 - 3:00 PM
Poster #41 in the Exhibit Hall

Evaluation of the PLEX-ID and Biothreat Assay Kit for use with Environmental Air Samples

Roberta Housley (rhousley@ibisbio.com)
Ibis Biosciences, An Abbott Company

It is critical that an assay used for biothreat monitoring be capable of detecting threats and, importantly, of not falsely identifying a threat. We will present data demonstrating the capability of the PLEX-ID Biothreat assay to accurately discriminate between target agent and near neighbors. The method identified each organism with which it was challenged and accurately identified threat strains as a threat; strains that are not considered a threat were correctly detected and differentiated. The validation data supports use of the Ibis PLEX-ID and the Biothreat Assay kit for detection of biological warfare agents in complex environmental matrices.

Tuesday 6:30 - 7:30 PM
Poster #42 in the Exhibit Hall
Correlation of Ethyl Glucuronide and Ethyl Sulfate as Bio-markers of Ethanol Use
Amadeo Pesce (apesce@becausepainmatters.com)
Millennium Research Institute
Ethanol use is often monitored by its metabolites, EtG and EtS, which have longer windows of detection than ethanol. However, these two biomarkers do not always correlate. This retrospective study examined 50,890 de-identified urine specimens that were collected between March 2008 and April 2010 from pain patients on opioid therapy and analyzed at Millennium Laboratories with LC-MS/MS. When both analytes are present, alcohol consumption is virtually certain. The presence of only one analyte was associated with concentrations below 5,000ng/mL. Sources for this observation may include microbiological agents, pharmacogenomic variance, non-alcoholic wine, and alcohol-containing hand sanitizers.

Tuesday 2:00 - 3:00 PM
Poster #43 in the Exhibit Hall
Implementation of Busulfan Testing in the Clinical Laboratory
Laura Bechtel (bechtell@mskcc.org) -- *Young Investigator Awardee*
Memorial Sloan Kettering Cancer Center
Implementing testing in a clinical hospital laboratory requires not only validation of busulfan assay and pharmacokinetic modeling software, but also requires monitoring multiple dose kinetics for quality assurance purposes. The analytical methodology uses TurboFlow™ LC-MS/MS. We also evaluated the pharmacokinetic software for calculating AUCs for Dose 1 and Dose 5 using “Trapezoidal Rule” vs an algorithmic one-compartment model with first-order absorption. Our results suggest 1) turbo-flow LC-MS/MS is a robust method; 2) monitoring Dose 1 and Dose 5 AUC determined by trapezoidal rule is a good method for demonstrating validity and of Dose 1 algorithmic AUCs and monitoring quality assessment.

Tuesday 2:00 - 3:00 PM
Poster #45 in the Exhibit Hall
Development of a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Assay for Total Testosterone
Deborah French (deborah.french@ucsf.edu) -- *Young Investigator Awardee*
University of California San Francisco
Background: Our institution currently sends female and pediatric samples for total testosterone testing to a reference laboratory. Objectives: Develop an accurate, sensitive and robust LC-MS/MS assay for total testosterone. Results: Six calibrators were used (0-1000 ng/dL), the concentrations of which were assigned based upon NIST SRM 971. The LOD=1 ng/dL and LOQ=2 ng/dL. Recovery was ~93% and ion suppression was ~5%. Precision studies yielded CVs <15% at LOQ and <10% through the linear range. Epitestosterone and DHEA were baseline separated from testosterone. Conclusions: An accurate, sensitive and robust LC-MS/MS assay for total testosterone was developed. Method comparison and cost-analysis studies are ongoing.

Tuesday 6:30 - 7:30 PM
Poster #46 in the Exhibit Hall
Enhancing trypsin digestion using a novel recombinant Lys-C protease, Arg-C protease and ProteaseMAX surfactant
Sergei Saveliev (sergei.saveliev@promega.com)
Promega Corporation
Owing to robust proteolysis, specificity and size of proteolytic peptides, trypsin is the most popular protease used in protein mass spec sample preparation. However, in some cases proteolysis is incomplete. Protein digestion is compromised if cleavage sites are not accessible due to protein conformation or if cleavage is compromised because of amino acid sequence constraint as well as other reasons. We show here that using a novel recombinant Lys-C protease, Arg-C protease and ProteaseMAX mass spec compatible surfactant the shortcomings associated with trypsin proteolysis are effectively addressed while retaining all the advantages of trypsin digestion.

