19th Knowledge Foundation International Detection Technologies Conference Series

Integrating Sample Preparation

of Chemical & Biological Agents, Threats & Pathogens into Detection, Identification & Analysis Technologies & Devices

KNOWLEDGE FOUNDATION
TECHNOLOGY COMMERCIALIZATION ALLIANCE

December 8-9, 2011
Washington, DC USA

Distinguished Faculty:

David S. Alburty,
InnovaPrep LLC

Michael Connolly, PhD,
Integrated Nano-Technologies, LLC

Sandra M. Da Silva, PhD,
National Institute of Standards and Technology

Tony Chen,
Taigen Bioscience Corporation, Taiwan

Claudia Gärtner, PhD,
microfluidic ChipShop GmbH, Germany

Vincent Gau, PhD,
Genefluidics

Darren S. Gray, PhD,
FLIR

Yousef Haj-Ahmad, PhD,
Norgen Biotek Corp., Canada

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

Ramzi Joseph Nasr,
Deton Corp.

R. Andrew McGill, PhD,
Naval Research Laboratory

Nathan Olson,
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Omar A. Oyarzabal, PhD,
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Ken Owen,
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Kamlesh Patel, PhD,
Sandia National Laboratories

Scott Perschke,
PathSensors, Inc.

Bradford Powell, PhD,
Cernomics Solutions, representing Pressure BioSciences, Inc.

Kurt E. Schaecher, PhD,
US Army

Annie Schnyder, PhD,
Chemspeed Technologies AG, Switzerland

Maxim Shusteff,
Lawrence Livermore National Laboratory

Nickolaj F. Starodub, DrSci,
National University of Life and Environmental Sciences, Ukraine

Carl P. Tripp, PhD,
University of Maine & OSS, Inc.

Peter J. White, PhD,
Defense Science and Technology Laboratory (DSTL) Porton Down, United Kingdom

Season Wong, PhD,
Lynntech, Inc.

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COMPREHENSIVE DOCUMENTATION AVAILABLE

Nothing can substitute the benefits derived from attending **Integrating Sample Preparation 2011**. But if your schedule prevents you from attending, this invaluable resource is available to you. Please allow 2-3 weeks after the conference date for delivery. *Note: Documentation is included with conference fee for registered delegates*

Integrating Sample Preparation

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CONFERENCE AGENDA

Thursday, December 8, 2011

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

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

9:00 **Ignoring the Detector: Fast, Modular, Agnostic Sample Preparation**

Maxim Shusteff, Center for Meso-, Micro- and Nano-Technologies, Lawrence Livermore National Laboratory

Lawrence Livermore National Lab has demonstrated a series of microfluidic devices to separate broad classes of particles from complex samples. We use acoustic focusing to extract large cells, dielectrophoresis (DEP) to filter bacteria, isotachophoresis (ITP) to concentrate and extract nucleic acids, and thermal on-chip lysis to release cell contents. The design is modular such that the devices can be configured to suit many types of input samples and a variety of downstream detection methods.

9:30 **Complete In-Cartridge Sample Preparation for Rapid Antimicrobial Susceptibility Testing**

Vincent Gau, PhD, CEO, President, CTO and Co-Founder, Genefluidics

An integrated diagnostic compact system to enable clinicians to direct point-of-care (POC), evidence-based selection of antibiotics for treatment of acute bacterial infections is presented to include sample/reagent delivery, mixing, lysing, 37°C incubation, stringency washing and electrochemical detection. The feasibility, accuracy and reproducibility of cartridgebased rapid antimicrobial susceptibility testing (AST) are demonstrated on raw urine samples. *Escherichia coli* spiked in urine and *Staphylococcus epidermidis* spiked in whole blood are loaded into cartridges under different antibiotics conditions. The AST culture time inside the cartridge can be as short as 75 minutes followed by the 30-min pathogen ID assay. All fluidic controls are automated by the reader/manifold system with a built-in multi-channel potentiostat.

10:00 **Integrated Lab-On-A-Chip for 8-Plex Pathogen Detection**

Claudia Gärtner, PhD, CEO, microfluidic ChipShop GmbH, Germany

We present the development of an 8-plex pathogen detection lab-on-a-chip system which integrates all steps from sample input to pathogen identification on a single microfluidic cartridge. It combines sample prep, amplification and on-chip reagent storage. The individual microfluidic functionalities can be combined for a wide range of applications.

