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Biodetection Technologies 2012

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Technological Advances in Detection & Identification of Biological Threats

June 28-29, 2012 • Washington, DC USA

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Thursday, June 28, 2012

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

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

JOINT BIODETECTION & BIOSURVEILLANCE PLENARY KEYNOTE SESSION

9:00 **Challenges and Lessons Learned Developing a National Biosurveillance System**

Robert Hooks, Director, CBRNE Technologies, TASC, Inc.;

former Deputy Assistant Secretary for WMD & BioDefense, U.S. Department of Homeland Security (DHS)

We will address the challenges and important considerations to operating a national biosurveillance system and a discussion of the experiences sharing and providing the information to decision makers using a series of real-world examples. The talk will also include laying out the fundamental building blocks of an effective biosurveillance system and an approach to successfully make progress in working this complex problem.

9:30 **Next Generation System Platform for Biodetection Technologies (title to be confirmed)**

Eric Van Gieson, PhD, Division Chief, Diagnostics & Disease Surveillance, U.S. Defense Threat Reduction Agency (DTRA)

10:00 **Title of Presentation to be Confirmed**

Nathaniel Head, Biological Countermeasures Unit, Federal Bureau of Investigations (FBI)

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

11:00 **Rapid Characterization of Pathogens in Clinical Specimens via Suppression of Host Background for Efficient Second Generation Sequencing Analyses**

Steven S. Branda, PhD, Senior Member of Technical Staff, Biotechnology and Bioengineering Department, Sandia National Laboratories

Effective response to an infectious disease outbreak critically depends upon rapid and accurate identification and characterization of the causative pathogen. Second Generation Sequencing (SGS) has enabled discovery of novel pathogens in clinical specimens, but brute-force sequencing is extremely inefficient because most nucleic acids (NA) in the specimens are derived from the host and its microbiome, rather than the pathogen. To focus sequencing bandwidth on potentially informative NA, we have developed molecular suppression techniques for selective, physical removal of host NA during library preparation of clinical specimens for SGS analysis. This approach can be applied to virtually any specimen type, and requires no *a priori* knowledge of the pathogen. We have successfully demonstrated the approach using benchscale methods, as well as a microfluidics-based automated molecular biology platform that enables rapid, precise, and reliable processing of small, precious clinical specimens. Getting more reads on target yields better pathogen genome coverage with less SGS bandwidth; this further speeds the characterization process, by enabling use of quick turnaround, low capacity SGS systems (e.g., Illumina's MiSeq), and by simplifying the bioinformatics required for identification and assembly of pathogen sequences. Taken together, our Rapid Threat Organism Recognition (RapTOR) system is a powerful new approach for highly efficient sequence analysis of pathogens in clinical specimens. Implementation of RapTOR will greatly accelerate identification and characterization of novel pathogens, and thereby support rational and effective response to infectious disease outbreaks. **In collaboration with: K.Patel, J.S.Schoeniger, S.A.Langevin, V.VanderNoot, H.Kim, Z.Bent, K.P.Williams, O.D.Solberg, P.Lane, D.Curtis, A.Sinha, M.Misra, N.Thaitrong, B.Carson, J.B.Ricken, E.La Bauve, R.F.Renzi, M.Bartsch, N.D.Pattengale, R.Meagher, E.May, A.J.Powell, T.W.Lane*

11:30 **Electrokinetic Device for Complete *In-situ* Sample to PCR Detection of Pathogens and Disease Biomarkers**

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

We have developed a unique sample to answer electrokinetic device that allows the complete *in-situ* isolation, cell lyses, DNA processing and PCR reaction to be carried out in the same device chamber. In previous work, we were able to carry out the *in-situ* (in the same device chamber) isolation and preparation of DNA from bacteria, and disease related cfc-DNA isolation from blood samples, with the final PCR being carried out in a separate component (tube or chamber). In the case of sample preparation of bacteria for DNA sequencing, it was possible to carry out the *in-situ* isolation of the bacteria, lyses of the cells, proteinase treatment, recapture of the extracted DNA and sequencing related tagmentation reactions *in-situ*; however the final PCR was done in a separate device. We now have been able to develop AC/DC electrokinetic microarray devices which allow all steps, including PCR to be carried out in the same chamber. This represents a major advancement in that isolated DNA/RNA does not have be removed and transported into a different component device. This limitation of efficient DNA sample preparation has plagued miniaturized lab on a chip device technologies, and limits the overall assay sensitivity for many detection technologies. **In collaboration with: A.Sonnenberg, UCSD; and R.Krishnan, Biological Dynamics*

