Knowledge Foundation's 7th Annual International Conference

SAMPLE PREP 2013

Sample Preparation for Virus, Toxin & Pathogen Detection & Identification

May 9-10, 2013
San Diego, CA USA

KNOWLEDGE FOUNDATION
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Richard Allen, PhD,
Luminex Corporation

Gregory Auner, PhD,
Wayne State University

Patrick Broyer, PhD,
bioMérieux SA, France

John C. Carrano, PhD,
Paratus Diagnostics, LLC

Mark W. Eshoo. PhD,
Ibis Biosciences Inc., an Abbott Company

Sarah Fakih, PhD,
QIAGEN GmbH, Germany

Shan Gao, PhD,
Wave 80 Biosciences, Inc.

Michael J. Heller, PhD,
University of California San Diego

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University Paris-Sud, France

Martin McDonnell, PhD,
Defense Science and Technology Laboratory - DSTL Porton Down, United Kingdom

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San Diego State University

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McMaster University, Canada

Numrin Thaitrong, PhD,
National Center for Genetic Engineering & Biotechnology / Sandia National Labs

Mark Tondra, PhD,
Diagnostic Biosensors

Season Wong, PhD,
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David Wright,
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Charles Young, PhD,
The Johns Hopkins University

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Coveniently Timed and Co-Located Short Course

SHORT COURSE
May 8, 2013
San Diego, CA USA

Applications of Detection & Estimation Theory

This course will enable you to assess and explain the performance of diagnostic tests, sensors, detectors, or any other type of system that is attempting to give, with some level of confidence, a determination of the presence or absence of a "target." In this case the term "target" may be a wide variety of types (e.g. a biological pathogen or chemical agent; or a physical target of some sort; or even just some electronic signal). We will rigorously cover the theory and mathematics underlying the construction of the "Receiver Operating Characteristic (ROC)" curve, including dichotomous test histograms, false positives, false negatives, sensitivity, specificity, predictive values, likelihood ratios, and total accuracy. An in-class exercise involving constructing a ROC curve from laboratory data is used to round-out the topic.

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Thursday, May 9, 2013

8:00 *Registration, Exhibit Viewing/Poster Setup, Coffee and Pastries*

8:50 **Organizer's Welcome and Opening Remarks**

9:00 **Implications of Next Generation Biosensor Development on Sample Preparation Approaches**

Charles Young, PhD, Principle Professional Staff, Asymetric Operations Dept, The Johns Hopkins University Applied Physics Laboratory; Assistant Research Professor, Dept of Geography and Environmental Engineering, The Johns Hopkins University

Biological sensors represent one facet of a system designed to identify a biological agent. In addition to the sensor, a detection system must account for sample acquisition, sample storage/transport, sample preparation, assay design/performance and data management. Therefore, a systems approach is critical for the development of next generation sensors since changes to one component can impact the entire system. Over the past 5 years, researchers at JHU/APL have been tracking biosensor development and building a database of commercial-off-the-shelf systems and their capabilities. In this presentation we will discuss the database, current sensor trends and their implication for sample preparation.

9:30 **Sample Preparation Approaches for Point of Need Diagnostics**

Richard Allen, PhD, Senior Scientist, Biodefense and Food Safety, Luminex Corporation

Complex biological states such as transmissible infectious disease involve a dynamic relationship between pathogenic organisms and their hosts. Accurate assessment of this relationship presents a unique instrumentation and assay challenge. To provide a more comprehensive snapshot of an evolving infection, efforts at Luminex Corporation include development of an automated, field portable diagnostic system based on the open architecture xMAP technology. A key aspect of this system is the ability to test for both the pathogen itself, through molecular techniques, and also host biomarkers that signal the course of infection via affinity capture reagents. Taken together, these data will yield a more accurate and timely treatment, leading to better clinical outcomes than either test alone. The challenge in developing such a system is to devise sample preparation protocols that 1) are versatile enough to encompass either technique, 2) can be deployed onto an automated, inexpensive disposable cartridge, and 3) are rapid enough to both inform clinical decisions and provide multiple measurements throughout disease progression. Luminex will provide an update on sample preparation techniques being investigated for this effort and provide data from initial testing.

