

Integrating
**Sample
Preparation**

*6th International Conference
in our Sample Prep Series*

Techniques & Applications

- Viruses, Toxins & Pathogens
- Point-of-Care Diagnostics
- Genomics Sampling
- Environmental & Water Sampling
- Food Safety
- Chemical & Biological Agents & Threats

**October 18-19, 2012
Baltimore, MD USA**



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Thursday, October 18, 2012

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

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

9:00 **Solving the Impedance Mismatch between Patient Sample and POC Diagnostic System**

John C. Carrano, PhD, President and CEO, Paratus Diagnostics, LLC

In this paper we posit that in order to realize pragmatic point-of-care medical diagnostic devices capable of meeting the rigorous requirements of CLIA waiver, that one must solve the inherent "impedance mismatch" between the clinical acquisition of a human patient sample and its delivery to the POC Dx system or device. We work from the assumption that specimen acquisition from the patient must follow standard clinical practice, and that the POC system (e.g. "device") will be designed to follow a yet to be determined international interface standard (similar in principle to the now ubiquitous Luer-Lock standard). We will present a design concept for a "specimen delivery system" and the associated proposed interface standard, along with experimental data from preliminary rapid prototype models.

9:30 **Automated Sample Preparation in a Portable Field Unit**

Michael Connolly, PhD, President and CEO, Integrated Nano-Technologies, LLC

Integrated Nano-Technologies has been developing a fully automated sample preparation system for use in the field or in laboratories. The system consist of a portable battery powered unit and a plastic disposable cartridge. The cartridge can automate sample disruption (chemical and ultrasonic bead beating), magnetic separation, filtration, size-exclusion chromatography, and PCR in a single cartridge. The disposable cartridge has more than 20 chambers and utilizes a revolver valve system to address chambers and move fluids. The process flow can be modified depending upon the needs of the user. Additional processing steps can be carried out in temperature controlled reaction chambers. Isolation of nucleic acids has been carried out from a wide variety of samples including blood, tissue, insects, soil and air filters. RNA, DNA or protein can be isolated from the samples with high efficiency. RNA and DNA isolated from samples has been PCR amplified and is also suitable for use in automated gene sequencing systems. The cartridges have also been designed with an archiving chamber to preserve part of the sample for alternative analytic processes if needed. A multi-channel system is also being built which can process 10 samples simultaneously.

10:00 **Addressing Real-World Genomics through Integrated Sample-To Answer Systems**

Dennis W. Harris, DPhil, Chief Scientific Officer, IntegenX, Inc.

IntegenX is leveraging MOVE™ valve technology to enable the integration of large volume, real-world samples with microfluidic chip-driven devices that for the first time allows true sample-in, answer-out solutions for genomics analysis. This eliminates the need for highly skilled operators and, opens up the possibility to apply sophisticated genomic analysis in non-laboratory settings. The RapidHIT 200 Human Identification system generates

actionable information in a usable time frame for law enforcement, anti-terrorism and immigration. This talk will focus on the results from deployment of the System in the context of law enforcement.

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

11:00 **A Microfluidic Toolbox for Integrated Sample Prep**

Claudia Gärtner, PhD, CEO, microfluidic ChipShop GmbH, Germany

We have developed a set of microfluidic functionalities for sample prep in a modular toolbox fashion. This allows to flexibly combining these modules for the integration of complex sample prep protocols into integrated microfluidics-based devices used for applications such as molecular diagnostics, multiplexed pathogen detection or human identification. Development and integration strategies for such devices will be presented.

11:30 **Low Cost Sample-to-Sequence Device for Human & Pathogen ID**

David Cohen, PhD, Program Manager, Advanced Liquid Logic

Advanced Liquid Logic has built and tested a sample-to-sequence microfluidic system. The system is comprised of a low-cost, disposable microfluidic cartridge and an analyzer based on the commercially-available R110 platform. The cartridge can accept up to 250uL of crude sample. Sequencing is accomplished using a modified version of the pyrosequencing reaction. To date, 22 STR loci (single tandem repeat) and 10 fungal pathogens have been sequenced on this platform.

