DIVISION OF ENVIRONMENTAL CHEMISTRY

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TUESDAY MORNING

Biological Detection and Surface Sampling for Biodefense

Cosponsored by AEESP and ANYL‡
M. G. Davenport, J. A. Rosati, and J. B. Morrow, *Organizers, Presiding*

8:30 — Introductory Remarks.

8:35 —42. New technology integration: Benefits of interlaboratory testing in DNA forensics. **P. M. Vallone**

9:05 —43. Antimicrobial peptides for detection of bacterial pathogens. **J. W. Soares**, S. H. North, L. A. Doherty, M. Slutsky, C. R. Taitt, C. M. Mello

9:25 —44. Effect of polyelectrolyte encapsulation on responsiveness of recombinant bacteria. **V. K. K. Upadhyayula**, D. M. Eby, S. Balkundi, Y. M. Lvov, G. R. Johnson

9:45 —45. Lanthanum-based concentration and microrespirometric detection of microbes in water. **Y. Zhang**, Z. Hu

10:05 — Intermission.

10:20 —46. Bioterrorism detection: Broad perspectives on sampling and detection. **S. R. Shah**

10:50 —47. Factors that determine the efficacy of different biological surface sampling methods. **J. W. Thornburg**

11:10 —48. Surface and bulk sampling to characterize the fate of *Bacillus thuringiensis* var. kurstaki (Btk) in urban environments. **K. M. Omberg**, S. Van Cuyk, A. Deshpande, A. Hollander, L. Ticknor, P. S. White

11:30 —49. Ambient aerosol characterization for improved understanding of bioaerosol diversity and fluctuation. **J. L. Santarpia**, D. J. Cunningham, J. U. Gilberry, S. Kim, E. E. Seay, S. A. Ratnesar-Shumate, J. J. Quizon

11:50 —50. Bioaerosol dispersion in a room: An experimental and computational study. J. Redrow, **J. A. Posada**, W. G. Lindsley, T. Pearce, I. Celik

ABSTRACTS

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New technology integration: Benefits of interlaboratory testing in DNA forensics

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The Human Identity project team at NIST has coordinated and participated in various interlaboratory tests over the past 15 years. Interlaboratory tests are a way for multiple laboratories to compare results and demonstrate that the methods or instrument platforms used in one's own laboratory are reproducible in another laboratory. This can also be applied to the area of evaluating new technologies. Parallels with microbial detection will be illustrated using an interlaboratory study for human DNA quantitation as an example.

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Antimicrobial peptides for detection of bacterial pathogens

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Our studies focus on discovering antimicrobial peptides

(AMPs) that discriminately bind target clinical organisms through rational peptide design, innovative immobilization, and high-throughput screening. Rationally designed/selected peptides include native AMPs, truncated AMPs (8-15 amino acids), peptide variants created by scanning alanine mutagensis, and AMP hybrids. The peptide candidates are covalently-attached to 96-well microplates through two approaches: site-directed and random. Both immobilization approaches facilitate high-throughput screening for discovery of peptides with discriminatory binding of target pathogen whole cells, lipopolysaccharide (LPS), and/or lipotechoic acid (LTA). Preliminary results suggest several peptide candidates preferentially bind LTA, while others preferentially bind LPS.

Furthermore, select candidates screened against *Staphyloccocus aureus* exhibit significant binding compared to others evaluated. The ability to discover first generation peptides exhibiting gram-specific binding behavior is a promising first step toward the use of peptides as molecular recognition elements in a biosensor platform array to detect target bacterial pathogens and biothreat agents in real-time.

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Effect of polyelectrolyte encapsulation on responsiveness of recombinant bacteria

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Electrostatic layer-by-layer (LbL) deposition of polyelectrolytes provides a method to build synthetic coats for encapsulation of materials, surfaces and even biological cells. In the present work, recombinant bacterial strains were coated with three different combinations of cationic and anionic polyelectrolyte polymers. Up to four bi-layers of the polyelectrolytes were used for surface modification. The recombinant strains carried plasmids encoding green fluorescent protein (GFP) that was either constitutively synthesized or the synthesis controlled by exposure to inducer molecules or treatments. Results of preliminary experiments demonstrated that the coatings did not eliminate fluorescence produced from the constitutive bacterial strain. Further experiments will define the responsiveness of coated cells to various environmental conditions. The modification may fortify the bacterial cells and help develop a robust system that may serve as an environmental biosensor.

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Lanthanum-based concentration and microrespirometric detection of microbes in water

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Lanthanum chloride was used to concentrate *E. coli* in water compared with traditional flocculants (ferric sulfate and aluminum sulfate). Turbidometric assay and microrespirometric assay were developed to enumerate the bacteria in water samples by monitoring the absorbance of bacteria and the fluorescence-based oxygen concentration. The results showed that turbidometric assay and microrespirometric assay can accurately enumerate the bacteria with the concentration of 10 - 10^9 cells/mL. Based on turbidometric assay, concentration efficiencies of three flocculants was 75% ($LaCl_3$), 40% ($FeCl_3$) and 33% ($Al_2(SO_4)_3$), while recovery efficiencies were 94%, 69% and 51%, respectively. From microrespirometric assay, concentration efficiencies were 85% ($LaCl_3$), 34% ($FeCl_3$) and 32% ($Al_2(SO_4)_3$), while recovery efficiencies were 43%, 52% and 30%, respectively. The results show that compared with traditional flocculants, $LaCl_3$ had the highest microbial concentration efficiency. Recovery efficiencies were lower when using oxygen-based microrespirometry, indicating that flocculation processes may have some negative impact on microbial activity.

