

Large-Volume Sample Preparation for Waterborne Pathogens

Workshop Summary Report



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Large-Volume Sample Preparation for Waterborne Pathogens Workshop Summary Report

**U.S. Environmental Protection Agency
Andrew W. Breidenbach Environmental Research Center, Cincinnati, OH**

April 4 and 5, 2006

SPEAKER LIST

Kevin Oshima, U.S. Environmental Protection Agency

“Characterization and Optimization of Ultrafiltration Processes for the
Efficient Recovery of Waterborne Pathogens”

Vincent Hill, U.S. Centers for Disease Control and Prevention

“Ultrafiltration for Simultaneous Recovery of Diverse Biological
Agents in Drinking Water”

Otto D. Simmons, University of North Carolina

“Improved Methods for Concentrating Microbial Pathogens from
Large Volumes of Water”

Jennifer Cashdollar, U.S. Environmental Protection Agency

“Reuse of Electropositive Cartridge Filters for Concentrating Viruses from Water”

Mark Borchardt, Marshfield Clinic Research Foundation

“Concentration of Enteroviruses, Adenoviruses, and Noroviruses from Drinking
Water with Glass Wool Filters”

Fred Tepper, Argonide Corporation

“High-Volume Sampling and Concentration”

Fu-Chih Hsu, Scientific Methods, Inc.

“Concentration of Viruses from Water Using Functionalized Silica Beads”

Jim Larkin, Scientific Methods, Inc.

“Continuous-Flow Centrifugation of Pathogens and Indicators from
Large-Volume Water Samples”

Suresh D. Pillai and Ali Beskok, Texas A&M University

“A Microfluidic Approach to Capturing and Concentrating Organisms from
Large Sample Volumes”

Mike Kent, Sandia National Laboratory

“A High-Volume Alternative to Filtration Based on Adsorption and Elution”

Blake Simmons, Sandia National Laboratory

“Design, Fabrication, and Testing of Polymeric Microfluidic Separators and
Concentrators Utilizing Insulator-Based Dielectrophoresis”

Mike Carpenter, Idaho National Laboratory

“Making a Lab Process Field Portable”

Large-Volume Sample Preparation for Waterborne Pathogens Workshop Summary Report

At the U.S. Environmental Protection Agency's (EPA) workshop entitled "Large-Volume Sample Preparation for Waterborne Pathogens," leading scientists from across the country gathered in Cincinnati, Ohio, on April 4 and 5, 2006, to discuss a wide variety of techniques for capturing and concentrating waterborne pathogens. Experts gave 12 presentations on a range of topics, including various filtration methods, continuous flow centrifugation, microfluidic devices that use electrophoresis or dielectrophoresis, and field-portable water concentrators.

Welcome and Workshop Objectives

Opening

Eric Bissonette, EPA

The conference began with an opening statement from Mr. Eric Bissonette, Deputy Director of the Technical Support Center of the Office of Ground Water and Drinking Water (OGWDW), in which he welcomed the workshop participants.

Workshop Background and Objectives—ORD's Perspective

Ann Grimm, EPA

Dr. Ann Grimm, Chief of the Biohazard Assessment Research Branch of the National Exposure Research Laboratory (NERL), explained the need for the workshop. Specifically, occurrence methods are critical to EPA because they allow the Agency to obtain information on the presence and concentration of waterborne pathogens. Such information is needed to determine the risk associated with these contaminants. In addition, these methods are important for determining the effectiveness of treatment methods.

Over the past several years, there have been dramatic improvements in the quality of detection methods for waterborne pathogens because of significant technological advances. For instance, there are now molecular methods that can detect waterborne pathogens in less than two hours. However, technologies often focus only on a single class of pathogen and can be both expensive and time consuming. Although more research is needed in the area of pathogen detection, overall, this component of occurrence methods is progressing rapidly. In contrast, the sample collection part of these methods has lagged behind. Although significant progress has recently been made in the technologies used for sample collection and concentration, much still needs to be done to incorporate these improvements into complete pathogen detection methods. EPA, therefore, is considering where it should invest its funding and research efforts in this area. Ultimately, the goal is to develop a method that can concentrate a variety of pathogens; has good recovery; and is rapid, relatively inexpensive, and easy to use.