12:00 **Development of a Combined ELISA Protein Microarray and Activity Assay Platform for the Sensitive and Quantitative Detection of Toxins**

Susan M. Varnum, PhD, Senior Research Scientist, Pacific Northwest National Laboratory

We are developing an ELISA microarray for the sensitive and quantitative detection of botulinum neurotoxins (serotypes A, B, C, D, E and F), ricin toxin, Shiga toxin and *Staphylococcal*

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enterotoxin B. Simultaneously we are developing a toxin activity assay that is specific for *botulinum* neurotoxin, ricin, and Shiga toxin. These assays will be integrated into a single platform allowing for the sensitive and specific detection and the determination of functional activity of *botulinum* neurotoxin, ricin, Shiga toxin and *Staphylococcal* enterotoxin B in clinical samples. The ELISA microarray portion of this project is nearing completion and has produced highly sensitive assays (LOD between 1-20pg/mL), while work to develop activity assays is ongoing.

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

2:00 **Computational Methods for Biosurveillance**

Robert Cottingham, Group Leader, Computational Biology and Bioinformatics; and

Richard Stouder, Director, Technology Development and Deployment, Global Security Directorate, Oak Ridge National Laboratory

Biosurveillance has been primarily based on PCR detection, and intelligence gathering and analysis. With the increasing potential for terrorist engineered threats, and the computational means to detect both natural and synthetic threats, bringing together experts in related capabilities (i.e., both biological and computational) to consider existing and proposed methods for computational biosurveillance will provide an integrated view of the state of the art, identify technical challenges, and will develop a vision for the future of computational biosurveillance. Real-time detection of biothreats and emerging and pathogenic diseases is the ultimate objective of biological defense. With the advent of synthetic biology and genetic engineering, the challenges for biodetection, let alone real-time detection, have been multiplied.

2:30 **Rheonix CARD® Technology Achieves Fully Automated Biothreat Detection through Microfluidics**

Richard A. Montagna, PhD, Senior Vice President, Rheonix, Inc.

In order to reduce the complexity of molecular detection of biothreat agents, the fully automated Rheonix CARD® technology was developed to rapidly and automatically process "raw" specimens to permit detection of a wide range of microbial targets. Once a "raw" sample is introduced, no further user intervention is required to achieve sample preparation, multiplex PCR and amplicon detection. The versatility of the technology platform is further evidenced by the ability to process both clinical and environmental specimens using either a bench top or a battery-operated version of the EncompassMDx™. Data will be presented demonstrating the system's "sample-to-results" capabilities for biothreat detection.

3:00 **Robust Process for Highly Specific and Sensitive Multiplexed Molecular Diagnostics**

R. Paul Schaudies, PhD, President and CEO, GenArraytion, Inc

Utiliziing DoD SBIR Phase II funding, we have developed two separate five-plex molecular assays for the identification of *Staphylococcus aureus* with further discrimination between the drug resistant MRSA and the more susceptible MSSA. We have validated the assay with over 150 clinical isolates form two different major medical centers. The Assays were developed using the Luminex magnetic beads and the LX-200 flow cytometer platform. The target regions were subsequently used for the development of a solution based real-time PCR on the ABI 7700 96-well platform. We have a database of over 35,000 laboratory validated molecular signature sequences for many biothreat and infectious disease causing organisms. We are developing custom molecular assays that are adaptable to almost any platform. We have demonstrated routine sensitivities in the 10s of genomic copies and lower.

