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Workshop Proceedings

ALLIANCE FOR COASTAL TECHNOLOGIES

Rapid Microbiological Indicator Methods

Seaside, California
May 14-16, 2003

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An ACT 2003 Workshop Report

A Workshop of Developers, Deliverers, and Users of Technologies for Monitoring Coastal Environments:

Rapid Microbiological Indicator Methods

Moss Landing, California
Seaside, California
May 14-16, 2003

Sponsored by the Alliance for Coastal Technologies (ACT) and NOAA's Center for Coastal Ocean Research in the National Ocean Service.

Hosted by ACT Partner organizations Moss Landing Marine Laboratories (MLML) and Monterey Bay Aquarium Research Institute (MBARI) and co-sponsored by the Southern California Coastal Water Research Project (SCCWRP) and the California State Water Resources Control Board (CSWRCB)

ACT is committed to develop an active partnership of technology developers, deliverers, and users within regional, state, and federal environmental management communities to establish a testbed for demonstrating, evaluating, and verifying innovative technologies in monitoring sensors, platforms, and software for use in coastal habitats.

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ACT WORKSHOP: RAPID MICROBIOLOGICAL INDICATOR METHODS

EXECUTIVE SUMMARY

The Rapid Microbiological Indicator Methods Workshop was held at Moss Landing and Seaside, California May 14-16, 2003. The workshop was sponsored by Alliance for Coastal Technologies partner organizations Moss Landing Marine Laboratories (MLML) and Monterey Bay Aquarium Research Institute (MBARI) and co-sponsored by the Southern California Coastal Water Research Project (SCCWRP) and the California State Water Resources Control Board (CSWRCB).

The goals of the workshop were to: (1) Exchange information about the present state of technologies targeting rapid (<4 h) and sensitive (<100 enterococci per 100 mL) detection of microbes in natural water samples, (2) Identify technical impediments to routine implementation and regulatory acceptance of new technologies, (3) Outline strategies for evaluating performance of emerging technologies and (4) Identify the principal non-technical impediments to commercial development of rapid microbiological indicator methods for application in water quality monitoring programs.

The first day of the workshop was open to the public and involved 16 presentations summarizing progress in development of rapid microbiological indicators. The second two days were devoted to small working groups of invited participants developing consensus about impediments to future application of new indicator technologies in water quality monitoring programs. Invited participants were selected to ensure representation from three segments of the community: researchers, commercial vendors and water quality managers. The working groups reached the following conclusions and recommendations:

- Current beach monitoring systems will undergo comprehensive evolution during the coming decade and this evolution will substantially enhance our ability to correctly and rapidly identify when recreational waters are contaminated with microorganisms pathogenic to humans.

- There are three broad classes of rapid methods that are under development and research investment in all three is warranted. The most advanced are those in the enzyme/substrate class because they are enhancements of currently approved methods. Molecular surface recognition and nucleic acid based methods are comparatively less well developed, but have potential for being even more rapid, more sensitive and adaptable to a wider class of indicators and pathogens.

- The biggest technical challenge is sensitivity. Present technology is able to accurately identify the microbial indicators of interest in a short time, but only at concentrations well

above water quality standards. Research investment should focus on developing preconcentration technology that will improve sensitivity.

- The biggest non-technical impediment is regulatory approval. The approval process is ill-defined and likely to be expensive relative to size of the market. EPA needs to better define the criteria for approval and develop a strategy for assisting vendors navigate this process.

- There is need for a market survey to define customer needs. The survey should address potential overlap among markets for monitoring of marine beaches, freshwater beaches, shellfish beds and drinking water, all of which use similar indicators.

ALLIANCE FOR COASTAL TECHNOLOGIES

There is widespread agreement that an Integrated Ocean Observing System is required to meet a wide range of the Nation's marine product and information service needs. There also is consensus that the successful implementation of the IOOS will require parallel efforts in instrument development and validation and improvements to technology so that promising new technology will be available to make the transition from research/ development to operational status when needed. Thus, the Alliance for Coastal Technologies (ACT) was established as a NOAA-funded partnership of research institutions, state and regional resource managers, and private sector companies interested in developing and applying sensor and sensor platform technologies for monitoring and studying coastal systems. ACT has been designed to serve as:

- An unbiased, third-party testbed for evaluating new and developing coastal sensor and sensor platform technologies,

- A comprehensive data and information clearinghouse on coastal technologies, and

- A forum for capacity building through a series of annual workshops and seminars on specific technologies or topics.

