

12th International Conference

Biodetection Technologies 2008

Technological Responses to Biological Threats

June 23-25, 2008 • Atlanta, GA USA



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12th International Conference

Biodetection Technologies 2008

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Industry and academic scientists are encouraged to submit poster titles for this event. One-page abstracts (8 1/2" x 11" with 1-inch margins) must be submitted no later than **May 26, 2008** for inclusion in conference documentation. Additional poster submissions will be accepted until **June 10, 2008** but may not be included in conference documentation. Note: If you are submitting a poster, you **MUST** be registered and paid in advance to ensure that a posterboard is reserved for you.

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Program Agenda

Monday, June 23, 2008

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

8:45 U.S. DEPARTMENT OF HOMELAND SECURITY

KEY NOTE ADDRESS:

The Value of Technology in Keeping the Homeland Secure

Robert Hooks, Deputy Assistant Secretary for WMD and Biodefens, The Department of Homeland Security

In biodefense area, DHS efforts focus on providing leadership by coordinating with other Federal partners, state / local / tribal jurisdictions, the private sector and the international community. In addition to developing technologies for Biowatch or NBIS, which are two DHS program areas, improved technology is equally important for our partners and is critical to improving the nation's capabilities in the biodefense arena.

PCR AND NON-PCR NUCLEIC ACIDS BASED DETECTION

9:15 SAFE: Sequencing for Avian Flu Epidemic

Niveen Mulholland, PhD, Senior Scientist, MRI-NCR, Midwest Research Institute*

We have developed a system for detecting mutations that would give rise to potential pandemic-causing influenza strains. The system, SAFE: Sequencing for Avian Flu Epidemic, first uses real time RT-PCR to detect H5, the highly pathogenic avian influenza most likely to cause a pandemic. We next use pyrosequencing to detect codon changes encoding amino acids known to define human versus avian influenza signatures. The SAFE real time RT-PCR assay specifically detects H5 in a multiplex reaction designed to detect a region of the Matrix gene common to all Influenza A subtypes. The H5 primer/probe set is specific; it does not cross react with other Influenza A subtypes tested. The M primer/probe set serves as an internal control, detecting all subtypes tested. Pyrosequencing assays were developed to screen, at the nucleotide level, for 52 amino acids changes defined by Chen et al (2006) to be avian- or human-specific. A library has been built to screen the sequence data generated and properly identify the strain in question as a potential threat. This surveillance system described here will allow the global community to monitor for H5N1 and for mutations that will render the virus more infective and virulent to humans. *In collaboration with: N.Waybright, E.Petrangelo, P.Lowary & J.Bogan

9:45 Next Generation Automated Multi-Target Detection Platform for Closer to Source Diagnostics

Todd Ritter, CEO of Applied Science & Technology, Idaho Technology Inc.

Idaho Technology has developed a highly multiplexed detection system capable of concurrently identifying and genetically discriminating dozens of viruses and bacteria. The syringe-loaded system utilizes a flexible plastic pouch combining automated sample preparation, reverse transcription for RNA viruses, and two-stage nested multiplex PCR performing 120 discreet analyses simultaneously. Capable of processing a variety of sample types, the small, lightweight diagnostic system represents a next generation in automated detection systems.

10:15 Multiplex Real-Time PCR Assay Design for Pathogen Detection, Quantification and Speciation

Robert S. Tebbs, PhD, Staff Scientist, Applied Biosystems*

A multiplex real-time PCR assay was designed to identify 3 species of *Vibrio* and an internal positive control using a four-dye configuration. Multiple strains were sequenced to identify target sites. Several individual assays within the multiplex contain more than one primer or probe due to strain to strain polymorphisms. *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus* were detected either individually or in mixtures at ≤ 25 genomic copies. The *Vibrio* multiplex assay showed 100% specificity to all targets analyzed and no detection of an exclusion panel that included nearest neighbors. Each assay in

the multiplex exhibited $100 \pm 10\%$ efficiency over a 5 log range. Multiplex real-time PCR can simplify pathogen detection and reduce costs since 3 species can be analyzed in a single reaction tube. *In collaboration with: P.M.Brzoska, S.Bit, M.R.Furtado, and O.V.Petrauskene