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

11:00 **Seamless Sample to Answer BioAnalytical and Diagnostic Systems**

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) device for *in-situ* isolation, processing and identification of disease biomarkers (cfc-DNA), cancer cells, bacteria and virus from complex samples (blood, etc.). For example, the device allows specific bacteria to be isolated, cell lysis and DNA extraction carried out, followed by PCR or other detection/analyses in same device chamber. Complex sample preparation for DNA sequencing applications can also be carried out. **In collaboration with: Avery Sonnenberg, UCSD*

11:30 **Field Laboratory Analysis, Perspectives from a Soldier Scientist**

Kurt E. Schaecher, PhD, Major, US Army

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

12:00 **Universal Sample Preparation with Target Enrichment**

Darren S. Gray, PhD, Principal Investigator, Chem-Bio Detection, FLIR

FLIR's universal sample preparation platform rapidly isolates nucleic acids and proteins from multiple input types without sample splitting. Threat levels are stratified by analyzing intact threats separately from free biomolecules. High sensitivity is achieved by concentrating target nucleic acids from pathogens, while excluding irrelevant nucleic acids from humans, plants, etc. Superior inhibitor removal, and options to collect viable cells or HMW nucleic acids, enables integration with a range of assays, from PCR and immunoassays to mass spec and NextGen sequencing.

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

2:00 **Practical Developments in Surface Sampling and Detection**

Peter J. White, PhD, Detection Department, Defense Science and Technology Laboratory (DSTL) Porton Down, United Kingdom

Surface sampling for hazardous material in deliberately contaminated environments presents many technical challenges to users at the frontline. This talk will highlight those challenges, from an end user perspective, and introduce two novel sampling technologies, developed at DSTL, that aim to provide simple cost effective practical solutions. First, the development of an ergonomic integrated sampling and detection device that uses lateral flow technology and secondly the verification of a sampling technique that uses soluble adhesive films for the efficient recovery of biological material from a range of surface types.

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2:30 **New Selective Vaporization Based Sample Preparation and Detection Method**

R. Andrew McGill, PhD, Section Head, Materials & Sensors, Naval Research Laboratory

We have developed a new approach which can selectively vaporize low vapor pressure chemicals present on surfaces to be immediately detected by a detector. We utilize a miniature quantum cascade laser (QCL) to direct infrared light of a particular wavelength to selectively couple light and heat the chemical on a surface. This raises the vapor pressure substantially with a low power small sized add on unit to the detector.

3:00 **Infrared Amenable Sampling Methods for Water and Surface Based Agent Detection**

Carl P. Tripp, PhD, President, OSS Inc, and Professor of Chemistry, University of Maine

This talk will discuss new sampling methods that are best described as solid phase infrared amenable extraction processes that enable detection of the concentrated agents via FTIR analysis. Traditional (non-infrared amenable) solid phase extraction methods are widely used for pre-concentrating agents from water, which are subsequently eluted with a reagent/solvent and injected into a detector. In our approach, both collection and detection are performed directly on the same device, thereby eliminating the need for eluting reagents and sample processing steps. Once fully extracted, the concentrated agent is simply detected via infrared spectral analysis directly through the device itself with no further sample handling.

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

4:00 **Parameters Affecting Bacterial Collection and Their Impact on Downstream Analysis**

Sandra M. Da Silva, PhD, Research Chemist, Biochemical Science Division, National Institute of Standards and Technology*

Reliable and precise methods for detection, quantification and decontamination of biological threats deposited on surfaces are fundamental to public health. A comprehensive review of surface sampling literature has demonstrated that surface sampling efficiency is impacted by numerous experimental parameters including variability in extraction methods and deposition techniques. In the current work, the effect of experimental conditions on the recovery of spores, gram negative and gram positive bacterial cells was investigated in order to optimize and better understand sources of variability in biological surface sampling performance. **In collaboration with: Autumn S. Downey, Nathanael Olson and Jayne B. Morrow, NIST*

4:30 **Integratable Bioparticle Concentrator for Autonomous Systems**

David S. Alburty, CEO, InnovaPrep LLC

InnovaPrep has introduced the first fully automated, efficient and robust sample prep instrument built for ease of integration into sample collection/detection systems. The InnovaPrep technology concentrates dilute particles in a liquid. Nowhere is this more important than for the preparation of biological samples for trace analysis. The system separates the particles from the fluid, and then delivers them in a highly concentrated form in a user-selected final fluid and volume format. The process is highly efficient and effective over a wide range of particle sizes from 5 nanometers to 20 microns. The InnovaPrep instruments use a concentration cell containing a hydrophilic hollow fiber membrane filters to capture biological agents and other particles from a relatively large volume of liquid. After collection, the particles are efficiently captured into volumes as small as 40 µL using a proprietary foam extraction method. Two InnovaPrep concentrators were used to process samples extracted from environmental filters, to determine the longevity and consistency of concentration cells scaled for the BioWatch Gen3 program. Membrane deterioration could be readily determined by monitoring base performance with time.