ACT Headquarters is located at the UMCES Chesapeake Biological Laboratory and is staffed by a Director, Chief Scientist, and several support personnel. There are currently seven ACT Partner Institutions around the country with sensor technology expertise, and that represent a broad range of environmental conditions for testing. The ACT Stakeholder Council is comprised of resource managers and industry representatives who ensure that ACT focuses on service-oriented activities. Finally, a larger body of Alliance Members has been created to provide advice to ACT and will be kept abreast of ACT activities.

The ACT workshops are designed to aid resource managers, coastal scientists, and private sector companies by identifying and discussing the current status, standardization, potential advancements, and obstacles in the development and use of new sensors and sensor platforms for monitoring and predicting the state of coastal waters. The workshop goals are to both help build consensus on the steps needed to develop useful tools while also facilitating the critical communications between the various groups of technology developers, manufacturers, and users.

ACT is committed to exploring the application of new technologies for monitoring coastal ecosystem and studying environmental stressors that are increasingly prevalent worldwide. For more information, please visit www.actonline.ws.

GOALS FOR THE MICROBIOLOGICAL INDICATORS WORKSHOP

Public health officials across the nation routinely rely on measurements of fecal indicator bacteria as the basis for assessing recreational water quality. These monitoring programs are compromised, though, because current methods of enumerating bacteria are too slow to provide full protection from exposure to waterborne pathogens. United States Environmental Protection Agency approved methods to evaluate recreational waters require an 18 to 96 hour incubation period, while several recent studies have shown that temporal changes in indicator bacteria levels in beach water occur much more rapidly. Thus, contaminated beaches remain open during the incubation period and are often clean by the time warnings are posted.

This methodological time lag also inhibits tracking of contamination sources since the signal has often dissipated before upstream tracking can be initiated. Lacking a more rapid method, investigators are unable to follow the trail of contamination back to its origin.

Present methods rely on culturing techniques that measure a metabolic endpoint after an extended incubation period, but new molecular methods that allow direct measurement of cellular properties without incubation are becoming available. These methods have the potential to improve monitoring by allowing public health warnings to be issued on the same day that samples are collected. To facilitate development of these new methods, the Alliance for Coastal Technologies, the State of California and the Southern California Coastal Water Research Project jointly sponsored a workshop that brought together experts in the field to exchange information about the current state of rapid microbiological indicator measurement technologies. The workshop was intended to address four goals:

(1) Exchange information about the present state of technologies targeting rapid (<4 h) and sensitive (<100 enterococci per 100 mL) detection of microbes in natural water samples

(2) Identify technical impediments to routine implementation and regulatory acceptance of new detection technologies

(3) Outline strategies for evaluating performance of emerging technologies

(4) Identify the principle non-technical impediments to commercial development of rapid microbiological indicator methods for application in water quality monitoring programs

WORKSHOP STRUCTURE

The Rapid Microbiological Indicator Workshop was held May 14-16, 2003 in Moss Landing and Seaside, California. The first day of the workshop was held at the Embassy Suites in Seaside and was open to the public. It was attended by approximately 100 people and involved 16 presentations summarizing progress in development of rapid microbiological indicators (Appendix A).

The second two days were held at the Monterey Bay Aquarium Research Institute in Moss Landing and were devoted to small working groups of invited participants to develop consensus about impediments to future application of new indicator technologies in water quality monitoring programs.

There were 33 invited participants (Appendix B), who were selected to include equal representation from three segments of the community: researchers (technology developers), commercial vendors (technology suppliers) and water quality managers (technology users). Participants were separated into three groups that included each of these communities during each breakout session and all groups were asked to address the same set of questions/issues. After each session, all participants were reconvened to compare recommendations among groups. The following sections of this report summarize the recommendations that evolved from those sessions.

OVERVIEW OF METHODS

The first presentation in the public session provided an overview of rapid indicator methods under development and suggested that there were two aspects to each method (Appendix C). One is capture technology, in which the desired bacterial group (or some molecular signature of the group) is removed, tagged or amplified to differentiate it from the remaining material in the sample. The other is detection technology, in which the captured, tagged or amplified material is counted. Three broad classes of capture technology were identified:

- Molecular surface recognition methods, which capture and/or label the target bacterium by binding to molecular structures on the exterior surface of bacterium or in its genetic material. These include immunoassay techniques, bacteriophage, or RNA probes.

- Nucleic acid detection methods that target genetic material of bacteria, viruses or protozoan indicators. These include polymerase chain reaction (PCR), quantitative PCR (QPCR), nucleic acid sequence based amplification (NASBA) and microarrays.