10:45 Refreshment Break, Exhibit/Poster Viewing

11:15 Rapid Multiplexed Nucleic Acid and Antibody Based Sensor for Biothreat Detection

Michael R. Meyer, Director of Laboratories, ICx Biosystems

RapidPlex is a fully automated, triggered confirmation system designed for detection and identification of bacteria, virus, and toxin threats in 10 minutes. The RapidPlex system provides simultaneous, multiplexed detection of protein and DNA/RNA markers through parallel antibody and nucleic acid-based assays. Antibody-based detection utilizes multiplexed sandwich assays on spectrally encoded microspheres followed by high resolution imaging of individual beads for detection of spores/cells captured on the bead surfaces as well as detection of toxins and viruses coating the bead surfaces. The nucleic acid-based process includes automated cell lysis and purification, followed by multiplexed DNA/RNA amplification and detection of the DNA amplicons on spectrally encoded microspheres.

11:45 Biological Agent Identification via Rapid DNA Sequencing

John P. Jakupciak, PhD, Technical Project Leader, Cipher Systems; Chemical, Biological, and Radiological, Technology Alliance (CBRTA)*

It has been shown over and over again that bacterial pathogens have variable genomes. For example, there are indigenous *Vibrio cholerae* living in Chesapeake Bay that do not cause disease. Detection systems should not alarm on these innocent strains. The genes coding for cholera toxins are mobile and can be transferred to other species. Indeed, some *Vibrio mimicus* strains, which do not have potential of being used for bio-terrorism, possess cholera toxin genes. So, alarming on *Vibrio cholerae* or cholera toxin gene may be false, and only creates confusion (Can we afford this?). DNA sequencing combined with rapid probabilistic matching is the only way to precisely detect the *Vibrio cholerae* strains with potential bio-terrorism capability. The same is true for most other pathogens. *In collaboration with: Rita Colwell, University of Maryland

12:15 Practical Uses of Blood RNA Carried by Microvesicles Originating from Specific Cells In Vivo

Zb J. Pietrzowski, PhD, Director of New Technology Development, SourcePharm, Inc.

Cells in vivo generate a number of microvesicles carrying RNA (mcRNA) and proteins specific to the originating cells. So far, it has been found that a number of pathological conditions or toxicating substances may induce generation of mcRNA in a cell type-specific manner. Therefore, microvesicle associated RNA isolated from serum is an attractive and novel blood based material for diagnostic and toxicology uses. mcRNA can be isolated and segregated per cell specific types. Subsequently, pools of cell-specific mcRNA can be analyzed further using RNA array technology. mcRNA may be advantageous over traditional circulating RNA methodologies due to the protective status against degradation afforded microvesicle associated RNA, the cellular origin identifying characteristic of micro-vesicles, and the possibility of multi-factorial relational analysis of the various cellular originating components associated with micro-vesicles. Results supporting practical uses of mcRNA will be presented.

12:45 Luncheon Sponsored by:
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REAGENT / REAGENTLESS OR BIOSENSOR BASED DETECTION

2:00 Multiplex Detection of Biothreat Agents by Fluidic Force Discrimination

Gary W. Long, PhD, Vice President and Senior Scientist, Tetracore, Inc.

Fluidic Force discrimination (FFD) has recently been applied to the detection of

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Biothreat agents. In this talk, we will describe the multiplex detection of 3 or more agents by an immunoassay developed for FFD, and demonstrate that sensitivity of detection is similar or greater than that obtained in lateral flow devices and by ELISA. We will also describe improvements to the sensitivity of detection of nucleic acids using this technology.

2:30 Use of CANARY™ for Rapid, Automated Collection and Analysis of Bioaerosol Samples

Thomas Hazel, PhD, Vice President Research, Innovative Biosensors, Inc.

CANARY™ is a cell-based technology that enables rapid identification of bacterial, viral, and toxin targets in liquid or aerosol samples. In this presentation we describe the testing and validation of our new BioFlash™ instrumentation, designed to provide 'detect-to-protect' capability for environmental monitoring and building security applications. This platform enables automated collection, detection, and simultaneous identification of up to 21 target aerosol agents with increased speed and sensitivity.

