

Knowledge Foundation International Conference

SAMPLE PREP 2011

Sample Preparation
for Virus, Toxin
& Pathogen Detection
& Identification

April 4-5, 2011
San Diego, CA USA

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Len Vanderbosch,
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Monday, April 4, 2011

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

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

9:00 **Development of IPF Automated Nucleic Acid Extraction System**

David Kelso, PhD, Center for Innovation in Global Health Technologies (CIGHT), Dept of Biomedical Engineering, Northwestern University

Pre-analytical processing typically involves nucleic acid capture on paramagnetic particles (PMPs), extensive washing to remove inhibitors and elution. We have developed an extraction method which replaces wash steps with transporting PMPs through an immiscible-phase filter (IPF) yielding nucleic acid as pure as that obtained from conventional purification. A flexible automated system applicable to a broad range of biological matrices will extract nucleic acids with minimal user input is under development.

9:30 **Nucleic Acid Sequence and Methylation Enrichment Using SCODA**

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

We have previously presented a novel electrophoretic concentration technology, named SCODA (Synchronous Coefficient of Drag Alteration) for efficiently purifying and concentrating nucleic acids. SCODA is able to purify DNA from a variety of complex matrices, including samples that contain 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. 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. Recent experiments show that SCODA can enrich for single nucleotide mutations by 10,000 fold compared to the wild type, and that it is capable of separating identical sequences that differ only in degree of methylation. This presentation will give a brief overview of the SCODA technology with emphasis on recent progress in sequence specific DNA concentration.

10:00 **Automated Nucleic Acid Library Preparation for Sequence-Based Unknown Pathogen Detection**

Hanyoung Kim, PhD, Research Scientist, Sandia National Laboratories

DNA sequencing technology is advancing at an unprecedented rate, but sample preparation relies on slow, labor-intensive, manual bench-top processes. We have developed an automated droplet-based platform coupled with multiple lab-on-a-chip modules to

process clinically-derived DNA samples by directly interfacing with next generation sequencers (NGS). This platform maximizes the sensitivity of NGS by enriching informative nucleic acid sequences (those derived from the pathogen) and suppressing background DNA (those from the host).

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

11:00 **Rapid Isolation and Detection of Virus Directly from Whole Blood Samples Using HC-DEP**

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

A high conductance dielectrophoresis (HC-DEP) procedure has been developed which allows rapid separation and detection of virus directly from "un-diluted whole blood" samples. Using a HC-DEP microarray device, red fluorescent Cherry T7 bacteriophage was separated from whole blood samples in minutes. The fluorescent T7 bacteriophage is held in high field areas while blood cells move to low field areas. All the blood cells are washed away, while the fluorescent T7 bacteriophage remains in the high field areas. The isolated, highly purified viruses are then ready for further analysis.

11:30 **Maximizing the Value of Clinical Biopsies for Monitoring Tissue Response to Biological Insults**

Sandra M. Gaston, PhD, Principal Investigator, Division of Surgical Research, Dept of Surgery, Beth Israel Deaconess Medical Center; Assistant Professor of Surgery, Harvard Medical School

Prostate, breast and colon cancer screening programs each include routine collection of tissue biopsies from relatively healthy individuals within the community. Conceptually, such samples could be useful in assessing population exposure to dietary or environmental toxins, provided that the primary goal of cancer detection is not compromised. We have developed a set of innovative "tissue print micropeel" technologies that allow us to obtain high quality RNA and DNA based molecular profiles without compromising the biopsies for surgical pathology. In addition to providing a valuable tool for cancer biomarker studies, biopsy tissue print RNA and DNA samples also reveal inflammatory and endocrine responses associated with non-malignant disease or with medications. Using this approach, with appropriate patient consent, biopsy tissues collected in the normal course of cancer surveillance could also provide important information about environmental exposures to pathogens or toxins that is otherwise unobtainable.

12:00 **Unravelling the Potential of Formalin-Fixed Paraffin-Embedded Tissue**

Martin Schlumpberger, Senior Scientist R&D, Qiagen GmbH, Germany

The vast archives of clinically annotated, formalin fixed paraffin-embedded (FFPE) tissue samples provide extremely valuable research potential. Unfortunately, quality of nucleic acids and

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proteins extracted from FFPE material strongly depends on the treatment before, during and after fixation and embedding as well as on the retrieval of usable analytes. Major difficulties are crosslinking and heavy fragmentation of biomolecules, as well as the limited amount of FFPE sample material. The purification procedure needs to be highly efficient to recover as much usable analytes as possible. This talk will provide insights into major problems and possible solutions for the isolation of the biomolecules as well as for suitable downstream analyses.

