18th International Conference

Biodetection Technologies 2011

Technological Advances in Detection & Identification of Biological Threats

June 23-24, 2011 • Washington, DC USA

Distinguished Faculty:

David Almassian,
Tetracore, Inc.

Amy L. Altman,
Luminex Corporation

Marie Binet,
EDF, France

Amos Danielli,
Washington University in St. Louis

Peter Emanuel,
US Army

Matthias Frank,
Lawrence Livermore National Laboratory

Julie Fruetel,
Sandia National Laboratories

Andrei Gindilis,
SHARP Corporation

Robert Haushalter,
Parallel Synthesis Technologies

Ryan Kim,
National Center for Genome Resources

Steve Jackinsky,
Wi, Inc.

Kiyoshi Nokihara,
HiPep Laboratories, Japan

Natasha Paul,
TriLink BioTechnologies

Dinakar Ramadurai,
Episensors, Inc.

Ze'ev Russak,
Azure PCR Limited, United Kingdom/Israel

Robert F. Standaert,
Oak Ridge National Laboratory

Felix von Stetten,
University of Freiburg, Germany

Yi-Wei Tang,
Vanderbilt University Medical Center

Willy A. Valdivia-Granda,
Orion Integrated Biosciences Inc.

Francisco Veas,
University Montpellier 1, France

Reinhold Wimberger-Friedl,
Philips Corporate Technologies, The Netherlands

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18th International Conference

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Conference Agenda

Thursday, June 23, 2011

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

9:20 **Organizer's Welcome and Opening Remarks**

9:30 **Rapid Detection and Identification of Biological Aerosols and Other Threat Materials with Single-Particle Aerosol Mass Spectrometry (SPAMS)**

Matthias Frank, PhD, Advanced Diagnostics and Instrumentation Group Leader, Lawrence Livermore National Laboratory*

At LLNL, we have developed a single-particle aerosol mass spectrometry (SPAMS) system that can rapidly analyze individual micrometer-sized biological aerosol particles or cells that are sampled directly from air or a lab-generated aerosol into a mass spectrometer. As particles enter the SPAMS system, their aerodynamic size and fluorescence properties are measured before mass spectra from both positive and negative ions created by matrix-free laser desorption and ionization are recorded. All the correlated data obtained from a particle can be analyzed and classified in real-time. Our present system is capable of discriminating, particle-by-particle, between bacterial spores, vegetative cells and other biological and non-biological background materials using the mass fingerprints obtained from those particles. In addition, selected species of bacteria can be discriminated from each other with this method. Here we will describe the most recent improvements in this system and the related algorithms. We will present the results from applying this technique to the analysis of biological agent simulants and aerosol background studies in large public facilities. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. **In collaboration with: K.Adams, K.Coffee, G.Farquar, E.Gard, A.M.Williams, V.Riot, P.Steele, B.Woods, LLNL; M.Bogan, Stanford Linear Accelerator Center; D.P.Fergenson, Livermore Instruments Inc.; H.Tobias, Cornell University*

10:00 **A Novel Biochip System Focusing on Protein Detection by the Use of Protein Finger Prints Generated by Arrays Consisting of Designed Peptide and Glycopeptide Libraries**

Kiyoshi Nokihara, DPharmSci, CEO and CSO, HiPep Laboratories, Japan

The major goal of biochips is on-site and real time detection. Focusing on biomedical applications we have proposed a novel biochip-concept for a protein detection system involving labeled structured peptides as capture molecules, which have been generated by peptide engineering, and the 'protein-fingerprint' method, which affords a barcode-like visualization, while the protein-protein interaction can be mimicked by a protein-peptide interaction. Three important factors for peptide micro-arrays have been constructed.