3:00 SAW RFID Biosensors for Ubiquitous Molecular Recognition

William D. Hunt, PhD, Professor, School of Electrical and Computer Engineering, Georgia Institute of Technology*

Research by Hunt's group at Georgia Tech has demonstrated that an acoustic wave biosensor with a biolayer coating can detect analytes such as Bascillus spores, cocaine, TNT/RDX in the gaseous phase. A new embodiment of this approach involves a SAW RFID/biosensor, which can passively sense and report on molecules in its environment. The SAW RFID/biosensor system involves a main interrogation unit transmitting an RF signal to a passive SAW structure located a short distance away. The device combined with the antenna re-transmits a reflected acoustic wave, which has been perturbed by the on-chip molecular recognition events. *In collaboration with: P.J.Edmonson, D.D.Stubbs, Zen Sensing LLC; D.Bertieri, Penn United Technologies Inc.

3:30 TIRF-EC Biosensors with Reagentless Bioassays for Rapid and Accurate Detection of Pathogens

Alexander N. Asanov, PhD, President & Scientific Director, TIRF Technologies

This presentation will describe revolutionary new portable and handheld biosensors based on Total Internal Reflection Fluorescence (TIRF) combined with ElectroChemistry (TIRF-EC). TIRF-EC sensors are capable of detecting hundreds of DNA/RNA and protein signatures of multiple pathogens in a matter of several seconds. The detection limit is at the level of single molecules. In conjunction with reagentless assays TIRF-EC sensors require no or minimum sample preparation stages. The sensors are suitable for a variety of biomedical point-of-care and biodefense field applications.

4:00 Refreshment Break, Exhibit/Poster Viewing

4:30 A True Real-Time, On-line Monitoring System for Waterborne Pathogen Surveillance

John Adams, PhD, Chief Scientist, Sensor Products Group, JMAR Technologies, Inc.*

Real time detection and classification of waterborne pathogen events can now be realized with an on line instrument using MALS (multi-angle light scattering) technology. A continuous slip stream of water passes through a flow cell in the instrument where a laser, focused perpendicular to the flow, strikes particles as they pass through the beam. Light scattering patterns are generated from each particle and are captured by photodetectors. Microorganisms produce unique patterns termed "bio-optical signatures" which are then compared to an on-board database where detection and classification occurs within minutes. Data from current installations and EPA testing will also be presented. *In collaboration with: David McCarty, JMAR

5:00 Cost-Efficient Technological Solutions for Warning-Type Bioaerosol Detection

Victor N. Morozov, PhD, Research Professor, Manager of a Laboratory of Detection & Diagnostics, George Mason University, The National Center for Biodefense and Infectious Diseases (NCBID)

Rapid ultra-sensitive assay of aerosolized pathogens presents a tremendous challenge in biodetection. NCBID has developed two key technologies solving

major "bottle-necks" in such detection: (i) energy-consuming collection of aerosol and (ii) slow, diffusion-controlled immunoassay. We demonstrated that collection of aerosol on water-soluble nano-filters combined with electrophoresis-assisted immunoassay and active detection of collected pathogens with magnetic beads enable detection of zeptomolar amounts of pathogens (150-500 molecules or viruses) in 2-3 minutes.

5:30 Optical Polarimetric Techniques to Detect and Identify Biological WMD's

Ezekiel Bahar, PhD, Professor of Electrical Engineering, University of Nebraska, Lincoln

Biological materials are identified by their optical activity which manifests itself by optical rotation and circular dichroism of polarized light. The optical activity of the biological material is characterized by constitutive relations in electromagnetic theory through its chirality. This chiral property leaves a distinct footprint on the polarization of light scattered by biological materials. Polarimetric scatterometers are used to measure the 16 elements of 4 x 4 Mueller matrixes that completely characterize scattered light, including magnitude, relative phase and the polarization state. We present a road map for optical polarimetric techniques to detect and identify biological WMD's based on measurements of specific Mueller matrix elements.