12:00 **Self-Powered Multiplex Immunoassay Platform**

Zhenyu Li, PhD, Assistant Professor of Electrical and Computer Engineering / Biomedical Engineering, The George Washington University

We have developed a general bead-based microfluidic platform for multiplex heterogeneous immunoassays such as ELISA and Fluorescence Immunoassays (FIA). The microfabricated device is driven by capillary force, thus eliminating centrifugal or magnetic washing steps, and its unique size-differentiating microfluidic trapping of multiple bead species enables multiplex (>10) immunoassays on a single device using only one enzymatic or fluorescent label. The device is ideally suited for handheld IVD systems if integrated with a handheld chemiluminescence or fluorescence reader enabled by the emerging optofluidic technology.

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

2:00 **The NCI Cancer Human Biobank (caHUB): A National Center for Biospecimen Science and Standards Development**

Latarsha Carithers, PhD, Project Manager, Office of Biorepositories and Biospecimen Research, National Cancer Institute

The Office of Biorepositories and Biospecimen Research (OBBR) at the National Cancer Institute (NCI) was established in 2005 to address the lack of high-quality human biospecimens available for disease research. The cancer Human Biobank (caHUB) is OBBR's main infrastructure for conducting biospecimen research and consists of a network of medical centers, a comprehensive data center, pathologists, and molecular analysis facilities. caHUB carries out specialized tissue and data procurements, within a stringent ethical, legal, and regulatory framework, using evidence-based protocols and a comprehensive quality program to generate high-quality and well annotated biospecimens.

2:30 No Culture, No Assembly, Direct Sequencing To Microbe Identification

John Jakupciak, PhD, Researcher, Cipher

We demonstrate Population-Direct-Analysis (PDA) the use of Next Generation sequencing (NGS) to characterize all genomes in a mixture, directly, without post-assembly, without culture purification, and distinguish between closely related populations. This research offers promise of bio informatics coupled with direct sequencing as a reliable approach to characterize pathogens (bacteria and viruses) in a sample. Author will cover the strengths and limitations of the immediate analysis of raw data output.

3:00 Use of a Novel Online Pretreatment and Tandem LCMS Approach for Removal of Anionic Interferences for Analysis of Hormone Active Compounds in Environmental Samples

Robert Classon, Shimadzu Scientific Instruments

Hormone compounds, such as naturally occurring estrogens, may be present in water. Analysis of these compounds can be difficult depending on the presence of contaminants such as humic acids. In this study, a new on-line pretreatment column (MASK) is used to reduce background noise and ion suppression by removing humic acid type contaminants from environmental samples at neutral pH. This MASK column also increases the life of the analytical column.

3:30 Networking Refreshment Break, Exhibit/Poster Viewing

4:00 Human Virus Detection by Tissue Culture and Molecular Methods for Reliable Detection of Infective Viral Particles in Sludge

Yossi Manor, Central Virology Laboratory, Sheba Medical Centre, Israel

Wastewater reclamation has become a critical issue as the demand for water increases. The growing number of sewage treatment facilities create large quantities of sludge as a byproduct of the process. The strict environmental regulations underscore the importance of finding a practical solution for this sludge. One logical solution is to use this high concentrated organic matter product as fertilizer for agriculture. Still, this approach poses public health threats. Regulations for the microbial contents of sludge for agriculture have been defined, yet the methodology for detection is still under consideration, especially for viruses. This work compares the classical tissue culture method to the real time PCR (rtPCR) method. Main conclusions of this work were: a) Tissue culture is a

reliable method to identify infective viral particles but is time-consuming and limited for the detection of cultivable viruses. b) RtPCR is a faster and more sensitive method than the tissue culture method. A pretreatment using nuclease enzyme for the detection and elimination of incomplete viral particles is needed. However, the pretreatment cannot ensure that all particles detected are infectious. c) Even though the rtPCR can detect non-cultivable viruses, it is still limited for viruses chosen to be analyzed. The development of a fast and reliable method is critical to reduce both false negative and positive viral detection in processed batches of sludge. By extension, this will increase the safety of resulting fertilizer and significantly reduce public health risk.