Workshop Background and Objectives—OW's Perspective

Sandhya Parshionikar, EPA

Dr. Sandhya Parshionikar of the Technical Support Center of OGWDW, elaborated on Dr. Grimm's comments, providing further background for the workshop. The Safe Drinking Water Act, enacted in 1974, mandates EPA to set national health-based standards for drinking water to protect consumers from contaminants. This includes the identification of new contaminants that may require regulation in the future. The Contaminant Candidate List (CCL) is the Agency's list of priority unregulated chemicals and microorganisms. This list, which includes several pathogens, is used to prioritize research and data collection efforts to determine if specific contaminants will need to be regulated. Data collection, which includes gathering accurate information on occurrence, disinfection properties, health effects, and dose response, requires robust detection methods for the pathogen of concern. Also, although alternate approaches exist, such as the use of indicators, they can be limited in their ability to accurately predict the presence of pathogens. Furthermore, other EPA-hosted workshops, held to determine the usefulness of molecular techniques in solving environmental problems, identified the pathogen capture and concentration methods as the bottleneck to detection. Low recovery efficiencies, high variability in recovery efficiencies, high costs, and the inability to simultaneously sample for all classes of pathogens, such as viruses, bacteria, and protozoa, are some of the major challenges to water sampling. This served as a crucial impetus for the workshop in Cincinnati. Therefore, the workshop objectives were to:

- summarize and evaluate the state of the science,
- identify research gaps in technologies useful for EPA, and
- support research in those technologies that can be useful for EPA in the near future.

Day 1: Invited Speaker Presentations

Characterization and Optimization of Ultrafiltration Processes for the Efficient Recovery of Waterborne Pathogens

Kevin Oshima, EPA

Dr. Kevin Oshima, formerly of the Department of Biology at New Mexico State University, described the challenges in trying to concentrate multiple pathogens from groundwater, surface water, drinking water, sewage effluent, and other water matrices. One of these challenges is the difficulty in obtaining consistent recoveries from water sources with differing water quality parameters. Therefore, a goal of this research has been to try to develop a methodology or process that can concentrate a range of contaminants including viral agents, bacterial agents, protozoans, and toxins.

For multiorganismal recoveries, ultrafiltration and microfiltration are well-established methodologies. There are significant differences between ultrafiltration and microfiltration; for instance, ultrafiltration can retain even the smallest viruses, whereas microfiltration usually stops at the ability to trap, remove, or retain bacterial agents and protozoa. Dr. Oshima's group has focused on researching ultrafiltration, in particular, the development of a rapid, user-friendly method. In one study, virus recoveries between small-scale and large-scale 50,000 molecular weight cutoff, hollow-fiber ultrafiltration systems was determined using spiked water samples. The recoveries of virus, bacteria, and *Cryptosporidium parvum* using this 10-L system were also determined. Consistent recoveries among viral, bacterial, and protozoan agents were obtained using this simultaneous approach.

In addition, Dr. Oshima has determined that:

- Ultrafiltration methods have been developed for multiorganism concentration from ground, surface, and drinking water that have greater than 50% recovery, except for *Cryptosporidium* oocysts.
- Ultrafilters can be reused at least 30 to 40 times without loss of performance.
- Pretreatment of the filters appears feasible and it can be done overnight. Once pretreated, the filter can either be used immediately or stored for up to one month.
- Concentrations of 100-L samples down to 15 to 20 mL can be completed in less than three hours.

In the future, his group plans to do additional work that includes completing final optimization of 100-L concentration procedure with low pathogen concentrations, and testing new concentration methods with a variety of environmental water samples; not just drinking water.

Ultrafiltration for Simultaneous Recovery of Diverse Biological Agents in Drinking Water

Vincent Hill, U.S. Centers for Disease Control and Prevention (CDC)

The CDC became interested in ultrafiltration because of its duty to respond to suspected bioterrorism events and waterborne disease outbreaks. In particular, Dr. Hill has been interested in finding techniques to detect biothreat agents in drinking water distribution systems.

The group's main goal has been to establish a single sampling technique for total microbe capture. A short response time is critical, as is high recovery efficiency. In addition, the technique needed to be field deployable and compatible with culture-, polymerase-chain-reaction- (PCR), and immunodiagnostic-based detection methods.

Dr. Hill described the filtration processes, provided a schematic for an ultrafiltration method, and outlined the secondary concentration techniques his group used. Among his project's results, a 50% recovery generally was achieved using just ultrafiltration (he did not incorporate secondary concentration data into his talk) for vegetative bacteria, bacterial spores, viruses, and parasites. The use of polyphosphate improved the recovery of most microbes but had negative effects on culturability for one type of bacteria (but not on vegetative bacteria in general); the group has been using divalent cations and organic media to counteract the effect.

In his work with low seeding levels, he detected pathogens consistently at concentrations of as low as 100 microbes per 100 L using PCR in conjunction with sample collection.

The processing times were 1.5 to 2 hours for ultrafiltration, 1 to 2 hours for secondary concentration, and 2 to 2.5 hours for nucleic acid extraction and real-time PCR. Consequently, the entire method could be completed in 4.5 to 6.5 hours, which was the group's goal for a rapid method.

The costs totaled about \$60 to \$80 per sample for single-use consumables. The system could be field deployable, making it effective for groundwater-related outbreak investigations. With this method, his group has detected various viruses, bacteria, and parasites in contaminated groundwater.