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

4:00 **Novel Strategies for Biodetection: Selective Detection of Viable Pathogens without Culture**

Harshini Mukundan, PhD, Scientist, Principal Investigator, Biosensor Group, Chemistry Division, Los Alamos National Laboratory

The sensor group at the Los Alamos National Laboratory has developed bench-top and fieldable sensor devices for the rapid detection of pathogens. We have developed novel transduction schemes, functional surfaces, robust ligands and reporters for use with bio-detection platforms, and developed assays for influenza, anthrax tuberculosis, breast cancer and others with exquisite specificity, sensitivity and speed. More recently, we have developed a conjugated siderophore-based strategy for the selective detection of viable bacteria in complex backgrounds, eliminating the need for culture. An overview of our work will be presented, with focus on the siderophore-based strategy for interrogation of viability.

4:30 **Simple Multiplexed Fluorescence Immunoassay System for Rapid, Low Cost Pathogen and Toxin Detection**

Charles Greef, PhD, Scientist, MBio Diagnostics, Inc.

MBio Diagnostics has developed a sensitive assay system that delivers quantitative analysis of up to 80 biomarkers simultaneously from a single < 50 microliter sample. The low cost system combines single-use disposable cartridges with a simple reader. Data will be presented demonstrating multiplexed analysis of a panel of blood viruses in human clinical samples, as well as sensitive detection of a panel of small molecule neurotoxins.

5:00 **By Any Means Necessary, (Re)Engineering Small Molecule Protein-Based Sensors**

Randall A. Hughes, PhD, Scientist, Applied Research Laboratories, The University of Texas at Austin

Nature has provided an abundance of high-affinity binding proteins to a myriad of small molecules ripe for potential diagnostic applications. However, adapting the exquisite specificity, plasticity, and affinity of these proteins to non-natural small molecule targets remains challenging. An interesting class of these proteins derived from bacteria, are called periplasmic binding proteins (PBPs). These proteins can be converted to fluorescent (optical) small molecule sensors via site-specific attachment of environmentally sensitive organic fluorophores. Herein, we report on our efforts to engineer the binding specificity of these protein-derived fluorescent sensors to non-native ligands using computational design and directed evolution.

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5:30 **Exhibitors and Sponsors Showcase Presentations and Selected Oral Poster Highlights - I**

6:00 *End of Day One*

Friday, June 29, 2012

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

9:00 **Biodetection Standardization: A Reality or Myth**

Mina Izadjoo, PhD, Director of the Diagnostics and Translational Research Center, Henry M. Jackson Foundation for the Advancement of Military Medicine

A major challenge confronting clinical and non-clinical diagnostic laboratories is the rapid detection and identification of organisms involved in intentional or natural exposures. Today, due to recent advances in the areas of genomics and biotechnology, many powerful diagnostic technologies have been developed. However, despite these advances, there is an unmet need for standardization of these technologies. Biodetection standardization will certainly improve our diagnostic capability and national readiness. Unfortunately, this type of research has fallen through the cracks. Platforms and reagents used for biodetection vary widely and there is a general lack of their standardization. Laboratories use different protocols leading to different results. With the aim of identifying a roadmap, challenges and potential approaches for biodetection standardization will be discussed.

9:30 **The Challenges of Selecting and Deploying Biodetection Technologies in a Mass Transit Environment**

Charles B. Burrus, DrPH, CIH, MTA/NYC Transit

MTA/NYC Transit is the largest mass transit system in the U.S. On an average weekday its ~48,000 employees transport >7 million customers using >6,000 subway railcars and >4,600 buses. The presentation will detail the many questions that must be answered before and after the decision to deploy a biodetection system. There can be significant ramifications associated with under or over reacting to a detected pathogen-of-concern. In the case of MTA/NYCT, the decisions can lead to major disruptions in service affecting millions of people. On the other hand, failing to act, or failing to act adequately, could result in potentially life-threatening exposures and extensive contamination of the mass transit system.