4:30 Late Night Pixie Dust Scavenger Hunt - Toys, Tools and Tricks Learned

Donn Zuroski, FOOSC, Emergency Response Section, Region 9, US Environmental Protection Agency

Our full-scale, real time environmental sample collection response exercises are devised to test and improve our procedures for rapid sample collection, preparation, and delivery to the CDC/LRN lab for analysis. Our aim is make these exercises as real as possible. Down Range teams have included EPA, FBI, USCG, CSTs, and local law enforcement and response agencies. Sample teams wear Level C as per CDC guidance. Full decon is performed. Sample preparation follows CDC guidance. Chain of custody follows FBI procedures. This presentation will include an overview of the preparedness effort as well as a detailed look at how lessons learned from workshops, drills and exercises have been utilized in multiple real world responses.

5:00 Exhibitors and Sponsors Showcase Presentations and Concluding Discussion

5:45 End of Day One

Friday, October 19, 2012

8:00 Exhibit/Poster Viewing, Coffee and Pastries

9:00 Quantitative Nanomechanical Diagnostics – Direct Label Free Noncoding RNA Detection from Serum

Martin Hegner, PhD, Professor, Centre for Research on Adaptive Nanostructures and Nanodevices, Trinity College Dublin, Ireland

Nanomechanical sensing platforms for label-free qualitative and quantitative bio-analytical measurements provide high-sensitivity, fast and specific bio-assay results. We will present measurements in the field of genomic sensing. We demonstrate that cantilever array sensors are capable to track the pharmacokinetics of therapeutic siRNA molecules and the early detection of miRNA biomarker molecules, which indicate organ pathology measured directly from serum. A prerequisite for these online measurements with ultimate sensitivity are optimized buffer systems and differential read-out. These new platforms pave the way for ultra-sensitive, selective, fast portable nanomechanical diagnostic devices.

9:30 Development and Characterization of a Rapid Pathogen Capture System for Direct Isolation of Microorganisms from Blood

Sergey A. Dryga, PhD, MBA, Vice President of Immunochemistry, nanoMR, Inc.

Blood-stream infections are a serious healthcare problem in US and other countries. Sepsis is the 10th leading cause of death in the US. Early detection and identification of BSI, and administration of correct treatment significantly improves health outcomes and reduces cost of treatment. Current standard of practice for the detection of BSI is blood culture, which usually takes 12 - 48 hr for the detection of infection, and an additional 12 - 24 hr for organism identification. No rapid, direct-from-blood, molecular tests with the required sensitivity have been successful, because such tests use small sample volumes which are statistically unlikely to contain pathogens present at the 1 CFU/mL load found in bacteremia. nanoMR has developed a rapid pathogen capture system (nanoMR PCS) that allows for the capture of live microorganisms from up to 10 mL of blood in less than 30 min and provides DNA for subsequent analysis by PCR or other molecular methods. Initial evaluation of analytical performance indicates that the system has limit of detection close to 1 CFU/mL. Analysis of clinical samples suggests that the system has sensitivity adequate for real-life use, moreover, in several cases nanoMR PCS isolated bacteria from blood culture false negative specimens.

10:00 Highly Efficient, Automated miRNA Isolation Method Using Solid Phase Reverse Immobilization (SPRI) Technology and Biomek

Bee-Na Lee, PhD, Senior Application Scientist, Beckman Coulter

Micro-RNAs are small ribonucleic acids that play an important role in biological and cellular responses. We developed an automated, robust and efficient miRNA isolation method using Solid Phase Reverse Immobilization technology. The two methodologies described are (1) miRNA extracted from FFPE samples using the Agencourt FormaPure Kit and (2) miRNA extracted from cell culture using the Agencourt RNAdvance Cell v2 kit.