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

11:00 **A Functional Blister-Pack LabDisk System for Rapid Field Testing of Biological Threats**

Felix von Stetten, PhD, Laboratory for MEMS Applications, Dept of Microsystems Engineering (IMTEK), University of Freiburg, Germany*

In case of a pathogenic threat, fast and automated field test systems are required. We present one candidate for a mobile nucleic acid and immunoassay based detection unit. Centerpiece of the detection system is a disposable LabDisk, fabricated as functional blister package, in which reagents are pre-stored and fluidic structures are integrated to perform all required liquid handling operations automatically in a centrifugal processing device. Based on this system, assays for the detection of bacterial pathogens such as *Bacillus anthracis* and *Yersinia pestis* and toxins such as ricin and botulinum toxin are being developed and field-tested. **In collaboration with: D.Mark, R.Zengerle, HSG-IMIT/IMTEK; T. van Oordt, HSG-IMIT; O.Strohmeier, IMTEK; D.Kosse, HSG-IMIT; J.Drexler, M.Eberhard, Qiagen Lake Constance GmbH*

11:30 **Meeting User Needs: Developing Field-Deployable Biodetection Systems Using a Microseparations Approach**

Julie Fruetel, PhD, Principal Member of the Technical Staff, Sandia National Laboratories

The challenges of developing field-deployable systems for biothreat identification are significant: not only must requirements typical of lab diagnostic systems be met - e.g., high sensitivity and specificity, ability to handle complex biological samples - but such an instrument must also be portable, low power, utilize stable reagents, and automate processing steps for autonomous operation or an untrained user. Sandia National Laboratories has developed a modular, automated biodetection capability that couples robust microscale sample prep with microseparations-based analysis for the detection of bacteria, viruses and toxins. This talk will describe the development of portable systems for environmental detection and other bioanalytical applications.

12:00 **Fully Automated System for the Multiplex Detection of Biothreat Agents and Emerging Infectious Diseases**

Amy L. Altman, PhD, Vice President, Luminex Corporation

Open-architecture xMAP® technology allows simultaneous detection of bacterial, viral and toxin agents, in a highly flexible, multiplexed architecture capable of protein and nucleic acid assay formats. In collaboration with Advanced Liquid Logic (ALL), Luminex is developing a rapid, automated system for multiplex detection of biological agents. The ALL technology, based on digital microfluidics (DMF), provides an ideal platform for sample and assay processing prior to detection on a LED-based Luminex analyzer. The integration of DMF and microsphere-based multiplex testing will have a profound impact on detection assays for biothreat agents and emerging infectious disease applications, where deployable, highly-

18th International Conference

Biodetection Technologies 2011

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Conference Agenda

automated instruments are required.

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

2:00 **Automated Sample Preparation for Highly Sensitive and Rapid Detection of Genomic Samples Using Next Generation Sequencing**

Ryan Kim, PhD, Director of Genome Center, National Center for Genome Resources

During the current genomic revolution with rapidly evolving second and third generation sequencing technologies and data analysis capabilities, originally envisioned impact of the Human Genome Project has been extended beyond medicine, such as agriculture and metagenome. Current sequencing technologies are now capable of generating vast numbers of reads in reasonably long read length within less than a few days, emerging as new tools for the rapid identification of microorganism and threat detection. Tremendous fold coverage of any given genome is possible with single run or even in a partial run with hundreds of indexed samples. Consequently, the bottleneck has moved from generation of the raw data to preparing samples to feed the machines as well as the downstream data analysis. Automated workflows have been developed for preparation of large numbers of sequencing libraries from genome, reduced genome, and environmental samples.

2:30 **Unbiased Known and Unknown Pathogen Detection and Characterization Using Next Generation Sequencing Technologies**

Willy A. Valdivia-Granda, PhD, CEO, Orion Integrated Biosciences Inc.

