

Knowledge Foundation International Conference

SAMPLE PREP 2010

Sample Preparation for Virus, Toxin & Pathogen Detection & Identification

- Collection
- (Pre-) Concentration
- Lysis
- Target Extraction

May 6-7, 2010
Baltimore, MD USA

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TECHNOLOGY COMMERCIALIZATION ALLIANCE

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Thursday, May 6, 2010

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

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

9:00 **KEY NOTE ADDRESS: Sample Preparation and Microfluidic Technologies**

Raymond P. Mariella Jr., PhD, Senior Scientist, Lawrence Livermore National Laboratory*

Current sample preparation procedures for assays that detect and identify unknown viruses in clinical samples are often laborious, inefficient, and irreproducible. We are developing a series of reconfigurable microfluidic modules that can isolate and concentrate analytes of interest, and be operated in an automated fashion. Our current focus is on isolating viruses in clinical samples but the modules are designed to be flexible and applicable to a broad range of samples and needs. One appealing aspect of microfluidics for sample preparation is its combination of small size and its ability to process microliter liquid volumes, potentially in an integrated series of process steps, minimizing the use of expensive reagents. However, real-world samples are often milliliter-scale, with low starting concentrations of the virus of interest. Since we need to process enough sample to pass many copies of each unknown pathogen species to its appropriate assay, we cannot process only a few microliters of the sample [Poisson statistics]. Thus, our design uses continuous-flow microfluidics to perform the sample preparation, so that, despite the microfluidics system containing only microliters of sample at any instant, the system can process milliliters on the time scale of 10 minutes. This presentation will report the work-in-progress for our current approach to this problem, including the serial use of standing-wave ultrasonics and variations of electrokinetic manipulations. This work performed under the auspices of the U.S. DoE by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. *In collaboration with: K.Rose, D.DeHlinger, M.Shusteff, E.Wheeler, K.Fisher, B.Jung, C.Bailey

9:45 **Point-Of-Test Sample Prep and Molecular Analysis**

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

Many components in biological matrices influence the result of an analysis, affecting assay sensitivity and reproducibility. Improved matrix management becomes critical as requirements for higher assay sensitivity and increased process throughput become more demanding. In microfluidics, effective matrix management is essential for developing fully integrated systems capable of meeting these requirements. Electrokinetic methods have the potential of handling whole blood samples and can be incorporated into point-of-test device for full automation.

10:15 **Automated Sandwich Immunoassay Preparation by a Magnetic Bead-Based Microfluidic Device**

Li (Julie) Zhu, PhD, Bioanalytical Chemist, Chemistry Technology and Material Characterization, GE Global Research, General Electric Company*

A microfluidic device was designed, fabricated and tested for the

automated preparation of a bead-based sandwich immunoassay. Bacteria were captured onto immuno-specific beads and subjected to a series of immuno-labeling steps in a flow through format. The complete assay protocol prior to detection was completed within thirty minutes, a significant advancement compared to the conventional manual assay. E. Coli and other bacteria assays were demonstrated using the system. The sensitivity and specificity achieved were comparable with conventional methods. *Work supported by NRL under contract #: N00173-08-2-C003

10:45 *Networking Refreshment Break, Exhibit/Poster Viewing*

11:15 **Development of an Integrated Sample Preparation System for Next Gen Whole Sequence Analysis of Trace Specimens**

Mark W. Eshoo, PhD, Director, Ibis Biosciences, Inc., a subsidiary of Abbott Molecular

Most next generation sequencing technologies require the input of microgram quantities of template DNA equivalent to the DNA from 106 human cells to 109 bacterial cells yet many samples of interest may consist of only a few uncultivable cells. Furthermore, the sample preparation processes for next generation sequencing are slow and laborious. To address these needs we have developed an integrated microfluidic sample prep card that lyses cells, extracts nucleic acids and whole genome amplifies the genetic material to generate sufficient template for whole genome sequence analysis. The resulting output can be used on a number of next generation sequencing platforms including Roche's 454 and Pacific Biosciences single molecule real time sequencing technologies.