Improved Methods for Concentrating Microbial Pathogens from Large Volumes of Water

Otto D. Simmons, University of North Carolina

Water quality is a significant concern because it is necessary for public health, and it is very important for there to be consumer confidence in the public water supply. In addition, with regard to microbial contaminants, there are societal and economic costs associated with microbial disease outbreaks, as evidenced in the loss of worker days and higher health care costs.

For more than 100 years, the primary way of assessing the microbial quality of water has been to look for indicator organisms. As detection methods for pathogens have improved, the question becomes whether it is better to use direct pathogen detection methodologies. To determine the risk posed by waterborne organisms, there are two possible approaches. One option is to be

reactive, as in doing retrospective epidemiological studies or by acting in response to ecological problems. In contrast, the second option is to take the proactive approach, which is to promote public health by monitoring source and finished waters or by improving biosecurity in response to potential terrorist events through the use of improved concentration, isolation, and detection methodologies for the pathogens of greatest concern. In order to implement a proactive approach, Dr. Simmons believed that direct pathogen detection would be necessary.

In joint research with Professors Mark Sobsey and Jan Vinje, it was determined that there were statistically significant differences in murine norovirus recovery between the Cuno Virosorb 1MDS pleated cartridge and 90 mm flat-disk filters. Overall recovery was 2.5% for cartridge filters and about 19% for flat filters. In these experimental trials, deionized reagent water was comparable to tap water. These comparisons are important to characterize because the smaller filters are less expensive and can process smaller water samples. This would be important when there are limited volumes of suspect water, such as from a retrospective outbreak investigation. If larger volumes of sample are available, it generally is advantageous to process these larger volumes, thus lowering the overall method detection limit and improving the probability of detecting viruses in the waters sampled.

Additional work by Dr. Simmons focused on alternative eluting solutions for 1MDS filters, specifically, ones made with arginine, asparagine, glycine, or lysine. These alternate elutants are advantageous because they are more defined and do not inhibit downstream virus detection methodologies, such as molecular methods. He saw several advantages in the Cuno Virosorb 1MDS filter method for microbial concentration. For instance, for the current EPA method to detect virus in water, no pretreatment of water is necessary prior to filtration, the filters are capable of processing large volumes of water in short periods of time, and there are multiple filter formats for different types and volumes of water. However, there are numerous disadvantages of the 1MDS filter, including the fact that they are very expensive and have demonstrated variable recoveries with different groups of viruses and water quality parameters. Also, humic and fulvic acids can interfere with virus adsorption to and elution from the surface of the filters.

Dr. Simmons has also been investigating the use of hollow-fiber ultrafiltration (HFUF) because this method is capable of concentrating multiple classes of pathogens using a single filter, which is inexpensive and made to the high standards of the medical industry. He also recognized these filters limitations, which include longer sample processing times and potential downstream problems during secondary concentration steps (they concentrate everything, including inhibitors that may interfere with molecular detection methodologies). To address the first issue, he hoped to develop ideas to decrease sample processing times. As for the second issue, he hoped to demonstrate that initial HFUF and secondary concentration steps are compatible with molecular- and cell-culture-based detection methods.

He concluded by noting that he and Mark Sobsey were recently awarded an EPA Star Grant, which gives them the funding to develop a new charge-modified filter during the next two years. They will then compare the developed filter's use with the currently used Cuno Virosorb 1MDS filter in a round-robin study with other laboratories familiar with the current method. This will ensure that the technology can adequately be transferred and will demonstrate that the new system works independently in other laboratories and with a variety of water types.

Reuse of Electropositive Cartridge Filters for Concentrating Viruses from Water

Jennifer Cashdollar, EPA

Ms. Cashdollar, who collaborated with EPA scientist Daniel Dahling on this project, identified the need for a robust method for virus recovery from large volumes of water that would result in high virus recovery while being cost effective and simple, so that most laboratories can perform it. Ideally, a desirable filter method would be one in which the testing laboratory would take the filter out to the field, hook it up, and walk away while the sample was being collected. She noted the advantages and disadvantages of electropositive filters, such as the 1 MDS filter used by the Agency to collect viruses. The biggest disadvantage is the cost of these filters (US \$150-\$180 each). This was one of the driving forces for investigating whether the filters could be used numerous times. She described previous scientists' regeneration of pleated electronegative filters and her group's attempt to adapt the method for use with electropositive cartridge filters. As data was being collected on filters that were treated and reused, it was noted that recovery efficiencies were comparable between filters that were treated (with 0.1 M sodium hydroxide) and those that were new. A new set of filters were then tested that were not treated prior to reuse to ascertain whether treatment was even necessary.

In the study, several factors were found to be statistically significant in virus recovery: water type (whether it was tap water or river water), filter type, and the volume of water filtered (although it was important to note that all 20-L volumes of water were tap water, which was statistically significant). There was no statistical difference in recovery between new filters, filters that were treated, or filters that were untreated. In addition, filters could be used one to three times without a statistically significant impact on percent recovery.