Next-generation sequencing (NGS) provides the technological basis for high resolution analysis of genetically heterogeneous samples. The coverage with these new platforms potentially allows for the detection of low-frequency variants and unknown species. However, there are bottlenecks in the disambiguation of this information which are due to the limitations of current sequence analysis systems and databases which fail assign an unknown sequence to a particular strain, subtype, or species. Here, we introduce a motif fingerprint (MF) scanning algorithm and advanced pathogen information database which provides an accurate and rapid genomic pathogen disambiguation pipeline capable of detecting and tracking rare genomic events during pathogen identification exercises. Once the genomic information from a particular sample is received, we can scan all known and unknown pathogen MFs through uncharacterized DNA reads. By keeping track of this process, we accurately assign the pathogen species, subtype, and strain, and exhaustively explore the sequence data space while quantifying the number of polymorphic haplotypes. Because our algorithms generate exhaustive searches we overcome the sensitivity limitations of heuristic bioinformatic applications to detect unknown strains and species as well reassortments, insertions, substitutions and deletions at the single sequence level. Benchmarking of false positives and false negatives rate and processing speed of different tools with our approach will be presented. A summary of the implications of next generation sequencing technologies and our known and unknown pathogen recognition approach for biodefense applications (attribution, forensics and intelligence) will be discussed.

3:00 **An Integrated Approach to Real-Time, Array-Based Molecular Diagnostics Enabled by Nano-Photonics**

Reinhold Wimberger-Friedl, PhD, Principal Scientist, Dept of Molecular Diagnostics, Philips Research, Philips Corporate Technologies, The Netherlands

Integrated solutions are an absolute necessity for bringing diagnostics to the point of need. For robust and low cost solutions, assays need to be integrated in closed, easy to use devices with on board reagents. At the same time also multiplexing, quantification and specificity demands need to be met. By making use of nano-photonics, real-time measurements can be performed even at high fluorescence background. This allows a reduction in the number of assay steps, while at the same time allowing for better quantification. By integrating thermal control and a compact fluorescence detector highly multiplexed specific and sensitive detection is achieved at affordable cost for the point of need.

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

4:00 **EpiSENSE™: A Rapid Detection Sensor for Air-Borne Biological Pathogens**

Dinakar Ramadurai, PhD, Scientist and Team Leader, Episensors, Inc.

A Fluorescent Resonance Energy Transfer (FRET) based technology has been developed for the rapid multiplexed detection of air-borne biological pathogens. This technology is based on a quantum dot (QD) immunoassay and has been incorporated into a light-weight, field-portable biosensor. The system performs the collection, detection and identification of air-borne biological contaminants, and demonstrated exceptional detection capabilities and performance under field testing at the US Army Dugway Proving Grounds.

4:30 **Technological Challenges for Rapid/Early/Specific Sensitive Detection**

Robert F. Standaert, PhD, Biological and Nanoscale Systems Group, Biosciences Division, Oak Ridge National Laboratory

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

5:00 **PANEL DISCUSSION: Creation, Assessment and Optimization of a Cost-Constrained Portfolio of Biodetection and Identification Technologies: Challenges and Solutions**

5:30 End of Day One

18th International Conference

Biodetection Technologies 2011

June 23-24, 2011 • Washington, DC USA

Conference Agenda

Friday, June 24, 2011

8:00 Exhibit/Poster Viewing, Coffee and Pastries

8:30 **KEYNOTE ADDRESS: CBRN Technology to Enable an Effective Biosurveillance Strategy**

Peter Emanuel, PhD, BioScience Division Chief, RDECOM Edgewood Chemical Biological Center, US Army

Presidential Policy Directive 2 which is titled the National Strategy for Countering the Proliferation of Biological Threats has launched an interagency effort to establish formalized programs to enable biosurveillance of the world health. Such efforts would enable rapid and proactive recognition of emerging pandemics and thus save lives and strengthen national security. This complex set of programs must be enabled through technology-both hardware and software. No single system will suffice but rather a family of systems that work with one another to share data and information in a complex global community. The DOD has worked with its interagency partners to conduct a large scale horizon scanning endeavor of all available technologies to enable the acquisition of a family of systems. The discussion will talk about what that effort found and what it may mean for the interagency needs for purchasing technologies in the coming years.