- Enzyme/substrate based methods, which pair chromogenic or fluorogenic substrate methods already in wide use with advanced optical or electronic detectors, greatly reducing the required incubation time.

The presentations following the overview were organized by class of method and provided detailed description of the methods, as well as their success in early testing (Appendix C). Within the molecular surface recognition class, one presentation described how fluorescently labeled antibodies or RNA probes are used to tag and enumerate indicator bacteria via flow cytometry. Several presentations described "antibody sandwich" technology, in which indicator bacteria are captured by a first set of antibodies, labeled with second set of antibodies containing a fluorophore, and detected via fiber optics. Another presentation described use of fluorescently labeled virus probes that are detected using fiber optics. The last presentation in this class described a method that uses antibodies to capture and separate the indicator bacteria, but accomplishes detection through a light-based ATP assay.

Three enzyme/substrate methods were presented next. Two of the methods were very similar in that they measure excitation and absorbance of the fluorescent metabolite using a standard fluorometer to enhance speed of detection. The third presentation described the use of dual-wavelength fluorimetry to simultaneously determine the rate at which the fluorescent substrate disappears with the rate of appearance of the fluorescent metabolite.

The last group of presentations dealt with nucleic acid-based methods. One presentation described a QPCR method currently being used in an epidemiological study by EPA. Others described the versatility of nucleic acid detection-based methods and their potential to rapidly measure multiple indicators or human pathogens simultaneously through such techniques as NASBA and optical sensor microarrays.

It was apparent during the presentations that some classes of technology are further along in the development process. The most advanced are those in the enzyme/substrate class because they are enhancements of currently approved methods. Molecular surface recognition and nucleic acid based methods are comparatively less well developed, but have greater potential for being even more rapid, more sensitive and adaptable to a wider class of indicators and pathogens. There was general consensus among the presenters that investment in rapid method development should continue to be broad-based, focusing on the most developed methods in the short term, while concurrently pursuing development of all promising technologies for the future.

A question that arose during the discussion following the presentations concerned the definition of "rapid". Workshop organizers provided a working definition, which was "a method that produces results within a time frame that allows managers to take action to protect public health the same day water samples are collected." In the best-case scenario, these results would be transmitted to decision makers almost instantaneously from the sampling site. A more practical definition of "rapid" is a method that takes less than four hours to obtain results, which allows for retesting of a site within a typical 8-hour workday. Participants adopted the definition of rapid as less than four hours for use in subsequent discussions.

TECHNICAL IMPEDIMENTS TO IMPLEMENTATION

Breakout group members agreed that the largest technical impediment to implementation of rapid methods is sensitivity and that detection, rather than capture technology is the limiting factor. Current detection levels for the majority of methods presented at the workshop are in the range of 103 -104 cells per 100 ml, which is inadequate to address current bathing water standards of 35 enterococci per 100 ml.

Participants suggested that there are two possible approaches to overcome inadequate sensitivity. The first is to improve detector technology, but this was not the preferred option. Most researchers already use the most advanced detectors available and more sensitive detector technology is not likely to be available in the near future because of the cost and extensive time necessary for further development.

The preferred option at the present time is to develop methods for preconcentration of target organisms, which can be achieved through a variety of techniques, such as centrifugation, filtration, and immunomagnetic separation. Preconcentration has the potential to increase sensitivity several fold by increasing the number of target bacteria per unit volume at a relatively modest cost.

The biggest drawback to preconcentration is the additional time it requires, potentially pushing some methods past the 4-hour criterion. Depending on the method employed, preconcentration could also result in partial loss of target organisms or the unintended concentration of environmental contaminants, both of which could have unpredictable effects on results. Still, workshop participants felt that these potential shortcomings could be overcome and that investment in preconcentration technology research would be a very effective means for advancing the field.

While workshop participants determined that detection technology was the largest technical impediment, they also identified a number of research opportunities for improving capture technology. Most of these were suggestions for ways to increase capture specificity, such as development of new antibodies for surface recognition or new primers for nucleic acid detection. More specific capture technology has the potential to enhance monitoring systems by allowing quantification of indicators, or even pathogens themselves, that have the best relationship to swimmer health.

METHOD EVALUATION CRITERIA FOR REGULATORY ACCEPTANCE

Workshop participants agreed that the most important criterion for evaluating whether a rapid method is acceptable as a replacement for current methods is a demonstrated relationship to human health risk. Participants further agreed that the best way for accomplishing this is by incorporating the new methods into epidemiological studies, as EPA is presently doing on a pilot

basis with a limited number of methods. The problem with epidemiological studies, though, is that they are difficult and expensive. EPA's present epidemiological study has a budget in excess of $5M.