Concentration of Enteroviruses, Adenoviruses, and Noroviruses from Drinking Water with Glass Wool Filters

Mark Borchardt, Marshfield Clinic Research Foundation

Dr. Mark Borchardt, his colleagues from the Marshfield Clinic, and researchers at the University of California-Davis, have been involved in a collaborative project that makes use of a novel glass wool filter. Dr. Borchardt noted that his group did not develop the glass wool filter method being used in this study, although the technique was validated in his laboratory. The impetus for the work came from a large groundwater epidemiological study called the Wisconsin Water and Health Trial for Enteric Risk (WAHTER) Study.

The goal of the project was to estimate the amount of illness occurring in children who drank municipal water served by a groundwater source. A secondary objective was to determine the source of the viruses in the communities.

One of the problems faced by the study was the cost of virus sampling. Specifically, there were 14 communities involved in the study, and for each, 12 samples needed to be taken every month for a year. Because each filter cost \$170, and the university charged a 50% indirect rate, the total filter cost would have been \$514,080. In contrast, it was determined that the total cost of glass wool filters for the project, including assembly labor plus the indirect cost, came to \$25,636. He

therefore adopted the glass wool filter method for the study. To evaluate the method, his group looked at the following variables: virus type, water matrix, pH, flow rate, virus seeding level, and filter size. Their goal was to concentrate the EPA CCL viruses at a flow rate of 4 L per minute.

When looking at factors important for glass wool recovery, his group had to use a linear mixed-effects model because of the day-to-day variation seen with this method. He found that flow rate and volume filtered, among other things, did not have a statistically significant impact on recovery. Variables that were significant for the glass wool method included virus type, water matrix, and the interaction between these two factors. Also, the pH of the sample is crucial for glass wool recovery, with high pH being associated with poorer recovery.

He emphasized the advantages of glass wool. The cost of the materials for one glass wool filter can be divided into a one-time cost of \$3.65, and an expendable cost of \$0.75. As for the labor for constructing glass wool filters, his estimate is 15 minutes per filter.

Dr. Borchardt concluded that the recovery efficiency of the glass wool method ranged from 8% to 98%, and that it depended on virus type and water matrix. Additionally, it was determined that the water pH must be less than 8.0 for effective concentration, and that it was possible to do continuous-time composite sampling. In summary, glass wool filtration is a cost-effective method for concentrating viruses from large sample volumes.

High-Volume Sampling and Concentration

Fred Tepper, Argonide Corporation

Mr. Tepper introduced attendees to another kind of filter called the NanoCeram® filter, which is relatively inexpensive and good at concentrating viruses out of water. Argonide, founded in 1994 to develop nanotechnology products, sells NanoCeram® filters, whose active ingredient is a highly electropositive nanoalumina (aluminum oxide hydroxide) fiber of only 2 nm in diameter. These nanoalumina fibers are distributed on the scaffolding of another fiber, e.g., glass. The filter media is manufactured using paper-making technology. During the several manufacturing steps, the nanofibers are dispersed and adhere to glass fibers.

For NanoCeram® filters, flow rates (flux) were determined to be ten-fold or greater than ultraporous membranes. Furthermore, NanoCeram® separates particles by charge rather than size and has higher retentivity for virus than “absolute” ultraporous membrane filters or other electropositive media. Additionally, filtration efficiency for micron-size particles exceeds 99.995%.

This filter was found to work well with matrices that contained a significant amount of particulates. For instance, when used with water samples, the filter was able to function in a muddy stream. In addition, the filter was very effective for removing viruses—at least at sampling levels—and bacteria. It operates effectively over pH 5-9.5 and in the presence of salt water.

Right now, the company’s principal thrust is the manufacture of filters as primary adsorbers (point of use at a faucet, point of entry for a whole house, and as a prefilter to extend the life of reverse osmosis membranes). The filters are being developed for portable (bottle) filter systems,

and the company is looking at biological warfare filters for the military, for hospitals, and for other critical buildings.

In conclusion, the NanoCeram® filter is formulated specifically for water and can effectively filter parasites, bacteria, and virus. Additionally, large-pore NanoCeram® filters are superior to HEPA filters for retention of dry aerosols, as well as of microbe-containing aerosols. It has major promise as an air sampling media. Microbes can be eluted from the media, although elution is far less efficient than the adsorption step. Finally, a novel multistage adsorption/desorption process for concentrating virus was described. He said that they demonstrated an enhancement factor of 2700 in one stage and a projection of 10^6 for two stages. A third stage may be needed to get from 100 L of sample down to microliter levels.

Note: In May 2008, Mr. Fred Tepper provided the following update on the NanoCeram® filter. NanoCeram® VS2.5-5 virus filters have been tested in many countries and found to exceed the performance of 1MDS sampling filters. Three U S groups, including the EPA, are developing methodology for sampling of virus in fresh and sea water. International R& D teams in Korea, the U K, Canada and France are utilizing existing extraction EPA-developed protocols and also modifying them for different viruses using NanoCeram® samplers. The main benefits of the NanoCeram unit are its much lower cost than the 1MDS, its capability to be used in alkaline water (without requiring pH neutralization), its utility in sea water and the reduction of complexity in extraction.