Tuesday 2:00 - 3:00 PM
Poster #47 in the Exhibit Hall

Evaluation of LC-MS/MS for the measurement of 25-OH vitamin D2/D3 in human serum employing isotopically labeled internal standards

Sail Chun (sailchun@amc.seoul.kr)
Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea

Linearity, precision, detection limit, carryover, and matrix effect of LC-MS/MS were evaluated according to CLSI guidelines. Accuracy was evaluated using SRM 972 from NIST. LC-MS/MS afforded good linearity in 25-OH vitamin D2/D3. Intra/inter-assay CVs were 6.2-9.1% for 25-OH vitamin D2 and 7.1-10.6% for 25-OH vitamin D3. LOD of 25-OH vitamin D2 and 25-OH vitamin D3 were 2.28 and 0.68 ug/L, respectively. Matrix effect was minimal. Results of 25-OH vitamin D2/D3 fell in target ranges of SRM 972 except one level. LC-MS/MS employing isotopically labeled internal standards provides reliable measurements of 25-OH vitamin D2/D3 levels over a broad range of concentrations.

Tuesday 2:00 - 3:00 PM
Poster #49 in the Exhibit Hall

Identifying qualitative and quantitative differences in recombinants and antibody enrichments for IGF1 using high resolution mass spectrometry.

Amol Prakash (amol.prakash@thermofisher.com)
Thermo Scientific

In many targeted analysis studies, key to effective assay development reside with a good quality recombinant protein coupled with a good quality antibody that can be used to enrich the protein of interest from complex mixtures. Unfortunately, subtle differences like mutations, clippages, and modifications within these bring significant differences in efficacy and degradation kinetics . IGF-1 is the dominant effector of growth hormone, thus is extremely relevant in most proteomics and biomarker studies. We analyzed recombinant IGF1 available from 6 vendors, and 5 commercially available antibodies against IGF1, and using high resolution mass spectrometry present an easy workflow to compare protein preparations in recombinants and antibody enrichments.

Tuesday 6:30 - 7:30 PM
Poster #50 in the Exhibit Hall

A Sensitive Method for the Quantification of Estrone and Estradiol in Serum by 2D-LC-MS/MS without Derivatization.

Bruno Casetta (bruno.casetta@absciex.com)
AB Sciex

In recent years there has been a considerable interest in the development of measurements of low serum concentrations of the estrogens estrone and estradiol. For male and especially for postmenopausal and paediatric samples a sensitivity of preferably less than 1 pg/mL is needed, which until now was seldom reached. An ultrasensitive LC-MS/MS method was developed for E2 and E1, which is convenient for large-scale studies and avoids undesirable derivatization steps. The assay relies on a serum extraction followed by direct measurement on 2D-LC-MS/MS. LOQ are below 1 pg/mL for E2 and E1, and recoveries for standard additions are excellent.

Tuesday 2:00 - 3:00 PM
Poster #51 in the Exhibit Hall

A Validated Method for the Quantification of Pyridoxal-5'-Phosphate in Whole Blood by Stable Isotope Dilution LC-ESI-MS/MS.

Bertrand van Zelst (b.vanzelst@erasmusmc.nl)
Erasmus MC, University Medical Center Rotterdam

In this study, a simple and fast LC-ESI-MS/MS method is described for the quantification of pyridoxal-5-phosphate, the biologically active form of vitamin B6. A linear gradient of 0.1% formic acid/methanol was used for separation on a Symmetry C18 column. The method was linear until 8000 nmol/l with an r2>0.999 at an LOQ of 4 nmol/l. Interday and intraday precision were <4.1% and <2.8%, respectively. The mean recovery of 20 different samples was 98%. After thorough examination, no matrix effects were detected. Method-comparison with an existing HPLC-method showed excellent correlation, y=1.11x+4.6, r2=0.94. Method- comparison with the Chromsystems method yielded, y=0.64x+9.7, r2=0.98.