5:00 **An Easy-to-Use, Hand-Held, Self-Contained Sample Preparation Module for Low-Resource Settings**

Season Wong, PhD, Senior Research Scientist, Lynntech, Inc.

This presentation will cover the development of Lynntech's Sample Preparation Module (SPM) which delivers high quality nucleic acid for molecular analysis. Our handheld SPM is powered by AA batteries or can be operated in a battery-less manual mode. The SPM is a closed-system that prevents cross-contamination. We will demonstrate its utilities using a range of samples. Rapid detection of nucleic acid in a non-laboratory setting will also be discussed.

5:30 **Selected Oral Poster Highlights – I**

5:45 *Concluding Discussion, End of Day One*

Friday, December 9, 2011

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

9:00 **Automating Molecular Biology for NGS Library Preparation**

Kamlesh Patel, PhD, Senior Member of the Technical Staff, Microfluidics Department, Sandia National Laboratories

DNA sample preparation for next generation sequencers still relies on slow, labor-intensive steps. We have developed a digital microfluidic (DMF) hub that interfaces multiple discrete sample processing modules to automate clinically-derived DNA library preparation. We will present our progress in integrating multi-step workflows to maximize the sensitivity of state-of-the-art NGS for unknown pathogen detection by enriching informative nucleic acid sequences (from the pathogen) and suppressing background DNA (from the host).

9:30 **Standardizing and Accelerating Analytical Sample Preparation - Flexible, Easy, and Rugged**

Annie Schnyder, PhD, Director HT Experimentation, and Michael Schneider, PhD, Senior VP Business Development, Chemspeed Technologies AG, Switzerland

The need for increased efficiency in the laboratory has kick-started

the development of innovative equipment for workflow automation. For this reason instruments are being used for sequential / parallel, unattended Sample Preparation, Pretreatment, Digestion and Analysis. Multiple dispensing of solid and liquid samples, sample treatment, work-up steps, centrifugation & SPE purification are only some of the workflow steps. Using a selection of case studies, this presentation shows how a variety of challenging parallel sample preparation workflows have been fully automated.

10:00 **Closing the Loop for On-Site Detection and High-Throughput Testing**

Ken Owen, CEO, Ag-Defense Systems

Automated, portable, real-time sample prep is the final necessary step for having on-site detection and closed-loop analytics and controls. This technology creates the critical data point needed for earliest detection and on-going assurance anywhere and all along the supply chain. This technology also relieves the primary bottleneck for next-generation hi-throughput testing facilities. We will present the developing architecture and technology that makes this work.

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

11:00 **An Integrated System for Sample Processing and Diagnostics for Field Applications**

Michael Connolly, PhD, President, Integrated Nano-Technologies, LLC

Integrated Nano-Technologies has now successfully integrated sample processing and diagnostics into a field-able system for rapid testing. The system is being used to detect pathogens in patient samples, insect vectors, and complex environmental samples. Currently the system is identifying nucleic acid sequences, but is being modified to also carry out immunodiagnostics.

11:30 **Sample Preparation in Resource-Limited Areas**

Yousef Haj-Ahmad, PhD, Professor, Founder, Norgen Biotek Corp., Canada

Current nucleic acid-based assays have limited use in resource-limited areas due to complexity and cost. With most research focused on amplification and detection, sample preparation remains a bottleneck in the development of point-of-care diagnostics. Norgen has developed a sample preparation method based on its technology which allows for the isolation of high quality analytes from biological samples and is well suited to nucleic acid-based assays in resource-limited settings.

12:00 **Pressure Cycling Technology (PCT) for Improved Preparation and Analysis of Biothreat Agents and Infectious Microbial Samples**

Bradford Powell, PhD, PMP, Founder & CSO, Cernomics Solutions, representing Pressure BioSciences, Inc.*

Biological threat agents and other infectious microbial samples present multiple challenges to maintaining safety during handling, preparation and analysis. The use of conventional methods to homogenize and extract dangerous bacteria, virus, and invertebrate vectors can create deadly aerosols. Unfortunately, traditional pre-treatment methods for biological inactivation (e.g., autoclave, ionizing radiation, oxidiziders) modify or destroy the biological molecules intended for analysis. Popular extraction procedures that isolate analytes by molecular class further diminish supply, which can be limited, and often confound strategies for multiplexing. Finally, the design of integrated test capabilities for use outside of the laboratory is complicated by *bona fide* specimen matrix issues (host tissues, soil substrate) and the lack of infrastructure (containment equipment, electricity, cold storage), which are heightened by the infectious nature of the samples. Aiming for safety, simplicity, quality, and robust fieldability, we have developed and demonstrated a novel pressure cycling technology (PCT)-based platform for simultaneous inactivation and preparation of dangerous biological samples, including arthropod-borne bacteria and viruses, and bacterial spores. Discussion will include background descriptions of the technology gap and proposed solution, with preliminary results for safe and convenient inactivation, extraction, and assay of protein and nucleic acid markers. **In collaboration with: Nathan Lawrence, Pressure BioSciences, Inc.*