10:00 **Concept for a Specimen Delivery System**

John C. Carrano, PhD, President and CEO, Paratus Diagnostics, LLC

In this paper we posit that in order to realize pragmatic point-of-care medical diagnostic devices capable of meeting the rigorous requirements of CLIA waiver, that one must solve the inherent "impedance mismatch" between the clinical acquisition of a human patient sample and its delivery to the POC Dx system or device. We work from the assumption that specimen acquisition from the patient must follow standard clinical practice, and that the POC system (e.g. "device") will be designed to follow a yet to be determined international interface standard (similar in principle to the now ubiquitous Luer-Lock standard). We will present a design concept for a "specimen delivery system" and the associated proposed interface standard, along with experimental data from preliminary rapid prototype models.

10:30 *Networking Refreshment Break, Exhibit/Poster Viewing*

11:00 **Real Time Metagenomics: From Coral Reefs to the Clinic**

Forest L. Rohwer, PhD, Professor of Biology, San Diego State University

Abstract is not available at time of publishing. Please visit www.KnowledgeFoundation.com for the latest Program updates.

11:30 **A Toolbox for Pathogen Live-Dead Viability PCR**

Sarah Fakih, PhD, Senior Scientist, Food Safety Testing R&D, QIAGEN GmbH, Germany

Nucleic acid detection methods, such as real-time PCR, provide fast and powerful tools to analyze samples for the presence of potentially harmful microbes, but also hold the risk of false positives by detecting nucleic acid from harmless dead cells. We addressed this by a universally applicable tool box for an advanced viability PCR, which centers on the DNA-masking compound Propidium monoazide (PMA), suppressing amplification signals from dead cell DNA. We have pre-developed viability PCR as a complete standardized system to be used with a new illumination device designed to catalyze the PMA reaction. The new tool box system, the illumination device and its function together in viability PCR applications are presented with multiple sets of user data.

12:00 **A Robust and Self-Contained Sample Preparation Cartridge for Point-of-Care Settings**

Season Wong, PhD, Co-Founder and Director, AI Biosciences, Inc.

This presentation will cover the development of AI Biosciences Inc.'s Sample Preparation Cartridge (SPC) which delivers high quality nucleic acids for point-of-care molecular analysis. Our SPC can be operated manually or hands-free using battery pack. The SPC is a closed-system that eliminates cross-

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contamination and provides hassle-free waste disposal. We will demonstrate its utilities using a wide range of samples. The detection of the derived nucleic acid in a non-laboratory setting will also be discussed.

12:30 *Luncheon Sponsored by the Knowledge Foundation Membership Program*

2:00 **Isolation and Detection of cfc-DNA Directly from CLL Patient Blood "Rapid Sample to Sequence"**

Michael J. Heller, PhD, Professor, Depts of Bioengineering and Nanoengineering, University of California San Diego*

We have developed a unique sample to answer dielectrophoretic (DEP) technology for rapid isolation and detection of cancer related cell free circulating DNA (cfc-DNA) biomarkers, bacteria and virus directly form blood and other complex biological samples. Using this DEP microarray device, cfc-DNA from chronic lymphocytic leukemia (CLL) patients could be isolated directly from 50uls of unprocessed whole blood in about 15 minutes. The cfc-DNA was then eluted from the DEP chip, PCR amplified using CLL specific primers and sequenced to determine CLL patient specific polymorphisms and mutations. Overall, the use of DEP devices for the rapid isolation of cfc-DNA from a small volume of blood will allow "noninvasive liquid biopsy" point of care (POC) detection and diagnosis of incipient, residual, and recurrent cancer and other diseases.**In collaboration with: Jennifer Marciniak and Avery Sonnenberg*

2:30 **Approaches to Generic Sample Preparation for the Detection of Biological Agents in the Environment**

Martin McDonnell, PhD, Principal Scientist, Detection Department, Defense Science and Technology Laboratory - DSTL Porton Down, United Kingdom