10:30 Networking Refreshment Break, Exhibit/Poster Viewing**11:00 Existing and Emerging Approaches for Pre-Analytical Sample Preparation in Food Microbiology**

Byron Brehm-Stecher, PhD, Professor, Rapid Microbial Detection and Control Laboratory, Iowa State University

Early and reliable detection of foodborne pathogens may be achieved, at least in part, through judicious application of existing or emerging sample prep approaches. Target microbes may be present in some foods in physically inaccessible niches, where effective diffusion of detection reagents may be limited. Other physical challenges may include sample bulk and/or low-level contamination with pathogens, high viscosity, fat or particulate content and the presence of other microorganisms, especially those that may be physiologically and genetically very similar to target cells. Physical structures such as biofilms may also present challenges to detection and must be disrupted for release of

individual cells or target molecules. Depending on the detection method used, foods may also present biochemical challenges. As an example, common food constituents such as divalent cations, fats, enzymes or structural proteins and plant polyphenols are often implicated as inhibitors of the polymerase chain reaction. We will provide an overview of the challenges inherent in pre-analytical processing of foods and will review existing and novel tools that can be leveraged to help researchers navigate the path from sample-to-result. Topics to be discussed will include new developments in cell capture reagents and alternative binders, novel solvent systems or "green" chemistry-based solutions, and the use of robust reagent systems or novel physical processes for minimizing the need for extensive sample prep using traditional methods.

11:30 Modification of PCR Sample Preparation to Delineate Live and Dead Foodborne Pathogens

Teshome Yehualaeshet, DVM, PhD, Associate Professor, College of Veterinary Medicine, Nursing and Allied Health, Tuskegee University

Pretreatment of the cells with DNA-intercalating chemicals can be of value with suppressing amplification of DNA from non-viable cells. Recently, a technique incorporating either *propidium monoazide* (PMA) or *ethidium bromide monoazide* (EMA) during the sample preparation steps was reported, in which chemicals selectively intercalate into DNA from dead cells (membrane compromised cells) but not DNA in intact live cells. As a result, amplification of DNA from dead cell will be blocked. Such techniques offer great promise in amplifying DNA extracted only from live bacteria by real-time PCR. Our lab elaborated the use of previously reported DNA-binding chemicals as well as we checked for 21 chemicals as alternate and find one chemical which is comparable to the previously reported compounds: The talk is part of our on-going research and will include: (a) The rationale and principle of PCR sample modification; (b) Explain the results of DNA-intercalating chemicals previously reported; advantages and disadvantages; (c) Explain the results of from the chemical what we worked with; advantages and disadvantages; (d) The experimental results of 5 foodborne pathogens using the previously reported and our chemical; (e) Future research directions to make PCR as a tool to delineate live and dead bacteria. The method presented will overcome the currently missing knowledge gap to detect only viable cells by PCR. The output of this novel approach will be extremely useful for the detection of only viable pathogens by PCR assay, which is demanding in food safety and other disciplines.

12:00 A Novel Membrane-Based System for Fractionation and Concentration of Biological Particles from Complex Environmental Matrices

Andy Page, President and CTO, InnovaPrep LLC

More powerful, automated sample preparation techniques are needed so problems currently associated with detection of rare biological particles in complex environmental samples can be overcome. Inhibition by environmental debris is a common problem due to the presence of highly-varied, complex mixtures of particle and chemical inhibitors. Ultra-high-throughput sequencing, qPCR, and other rapid detection techniques can also fail due to high levels of background environmental clutter. There is also a significant need to be able to differentiate between detection events that are associated with whole viable cells and those only associated with free DNA or free proteins. Ongoing development of a novel, integratable, membrane-based fractionation and concentration

system, under a Department of Homeland Security Phase II SBIR, is presented.