12:30 *Lunch on Your Own*

2:00 **Improving the Methodologies for Identification of *Campylobacter spp.* from Foods by Molecular Techniques**

Omar A. Oyarzabal, PhD, Associate Professor of Microbiology, Dept of Biological Sciences, Alabama State University

Campylobacter spp. are bacterial foodborne pathogens of major importance in public health. These bacteria are in low numbers in retail broiler meat and the enrichment of the samples is necessary for isolation and further identification. This presentation will summarize our work for several years to improve and simplify the isolation of these bacteria from food samples. Different molecular techniques to detect *Campylobacter spp.* will also be reviewed.

2:30 **Evaluation of DNA Extraction Efficiency for a Suite of Organisms Using 5 Different Extraction Methods**

Nathan Olson, Multiplexed Biomolecular Science Group, Biochemical Science Division, National Institute of Standards and Technology

Identifying an optimal method for DNA extraction of a specific sample type is challenging due to the breadth of available methods and limited guidance on characterizing performance. We processed five organisms including gram positive and negative, spores, and yeast with five different extraction methods. Extraction efficiency was evaluated based on lysis (cell counts), DNA purity (UV abs, qPCR inhibition), DNA fragmentation (gel electrophoresis), and DNA yield (Abs 260nm, fluorometry).

3:00 **Aerosol or Liquid Collection and Detection in Minutes with BioFlash**

Scott Perschke, Chief Scientific Officer, PathSensors, Inc.

The BioFlash-E® collects and analyzes air samples for the presence of up to 16 different biological agents simultaneously, with a time to

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result of less than 5 minutes. Liquid samples can also be analyzed with similar throughput. CANARY® biosensors which are incorporated in the platform provide high sensitivity and specificity to the detection of toxins and pathogens.

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

4:00 **Cough Analyzer of Airborne Bacteria for Lung Infection Diagnosis**

Ramzi Joseph Nasr, Chief Technical Officer, Deton Corp.

Multiple diagnostics for lung infections rely on sputum samples. Sputum samples are difficult to collect and are contaminated by saliva, lowering their quality. With Deton's device, a patient wears a disposable mask and coughs naturally into a novel impactor that breaks up the cough droplets in air and collects their DNA. The device serves as a point-of-care diagnostic or SSM replacement with an estimated available yearly market of 280 million tests.

4:30 **Time Automation for DNA / RNA Purificatoin**

Tony Chen, Taigen Bioscience Corporation, Taiwan

LabTurbo 48 Compact System, by using membrane column vacuum technology, can fully automate DNA/RNA extraction up to 48 samples in 90 minutes from raw sample to nucleic acid elution in unibody (WxDxH) 66 x 64 x 160 cm; the sample size are loaded up to 5 ml for cell-free fluids (serum/plasma) or up to 1 ml for whole blood, or up to 0.5 ml buffy coat; the elution volumes are collected as low as 60 µl; the performances are excellent on recovery, purity, sensitivity, and cross contamination free.

5:00 **Sample Preparation and Instrumental Analytical Approaches for the Express Revealing of Bacterial Contamination of Environmental Objects**

Nickolaj F. Starodub, DrSci, Professor, National University of Life and Environmental Sciences, Ukraine*

We analyze the results of application of optical immune biosensors based on SPR (stationary and portable versions of the device) and total internal reflection ellipsometry (TIRE) as well as electrochemical ones based on the IsFETs (in particular, with cerium oxide gate for the increasing stability analytical chip) and planar electrodes with amperometrical registration of generated signal. Analytical procedures for sample preparation based on the preliminary immune affine chromatography gives possibility to increase sensitivity of analysis up to one order, transducer surface preparation, algorithm of measurements, sensitivity of the analyte determination, reusability and others issues will be discussed in detail. Combination of the above mentioned procedures allows completing the response to all practical requirements with respect to sensitivity and express revealing bacteria in the contaminated water and food. **In collaboration with: Ju.A.Ogorodniichuk, I.M.Kushnir, I.Ja.Kots'umbas*

5:30 **Exhibitor Showcase Presentations & Selected Oral Poster Highlights – II**

5:45 *Concluding Remarks, End of Conference*

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