11:45 **Single-Molecule Science with a Nanopore: Inspiration from Nature**

Liviu Movileanu, PhD, Dept of Physics; Structural Biology, Biochemistry, and Biophysics Program; and Syracuse BioMaterials Institute, Syracuse University

A nanopore may act as an amazingly versatile single-molecule probe that can be employed to reveal several important features of nucleic acids and proteins. The underlying principle of nanopore probe techniques is simple: the application of a voltage bias across an electrically insulated membrane enables the measurement of a tiny picoamp-scale transmembrane current through a single hole of nanometer size, called a nanopore. Each molecule, translocating through the nanopore, produces a distinctive current blockade, the nature of which depends on its biophysical properties as well as the molecule-nanopore interaction. Such an approach proves to be quite powerful, because single small molecules and biopolymers are examined at very high spatial and temporal resolutions. I will discuss our recent work that provided a mechanistic understanding of the forces that drive protein translocation through a nanopore. These measurements facilitate the detection and exploration of the conformational fluctuations of single molecules and the energetic requirements for their transition from one state to another. I will also describe our recent strategies for engineering new functional nanopores, in both organic and silicon-based materials, with properties that are not encountered in nature. From a practical point of view, this methodology shows promise for the integration of engineered nanopores into nanofluidic devices, which would provide a new generation of research tools in nanomedicine and high-throughput devices for molecular biomedical diagnosis.

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12:15 **Sample Preparation as Part of an Integrated Fluidic Process for Rapid Diagnostics**

John Clarkson, PhD, Chief Executive Officer, Atlas Genetics Limited, United Kingdom

Atlas Genetics has integrated sample preparation, target amplification and a novel form of electrochemical detection onto a single fluidic cartridge for use in point-of-care settings. Focusing on sample preparation, this presentation will describe chemistries and device integration with particular respect to the rapid diagnosis of sexually transmitted diseases. In addition, methodologies and limit of detection data for bacterial pathogens that are difficult to lyse will be presented. These present particular challenges to device integration, and novel approaches will be discussed.

12:45 *Luncheon Sponsored by the Knowledge Foundation Membership Program*

2:00 **Advanced Sample Preparation Methods and Technology Requirements: Current Status and Perspective**

Jennifer McLaughlin, Chemical Biological Medical Systems Joint Project Management Office (CBMS-JPMO), JPEO-CBD - Joint Program Executive Office for Chemical and Biological Defense, US Army

The detection and identification of biological warfare agents relies heavily on the ability to purify, enrich, and concentrate molecular targets prior to analysis. The use of analytical technologies in the field is complicated or limited by available methods for processing a wide range of sample types into a form compatible with multiple analytical methodologies. Here we describe current sample preparation methods, recent improvements to traditional methods, and planned future directions.

2:30 **Electrophoretic Nucleic Acid Sample Prep with Capacity for Integrated Sequence Enrichment**

Andre Marziali, PhD, President and CSO, Boreal Genomics; Director, Engineering Physics, University of British Columbia, Canada

We have developed a novel electrophoretic concentration technology, named SCODA (Synchronous Coefficient of Drag Alteration) for efficiently purifying and concentrating nucleic acids. SCODA excels in applications where common extraction techniques can fail by offering unique advantages including exceptional contaminant rejection, an unparalleled ability to enrich for low abundance nucleic acids, and minimal mechanical disturbance of samples enabling reduced cell lysis or recovery of high molecular weight DNA when desired. Due to the flexibility of the SCODA system, we are able to process samples in a variety of complex matrices, including samples that contain particulates or strong PCR inhibitors. We are also able to recover nucleic acids from extremely dilute samples, with successful concentration from starting DNA concentrations in the zeptomolar range. The non-mechanical nature of the process has allowed recovery of intact high molecular weight DNA over 1Mb. More recently we have demonstrated that SCODA can be made specific to the sequence of DNA targets to be concentrated, opening the opportunity for sequence enrichment

applications. This presentation will give a brief overview of the SCODA technology with emphasis on recent progress in sequence specific DNA concentration.

3:00 **Isolation of Nucleic Acids and Proteins in Resource-Limited Settings**

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

In comparison to nucleic acid sequence amplifications and signal detection, little attention has been devoted to the area of specimen processing, especially in resource-limited areas. Currently, there are no products for the purification of nucleic acids or proteins without the use of instrumentation. This presentation presents data on the success and challenges that our research group has encountered in our quest to produce kits for the isolation of nucleic acids and proteins in resource-limited settings.

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

4:00 **A Microfabricated Polymer Filter with a PCR Compatible Viability Assay for Isolating and Detecting Pathogens from Liters of Water**

Haiqing Gong, PhD, Director, BioMEMS Laboratory, School of Mechanical and Aerospace Engineering, Nanyang Technological University, Singapore

Waterborne pathogen monitoring is extremely challenging due to the large sample volume (1-1000 liters) and dirt particles in water sample. We present a microfabricated polymer filter to capture waterborne pathogens and a rapid viability assay which is PCR compatible. The genetic testing method we developed is based on a microfluidic scheme for loading and sealing a large number of micro/nano liter PCR reactors on a chip. This microfluidic scheme is integrable with the microfabricated filter to achieve the simultaneous detection of multiple viable pathogens.