6:00 End of Day One

Tuesday, June 24, 2008

8:00 Exhibit/Poster Viewing, Coffee and Pastries

POINT-OF-CARE METHODS. TOXIN AND PATHOGEN DETECTION

8:45 Bridging Between the Worlds: When 'Proteomics', 'Ionics', and 'Electronics' Meet

Ilan Levy, PhD, Bioelectronic Chip Research Project, Intel Research Israel, Corporate Technology Group, Intel Corporation, Israel

Over the years, significant inflection points in understanding and delivering health care advances have occurred, particularly with the convergence of technologies. Today's ecosystem of aging populations, increased number of chronic patients, a global shortage of physicians, nurses and hospital beds, enforces major market shift towards home health care. While some initial technologies for remote patient monitoring are already in trials, fuller and more elaborated solutions are sought for. Cost effective medical diagnostics techniques can be executed at point of care (PoC), deliver immediate results, and be connected to the rest of the health care information systems. It will enable quality health care services that will not only overcome the shortages described above, but will also enable more precise and personalized medicine. The unique technologies developed at Intel have the potential of redefining modern health care, thus enabling the broad range implementation of personalized medicine and PoC practice.

9:15 Activity Detector for Botulinum Neurotoxins (BoNT)

Avi Rasooly, PhD, Center for Devices & Radiological Health, Office of Science & Engineering Labs, Division of Biological Sciences, US FDA; and the Nat'l Cancer Inst, Cancer Diagnosis Program

We developed an activity detector for Botulinum Neurotoxins (BoNT) based on cleavage of a specific target (SNARE peptide such as SNAP-25) as an alternative to the existing FDA mouse bioassay. We tested this assay with a portable microfluidics chip and a CCD-based detector developed to carry out BoNT activity assays. The detector consists of two modules: (1) the detection module which houses the CCD camera and emission filters; and (2) the excitation and sample module, containing the illumination source, the excitation filter, and the 9-well sample chip. The FRET activity assay used in this study utilized a FITC/DABCYL-SNAP-25 peptide substrate cleavage of the substrate by BoNT/A produced an increase in fluorescence emission, measured using the EL-CCD

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detector platform. Limits of detection (LODs) were between 0.625 and 1.25 nM (31 - 62 ng/ml) for LcA and 0.313 nM (45 ng/ml) for the full toxin, BoNT/A, results that are similar to standard fluorescent plate reader measurements.

9:45 Protein Based Assays for Novel Viral Detection and Serologic Assays for Detection of Poxviruses Including Smallpox Virus; Applications for Outbreak Response and Surveillance

Kevin L. Karem, PhD, Microbiologist, National Center for Zoonotic, Vector-Borne, and Enteric Diseases (ZVED), Division of Viral and Rickettsial Disease, Centers for Disease Control and Prevention (CDC)

Since the eradication of Smallpox in 1980, concern over the potential use of Smallpox virus (Variola) as an agent of bio-warfare, or terrorism has driven research for new vaccination methods, treatments and detection methods. We have developed and characterized monoclonal antibodies for use in novel viral detection assays based on antigen (protein) recognition, viral protein recognition and insight into detection and recognition of live virus. Detection of virus exposure in the absence of traditional laboratory equipment has also been piloted as a rapid point of care test.

10:15 Pathogen Detection with a Microflow Cytometer on a Chip

Lisa Shriver-Lake, Research Scientist, Center for Bio/Molecular Science & Engineering, Naval Research Laboratory*

A rapid, automated, multi-analyte microflow cytometer is being developed as a portable, field-deployable rapid sensor for on-site diagnosis of biothreat agent exposure and environmental monitoring. The technology relies on a unique method for ensheathing a sample stream in continuous flow past an illuminated interrogation region. This microfluidic approach avoids clogging by complex samples and provides for subsequent separation of the core and sheath fluids in order to capture the target for orthogonal confirmatory assays. Coded microspheres provide the capability for highly multiplexed assays in a few minutes. *In collaboration with: L.Hilliard, J.Erickson, P.J.Howell, J.P.Golden, G.P.Anderson, D.Ateya, A.Thangawng, M.Nasir, and Frances S. Ligler