9:00 **Assessment of *Clostridium Difficile* Infections by Quantitative Detection of tcdB Toxin on Real Time Cell Analysis System**

Yi-Wei Tang, MD, PhD, Professor of Pathology and Medicine, Director, Molecular Infectious Diseases Laboratory, Vanderbilt University Medical Center

We explored the use of a real-time cell analysis (RTCA) system for the assessment of *Clostridium difficile* toxins in human stool specimens by monitoring the dynamic responses of the HS27 cells to tcdB toxins. The *C. difficile* toxin caused cytotoxic effects on the cells, which resulted in a dose-dependent and time-dependent decrease in cell impedance. The RTCA assay possessed an analytical sensitivity of 0.2 ng/ml for *C. difficile* toxin B with no cross-reactions with other enterotoxins, non-toxigenic *C. difficile* and other *Clostridum* species. Clinical validation was performed on 300 consecutively collected stool specimens from patients with suspected *C. difficile* infection (CDI). Each stool specimen was tested in parallel by a real-time PCR assay (PCR), a dual glutamate dehydrogenase and toxins A/B immunoassay (EIA), and the RTCA assay. In comparison to a reference standard in combination of the three assays, the RTCA had a specificity of 99.6% and a sensitivity of 87.5% (28/32), which was higher than the EIA (p=0.005) but lower than the PCR (p=0.057). In addition, the RTCA assay allowed for quantification of toxin protein concentration in a given specimen. Among RTCA-positive specimens collected prior to treatment with metronidozole and/or vancomycin, a significant correlation between toxin protein concentrations and clinical CDI severities was observed (R2=0.732, p=0.0004). Toxin concentrations after treatment (0.89 ng/mL) were significantly lower than those prior to the treatment (15.68 ng/mL, Wilcoxon p=0.01). The study demonstrates that the RTCA assay provides a functional tool for the potential assessment of *C. difficile* infections.

9:30 **Hot Start dNTPs - Novel Chemistries for Use in Advanced PCR Applications**

Natasha Paul, PhD, Scientific Investigator, TriLink BioTechnologies, Inc.

Recently developed Hot Start dNTPs are a distinct approach to Hot Start activation in PCR that employs modified nucleoside triphosphates with a thermolabile protecting group at the 3′-hydroxyl. This thermolabile chemistry can be applied to dNTP analogs such as dUTP for UNG decontamination methods and 7-deaza-dGTP for GC-rich target amplification. In addition, further studies have led to the development of 3′-protecting groups that deprotect more quickly than the current 3′-modification group, allowing application to fast PCR. With the evolving chemistry of Hot Start dNTPs, the areas of application benefiting from the versatility and flexibility of this technology continue to grow.

10:00 **ApoH Technology, a Solution for Ultrasensitive Detection of Viruses and Bacteria from Different Origins**

Francisco Veas, PhD, Research Director, Head of the Comparative Molecular Immuno-Physiopathology Lab, Faculty of Pharmacy, University Montpellier 1, France*

Apolipoprotein H (ApoH) is a human protein that captures and concentrates pathogens such as viruses and bacteria for their ultrasensitive detection. An important set of patents has been granted for the use of the protein in various diagnostic settings. The technology is a platform of solutions to avoid false negatives, adapted to handle numerous diagnostic systems and applications in the clinical, veterinary, food and plant diagnostic fields. Samples can be solid or liquid, such as stool, tissues, whole blood, serum, food or milk and mixed with the ApoH-coated substrate of choice for rapid trapping of the microbes of interest. The sample can then be removed of potential interfering substances, thus leaving a concentrated clean specimen for further processing. This can be done through ELISA, qPCR, cultivation or any other methodology. **In collaboration with: Ilias Stefas*

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

11:00 **Impedimetric Platform for Real-Time Bio-Affinity Detection: Assay Procedure and Data Analysis**

Andrei Gindilis, PhD, SHARP Laboratories of America, SHARP Corporation

An all electronic, impedimetric system allows for real-time, label-free detection of a wide variety of specific bio-affinity interactions. Assay procedures coupled with automatic mathematical data analysis have been developed to extract

18th International Conference

Biodetection Technologies 2011

June 23-24, 2011 • Washington, DC USA

Conference Agenda

reaction parameters from raw binding curves. The analysis algorithm allows efficient discrimination of specific and non-specific bio-affinity interactions as well as direct observation and analysis of fundamental binding kinetics.