High confidence detection of potentially hazardous pathogenic micro-organisms and protein toxins in the environment requires effective and rapid sample prep. As the consequences of a positive alert in either military or civilian settings could be severe, it is desirable that monitoring is performed using at least two very different detection technologies, including, for example immunoassay, nucleic acid or mass spectrometry-based methods. To meet this requirement, the sample prep should ideally be generic, with an output sample suitable for a broad range of sensor types. When added to the high variability of environmental backgrounds, including both biological and non-biological materials, and the need to deal with multiple sample inputs including, for example aerosols, soils, vegetation, or waste water, the development of such a system represents a significant technical challenge. In order to perform its required role, the sample prep system may perform sample maceration and agent extraction, selective background and interferent removal, agent clean-up, concentration and lysis for antigen and nucleic acid release. This requires the use of a range of processing technologies which must be capable of integration into a fully automated system capable of being used in the field by non-expert users. This presentation will describe progress in the development of such a generic integrated sample prep system in a project involving Dstl, academia and industry.

3:00 **Rapid Bacterial Sample Preparation from Blood**

Alexis Sauer-Budge, PhD, Senior Research Scientist, Fraunhofer Center for Manufacturing Innovation, Fraunhofer USA

Traditionally, bacterial pathogens in the blood have been identified using culture-based methods that can take several days to obtain results. This can lead to physicians making treatment decisions based on an incomplete diagnosis contributing to patient morbidity. To decrease diagnosis time, we are developing a novel sample preparation device for isolating and concentrating dilute bacteria from blood. The device is designed to be a single-use disposable that can be used manually or with a fully automated instrument.

3:30 *Networking Refreshment Break, Exhibit/Poster Viewing*

4:00 **Rapid Reagentless Identification of Microbial Contamination in Water by Raman Spectroscopy**

Gregory Auner, PhD, Professor, Electrical and Computer Engineering, SSIM Director, Wayne State University

We are developing a reliable and rapid method to assess microbial contamination in water. Raman Spectroscopy is a reagentless, non-destructive, technique that provides a unique spectral fingerprint of bacteria without sample preparation and is conducive to field application since it can provide both qualitative and quantitative analysis. We will present Raman identification of *E. coli, Salmonella, Rahnella aquatilis, Enterobacter, Vibrio fluvialis, Pseudomonas, Bacillus subtilis, Listeria, Staphylococcus,* and *Streptococcus.*

4:30 **Label-Free Electrochemical Aptasensor of Human Cellular Prion Based On MWCNTs Modified with Dendrimers**

Hafsa Korri-Youssoufi, PhD, Researcher, Institut de Chimie Moléculaire et des Matériaux d'Orsay – ICMMO, University Paris-Sud, France

The present work aims to develop an electrochemical aptamer based biosensor able to detect human cellular prions PrPC as a model biomarker of prion disease with high sensitivity. We designed biosensor using multiwalled carbon nanotubes (MWCNTs) modified with polyamidoamine dendrimers (PAMAM) of fourth generation (G4) able by their amino group to attach ferrocenyl redox marker and the DNA aptamer as bioreceptor. MWCNTs, thanks to their nanostructure organization and electrical properties, allow the distribution of aptamer and redox markers over the electrode surface. We demonstrated that the interaction between aptamer and prion protein leads to variation in electrochemical signal of the ferrocenyl group. High sensitivity detection limit of 0.5 pM and

wide linear range of detection from 1 pM to 10 µM has been demonstrated. Detection of PrPC in spiked blood plasma has been achieved and demonstrated a recovery of 85%.

5:00 **No-Spin Sample Preparation for Cartridge-Format Point-of-Care HIV Viral Load Testing**

Shan Gao, PhD, Senior Chemical Engineer, Wave 80 Biosciences, Inc.*

Porous polymer monoliths (PPMs) were developed to prepare nucleic acids from HIV virion-spiked whole blood or plasma samples for quantification of viral RNA using both standard qRT-PCR assays and a novel high-sensitivity bipartite signal amplification assay (BSAA). The efficiency of optimized in-cartridge, glycogen-mediated and monolith-based extraction of viral RNA was approximately 50%. This is comparable to standard centrifuge-based methods involving either phenol-chloroform extraction s or commercial spin columns. The new sample preparation method is particularly well suited for the development of point-of-care HIV viral load testing systems with sophisticated functionality and low cost.**In collaboration with: F.Wang, H.Negussie, J.Frey, A.Arsham, A.Droitcour, L.Mazzola, D.Laser, Wave 80 Biosciences; and A.Fan, C.Klapperich, Boston University*

5:30 **Exhibitor/Sponsor Showcase Presentations**

6:00 *End of Day One*

Friday, May 10, 2013

8:00 *Exhibit/Poster Viewing, Coffee and Pastries*

9:00 **Fluid-on-Board**

David Wright, CEO, Wi Medical Device Development, Inc.