Concentration of Viruses from Water Using Functionalized Silica Beads

Fu-Chih Hsu, Scientific Methods Incorporated

Today there are many more tools available to collect and concentrate pathogens (including hollow-fiber ultrafiltration, nanoceramic filters, and glass wool filters) than there were in the past. Dr. Hsu described an alternative approach to sample concentration, which involved the use of functionalized silica beads to capture and remove viruses from water.

The goal of this project was to develop an electropositive silica matrix using functional chemical groups to characterize the virus adsorption properties of each silica matrix using model viruses, to develop an optimized surfactant-based elution buffer, and to validate the adsorption and elution procedures for sampling large volumes of tap water.

Silica beads had many properties that may make them especially useful for pathogen collection. For instance, silica chemistry is well established, a diversity of functional chemical groups is available to attach to the surface of the silica beads, and silica beads provide good flexibility and diversity because they are not uniform in size and shape. His group conducted many experiments, such as evaluating the capture and elution efficiency of bacteriophage MS-2 by prototype silica beads. For that, the capture efficiency was very good (reaching 100% for several types of functionalized silica beads, including APSI, APSII, and SLA), although elution reached only 9%. However, as for the capture and elution of bacteriophage PRD-1 by prototype silica beads, the best results were with APSII, which obtained an elution of 69.5%.

As a result of this work, the researchers concluded that silica beads could be functionalized with an assortment of chemical groups, and higher zeta potentials were associated with higher virus

capture efficiency. Combinations of functional group chain length and density yielded capture efficiencies exceeding 95%. Functionalized silica beads could be “tuned” to achieve high efficiency concentration for different virus groups. Thus far, his group tested only MS-2 and PRD-1 but plans to test other viruses.

Furthermore, it was observed that overall recoveries of viruses from functionalized DPS silica beads range from 20% to 90% with alkaline beef extract or amino acid eluants (beef extract, amino acids, and surfactant) and coliphage MS-2 could be recovered with efficiencies of 70% to 80% from 20-L seeded volumes. The Scientific Methods group will continue to optimize the functionalized silica beads and try to optimize the column geometry for large-volume samples. This will include studying the column size, length, diameter, and flow rate.

Continuous Flow Centrifugation of Pathogens and Indicators from Large-Volume Water Samples

Jim Larkin, Scientific Methods Incorporated

Dr. Larkin first noted that centrifugation is actually a very old and basic technique, developed as a blood separation method called “blood fractionation”, by Jack Latham of Arthur D. Little more than 50 years ago. For CFC, the company Haemonetics has been central in developing this technology. One significant modification was the development of a disposable plastic bowl to be used for CFC. Dr. Larkin then noted other innovators in environmental microbiology who have worked with centrifugation, including Jakubowski in 1982, Borchardt in the mid-90s to 2001, and Tzipori in the mid-90s.

One important issue being addressed at the workshop is the fact that different matrices have an impact on these different collection and concentration techniques. Another important issue is the need for multiple pathogen concentration. Dr. Larkin showed an example of *Giardia*, *Cryptosporidium*, and *Microsporidia* being concentrated from the same samples by CFC.

He drew numerous conclusions about portable continuous-flow centrifuge (PCFC), including that PCFC can concentrate protozoa from large volumes of water (10 to 1,000 L). PCFC also can concentrate organisms from different types of water (i.e., finished, groundwater, surface water, secondary wastewater), and it can concentrate multiple species of pathogenic protozoa.

As a result of his experiments, he concluded that it is possible to concentrate multiple microorganisms using CFC. *Cryptosporidium*, *Giardia*, bacteria, and algae can be concentrated inside the CFC bowl. Additionally, viruses can be captured and eluted by positively-charged filters after CFC.

In the future, Dr. Larkin’s group intends to concentrate multiple pathogens with CFC by looking at the following: large-volume water samples, continuous monitoring for pathogens and indicators, sampling a variety of water types, capturing rare organisms that occur at low densities, and investigating oligotrophic waters.

Day 2: Invited Speaker Presentations and Panel Discussion

Summary of Previous Presentations

Ann Grimm, EPA

Dr. Ann Grimm provided an introduction for workshop attendees for the second day of the meeting. She noted that in the workshop's first day there were interesting discussions pertaining to ultrafiltration, the 1MDS electropositive filter and the potential for reusing the filter, glass wool filters, nanoceramic filters, silica beads, and CFC. In the second day, discussion would focus on microfluidics, alternatives to filtration, the iDEP technology, and field portability. She observed that the talks have shown that the field has been advancing, and there appear to be potential opportunities for EPA to invest and collaborate.