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Bioterrorism detection: Broad perspectives on sampling and detection

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Significant progress has been made in the preparedness for defense against bioterrorism events since the 2001 anthrax spore-contaminated letter incidents. This has been possible due to aggressive efforts by different government agencies, industries, and academia. As per various Homeland Security Presidential Directives (HSPDs) and under the umbrella of the Integrated Consortium of Laboratory Networks (ICLN), roles and responsibilities of different government agencies have been defined for a coordinated response to bioterrorism incidents. To address the environmental side, the EPA is responsible for water and water infrastructure, and remediation for buildings and other infrastructures (indoor and outdoor). This entails determination of extent of contamination, decontamination, and post-decontamination sample analysis. Along with robust bioagent detection technologies and analytical methods, the overall sampling strategies and uncertainties in sampling methodologies must be determined in order to establish confidence in the reported results. Many efforts are underway to increase the confidence in our ability to detect potential biological contamination events and understand the challenges of integrating sampling methodologies with detector performance parameters. Some of the successes and challenges will be discussed.

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Factors that determine the efficacy of different biological surface sampling methods

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Selection of the best biological sample collection method from surfaces depends on the surface and biological particle characteristics. First, the need for qualitative (presence/absence) or quantitative (spore per unit area) data is needed. Then, potentially contaminated surfaces need to be identified. The type of biological release, location, and time elapsed are important factors. The interaction between the type of biological particle released and the characteristics of the contaminated surface are important. Chemical and physical attributes of the particles and surfaces determine the magnitude of the adhesion force. The van der Waals, electrostatic, and capillary forces work in conjunction with asperities on both surfaces to determine the total adhesion force. The sampling method selected should consider the influences of these factors on the efficacy of the different sampling methods available. This presentation will summarize findings from multiple projects to present an integrated biological surface sampling strategy accounting for these factors.

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Surface and bulk sampling to characterize the fate of *Bacillus thuringiensis* var. kurstaki (Btk) in urban environments

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Los Alamos National Laboratory (LANL) has conducted field experiments in Seattle, WA and Fairfax County, VA to study agent fate in urban environments for the Interagency Biological Restoration Demonstration. As part of their gypsy moth suppression efforts, Washington State and Fairfax County have sprayed *Bacillus thuringiensis* var. *kurstaki*, a common organic pesticide with properties

similar to *Bacillus anthracis*, for decades. Many of the spray zones have been in or near urban areas. LANL has collected and analyzed more than 1500 surface and bulk samples from historical Seattle spray zones to characterize how long Btk persists at detectable levels in the environment, and how long it remains viable in different environmental matrices. In conjunction with Fairfax's 2008 spray efforts, LANL has also collected more than 1250 surface, bulk and air samples. Results indicate Btk remains viable in the environment for at least four years, and is most easily detected in bulk (soil or water) samples.

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Ambient aerosol characterization for improved understanding of bioaerosol diversity and fluctuation

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Time-resolved characterization of the ambient aerosol background, including bio-aerosols, is important both for understanding environmental processes that affect biological aerosols and for determining realistic test conditions for the evaluation of bioaerosol detection systems. There exist very few data-sets with the resolution to meet the testing needs of new biological agent detection systems and test facilities or to provide an understanding of the temporal fluctuations in aerosol concentration and bioaerosol concentration. To provide a more comprehensive view of the ambient aerosol environment, several complementary techniques were used to measure the aerosol. Aerosol concentrations and size distributions were measured from 0.014 to 32 microns, bioaerosol concentrations were measured *via* laser-induced fluorescence, and meteorological data were recorded. Collected aerosol samples were cultured for viable, aerobic bacteria and fungi, and were screened for 16S rRNA diversity using microarray analysis. These data indicate that meteorological conditions, growth processes observed in submicron aerosol and the source of the air mass may affect the concentration and diversity of the biological aerosol in an environment.

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Bioaerosol dispersion in a room: An experimental and computational study

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The dispersion of cough-generated bioaerosols was studied in an environmental chamber using a system that reproduces a typical human cough. Five aerosol optical particle counters were used to measure the number of particles and their size distribution over time after each cough. The chamber experiments were numerically simulated using the commercial CFD code, Ansys/Fluent. Particles were tracked using Lagrangian and Eulerian methodologies. Results from the models were compared to the experimental data to assess the performance of each method. The best-performing models were then used to create a numerical simulation of air and particle movement in the waiting room and examination rooms of the West Virginia University Urgent Care clinic. The flow field predicted by the simulations was used to estimate the best locations for bioaerosol sampling during a subsequent study of airborne influenza transmission.