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

2:00 **Sample Preparation - From Technologies to Devices**

Sébastien Ribault, PhD, Predevelopment-Technology-Collaboration R&D Manager, BioMonitoring, Lab Solutions, Merck Millipore, Millipore SAS, France

Sample preparation prior to molecular detection has to accommodate a broad range of matrices originating from biopharmaceutical industry, medical diagnostic, and environmental monitoring. Sample volume reduction is a key for sensitivity as well as removal of inhibitors, including proteins and undesired nucleic acids. We have developed technologies allowing positive selection of microbial contaminants nucleic acids versus eukaryotic ones, and proteins removal prior to concentration and final analysis. We incorporated the selected options in a device for contaminants testing focusing on high-volume processing, ease-of-use, reduced hands-on time, and false positive risk management. The efficiency of the method was proven for bacteria detection in hospital (urine, plasma, cerebrospinal fluid) and in biotech (bioreactor cell culture) samples. A great deal of effort was spent on the DNA-free topic for reagents and disposables, in order to avoid false positives with universal amplification assays.

2:30 **Triggered Bioaerosol Sampling onto Dry Electret Filters; Wanted: Dead or Alive**

David S. Alburty, CEO, InnovaPrep LLC

Tactical detect-to-warn bioaerosol systems must provide a physical sample for verification of a detected threat. A combined detector/collector developed by Areté Associates and InnovaPrep collects such samples on a dry electret filter at a rate of 200 LPM. The sample is rapidly eluted from the filters using a simple hand-held device, and is then concentrated and prepared for identification. Dry collection can reduce the viability of collected organisms. The effects of sampling and preparation on the post-sampling viability of vegetative bacteria will be compared to that of collected spores.

3:00 **Inexpensive Sample Preparation and Storage for HIV Viral Load Testing**

Catherine Klapperich, PhD, Associate Professor, Dept of Biomedical Engineering, Boston University

We have built and begun testing a small, portable tool that could be used to test a patient for HIV on the spot, or at "point-of-care". The tool, which is about the size of a student microscope, doesn't

require a power supply. The sample pops out of the tool so it can be processed and then shipped and stored. The input sample is whole blood and the output is dried, preserved RNA that can be stored at room temperature for later testing.

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

4:00 **Improving Process Workflow in Molecular Diagnostic Testing**

Len Vanderbosch, General Manager Invitex USA, Invitex GmbH

The workflow process in molecular diagnostic testing, from sample collection to final data analysis and reporting, involves a number of coordinated steps that requires a variety of interactions and manipulations of samples. Reducing the number of necessary steps while introducing the capability to perform multiplex analysis represent opportunities to simultaneously minimize potential for errors and improve processing timelines. The RTP® technology available from Invitex GmbH provides sample processing capabilities that will eliminate specific steps required for extracting and purifying nucleic acids from a variety of samples. The INFINITI® Analyzer from AutoGenomics, an automated multiplexing microarray platform for the analysis of DNA samples provides the ability to sort and measure multiple signals from a single sample. When used in conjunction, these two technologies can significantly improve the efficiency and effectiveness of any molecular diagnostic laboratory.

4:30 **Rapid Detection of Pathogens in Complex Food Matrices**

Byron Brehm-Stecher, PhD, Assistant Professor, Rapid Microbial Detection and Control Laboratory, Dept of Food Science & Human Nutrition, Iowa State University

Our ability to accurately screen for and detect pathogens in foods is crucial to maintaining the safety of these products. Much effort has been spent in both academia and industry on research and development aimed at improving the final step - detection. However, considered from a more holistic standpoint, the process of screening our foods for pathogens (or spoilage organisms) typically involves several interlinked steps. These include the initial sampling step, separation and concentration of microbial cells from complex food matrices, followed by labeling and detection of target cells. While the elements "upstream" from the detection step have traditionally been neglected or undervalued, all are critical to the final output and all must work in concert to yield optimal results. In this talk, we will provide examples of our recent work on detection of pathogens in complex foods and related matrices. Parallels between food, environmental and clinical diagnostics will be discussed, including how the same set of tools we have used in foods can also be applied to clinical problems such as detection of *Candida albicans* in blood.

5:00 **Mentor-100: A Fully-Automated Stand-Alone Environmental Bio-Detector**

Loganathan Doraisamy, PhD, Senior Systems Engineer, Menon & Associates, Inc.