10:45 Refreshment Break, Exhibit/Poster Viewing

A SPECIAL SESSION ON: EXPLOITING BACTERIOPHAGE FOR BIOLOGICAL THREAT RESPONSE

11:15 Bacteriophage Based Signal Amplification: The Solution to Noise Pollution

Lawrence D. Goodridge, PhD, Assistant Professor of Food Microbiology, Dept of Animal Sciences, Colorado State University

Signal amplification may be defined as the use of specific detection methodologies to directly increase the signal to noise ratio in proportion to the amount of target in the reaction. Here, we take advantage of the fact that phages produce multiple progeny during infection to develop a rapid and sensitive assay for detection of bacterial pathogens. We also demonstrate the use of phage amplification to develop an assay in which the phages themselves are the target organism, as indicators of biological water quality.

11:45 Utilization of Phage and Phage Lytic Enzyme for Rapid Field Identification of Bacteria

David Trudil, PhD, Executive Vice President, New Horizons Diagnostics Corporation

Bacteriophage has been utilized for treatments and preventatives in Eastern Europe for over 80 years. In the last decade the phage methods have been incorporated in various detection formats, including gamma phage for confirmation of anthrax. More recently new uses for the age old system has been refined and utilized for rapid field identification of specific bacteria. An update of these novel applications, both current and future potential uses, will be addressed. Included will be the utilization of luminescence and fluorescence with the lytic enzyme approach to address needs in the bio defense, food safety and water testing markets.

12:15 A Recombinant Bacteriophage-Based Assay for Discriminative Detection of Culturable and Viable but Nonculturable Escherichia coli O157:H7

Yasunori Tanji, PhD, Associate Professor, Dept of Biotechnology, Tokyo Institute of Technology, Japan

Green fluorescent protein (GFP) labeled bacteriophage, specific to Escherichia coli O157:H7 was used to construct lysozyme- inactivated GFP labeled phage. The new recombinant phage lack lytic activity due to inactivation of gene e, which produces lysozyme responsible for cell lysis. This system allowed the discriminative detection of culturable, viable but nonculturable (VBNC) and dead cells in the stress-induced environment.

12:45 Lunch on Your Own

A SPECIAL SESSION ON: EXPLOITING BACTERIOPHAGE FOR BIOLOGICAL THREAT RESPONSE (CONT'D)

2:00 Landscape Phage-Derived Recognition Interfaces for Detection Devices

Valery A. Petrenko, PhD, DSci, Professor, Dept of Pathobiology, Auburn University

Filamentous phages are thread-shaped bacterial viruses. Their outer coat is a tube formed by thousands equal copies of the major coat protein pVIII. Libraries of random peptides fused to pVIII domains were used for selection of phages probes specific for a panel of test antigens and biological threat agents. Because the viral carrier in the phage borne bio-selective probes is infective, they can be cloned individually and propagated indefinitely without needs of their chemical synthesis or reconstructing. As a new bioselective material, landscape phages combine unique characteristics of affinity reagents and self assembling proteins. Biorecognition layers formed by the phage-derived probes bind biological agents with high affinity and specificity and generate detectable signals in analytical platforms. The performance of phage-derived materials as biorecognition interface was illustrated by detection of Bacillus anthracis spores and Salmonella typhimurium cells. With further refinement, the phage-derived analytical platforms for detecting and monitoring of numerous threat agents may be developed, since phage interface against any bacteria, virus or toxin may be readily selected from the landscape phage libraries. As an interface in the field-use detectors, they may be superior to antibodies, since they are inexpensive, highly specific and strong binders, resistant to high temperatures and environmental stresses.