10:00 **RIGEL: Integrated Genomic-Based Computational Architecture for Rapid Characterization of Known and Unknown Biothreats and Interoperable Detection Assay Development.**

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

To address existing limitations of biothreat and pathogen detection and characterization initiatives, we have begun the implementation of a paradigm-shifting genomic-based known-and-unknown microbial discrimination system, named RIGEL that combines genomic and metagenomic analysis algorithms and object-relational database management systems to identify, store and update genomic signatures and motif fingerprints specific to a given strain, species, genus or family. Our implementation performs exhaustive searchers across all genomic information available up-to-date for more than 160,000 organisms. The results of this iterative search process are stored into a database where evidentiary information is logically linked to genomic data. Because its analysis are not dependent of probabilistic, heuristic, frequency or distance function algorithms, RIGEL provides an unparalleled and robust capability for forensics, attribution and situational awareness. RIGEL is used to: 1) Determine locations around the world that need to be sampled in order to resolve the geographical origin of pathogens of interest. 2) Establish the minimum number of microbial genomes that need to be sequenced in order to achieve full discriminatory resolution at a given taxonomic level. 3) Identify the minimum number of genomic signatures and/or motif fingerprints required to exclude known pathogens and discriminate unknown variants. 4) Disambiguate sequence information metadata improperly assigned to a particular taxonomy by using molecular membership analysis.

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

11:00 **Microbial Detection Array Applied to Public Health and Product Safety**

Crystal Jaing, PhD, Group Leader, Applied Genomics, Physical Life Sciences Directorate, Lawrence Livermore National Laboratory

Rapid detection and characterization of bacterial and viral pathogens is important for public health and product safety. We designed a Lawrence Livermore Microbial Detection Array (LLMDA) that contains 388,000 DNA probes. This array can detect any of the sequenced viruses or bacteria within 24 hours. We recently used this array to identify a contaminating pig virus from a rotavirus vaccine and to detect viral infections from various human clinical samples. The LLMDA is a comprehensive and cost-effective tool to rapidly detect and characterize different viral and bacterial pathogens for public health, product and food safety and global disease surveillance applications.

11:30 **Microbial Forensics at the Ultra-Rare Variant Level**

Viacheslav Fofanov, PhD, Director of Bioinformatics, Eureka Genomics Corp.

The presence of rare variants in bacterial samples could act as a sample's fingerprint and be important in investigation and prosecution of bioterrorism events. Next Generation Sequencing (NGS) can, in a cost acceptable manner, produce sufficient sequence data for the detection of ultra-rare variants (present in 0.1% of the sample). However, distinguishing true ultra-rare variant SNPs from false positive SNPs is a major limiting factor when the variant SNP is present at <1%. Corrections for systematic and random errors have been developed and are required to increase the specificity of ultra-rare variant detection from NGS data.

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12:00 **Laser-Induced Breakdown Spectroscopy as a Rapid, *In-situ* Bio Diagnostic**

Rosalie Multari, PhD, Scientist, Applied Research Associates, Inc.

The ability to detect and differentiate biological targets using laser-induced breakdown spectroscopy (LIBS) has become of increasing interest in recent years. Here the use of LIBS to detect CDC category A pathogens, category C viruses and other pathogens in complex matrices is discussed. Data will be presented demonstrating LIBS capability to differentiate various bio targets from each other and from uncontaminated samples using methods based on chemometric analysis of spectral data. Using this method of analysis, results are available within seconds to minutes. LIBS based instruments may be stationary or portable and may be operated with very little training.

12:30 *Lunch on Your Own*

2:00 **SpinDx: A Rapid, Sensitive, and Deployable Platform for Broad-Spectrum Pathogen Diagnostics**

Chung-Yan Koh, PhD, Sandia National Laboratories

There remains a need for deployable, diagnostic platforms that are rapid, sensitive, and amenable to biosurveillance applications in low-resource areas. Existing technologies tend to be fast and portable (e.g. lateral flow) or sensitive and quantitative (e.g. ELISA, qPCR) but not both. We present SpinDx, a sedimentation-based centrifugal microfluidic platform which is both fast (<20 minute) and sensitive (pg/mL limit of detection) for multiplexed protein and nucleic acid detection directly from complex samples with no sample preparation required.