This talk is aimed at the methods of designing a fully integrated self-contained IVD cartridge. The evolving world of complex IVD test cartridges is moving towards a self-contained format, where the fluidic assay is contained, from sample pre to waist containment. The need for this format is based on taking complex assays from the lab into the field, where persons of moderate skills may execute a test with confidence, in a short period of time, at acceptable cost. The world of diagnostics is changing the world of applied therapy, and thusly, the cost of healthcare.

9:30 **Improved Sample Prep and Target Enrichment for the Direct Molecular Detection of Borrelia burgdorferi from Clinical Specimens**

Mark W. Eshoo, PhD, Director of New Technology Development, Ibis Biosciences Inc., an Abbott Company

Early detection and treatment of Lyme disease is crucial to prevent late sequelae and to improve long-term prognosis. However, infection is often difficult to diagnose because of the variability of clinical manifestations and the biologically delayed antibody production upon which current serologic tests are based. Direct molecular tests for early Lyme disease have largely been unsuccessful possibly due to the low levels of circulating pathogen. To address this challenge, we have developed an "ultra-sensitive" molecular assay designed to detect B. burgdorferi directly from whole blood, CSF or other clinical specimens collected as early as the initial patient visit and to detect less than a single genome of Borrelia DNA in a clinical specimen. Furthermore this approach also has the potential to detect Borrelia in patients that have already been treated for Lyme and are sero-positive. To achieve our increased sensitivity we employed a larger than typical volume of samples. Secondly we performed an isothermal pre-amplification of the Borrelia DNA to increase the copies of Borrelia DNA in the sample. Thirdly we detected the enriched Borrelia DNA with a broad-range PCR and electro-spray ionization mass spectrometry assay that targets 8 Borrelia loci each of which is diagnostic for the presence of Borrelia DNA. We compared results of our assay with 2-tiered serology paired specimens collected from 21 endemic-area patients with both clinically and serologically defined early Lyme disease. Results of this study demonstrated the ability to detect B. burgdorferi in early Lyme disease directly from whole blood specimens prior to seroconversion.

10:00 **MEMS – Microfluidics Integration Standards and Design; Examples in Magnetic Bead Sample Concentration and Detection**

Mark Tondra, PhD, President, Diagnostic Biosensors

Commercialization of Point of Care (POC) and integrated Lab on a Chip (LOC) products is advancing. One enabling factor is industry standards for microfluidic interfaces between two devices, like a detector chip and a concentrator chip. These standards facilitate the design of higher-level micro-scale systems using plug-and-play architectures. The state-of the art in MEMS-Microfluidics industry standards will be presented. Our own use of these standards in design of multi-tube fluidics interfaces in our biosensor products will be shown, as will applications of devices using magnetic microbeads for sample concentration and manipulation in microfluidic LOC systems.

10:30 *Networking Refreshment Break, Exhibit/Poster Viewing*

11:00 **Fully Automated Sample Preparation for Rapid Pathogens Identification and Resistance Prediction by LC-ESI MS/MS Analysis**

Patrick Broyer, PhD, Senior Scientist, bioMérieux SA, France

This talk will present a new automated sample prep solution for extraction of proteins and peptides dedicated to LC-ESI-MS analysis. Protocol simplifications, new filtration device for urine and blood & automation of peptide digestion will be described

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to show the ability of this solution to move to a long 18h protocol to a short 1h fully automated protocol. Results will be showed demonstrating the capabilities to correctly identify resistant microorganisms from positive blood culture. The sample preparation automated prototype is already used in our laboratory providing within 1 hour peptides solution ready to be analyzed by LC-ESI-MS by batches of 30 samples per run.