2:30 Rapid Label-Free Phage-Based Biosensors for Bacterial Detection

Rosemonde Mandeville, PhD, President and CEO, Biophage Pharma Inc, Canada*

Biophage Pharma Inc. is actively involved in the development and commercialization of environmentally safe solutions for the management of bacterial contamination. Our first commercially available product, the PDS[®]-16 biosensor can be used for the continuous on-line monitoring of the total bacterial load in biological fluids as well as clinical sensitivity to antibiotics with outstanding reproducibility and sensitivity. The PDS[®]-16 biosensor can also detect the presence of anthrax spores in a biological sample. This presentation will cover Biophage's new generation of biosensors using phages as recognition receptors for specific and rapid detection of pathogenic bacteria in field samples. *In collaboration with: M.Zourob, A.Shabani, B.Allain, A.Martineau

3:00 SEPTIC - Sensing of Phage-Triggered Ion Cascade

Maria D. King, PhD, Research Scientist, Dept of Mechanical Engineering, Texas A&M University

In the era of potential bioterrorism and pandemics of antibiotic-resistant microbes, bacterial contamination is a major concern. We have recently developed and demonstrated an entirely new nanoscale technology, called SEPTIC, for Sensing of Phage-Triggered Ion Cascade. Based on a nanowell chip, SEPTIC has already been shown to be capable of unambiguous identification of live bacteria on a time scale of seconds to minutes. The technology is based on using nanoscale fluctuation analysis to detect the massive ionic fluxes associated with the initial step of bacteriophage infection, the injection of the phage DNA into the cell. *In collaboration with: Laszlo B. Kish, TAMU

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3:30 Refreshment Break, Exhibit/Poster Viewing

4:00 **Engineered Phage Banks: A Functional Answer to Bacteriological Threats**

Flavie Pouillot, PhD, Research Program Manager, Pherecydes Pharma; and Manuel Gea, CEO and Co-Founder, Bio-Modeling Systems, France

Infectious diseases due to microbes of high pathogenic potential remain a constant and variable threat for human and animal health. The emergence of new diseases or the re-emergence of diseases that were previously under control together with ever increasing levels of antibiotic multi-resistances complicates the situation. Bacteriophages have begun to provide solutions to combat these problems. However, bacteria have developed very efficient mechanisms of molecular evolution and genetic flexibility allowing rapid and very effective escape from recurrent phage attacks. We have developed a set of large-scale phage engineering technologies allowing to systematically counter the effects of bacterial escape strategies based on molecular evolution. Through the production of very large engineered phage banks in which each individual differs from all others for any number of selected proteins, this approach allows the rapid isolation and amplification of phage subsets capable of detecting and eradicating any given bacterial population, including emergent pathogens, while systematically preceding bacterial escape strategies.

4:30 **Bacteriophage Amplification for the Detection of *Yersinia pestis***

Christopher R. Cox, PhD, and Kent J. Voorhees, PhD, Dept of Chemistry, Colorado School of Mines

Bacteriophage amplification is a new approach for rapid diagnosis of bacterial agents. In the process, a bacterial organism is infected with a bacteriophage and allowed to progress through the infection process resulting in the release of progeny phage. With each progeny phage produced, there is an amplification of both proteins and nucleic acid content. Immuno-detectors have been designed using antibodies specifically raised against the ϕ A1122 phage proteins for secondary identification of *Yersinia pestis*.

5:00 **Biosensing Bacterial Pathogens with Reporter Bacteriophage**

Steven A. Ripp, PhD, Associate Research Professor, Center for Environmental Biotechnology, University of Tennessee

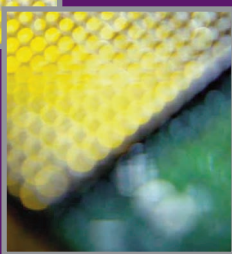
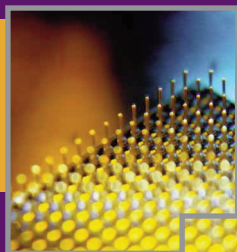
When contemplating approaches for the detection of chemical agents, nothing is more diverse, sensitive, robust, manipulatable, or cost-effective than the bacterial cell, and lux-based bioluminescent bioreporters have for over a decade taken acute advantage of this diversity to sense and respond to specific chemical targets. To add biological targets to their sensing repertoire, bioluminescent bioreporters have been linked to bacteriophage/host cell specificity through quorum sensing autoamplification to yield new bioreporter assays capable of detecting distinct bacterial pathogens such as *Escherichia coli* O157:H7. The integration of these assays with integrated circuit microluminometers, nanofabricated interfaces, and CCD imagers provides both miniaturized lab-on-a-chip and high-throughput real-time monitoring of biological exposures.