12:30 *Lunch on Your Own*

2:00 Simple, Non-Toxic, Automation-Compatible Nucleic Extraction Using A Thermophilic Proteinase

Jeffrey A. Hickey, PhD, Director of Reagents Development for Forensics, ZyGEM, Inc.*

ZyGEM's EA1 proteinase is a highly-active metallo-endo-proteinase isolated from a volcanic vent near Mount Erebus in Antarctica. With optimal activity at 75°C, EA1 proteinase requires limited chemical assistance in liberating nucleic acids from challenging samples, and in form that is PCR-ready. The ZyGEM method has been developed into macroscale kits (Eppendorf tubes) that have been validated for both forensic (swabs, blood, FTA cards, etc.) and research applications, and these are used worldwide for extracting of nucleic acids from a broad range of substrates. The ability to liberate DNA or RNA from crude samples in a PCR-ready manner means that it can be exploited directly for downstream PCR-based applications. This makes it ideal for integration in microfluidic systems where sample preparation is seamlessly mated with an analytical step for genomic analysis. *In collaboration with: D.Saul, A.Tsuej, I.Silber, ZyGEM; J.Lounsbury, U.Virginia; J.Landers, U.Virginia & MicroLab Diagnostics

2:30 Use of the KIM System for Rapid Bio-Detection and Analysis of BWA

Vanessa Yu, PhD, Field Scientist, Bertin Corporation

Bertin Technologies has developed a ruggedized bio-detection kit for military field use that allows for rapid analysis and identification of BWA such as toxins or bacteria in liquid or powder format. Antigen capture is facilitated by antibody grafted magnetic beads, and the application of a magnetic field to this immunoassay will decrease analysis time to 10 minutes. This also allows for highly sensitive detection (LOD<100 pM) and the omission of washing steps during sample preparation, which makes this process very user-friendly. The KIM can be integrated with our Coriolis biological air samplers and function together as a complete air sampling and bio-detection system.

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

3:30 Integration of PureLyse® Sample Preparation

Bruce Irvine, Chief Technology Officer, Claremont BioSolutions LLC

Claremont BioSolutions has created a novel method of rapid sample preparation that is entirely disposable; it requires no instrument. It provides mechanical cell lysis and simultaneously extracts nucleic acids or proteins. Nucleic acid can be extracted in three to five minutes with two to three steps. The disposable PureLyse® device is comprised of a micro-motor and vane that agitate particles at unusually high shear forces. As cells are lysed, nucleic acid binds to the lysing particles. The flow-through configuration of the PureLyse® cartridge enables its use for a wide range of sample volumes. 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*. This system delivers high yield RNA preparation as well. The miniature disposable nature of this flow-through cartridge lends itself to integration. Our flagship approach to integration is the OmniValve™ fluidic system, where we have embedded the PureLyse® chamber within a valve with up to six ports connecting the lysis and extraction cartridge to other chambers. This facilitates integration of DNA extraction with sample introduction, pre-filters, wash, elution, and amplification. This approach was very successfully applied to an NIH funded SBIR project developing a semi-integrated system for detecting *Clostridium difficile*, resulting in 100 % specificity and 96% sensitivity as compared to standard enzyme immunoassay of stool samples.

4:00 Mass Genotyping by Sequencing Technology

John Curry, Senior Scientist, Eureka Genomics

We demonstrate a next generation sequencing technology that genotypes 1000 DNA samples for 100 loci, a mass genotyping by sequencing technology (MGST). Barcodes contained within the ligated DNA hybridization probes create a highly multiplexed library that is sequenced on Illumina platforms. With MGST we have obtained 99.2% concordance for ~100 loci in 1080 *Bos taurus* samples that were previously genotyped using the Illumina 54K Bovine bead chip system.

4:30 Selected Oral Poster Highlights

5:00 *Concluding Remarks, End of Conference*

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