Because the cost for epidemiological studies is high, participants agreed that an alternative way to establish a health risk relationship is through demonstrated equivalency with an existing USEPA-approved methodology. The equivalency approach will be most applicable to methods that are enhancements of existing measurement approaches, such as the enzyme/substrate class of methods, since these incorporate a measure of cell viability. Other new methods that measure subcellular structures are likely to require epidemiology studies since the attribute they measure is markedly different than that of existing measures, but equivalency studies are still a cost-effective first step for evaluating these methods.

Workshop participants proceeded to formulate recommendations for a four-tier study design for assessing equivalency. The new methods would be used simultaneously with EPA-approved methods throughout all phases, and multiple replicates would be included to test for variability within and across methods.

- The first tier would be designed to determine the sensitivity and comparability to existing methods over a range of concentrations. This would be quantified using blind samples created with known concentrations of laboratory strains of the target organism suspended in a simple sterile water matrix.

- The second tier would test the stability of each method when environmental contaminants such as salt, turbidity, or organic inhibitors are added to the matrix. In this phase, concentrations of target organisms in blind water samples would be kept within the detection range of each method, as determined during the first tier of the study. Concentrations of environmental contaminants would start at zero and increase sequentially to the highest levels commonly found in surface waters.

- The third tier would further test matrix stability using environmental water samples. Use of environmental samples would also allow assessment of whether the method is confounded by non-laboratory strains or other bacterial species that might be present. Blind water samples would be obtained from historically polluted locations in a variety of habitats, including rural and urban freshwater runoff, estuarine water, and marine surface waters.

- The fourth tier would test the ability of each method to discriminate between viable and non-viable cells. Replicate sets of water samples collected in tier three would be subjected to varying amounts of gamma radiation designed to injure or kill bacteria while leaving the cell structures intact.

Participants also discussed that the comparability and sensitivity requirements for new methods depends on the context in which they are applied. For instance, a rapid method that is not sensitive enough to measure bacterial concentrations below water quality standards would still be useful to screen quickly for large spill events, provided a second, slower but more sensitive,

method is also used to assess lower level chronic problems. Similarly, an inexpensive rapid method that generates some false positives would still provide good early warning, provided that monitoring personnel are positioned to respond with a more expensive method for validation before they issue warnings.

NON-TECHNICAL IMPEDIMENTS TO COMMERCIAL DEVELOPMENTS OF RAPID METHOD

Workshop participants identified regulatory approval as the most difficult non-technical obstacle to commercial development of rapid methods. The market for rapid indicators consists primarily of NPDES dischargers who monitor in response to requests from regulatory agencies, or local health departments that monitor under the supervision, or with funding from, state and federal oversight agencies. In both cases, the user community will not readily adopt a new method without approval of the oversight agency. In most cases, the oversight agency is EPA.

The difficulty is that the market is perceived as being too small for individual developers to encumber the expense of studies to achieve regulatory approval. Epidemiological studies are multi-million dollar endeavors that can take years to complete. Moreover, the approval process is ill-defined, so even a well-conducted study might not lead to immediate approval. Because the approval process is ill-defined, many of the participants from the commercial sector expressed hesitancy to undertake even the less expensive equivalency tests without some form of incentive.

Workshop participants agreed that government assistance would likely be required to overcome this impediment. The group suggested that EPA should conduct periodic epidemiological studies and provide the opportunity for minimal-cost participation by commercial interests that would like to see their new methods evaluated. Participants also recommended that EPA take a more proactive role in moving toward standardization of regulations from state-to-state. Such regulatory uniformity would help companies by alleviating the additional costs associated with gaining both federal and state approval.

Workshop participants also cited a poorly defined market for rapid methods as a non-technical impediment to commercial development. It was unclear to many participants how much overlap exists among potential markets for monitoring of marine beaches, freshwater beaches, shellfish beds and drinking water. All of these industries use similar indicators, which could serve to increase market size and stimulate investment, but there may be substantial differences in needs or cost structure that these other markets could support. Workshop participants recommended that an independent market survey be undertaken. This survey, which could be conducted under the auspices of the Alliance for Coastal Technology, would also help to determine the amount of public and private resources necessary to support implementation of rapid methods.

Workshop participants suggested that non-technical hurdles might require a financially disinterested champion to take up the cause. This champion would serve to identify specific needs and then promote the technology to both policy makers and the public. Participants

suggested California's Beach Water Quality Work Group as a likely candidate to fill this role because the size of the California's beach monitoring market is the largest in the country and the BWQWG has already been proactive in adopting new methods. Participants lauded California for starting down this path by funding the necessary research, conducting comparative testing and co-sponsoring this rapid microbiological indicator methods workshop.