11:30 **Development of a Novel Membrane-Based System for Fractionation and Concentration of Biological Particles from Complex Environmental Matrices**

Andy Page, President & CTO, InnovaPrep LLC

InnovaPrep LLC will present an update on development of a novel, integratable, membrane-based fractionation and concentration system, under a current Department of Homeland Security Phase II SBIR. Powerful, automated sample preparation techniques are needed for improved detection and identification of rare biological particles in complex environmental samples by autonomous biodetection systems. A novel cartridge containing a series of sharp-cut membrane filters in order of decreasing pore size, along with integrated pneumatic valves and fluid paths, are used to rapidly separate particles present in up to 20 mL of environmental sample by size into separate fractions containing large environmental particles, bacteria, viruses and DNA, and proteins. Each captured fraction is then eluted into separate concentrated volumes of less than 200 uL. Program goals, hurdles, and performance data will be presented.

12:00 **Automated Sample Preparation and Analysis for Next-Generation Sequencing**

Numrin Thaitrong, PhD, Researcher, National Center for Genetic Engineering and Biotechnology / Sandia National Labs

Thorough characterization of next-generation sequencing library is a required process in the sample preparation workflow. However, this built-in quality control feature is currently not available for small-scale library construction platforms. We have developed an integrated droplet-based digital microfluidics (DMF) architecture with capillary interface to handle sub-microliter sample processing and analysis. The platform providing size distribution and quantization of the library can be coupled to the existing modular DMF "hub" to automate start-to-finish library construction.

12:30 *Lunch on Your Own*

2:00 **Improving Clinical Sample Collection, Transport and Preparation**

Marek Smieja, MD, PhD, Associate Professor, Dept of Pathology and Molecular Medicine, McMaster University, Canada*

The development of point of care diagnostic tests for common infectious diseases needs parallel improvements in the ease of collection of clinical samples, and appropriate transport media for in-tube sample stabilization and/or extraction. We studied the effectiveness and reproducibility of Copan's FLOQSwabs™ for sample collection for respiratory, gastrointestinal, and genitourinary diseases. We determined the feasibility and performance characteristics of self-collected, less-invasive diagnostic samples; evaluated the robustness of transport media (dry swabs, UTM, eNAT, ESwab, MSwab, Cymol) under differing temperature and time conditions; and determined the performance characteristics of various media for antigen-based and molecular diagnostic tests.**In collaboration with: Copan Diagnostics*

2:30 **Integrated PureLyse® Sample Preparation**

Bruce Irvine, CTO, Claremont BioSolutions LLC

Claremont BioSolutions has created a novel method of rapid sample preparation that is entirely disposable. It provides mechanical cell lysis and simultaneously extracts nucleic acids or proteins. Nucleic acid can be extracted in three to five minutes with two to three steps. The disposable PureLyse® device is comprised of a micro-motor and vane that agitate particles at unusually high shear forces. As cells are lysed, nucleic acid binds to the lysing particles. This system delivers high yield RNA preparation as well. This system is also used to very effectively homogenize tissue. The miniature disposable nature of this flow-through cartridge lends itself to integration. Our flagship approach to integration is the OmniValve™ fluidic system, where we have embedded the PureLyse® chamber within a valve with up to six ports connecting the lysis and extraction cartridge to other chambers. This facilitates integration of DNA extraction with sample introduction, pre-filters, wash, elution, and amplification. This approach was very successfully applied to an NIH funded SBIR project developing a semi-integrated system for detecting Clostridium difficile, resulting in 100 % specificity and 96% sensitivity ascompared to standard enzyme immunoassay of stool samples.

3:00 **Stabilization of Gene Expression in Whole Blood: High Quality RNA with Room Temperature Handling of Human Blood Samples**

Vasco Liberal, PhD, Scientist, Biomatrica, Inc.

Expression profiles from blood samples are increasingly used to diagnose, monitor and treat diseases, requiring reliable RNA preservation during sample collection, transport and storage. Transcription profiles can change rapidly, potentially dictating inadequate treatment. Biomatrica has developed a blood collection device that stabilizes RNA in blood cells during ambient temperature sample handling and storage. With its coupled RNA purification kit, it provides a complete solution from blood collection to RNA purification, achieving high yields and great RNA quality, with unaltered gene expression for 14 days of room temperature storage, which can prove of extreme value for better patient treatment.

3:30 **Exhibitors and Sponsors Showcase Presentations / Selected Oral Poster Highlights**

4:00 *Concluding Remarks, End of Conference*

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