A Microfluidic Approach to Capturing and Concentrating Organisms from Large Sample Volumes

Suresh D. Pillai and Ali Beskok, Texas A & M University

At the time of the conference, Dr. Suresh D. Pillai and Dr. Ali Beskok of the Texas A&M University Bio-Micro-Fluidics Laboratory were completing an 18-month project funded by the National Aeronautics and Space Administration. Dr. Pillai began the presentation by describing the importance of concentration. Robust technology able to collect and concentrate a range of pathogens is needed for water quality monitoring. It is also vital that it be compatible with current and future detection technologies, e.g., PCR, biosensors, arrays.

In a clinical sample, it is much easier to detect an organism because the numbers of organisms per unit sample are much higher, whereas, in a water sample, the concentration of organisms is often only between 1 and 10 organisms per liter. Therefore, to develop a very robust system it is necessary to understand the first principles of how organisms attach to matrices. Until this is done, everything else is simply observation. If only recovery and capture efficiencies are considered, it might not be possible to understand why a system might fail. Consequently, some components of this work are outside the core competencies of microbiologists, and so engineers and physicists could play a critical role in developing effective collection and concentration methods.

Dr. Beskok then discussed the use of electrophoresis in pathogen isolation. His group looked into the concentration of bacteria and viruses using this technology, noting that these organisms mostly are negatively charged, even over the wide range of pH seen in various water matrices. Consequently, the group decided to use electrophoretic transport in a microfluidic channel to transport microorganisms toward electrode surfaces and to capture the microorganisms using electrostatic mechanisms.

Finally, Dr. Pillai noted that even if an organism is captured on a positively charged surface, eluting the organism can be a challenge. He therefore is trying to embed sensors into the surface, integrating sensors with capture. His group wants to optimize this real-time detection on the

capture unit. Also, his group recently signed a working agreement with Luna Innovations, which is working with off-the-shelf detection sensors. The group plans to, among other things, explore terahertz sensors to detect the capture of organisms.

Note: In May 2008, Dr. Suresh D. Pillai and Dr. Ali Beskok provided the following publications:

Pillai, SD., Beskok, A., Balasubramanian, AK., and Soni, KA. 2006. A microfluidic device for capture and concentration of microorganisms in recycled water. *Habitation* 10: 224.

Balasubramanian, AK, Beskok, A., and Pillai, SD. 2007. *In-situ* analysis of bacterial capture in a microfluidic channel. *Journal of Micromechanics and Microengineering* 17:1467-1478.

Balasubramanian, AK., Soni, KA., Pillai, S.D. and Beskok, A. 2007. A microfluidic device for continuous capture and concentration of pathogens from potable water. *Lab on a Chip* 7:1315-1321.

Soni, KA., Balasubramanian, AK., Beskok, A., and Pillai SD. 2008. Zeta potential of selected bacteria in drinking water when dead, starved, or exposed to minimal and rich culture media. *Current Microbiology* 56: 93-97.

A High-Volume Alternative to Filtration Based on Adsorption and Elution ***Mike Kent, Sandia National Laboratory***

Dr. Kent's project involved the development of a method for concentrating bacteria and viruses from streams. It was done with the support of a biosensor developed at Sandia by other groups. The handheld sensor can detect a range of different organisms within 15 minutes, and it is based on protein fingerprinting. The goal of the project was to detect 10^3 cells/mL by preconcentrating to 10^7 cells/mL, but, after listening to the workshop discussion, it was apparent that this is a relatively concentrated level of pathogens, and that participants are interested in detecting 1 cell per liter. This method had to be portable, low cost, low power, and consistent with the detection scheme, so that no proteins could be introduced. For this work, two approaches were pursued. One was to use temperature-responsive polymer, PNIPAM, to reversibly block and open pores in a column or a micro capillary. The other—the focus of his talk—was to adsorb bacteria and viruses from solution onto a high-surface-area media through nonspecific interactions and release by elution. To do this, he used glass beads that generally ranged from 300 to 400 μm . Dr. Kent noted that his group chose to use hydrophobic surfaces to bind the pathogens, placing emphasis on looking for chemical agents that maximized the efficiency of elution. He found one of the best elution agents to be sodium dodecyl sulphate (SDS). Dr. Kent indicated that SDS can be disadvantageous in that it lyses vegetative cells, but that this was not an issue for his group because the cells have to be lysed anyway in their detection method, which is based on protein fingerprinting (although that made it difficult to count). He added that the group has found efficient elution agents similar in structure to SDS that do not lyse the cells, and these are based on lipids (where one can use plate counts).

Dr. Kent's group spent a lot of time doing flow cell experiments looking at the efficiency of release of cells from the surface. They examined two organisms: (1) *Bacillus atrophaeus* and (2) *Chattonella marina*, and most of the work was with SDS, using nucleic acid extraction and elution with SDS. The capture for the two organisms in the vegetative state has generally been in the range of 70% to 90%. Other experiments, including those with spores of *Bacillus atrophaeus*,

showed that they routinely got elution peaks that were several orders of magnitude above background. Recovery efficiencies varied from 50% to below 10%, which are low values compared to results reported in Day 1 of the workshop. He closed by noting that he has done elution experiments with lipids, and they obtained efficient elution, but again, had low recovery efficiencies. In sum, with hydrophobic surfaces, one can maximize the elution efficiency and adsorption for a couple of species. Flow rate was not extensively examined.