Tuesday 2:00 - 3:00 PM
Poster #53 in the Exhibit Hall

Accurate Mass Analysis of Components Eluting from Dried Blood Spots

Robert Seward (robert.seward@perkinelmer.com)
PerkinElmer Health Sciences

Quantitative assays from the dried blood spot format historically use MRM methods. Other components eluting from the DBS are not detected but may interfere with assays by suppressing ionization of the compounds of interest and have a detrimental effect on the robustness of the assay. A prototype DBS clamping device (Spark Holland) was used to elute components from DBS cards to an SPE trapping cartridge, followed by gradient chromatography to an accurate mass TOF-MS. The profiles of peptides, proteins, lipids and other compound classes from dried blood spots were compared for different elution conditions.

Tuesday 6:30 - 7:30 PM
Poster #54 in the Exhibit Hall

An Herbal Biomarker Panel Using LC-TOF/MS

Tab Toochinda (toochinda.tab@gmail.com)
San Francisco General Hospital

Background: Today over one-third of Americans use herbal supplements. Despite this high prevalence, the FDA does not currently regulate the testing or manufacture of these medications. Because of this, different formulations of the same herb can vary, often widely, among manufacturers. This has important implications with respect to therapeutic dosing, toxicity, and drug-drug interactions. Herein we report on our initial studies into the development and validation of an herbal biomarker panel consisting of twenty commonly used herbal medications, using an Agilent LC-TOF/MS 6230. We subsequently applied this panel towards the analysis of three popular brands of nine supplements.

Tuesday 2:00 - 3:00 PM
Poster #55 in the Exhibit Hall

Rapid Quantitative Analysis of 25-Hydroxy Vitamin D2 and D3 in Plasma with Online Sample Cleanup by LC-MS/MS

Hyun-Jin Jung (hyun-jin_jung@agilent.com)
Agilent Technologies

This study has been developed to minimize sample preparation for the analysis of 25-OH-vitamin D2 and D3 using a triple quadruple mass spectrometer. The goal was to create an efficient, rapid, simple method while avoiding costly reagents and consumables. To achieve this goal, heart-cut column switching was implemented for online sample cleanup. Linearity, matrix effects and reproducibility were analyzed and exceeded required specifications.

Tuesday 2:00 - 3:00 PM
Poster #57 in the Exhibit Hall

A new UPLC-ESI-MS/MS based stable isotope dilution method for the detection and quantification of methotrexate in plasma

Ethan den Boer (e.denboer@erasmusmc.nl) -- *Young Investigator Awardee*
Department of clinical chemistry, Erasmus medical centre

High dose methotrexate is used in the treatment of proliferative diseases such as acute lymphoblastic leukemia. Therapeutic drug monitoring of plasma methotrexate is important to monitor efficacy and adverse events. The analysis consisted of simple sample preparation and fast, 3min. run time. A linear range of 0-50 umol/L was obtained (r2>0.99). A coefficient of variability (CV) of <6% for intraday and <10% was found for interday precision. Average recovery was 99% with a <6% CV. The LLOQ, defined as the lowest concentration where CV<20% and S/N>1:10, was 5 nmol/L. Method comparison with the Abbott AxSym FPIA immunoassay showed excellent agreement (LC-MS/MS=0.98*FPIA-7.3).

Tuesday 6:30 - 7:30 PM
Poster #58 in the Exhibit Hall

Quantification of ethyl glucuronide (ETG) in hair by LC/MS/MS as a marker for chronic excessive alcohol consumption

Stephen Lock (stephen.lock@absciex.com)
ABSCIEX

Ethyl glucuronide (or "EtG") is a metabolite produced when alcohol is in the bloodstream. EtG lasts for up to five days in urine and it's presence and detection in hair now provides a window of detection of up to three months in line with current Society of Hair Testing (SoHT) recommendations. Both the UK and international courts recognise the presence of EtG as a marker of chronic excessive alcohol consumption. In this poster we present a method to detect ETG in hair using LC/MS/MS analysis which has been developed in accordance with UKAS standards.