4:30 **Xisyl Sample Preparation System: A Completely Automated Random Access Sample Preparation Instrument for Lysis, Concentration, and Purification of Viral and Bacterial Analytes**

Jay A.A. West, PhD, Chief Technology Officer, Founder, Arcxis Biotechnologies

Molecular diagnostic tests are critically dependent on the availability of highly purified intracellular macromolecules to enable reliable downstream assay results. We report the development of a highly flexible automated sample preparation consumable and instrument system that enables streamlined laboratory workflow in the preparation of viral and bacterial nucleic acid analytes from a variety of sample matrices such as water, whole blood and plasma. The Xisyl sample preparation system will allow the non-skilled user to process large volume raw samples in a rapid, simple, three step process, enabling push button automated lysis, concentration and purification of target nucleic acids. Further discussed will be the integration of sequence selective capture materials into the Xisyl system for the isolation of disease specific RNA and DNA, and proteins.

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5:00 **Large-Volume Centrifugal Microfluidic Device for Whole Blood NA Analysis Sample Preparation**

Mary Amasia, PhD, Researcher, BioMEMS Lab, University of California, Irvine

While point-of-care devices are trending down in size, there remains a need for devices that process samples on the order of several milliliters. For example, *Bacillus anthracis* is present in infected whole blood at 10 copies per milliliter: the minimum number of copies needed for detection by standard nucleic acid analysis techniques. We present on a centrifugal microfluidic device that automates the classical blood separation technique of sedimentation, allows for the integration with downstream amplification and detection steps of nucleic acid analysis, and yields results with high sample purity in less than half the time of gold standard hospital techniques.

5:30 **A Fully Integrated System for Nucleic Acid Based Detection of Bacteria and Viruses in Biological Samples at the Point of Care**

Haim H. Bau, PhD, Professor of Mechanical Engineering and Applied Mechanics, University of Pennsylvania

We report on a self-contained, integrated, disposable, sample-to-answer system consisting of a low cost, disposable, pouch-based cassette and a portable analyzer. The cassette accepts a sample of biological fluid and performs lysis; nucleic acid isolation, concentration, and purification; enzymatic amplification; and amplicon labeling and detection. The cassette stores on-board all the required buffers and dry reagents, as well as, provides on-chip pumping and flow control. Detection is facilitated either in real time or with lateral flow strip. The analyzer provides mechanical and thermal actuation, and detection (if desired). The system can be operated by untrained personnel. The performance of the device was tested by identifying the presence of pathogenic bacterial *B. cereus*, Armored RNA, and HIV virus in spiked samples of oral fluids.

6:00 *End of Day One*

Friday, May 7, 2010

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

9:00 **Rapid and Sequential Isolation of Protein and Nucleic Acids**

David R. Pawlowski, PhD, Senior Research Scientist, CUBRC, Inc.

CUBRC, Inc. has developed a fast yet robust method to sequentially purify PCR-ready nucleic acids and immuno-reactive protein content from virtually any matrix without the need for typical laboratory equipment. These processes are in-line, do not require sample splitting, can be performed in less than 20 minutes, and are easily automated. Methods: The nucleic acid fraction of a sample is isolated using Akonni Biosystems™ TruTip® isolation procedure.

The protein fraction is isolated from the waste of the nucleic acid extraction using the TruTip or other silica matrix. Isolated products were identified by a number of methods including rtPCR and hybridization for nucleic acids and hand-held and ELISA assays for protein. The isolated nucleic acids performed as well as the gold standard isolation techniques from Qiagen using any identification or amplification method. The isolated protein was immuno-reactive to antibodies purchased from the Critical Reagents Program and other DoD suppliers. The development of a sample processing method that provides two analyte types from a single, sample provides the Warfighter or other end user the unique ability to directly identify an agent using multiple methods, thus increasing confidence in results. This method can also be adapted to laboratory settings for genomic and proteomic analysis of samples.

9:30 **A Novel Pre-Analytic System for Rapid, Quantitative Diagnostics**

Shawn R. Feaster, PhD, Senior Research Scientist, Sensors & Indicators, Corporate Research & Engineering, Kimberly-Clark Corporation

Since 1872, Kimberly-Clark has relied on innovation and technology to build some of the best known and most respected brands in the world. In keeping with our long standing history of innovation, Kimberly-Clark has developed a diagnostic platform capable of rapidly quantifying lateral flow test strips. At the heart of this innovation lies a pre-analytic system (PAS) that acquires, processes, and meters a tiny volume of whole blood with a simple push of a button. Typical input volumes between three and five microliters reliably result in one microliter of serum/plasma being applied to a lateral flow test strip. Data will be shared from a lateral flow-based test for C-reactive protein, demonstrating the performance and the benefits of the PAS.