Design, Fabrication, and Testing of Polymeric Microfluidic Separators and Concentrators Utilizing Insulator-Based Dielectrophoresis (iDEP)

Blake Simmons, Sandia National Laboratory

Sandia National Laboratory has been conducting research on insulator-based dielectrophoresis over the past five years. The motivation for this work has been the need for pathogen monitoring, which is a global health and security issue. There has been a growing interest, both from a homeland security standpoint and from a water quality monitoring standpoint, to be able to effectively monitor large volumes of water for very low concentrations of organisms—ideally one organism per liter. Traditional water analysis methods typically involve lengthy steps, such as culturing or mechanical filtration, and there are a lot of problems associated with the recovery and efficiency of those systems. The need for the selective concentration, as well as sensitive detection of pathogens, is therefore very great. However, there is usually an inverse relationship between selectivity and sensitivity. Thus, the question is, how can a system that provides both sensitivity and selectivity be engineered?

Sandia National Laboratory has a number of multiuse technologies relevant to water monitoring (examples include a Liquid microChemLab and a Gas microChemLab). In this presentation, he focused on Sandia's biological preconcentrator; it is an insulator dielectrophoresis to sort and preconcentrate bacteria.

The goal of his group was to develop a selective concentrator with significant flow rates. He noted that dielectrophoresis is a physically selective nonlinear electrostatic transport mechanism. He said such technology can be a highly sensitive sorting technique.

Dr. Blake Simmons said that Sandia developed a system that is very powerful, robust, and simple. It is a device that can separate particles from a background of various matrices in multiple formats. The polymer chip performance is comparable to the glass devices. Sandia also had initiated an integrated systems engineering approach to couple particle sorting and detection in one platform to provide the sensitivity and selectivity desired. Additionally, the group's experience indicated that the technology could be scaled from nanoliters per minute to milliliters per hour.

Sandia was exploring other techniques to produce sensitive and selective devices, alternate device geometries, and different configurations. Also, Sandia had a path forward to commercialization, with a couple of Cooperative Research and Development Agreements and licensing agreements to develop the iDEP technology for aerosol monitoring. Finally, it has been moving to alternative device architectures, such as continuous flow. Sandia recently developed a design that it hopes will allow for a flow rate of multiple liters per hour, as opposed to less than a liter per hour.

Making a Lab Process Field Portable

Michael Carpenter, Idaho National Laboratory

This project represented collaboration between a developing EPA laboratory process and an INL concept designed to embrace a new sample concentration paradigm, bringing the laboratory to the field.

The EPA has identified the need for a portable, automated field sample concentrator. The current concentrator exists as a laboratory scale process, and Dr. Carpenter said that INL was tasked to produce an automated prototype, man-portable field system. This concentrator system will be applied to monitoring municipal water supplies, public event potable water supply screening activities, and first-responder sample collection.

The project's objectives are to achieve a 400-fold initial sample reduction from 100 L to 250 mL; to reduce and control the sample collection rate variability; to make the system portable, simple to use, lightweight, rugged, and partially disposable (wet parts must be disposable and low-cost); and to maintain biohazard containment.

Phase-1 development of the control software for the prototype automated system had been done, and the prototype-1 system was complete. EPA will use the first prototype to evaluate recovery rates, variability between system runs, various filter model recovery performance, and various components of the system. The test information from the prototype will be used to design a second prototype, as well as subsequent designs.

Various obstacles in moving the work from a laboratory-based manual system to an automated portable field system have been encountered. The overall goal of the work has been to automate operation of the system to the greatest extent possible, which signifies a balancing act between expediency and technical necessity.

With regard to the process undertaken to pursue this project, the INL evaluated the benchtop ultrafiltrator concentrator (UC) process that required manual intervention to adjust operational parameters such as flow rates, pressures, elution, and back-flush operations. Working closely with EPA, INL proposed the initial system concept to EPA. After some review and changes, INL set up a benchtop system very similar to the current EPA method and introduced initial automated features. INL developed proprietary software executable to automatically operate the UC concept system within initially established parameters.

The reality of water security threats to long-standing infrastructure requires a paradigm shift in traditional laboratory methods. The laboratory simply has to be where the problem is, and the ultrafiltration concentrator allows a laboratory process to be taken to the field.

In the future, the group will continue testing concentrators in broader applications. Additionally, they will plan production and distribution of concentrators to municipalities and to County, State, and Federal agencies. INL Environmental Engineering will manage the subcontract manufacturing of multiple units in those contracts, and INL will provide technical support and system control training to the user base. The design will evolve as concentrator requirements evolve.