Tuesday 2:00 - 3:00 PM
Poster #59 in the Exhibit Hall

Determination of Sugars in Tobacco Filler using Liquid Chromatography/Tandem Mass Spectrometry

Lanqing Wang (lfw3@cdc.gov)
CDC

We developed a new method for the simultaneous determination of sugars, alditols, and humectants in tobacco fillers, using liquid chromatography/electrospray ionization tandem mass spectrometry. The amounts of D-fructose, D-glucose, sucrose, D-maltose, D-mannose, D-sorbitol, xylitol, myo-inositol, propylene glycol, glycerol, and triethylene glycol were measured in the tobacco filler from 50 domestic cigarette brands and related variants. The method utilizes UPLC HILIC separation, negative ion electrospray ionization, multiple reactions monitoring detection, and labeled internal standard calibration. The method provided sufficient selectivity and specificity, demonstrated analytical precision and accuracy, and had high sensitivity and a large dynamic range for routine cigarette analysis.

Tuesday 2:00 - 3:00 PM
Poster #61 in the Exhibit Hall

Automated Online Solid Phase Extraction UPLC/MS/MS for Simultaneous Analysis of Cyclosporin A, Tacrolimus, Sirolimus and Everolimus

Martin Eastwood (martin_eastwood@waters.com)
Waters Corporation

Therapeutic drug monitoring of immunosuppressants is an important requirement for the management of transplant patients. To streamline laboratory workflow there is a demand for the simultaneous measurement of multiple analytes. Here we successfully demonstrate the potential of online solid phase extraction (SPE) coupled to UltraPerformance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) for the automated sample preparation and simultaneous analysis of cyclosporin A, tacrolimus, sirolimus and everolimus. This assay displays good linearity, precision and accuracy with minimal ion suppression.

Tuesday 6:30 - 7:30 PM
Poster #62 in the Exhibit Hall

Orbitrap Mass Spectrometry: Ultra-high Resolution for Every Lab

Thomas Moehring (thomas.moehring@thermofisher.com)
Thermo Fisher

The advent of pulsed injection from an external ion storage device has allowed the Orbitrap analyzer to enter mainstream mass spectrometry as a part of a hybrid instrument with additional capabilities such as higher-energy dissociation (HCD), ETD, FAIMS and MALDI ionization. As a result, Orbitrap-based mass spectrometers are emerging as a platform for both ultra-high resolution proteomic applications as well as routine clinical analysis. Ultra-high resolution enables effective separation and unambiguous identification of analytes from chemical noise by utilizing extremely accurate m/z measurement. Examples of the analytical utility of high resolution and high mass accuracy for clinical analytes will be presented.

Tuesday 2:00 - 3:00 PM
Poster #63 in the Exhibit Hall

Analysis of 25-Hydroxy Vitamin-D3 (25OHD3) and of 25-Hydroxy Vitamin-D2 (25OHD2) in Urine using LC-MS/MS

Dean Carlow (carlow@email.chop.edu)
Children's Hospital of Philadelphia

Patients with significant proteinuria represent a unique population with respect to vitamin D status due to the urinary losses of vitamin D-binding protein and albumin. We have developed an assay by HPLC-MS/MS to measure vitamin D in urine. The method involves the addition of internal standards, SPE, RP-HPLC and tandem mass spectrometry. The limit of detection (LOD) for both 25OHD2 and 25OHD3 was 20 pg/mL and the linear range was 20-1500 pg/mL. Recovery and the within-run and between-run precision were excellent. In summary, we have developed a simple and sensitive method to quantify 25OHD2 and 25OHD3 in urine specimens.

Tuesday 2:00 - 3:00 PM
Poster #65 in the Exhibit Hall

Clinical Diagnosis of Erythropoietic Porphyria using Tandem Mass Spectrometry

John Choiniere (john.choiniere.chemistry@gmail.com)
Department of Chemistry, University of Washington

Heme, a necessary component of many biologically important molecules, is naturally synthesized by the body in an eight-step enzymatic pathway. However, genetic mutations are known that can cause deficiencies in these enzymes; in seven of eight cases, deficiency causes one of a set of diseases called the porphyrias. Ferrochelatase, the last enzyme in the pathway, incorporates a ferrous iron ion into protoporphyrin IX to form the complete heme molecule. Deficiency in ferrochelatase causes erythropoietic protoporphyria; here, a clinical assay to detect this deficiency by tandem mass spectrometry, utilizing cobalt and mesoporphyrin IX as enzymatic substrates, is described.