5:30 **Concluding Discussion: Pushing the Envelope or Revisiting the Past? Bacteriophage as Countermeasures to Biological Threat Agents**

Moderator: Steven A. Ripp, PhD, Center for Environmental Biotechnology, University of Tennessee

- Can bacteriophage really serve as a biodetection and/or biocontrol strategy?
- What about phage resistance mechanisms in bacteria that make them immune to attachment or infection?
- Are phage-based assays proficient enough to uniquely identify living cells, dead cells, viable but nonculturable cells, and spores in air, liquid, and solid samples?
- And how do we concentrate biological targets to ensure phage/host interaction?
- Is there a sufficient research and commercialization future for phage biodetection/biocontrol technology?
- Perhaps the deciding factor is public perception - how will the public react to "viruses" or worse yet "genetically engineered viruses" in, say, food products?

5:45 Closing Remarks, End of Conference



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Sample Prep 2008

**Sample Preparation for Toxin
& Pathogen Detection:**

Collection • (Pre-) Concentration
• Lysis • Extraction

One-Day Symposium Agenda

Wednesday, June 25, 2008

9:15 *Registration and Refreshments*

9:45 Sample Preparation for Rapid Microorganisms Detection - Current Perspective and Future Challenges

George Hong, PhD, R&D Manager, Technology and Pre-Development, Millipore Corporation

Since the discovery of nucleic acids, proteins, or cellular components as targets for microbial identification, this field has advanced from culture growth-dependent verification to rapid culture-independent analysis. However, no matter how sophisticated the field has progressed, these innovative technologies often rely heavily on robust sample preparation methods to achieve consistent and accurate performance. Here, we present an overview of major sample preparation methods, which encompass collection, concentration, and purifications of the target of interest for microbial identification. Also in this presentation, we will touch on major future challenges for the advancement of rapid sample-preparation for the field of microbial identification.

10:15 Bio-Aerosol Collection for Indoor and Outdoor Applications

Charles Call, PhD, CEO Biodefense, ICx Technologies, Inc.

Technologies for the collection of aerosolized pathogens and other bio-threat agents will be reviewed. Approaches which will be covered include standard and cascade impactors, rotating impactors, virtual impactors, wet-walled cyclones, and dry filters. Performance metrics for bio-aerosol collectors will be addressed. Applications of these technologies will be described in detail for both indoor and outdoor environments. Methods of characterizing the performance of aerosol collectors will be presented. The session will wrap up with a short review of approaches and products for surface sampling for pathogens.

10:45 A Water Condensation Method for the Concentration and Capture of Airborne Particles

Susanne V. Hering, PhD, Founder & President, Aerosol Dynamics Inc.

Key to the effective surveillance of airborne pathogens is the collection and delivery of these pathogens to the detection system. Generally, this requires the concentration of airborne particles from large sample airflows into a small volume of water solution.

Aerodynamic concentration methods such as virtual impactors can be effective for particles from 2 μm to 10 μm , yet many bacteria are below 2 μm , and nearly all viruses are below 0.5 μm . Described here is a new method that uses condensational growth to encapsulate submicrometer and supermicrometer particles within a supermicrometer water droplet, with subsequent collection in small liquid volumes.

11:15 *Networking Refreshment Break*

11:35 BEADS: A Modular Sample Preparation System for Pathogen Detection

Richard M. Ozanich, PhD, Senior Research Scientist, Chemical & Biological Sciences Group, National Security Directorate, Pacific Northwest National Laboratory*

Sample preparation is a limiting technology for developing fully integrated, autonomous, and portable biodetection systems. BEADS (Biodetection Enabling Analyte Delivery System) is a modular fluidics approach that bridges the gap between large volume liquid samples generated from environmental matrices (aerosol collectors, soils, water, etc.) and microscale detectors that require small, clean samples. The central components of BEADS sample preparation systems are derivatized particles, which are used for concentrating and purifying pathogens and pathogen biomarkers. The particles are automatically introduced, trapped and released in specialized flow cells. Fouling of the critical bio-interactive surface does not occur because new particles, and therefore new interactive surfaces are automatically introduced for each sample. This presentation will include a summary of sample preparation modules and results for trace detection of pathogens and toxins in complex samples and large samples (e.g., 1 liter water), an ultrasonic flow-through lysis module to increase the availability of DNA from *Bacillus* spores for detection, and integration of sample preparation and multiplexed detection modules. *In collaboration with: C.Bruckner-Lea, T.Straub, and C.Warner