2:30 **Nanoparticle Characterization Using a Size-Tunable Pore Sensor**

Kristoffer Bolen, Director at Izon USA, Izon Science Ltd.

Promising nanoscale structures of a range of engineered and biological particle types, such as contrast agent microcapsules, liposomes and viruses, can be monitored, analysed and quantified by a novel particle analysis tool. Particles are transported through a size-tunable nanopore via convection and electric field, for rapid and detailed characterization of particle size distribution, concentration, aggregate formation, and relative surface charge distribution, all determined simultaneously. Furthermore, real time monitoring of ionic current flow across the pore at different aperture settings enables detection and discrimination of individual nanoparticle populations in mixed multimodal suspensions.

3:00 **Tetracore T-COR 8 Handheld Real-Time PCR Thermocycler**

David Almassian, Senior Product Manager, Tetracore, Inc.

Tetracore's T-COR 8 Handheld Real-Time PCR Thermocycler can process eight independent samples and analyze up to four targets per sample. The T-COR 8 has a footprint of 11.8" x 10.7" x 3.2", weighs less than 10 pounds, and completes over six PCR runs off battery power. Tetracore's innovate approach does not require software installation, allowing the user to interact with the device using Google Chrome or Internet Explorer. An on board bar code reader reduces the chance of user error, and results can be communicated to existing networks using built-in Wi-Fi. The T-COR 8 is an open system capable of running any customer's PCR or isothermal 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.

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

4:00 **Collagen-Like Genes as Targets for PCR-Based Detection of Infectious Agents**

Slawomir Lukomski, PhD, Associate Professor, Dept Microbiology, Immunology & Cell Biology, West Virginia University HSC

bcl-gene polymorphisms as basis for *B. anthracis* detection and fingerprinting: A major challenge to defend against anthrax is the ability to rapidly and accurately distinguish infection by *B. anthracis* from more benign infections with other members of the *B. cereus* group. Here, we evaluate sequence polymorphisms of the *Bacillus collagen*-like genes, *bcl A-E*, as a basis for *B. anthracis* detection and fingerprinting. First, we identified sequence polymorphisms within *bclB* alleles that allowed for the specific detection of *B. anthracis* strains by PCR using both purified DNA and spores as templates. Next, we demonstrated that the combined *bclA-E* PCR products generate markedly different fingerprints, thus creating unique signatures for *B. anthracis* strains. Altogether, we present a new diagnostic concept for anthrax detection and fingerprinting. *acl* genes of the pathogenic mold *Aspergillus fumigatus* as biomarkers for early diagnosis of invasive *aspergillosis: A. fumigatus* is an etiological agent of invasive aspergillosis, a nosocomial infection that yields a mortality rate of up to 90 percent if undetected in early onset. To date, species-specific biomarkers of exposure are not available in diagnostic assays in the United States. *Aspergillus* collagen-like genes, designated aclF, were identified in *A. fumigatus* genome. PCR amplification of the 5' and 3' regions of *aclF1* gene yielded predicted amplicons in all *A. fumigatus* samples, but not in the control samples. This pilot study identifies *aclF1* gene as a candidate biomarker for species-specific detection of *A. fumigatus* infections in humans.

4:30 **Using Computers to Identify Microbes in Minutes versus Hours**

David Chiang, CEO, Sage-N Research, Inc.

Detection and identification of pathogenic microorganisms continues to be an area of high concern especially for Biodetection. Current methods rely on PCR (RT-PCR). The shortcoming of such an approach is that it requires known sequence information for detection. For example, RT-PCR uses probes that hybridize to a known sequence. If a significant mutation occurs in a viral target, the known effective probes are rendered useless, as they will be unable to hybridize to a mutant sequence. Data will be presented demonstrating a mass spec proteomics approach that does not require any knowledge of what is contained within the mixture, using the power of computational proteomics.

5:00 **Selected Oral Poster Highlights-II and Concluding Discussion**

5:15 *End of Conference*

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