10:00 **Autonomous Sample Prep in Ocean Environments**

Chris Melancon, President & CEO, Spyglass Biosecurity, Inc.

The Spyglass ESP System is a fully automated platform that collects water samples, concentrates microorganisms, analyzes the sample using molecular techniques and transmits the results to the end user. The system also enables sample archiving for additional analysis in a laboratory environment. This talk will offer results from deployments in the Monterey Bay and the Gulf of Maine from 2001 through 2009 at ocean depths from 10 to 900 meters.

10:15 **Networking Refreshment Break, Exhibit/Poster Viewing**

10:45 **Rapid Automated Sample Concentration**

David S. Alburty, Chief Executive Officer, InnovaPrep LLC

InnovaPrep is commercializing rapid sample preparation for biological identification based on wet-foam elution and is developing a suite of instruments and integrable processes. InnovaPrep technologies can provide automated sample preparation and concentration, sample extraction from filters and surfaces, matrix exchange, and pre-determination of the final sample volume. Two applications of the technology will be covered; extraction of biological particles from filters and direct concentration of biological particles from liquid samples.

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11:15 **Fully Integrated Microfluidic CARD™ for Molecular Diagnosis**

Peng Zhou, PhD, Senior VP for R&D/CSO, Rheonix Inc.

We have developed a fully integrated microfluidic device for the automatic performance of molecular diagnostic assays. Using our patented solvent lamination process, three polymer layers are assembled in our CARD™ (Chemistry and Reagent Device) to form all functional components (e.g., pumps, valves, reaction chambers, fluidic network etc.) that allow a "raw" sample to be introduced and automatically processed without any user intervention. Thus far, clinical samples such as whole blood, plasma, serum, saliva and vaginal swabs have been successfully processed and analyzed on-CARD™ using PCR, RT-PCR and NASBA.

11:45 **Selected Oral Poster Highlights and Discussion**

12:15 *Lunch on Your Own*

2:00 **Sample Collection and Preparation for Virus and Pathogen Detection with Alternatives Methods**

Quitterie Desjonquères, Biotech Engineer - Product Manager, Bertin Technologies, France

In the context of environmental contamination control and bio-sample preparation, Bertin Technologies designs a range of laboratory equipment based on new technologies. Coriolis® is dedicated to the monitoring of bioaerosols. This cyclonic technology ensures a sampling method supplying a liquid sample compatible with Rapid Microbiological Methods (RMM) in order to get rapid, reliable and specific data on airborne microorganisms and to go beyond impaction method limits. Precellys®24 is dedicated to the sample preparation and cell lysis, to homogenize and grind soft and hard biological materials. This bead beating technology improves the first critical step in any molecular biology process and ensures high throughput, reproducibility, and time saving. Both Coriolis® and Precellys®24 combined with molecular diagnostic methods, provide sensitive, accurate and quantitative data about several airborne pathogens (e.g. warfare agents) as spores of pathogens, non cultivable microorganisms or even viruses. The combination of these devices, Coriolis® with Precellys®24, with advances in molecular bio-analysis have now made it possible to rapidly detect, identify and accurately quantify airborne microorganisms and viruses. The limitations in monitoring and identifying pathogens in bioaerosols by microscopy or cultural methods can now be addressed by combining cyclonic air sampling, Coriolis®, bead-beating lysing cells, Precellys®24, with molecular diagnostic methods to provide sensitive, accurate and quantitative data on several pathogens. Several studies carried out with partners have shown interesting results and proved the efficiency of Coriolis in airborne viruses and pathogens' collection and could be presented.

2:30 **A Case for Industrial Lab Automation in Sample Preparation and Handling**

Yakov Kaplan, PhD, Invetech, Australia*

Despite the recent advances in pathogen detection, there still exist many challenges and opportunities to improve the current technology. There are a number of novel biosensors, immunoassays, molecular biology techniques that had been successfully employed in modern labs. However a challenge of

sample preparation and associated material and liquid handling are often left untouched due to a significant challenge of sample preparation logistic. Quite often a team of skilled operators and a hefty process guidelines are the answers to this challenge. Invetech in working with various partners over the past 10 years has been tasked with helping to develop commercial scale sample handling systems that are ranging from tabletop systems through to stand alone high-end automation solutions. Invetech has successfully adapted principles and methods of industrial automation to serve common lab practices.*In collaboration with: A.Donath, R.Speziale

3:00 **Fieldable Automated Nucleic Acid and Protein Sample Preparation and Processing** *Speaker to be confirmed*

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

3:30 *Concluding Discussion, End of Conference*

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