12:05 Sample Collection and Processing: Experiences from the Lab to the Field

Andrew Cannons, PhD, Scientific Director, University of South Florida Center for Biological Defense

Preparation and processing of samples for the detection and characterization of bioterrorism (BT) agents are essential elements for the early recognition of a BT event. This phase includes sample collection in the field to rapid processing of varied matrices in the lab followed by detection and analysis. Here we report on some of our efforts in sample preparation and processing, including sample collection training for emergency

Sample Prep 2008

Sample Preparation for Toxin & Pathogen Detection:

Collection • (Pre-) Concentration
• Lysis • Extraction

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responders and processing different real life samples (food, soil, powders) for rapid pathogen characterization.

12:35 Lunch

2:00 Development of an Automated Sample Preparation Device for Use on the BioSeeq®-Vet Portable PCR System

John W. Czajka, PhD, MBA, Vice President of Technology Acquisition, Smiths Detection

Smiths Detection has developed the BioSeeq®-Vet PCR System, a portable PCR instrument that performs automated sample preparation and PCR analysis.

Nucleic acids are extracted and purified from samples by the Sample Preparation Unit (SPU), a single-use, self-contained sample preparation and PCR consumable. All enzymes and reagents needed by the unit are preloaded into the SPU and are temperature stable. The SPU was designed to purify DNA or RNA from a wide range of sample types and is driven by the BioSeeq®-Vet instrument.

2:30 Electric Field Driven Sample Collection, Concentration and Preparation Technologies

Kapil Pant, PhD, Manager, Microsystems and Nanosystems, CFD Research Corporation

CFD Research Corporation (CFDRC) has developed a suite of sample collection, concentration and preparation technologies that harness electric fields to implement various steps involved in integrated biodetection. CFDRC-developed technologies include (1) high efficiency, "gentle" aerosol sampling and concentration using electrostatics, (2) intelligent discrimination of target cells and spores from contaminant particles using dielectrophoresis, (3) rapid, reagent-less lysis of cells and spores using AC electric fields, (4) fast target extraction in a microsphere-based format with active, electrokinetic mixing, and (5) nano-electrokinetic sample pre-concentration for enhanced assay sensitivity.

3:00 Networking Refreshment Break

3:20 High Affinity Chemicals Used in Sample Preparation

X. James Li, PhD, Chief Scientific Officer, Cellex, Inc.

Isolation and enrichment of detection materials (nucleic acids and proteins) from pathogens are the first and crucial step for successful detection of the pathogens. We will describe two sample preparation approaches. The first approach is a virus capture based approach; the captured virus can be directly used for downstream detection. The second approach involves isolation and enrichment of nucleic acids from highly diluted samples using a class of high affinity chemicals.

3:50 A Filtration System for Separation, Storage and Efficient Rapid Release of Plasma from Clinical Samples of Blood

Galina Fomovska, PhD, Senior Principal Scientist, Pall Lifesciences, Molecular Media R&D, Pall Corporation

We will demonstrate the effectiveness of a novel membrane system composed of asymmetric polysulfone BTS-SP 300 membrane and Accuwik® Ultra hydroxylated polyester media, for the rapid separation, collection, storage and efficient release of plasma from whole blood directly from a donor without the use of equipment. We will demonstrate that concentrations of important clinical biomolecules, lipids, proteins, and hormones, in plasma samples separated, from whole blood, stored, and reconstructed using the system are practically identical to those generated by centrifugation.

4:20 ROUND TABLE DISCUSSION: Current Needs and Future Directions in Sample Preparation for Rapid Microbial Detection

Moderator - George Hong, PhD, Millipore Corporation

5:00 Concluding Remarks, End of Symposium

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