Tuesday 6:30 - 7:30 PM
Poster #66 in the Exhibit Hall

Automation of a Method for the Analysis of a Pain Panel by LC-MS/MS: From Sample to Report

Adrian Taylor (adrian.taylor@absciex.com)
AB SCIEX

In the absence of complete method automation, LC-MS/MS results are susceptible to human error at many different stages, including preparation of calibration standards, sample preparation, and data processing. In this work, an LC-MS/MS method for the analysis of a pain panel comprising 15 analytes has been developed, and automated from start to finish. Calibration standards were automatically prepared using a Tecan Freedom Evo 150 liquid handling system, and all sample preparation was performed on the liquid handling system. The analysis was performed using a 4000 QTRAP® LC/MS/MS system, and data acquisition, processing, and reporting was automated using the Cliquid® software.

Tuesday 2:00 - 3:00 PM
Poster #67 in the Exhibit Hall

Mobile phase effects on peptide electrospray efficiency

Jesse Meyer (jgmeyer@ucsd.edu)
UCSD

Mass spectrometry based protein sequencing has traditionally relied on tryptic peptides. Collision induced dissociation (CID) of non-tryptic digests results in significantly fewer total protein and peptide identifications, when compared to tryptic digests of the same sample. Furthermore, CID of non-tryptic peptides results in an identification bias towards peptides with arginine and lysine in the P2 or P3 position. We report the effects of mobile phase modifiers on precursor ion signal, peptide charge, chromatographic resolution, and the number of peptide identifications.

Tuesday 2:00 - 3:00 PM
Poster #69 in the Exhibit Hall

Isotope-labeled differential profiling of amine-containing metabolites in cultured cells by high resolution mass spectrometry

Lekha Sleno (sleno.lekha@uqam.ca)
UQAM

Differential labeling experiments have previously been employed for relative quantitation experiments in proteomics. This involves isotopically-tagged reagents for the simultaneous analysis of protein samples. A similar strategy is proposed for labeling small molecules for the relative quantitation of metabolites. In order to assess relative metabolite levels, we have designed a strategy for differential analysis based on isotopic labeling of cell cultures using a specific chemical reaction modifying free amine groups. In this study, metabolites were extracted from cells under normal and hypoxic conditions, chemically derivatized with isotopically-coded tags, combined together, and analyzed simultaneously by LC-MS to directly compare relative amounts of these compounds.

Tuesday 6:30 - 7:30 PM
Poster #70 in the Exhibit Hall

Get more trustworthy results for your clients: Monitor the matrix effect in the routine clinical sample

Min Chang (min.s.chang@hotmail.com)
In Transition

A method to monitor LC-MS/MS matrix effect in routine clinical sample is presented. A concentrate analyte surrogate for matrix effect evaluation in 50% isopropranol was infused post-column at a rate of 10-50 µL/minute using the analytical HPLC pump fitted with a capillary flow restrictor to reduce flow variation. The target response of the surrogate should be between 2E4 to 3E5 CPS to avoid suppression of analyte signal but are strong /stable enough to measure suppression/enhancement. The method detect matrix effect from all sources.

Tuesday 2:00 - 3:00 PM
Poster #71 in the Exhibit Hall

Clinical Proteomics to Laboratory Medicine

Song Sang Hoon (cloak21@snu.ac.kr)
Seoul National University Hospital

Proteomics technologies are moving towards gel-free and mass spectrometry-based techniques. In this presentation, I will present some experience of ESI-Q-TOF based clinical proteomics experiments. They are verification of a variant protein, profiling of serum proteins of patients with tuberculosis, and semi-quantitation of red blood cell membrane proteins. After brief introduction of our lab's results, I will discuss the current status and barriers of, and future directions for implementing clinical proteomics technologies in clinical laboratories. As a laboratory physician's view, those points will cover pre-analytical, analytical, and post-analytical phases.

Index

9909 Hibert St. Suite C, San Diego, CA 92131
www.msacl.org