Summary

James Sinclair, EPA

At the end of all the presentations, James Sinclair, of the EPA OGWDW's Technical Support Center, summarized the presentations as follows:

- There were a range of interesting talks on a variety of different techniques, from continuous flow centrifugation, to hollow-fiber filters, to silica beads. Participants exchanged a great deal of data on the recovery efficiencies of the different technologies.
- The technologies all were demonstrated to offer noteworthy advantages, including the following highlights:
 - Hollow-fiber filters had a major benefit in that they allowed for the simultaneous concentration of viruses, bacteria, and protozoa.
 - Virosorb 1 MDS virus cartridge filters could be reused three times since there was no viral carryover after treatment. Also, filters could receive additional treatment to remove nucleic acids.
 - Glass wool filters were sufficiently inexpensive that they allowed for a large occurrence study that would not otherwise be feasible. The filters worked with large volumes of water, and different viruses and water types yielded between 8% and 98% recovery.
 - Electropositive nanoaluminum-fiber filters were shown to allow high flow rates. They also could hold a large amount of particulate material and retain a variety of microorganisms well.
 - Silica bead virus filters also had good recovery. The addition of different functional groups increased the capture of the viruses as compared with the uncoated silica beads alone. The capture efficiency could be optimized for different viruses.
 - Continuous-flow centrifugation was another valuable technique and could be used for bacteria and protozoa. It was inexpensive as well, with consumables costing about \$50. The flow rate was between 100 and 700 mL/minute, but better recoveries were obtained with the lower flow rates and higher centrifugation speeds.
 - A chemical affinity based approach on hydrophobic interactions was demonstrated be able to concentrate bacteria and viruses. Different eluting agents were used, but SDS was found to be the best. Adsorption and elution occurred efficiently, but bacteria appeared to be trapped on the thread, so more optimization was needed.
 - Electrophoresis was illustrated to transport and trap bacteria and viruses in a microfluidic device. Microorganisms are separated in a microchannel based on their charge and mobility under an electric field. The device was tested with different bacteria, viruses, and water types and under different operating conditions. Recoveries often exceeded 90%.
 - Dielectrophoresis, which is based on the movement of particles from polarization in a nonuniform electrical field, could separate particles based on characteristics other than size.
 - Portable sampling systems will be important for more extensive field testing.

Panel Discussion

An open-panel discussion followed the presentations. Dr. Parshionikar first asked the panel which technology, in their opinion, was the most promising that EPA could pursue. Dr. Suresh Pillai was of the opinion that continuous-flow centrifugation was promising simply because of its ease and simplicity. He also thought that microfluidics was a promising field that could be scaled up to meet the requirements of large-volume water sampling. He noted that it was important to understand how pathogens interacted with their matrices, and that it was not enough to simply study capture and recovery efficiencies of pathogens. Dr. Parshionikar then asked Dr. Simmons for his thoughts on whether the iDEP technology could be used to distinguish between live and dead viruses. She wondered if, after the initial elution and concentration step, the viral concentrate could be passed through the microfluidic channels of an iDEP and observe a differential migration pattern between dead and live viruses. Dr. Simmons answered that it had not been tried, but thought it might work, and was open to collaborative opportunities. Dr. Gerard Stelma asked the panel why large volumes of water had to be used when sampling for viruses. The panel answered that the viruses and parasites often are thought to be present in low concentrations in contaminated water but could pose a health risk, as some early studies have shown the infectious dose of viruses to be very low. It therefore was thought that the larger the volume of water, the more useful the data can be. Eunice Varughese asked how we could address the issue of pathogen attachment to solid particles, as this may affect recovery efficiencies. Dr. Hill answered that this indeed was a problem that he has had to deal with, but as yet, did not have a solution. Dr. Parshionikar asked the panel if the nanoceramic particles mentioned in Dr. Tepper's talk had different binding efficiencies to nucleic acids and whole organisms. The panel answered that the binding efficiency was the same for the two.

In summary, the following themes emerged from the panel discussion (please note that these themes do not constitute recommendations):

- There is a need for a method that could simultaneously recover multiple pathogens.
- The method must work with current and future detection methods such as PCR, microarrays, and biosensors.
- Variability in recovery efficiency is a major problem with many filter technologies.
- Microfluidics seem to hold promise if the technology could be scaled up for large sample volumes.

Closing Statement

Sandhya Parshionikar, EPA

Dr. Sandhya Parshionikar thanked the presenters and participants and acknowledged that pathogen capture and detection has come a long way since its very primitive days in the 1960s. She noted that the technologies presented at the workshop look promising, but it remains to be seen if they can withstand the demands of downstream molecular detection. One option for the future could be using a combined approach, in which different technologies could be combined to come up with a complete, optimized concentration method. Despite all the efforts that still need to be made in this field, the future seems promising.

List of Participants

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