11:30 **Is Normalization Of Raw Data Necessary? Truly Automated qPCR Data Analysis Using the AzurePCR Method**

Ze'ev Russak, CTO, Azure PCR Limited, United Kingdom/Israel

Azure PCR will demonstrate a novel and unique externally validated method for automated analysis of real-time (qPCR) data, including classification and quantification. Unlike existing software included within PCR cyclers, the AzurePCR™ automated analysis method is assumption-free and does not require setting of user parameters and, thus delivering time savings for both the researcher and the clinician. This automated analysis method demonstrates near 100% to near-100% accuracy of detection, as confirmed by recent validation studies.

12:00 **Deeply Multiplexed PCR**

Robert Haushalter, PhD, President and Founder, Parallel Synthesis Technologies

By isolating a given forward and reverse primer pair onto a Parallume encoded bead such that each primer pair is associated with a unique optical code, arbitrary numbers of on-bead PCR analyses may be performed in one tube. Upon primer extension in the presence of Target, the amount and rate at which double strand DNA is produced on the bead is proportional to the amount of Target present. Since primer-primer interactions occur only between a single pair on a bead, primer design is greatly simplified.

12:30 *Lunch on Your Own*

2:00 **T-COR 4 Handheld Real-Time PCR Thermocycler**

David Almassian, Senior Product Manager, Tetracore, Inc.

Tetracore's T-COR 4 Handheld Real-Time PCR Thermocycler can process four independent samples and is capable of analyzing two targets per sample. The T-COR 4 has a footprint of 9" x 7.5" x 3", weighs 6 pounds, and runs continuously off battery power for 8 hours. The user can run the device in either stand alone mode or connected to a PC. The T-COR 4 is an open system capable of running any customer's assay, in either a wet or dry formulation. The system is designed for use by both first responders in the field and biologists in the laboratory.

2:30 **Diagnostic MicroFluidics Assays: Lab Benchtop to Lab-on-Card**

Steve Jackinsky, Director of Diagnostics, Wi, Inc.

Microfluidic devices can be designed to closely match benchtop assays. In the development process are decision-making steps which have dramatic affects on the final launch of a diagnostic product. The general process of transferring a benchtop assay to a lab-on-card microfluidic will be presented. Selected topics will be presented to offer guidance on common development hurdles and methods to manage risk as the benchtop assay transforms into a Lab-on-Card diagnostic.

3:00 *Networking Refreshment Break, Exhibit/Poster Viewing*

3:15 **Development of a Rapid Method for the Quantification of *Legionella spp* in Environmental Waters in 48 Hours by FISH- ScanRDI™**

Marie Binet, Research Engineer - MICENE Project Manager, EDF, France

Monitoring of *Legionella* concentrations by standard cultural method (results in 10-12 days) is an important task in *Legionella* risk assessment international guidelines. To monitor *Legionella* colonization in man-made water cooling circuits, a rapid method was developed, based on Fluorescent *In Situ* Hybridization (FISH) coupled to solid-phase cytometer ScanRDI™ (AES-Chemunex) and gives definitive concentrations in 24 to 48h. This new method could be very useful as a new tool to monitor *Legionella* colonization in complex water systems.

3:45 **Magnetic Modulation Biosensing for Rapid Detection of Specific DNA Sequences and Proteins at Low Concentrations**

Amos Danielli, PhD, Department of Biomedical Engineering, Washington University in St. Louis

Magnetic modulation biosensing (MMB) is a novel technology for rapid, simple and sensitive detection of biological targets in homogeneous solution at low concentrations. In general, any assay that produces fluorescent light as a result of the biorecognition event can utilize the MMB system. MMB relies on coupling magnetic beads to the fluorescent-labeled probes and applying an alternating magnetic field gradient to condense the probes into the detection area and set them in a 1-D periodic motion, in and out of an orthogonal laser beam. Phase-locked detection of the resulted periodic fluorescent light provides high sensitivity without any washing or separation steps. Rapid detection of the Ibaraki virus without biological amplification and rapid detection of a protein (0.48 pg of human interleukin-8) will be presented.

4:15 **Selected Oral Poster Highlights and Open Discussion**

4:45 *Concluding Remarks, End of Conference*

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