Mentor-100, a portable, fully-automated bio-detector, uses a

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patented Nuclear Magnetic Resonance(NMR) based detection method to quickly and sensitively detect pathogens in the environment. The Mentor-100 is decontaminable and fully reusable, with sub-systems for aerosol collection, sample preparation, and NMR. This bio-detector is capable of unattended operation using its wireless capability to communicate with a control center. The development and qualification of the overall system has been supported by the Department of Homeland Security (DHS) and the Defense Threat Reduction Agency (DTRA).

5:30 *Concluding Discussion, End of Day One*

Tuesday, April 5, 2011

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

9:00 **Nano-Immunomagnetic Capture of *B. Anthracis* Spores in Food**

Kingsley Amoako, PhD, Research Scientist, Canadian Food Inspection Agency, Canada

Food is vulnerable to potential bioterrorist attacks and critical to a mitigation strategy, is the rapid concentration and detection of biothreat agents from food matrices. Magnetic nanoparticles offer a unique advantage in that they have a large surface area for efficient capture of cells. We have demonstrated the efficient capture and concentration of *B. anthracis* spores using nano-immunomagnetic particles and this is being explored for a potential food application to enhance sensitivity of downstream detection technologies. *In collaboration with: N.Goji, K.Hahn, T.Janzen, M.Shields

9:30 **Sample Preparation Integrated Waterborne Pathogen Monitoring System**

Thomas Haiqing Gong, PhD, Associate Professor, Thermal and Fluids Engineering Division, School of Mechanical and Aerospace Engineering, Nanyang Technological University, Singapore

Waterborne pathogen monitoring of drinking water is extremely challenging due to the large sample volume (1-1000 liters of water) and dirt particles in water sample. We present a sample preparation method for processing a large volume of water which is also integrated with a PCR array for rapid detection of waterborne pathogens. We also present an instrument which automatically processes the captured pathogens from cell capture to cell lysis, DNA extraction, purification and sample loading into a qPCR array with control reactions.

10:00 **Universal Sample Prep for Third Generation Sequencing**

Milena Iacobelli Martinez, PhD, Senior Laboratory Scientist, FLIR Systems, Inc.

Third generation sequencing technologies require purified HMW DNA to attain read lengths of tens of thousands of bases and beyond for optimal sequencing output. Therefore, ICx Biosystems

has developed a universal sample prep device capable of isolating HMW DNA suitable for sequencing from environmental samples including spores, bacterial cells, and viruses. The integrated SCODA technology allows for specific concentration of HMW DNA and removal of contaminants that are detrimental to downstream processes.

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

11:00 **Integrated Bioparticle Concentration for Improved Detection in Autonomous Systems**

Andrew E. Page, President & CTO, InnovaPrep LLC

Autonomous detection systems are one cornerstone in protecting our nation and troops from biological threats. InnovaPrep has developed an automated concentration system that fills the need for an interface between large sample volumes and submilliliter volumes required by sample preparation and detection modules. The system provides orders of magnitude improvement in detection limits and is currently under evaluation in two DHS aerosol detection programs (e.g. MBAND-Biowatch Gen3, AESaP) and a DTRA-funded water monitoring application. Bioparticle fractionation, concentration, and integration hurdles will be discussed.

11:30 **A Nano-Technology Enabled, Automated, Portable Sample Preparation System**

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

Biological samples are often in complex matrices containing substances that inhibit nucleic acid and/or immunological identification assays. In addition, relevant biomarkers are present at very low concentrations. Therefore, the sample must be isolated, cleaned, and concentrated prior to analysis. With current methods, cleaning and separating whole cells, nucleic acids, and proteins is an involved manual and time consuming process that requires a wide range of laboratory equipment. Integrated Nano-Technologies (INT) is developing a universal automated sample preparation approach for environmental and clinical samples including air filter material, soil, blood, tissue, and sputum. This universal automated sample preparation approach could be used as part of an integrated diagnostic system or as a stand-alone unit to provide analysis-ready purified samples for use in any other diagnostics systems. The preparation protocol relies on novel variations of ultrasonication and magnetic separation techniques. The sample preparation process is being automated in a novel rotary valve fluidic cartridge which has been developed and tested by INT. The unit being developed is a portable battery-powered device (under five pounds), using the disposable cartridge, which can run all steps of sample preparation in the field with minimal training. The simple operation of the system should allow for a CLIA waiver for medical diagnostics.

12:00 **Filling the Critical Gap in Sample Preparation Technology**

Robert E. Carlson, PhD, President and Chief Science Officer, Receptors LLC

Sample preparation is commonly the "Achilles Heel" of many

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detection and diagnostic systems. Generic methods like reverse phase media are limited by poor specificity while the analyte specific, antibody based methods are limited by cost and stability. RECEPTORS™ AFFINITY by DESIGN™ technology fills this significant simplicity versus specificity gap. Our CARA™ SMART MATERIALS™ create the right balance of analyte concentration and purification using a scalable, cost effective technology.

12:30 *Lunch on Your Own*

2:00 **The Need to Improve Sample Preparation to Advance Sensing of Microbial Contaminants in Marine Environments**

Kelly D. Goodwin, PhD, Microbiologist, National Oceanic & Atmospheric Administration (NOAA)

Biological sensing, coupled with environmental data, is needed to produce assessments and forecasts related to marine ecoservices. NOAA is engaged in projects to develop marine sensors for detection of microbial contaminants and harmful algae in order to improve decisions about the closings of fisheries and recreational waters, to assess the health of marine ecosystems, and to ascertain threats to public health. Advanced technologies are at hand, including automated platforms for in-situ sampling and genetic analysis. However, such technologies must be enhanced for use in routine management, particularly in the realm of sample preparation. In particular, many current methods are hampered by poor and variable nucleic acid extraction efficiencies and co-extraction of inhibitory substances. This problem impedes true quantification and is acute for sensing rare targets such as pathogens or source-tracking markers. Biological sensing of seawater represents a particular challenge because of high salinity, inclusion of interfering compounds including humics, a high level of background organisms relative to the target contaminant(s), the uncharted molecular diversity of the indigenous microbes, and the need for target quantification within a regulatory framework. Approaches to solve sample preparation issues, including electrophoretic approaches, will be discussed.

2:30 **PureLyse™ Sample Preparation**

Bruce Irvine, Chief Technology Officer, Claremont BioSolutions LLC

ClaremontBio has created a novel method of rapid sample preparation that is entirely disposable; it requires no instrument. It provides mechanical cell lysis that can simultaneously extract nucleic acids or proteins. Nucleic acid can be extracted in three to five minutes within two steps, binding and elution. Because lysis occurs mechanically rather than chemically harsh reagents are avoided that require multiple wash steps, the use of alcohols and drying steps. This system has been used to lyse and extract nucleic acids from *E.coli*, *Bacillus subtilis* vegetative cells and spores, *Mycobacterium bovis*, and *Clostridium difficile*. The disposable PureLyseR device is comprised of a micro-motor and vane that beat particles at unusually high shear forces. As the shear forces lyse cells nucleic acid binds to the lysing particles. The sample is allowed to flow through the lysing chamber during lysis and binding, enabling a wide range of sample volumes to be processed and binding up to 10 µg of DNA. Alternatively the same cartridge can be used for cell lysis only, the OmniLyseR cartridge, and the lysed sample can then be delivered for further processing. The lysing particles can also be functionalized for affinity capture of proteins. This has been done for His-tagged proteins using the HIS-

PureLyseR version of the PureLyseR system. Proteins are extracted and purified in less than five minutes. Alternatively the lysate is easily adapted to the Claremont Bio HIS-ExpressR column for an increase in binding capacity up to up to 1.6 mg and 95% purity.

3:00 **Cough Analyzer of Airborne Bacteria for Tuberculosis Diagnosis**

Patrick Sislian, PhD, Principal, Deton Corp

Active Tuberculosis (TB) causes approximately 2 million annual deaths. An estimated 9.3 million people worldwide develop TB every year, of which ~4.4 million are undiagnosed. Improved TB diagnostic tests for developing countries will reduce the spread of infection and result in ~625,000 annually adjusted lives saved. In industrialized countries (e.g. US), the goal is TB elimination through diagnosis of multi-drug resistant TB (MDR-TB) and non-resistant TB in high-prevalence and high-risk populations. Most current diagnostics rely on sputum samples which are difficult to collect and are usually contaminated by saliva, lowering their quality. Furthermore, they inherently have a tradeoff between practicality (time, resources, training, cost) and performance (sensitivity and specificity). Deton's proposed device is practical. A patient wears a disposable mask and coughs naturally into a novel impactor that breaks up the cough droplets in air and collects their DNA. The impactor avoids both sputum samples and microfluidics for lysing cells. The proposed device's high performance is based on the automation of the collected DNA with a nucleic acid amplification test (NAAT) specific to TB and multi-drug resistant TB (MDR-TB). It serves as a point-of-care diagnostic or SSM replacement with an estimated total available yearly market of 280 million tests.

3:30 **Selected Oral Poster Highlights and Open Discussion**

4:00 *Concluding Remarks, End of Conference*

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