WORKSHOP RECOMMENDATIONS

- Research investment should continue in all three classes of rapid methods that are under development. The enzyme/substrate class is likely to be ready for application in the shortest time, but the molecular surface recognition and nucleic acid based methods have potential for being even more rapid, more sensitive and adaptable to a wider class of indicators and pathogens.

- Research investment should focus on developing preconcentration technology that will improve sensitivity.

- There needs to be better defined criteria for regulatory approval of new methods and financial assistance for vendors to navigate this process.

- A market survey to define market size and customer needs should be conducted.

CONCLUSIONS

Development of rapid microbial indicators is moving quickly and will likely come to fruition within the next year, allowing managers to take action toward protecting swimmers from exposure to waterborne pathogens on the same day. Sensitivity is the major technological hurdle facing all three classes of rapid methods presented at the workshop, but may be overcome through a preconcentration step, which has the potential to boost sensitivity without the need to develop new detector technology.

In the short term, available methods are likely to be based on technological improvements to existing enzyme/substrate-based methods that reduce incubation times from overnight to a few hours and can be approved for use based on demonstrated equivalency with current EPA approved methods. Longer term, molecular surface recognition-based methods will also come on-line, but may require inclusion in an epidemiological study to gain EPA approval. Eventually, rapid methods will gravitate toward newer nucleic acid technologies, which are more versatile and sensitive, but are still early in their development and will also require inclusion in an epidemiological study for approval.

The process set in motion in California will add impetus to drive development of rapid methods for use in water quality monitoring and help to overcome the hurdles to widespread acceptance of these methods. Workshop participants were enthusiastic about the value of the workshop and suggested that the field is developing quickly enough that ACT should conduct future similar workshops.

APPENDIX A. OVERVIEW SESSION AGENDA

8:30 a.m. Welcome
Dr. Mario Tamburri - Alliance for Coastal Technology

8:40 a.m. Background and Workshop Goals
Dr. Stephen Weisberg - Southern California Coastal Water Research Project

9:00 a.m. Impacts of Anthropogenic Inputs on Water Quality: New approaches showing the way.
Dr. Rachel Noble - Univ. of North Carolina

9:30 a.m. Development and Evaluation of a Rapid (<4 hour) Test for Enumerating E. coli and Enterococcus from Beach Water Samples
Dr. Kristi Harkins - AATI

9:50 a.m. Development of a Portable Instrument for the Rapid Enumeration of Bacterial Indicators
Dr. David McCrae - Research International

10:10 a.m. Break

10:40 a.m. RAPTOR - A Portable Biosensor for Environmental Monitoring
Dr. George Anderson - US Navy

11:00 a.m. Rapid Biosensor Detection of Microbial Pathogens
Dr. Daniel Lim - Univ. of South Florida

11:20 a.m. Rapid Detection of Bacteria using IMS and ATP Bioluminescence
Dr. Rolf Deininger - Univ. of Michigan

11:40 a.m. Optical Sensors for Classification and Identification of Biological Material
Dr. Mary Beth Tabacco - Echo Technologies

12:00 p.m. Lunch
Lunch Talk: Industrial/Academic Synergy: The Journey from the Drawing Board to the Final Product
Dr. Mike Dziewatkoski - YSI Environmental

1:30 p.m. Rapid Detection of Indicator Bacteria with Dual Wavelength Fluorimetry
Dr. Claudia Koerting- University of Connecticut

APPENDIX A. OVERVIEW SESSION AGENDA (CONTINUED)

1:50 p.m. Rapid in situ Coastal Monitoring of Indicator Bacteria Based on a Fluorescent Enzymatic Assay
Dr. Michael P. Shiaris - University of Massachusetts

2:10 p.m. Development of an Instrumental Technology for the in situ Detection and Rapid Near Real Time Reporting of Fecal Contamination in Marine Waters
Heather Saffert - University of Rhode Island

2:30 p.m. Rapid Measurement of Bacterial Fecal Indicators in Surface Waters by Quantitative Polymerase Chain Reaction (QPCR) Analysis
Dr. Richard Haugland - US EPA

2:50 p.m. Rapid, Automated PCR Methods for Microbial Detection
Dr. Jeffrey Ryan - Cepheid

3:10 p.m. Break

3:40 p.m. Optical Sensor Microarrays for Rapid Detection
Dr. David Walt - Tufts University

4:00 p.m. The Future of Microarrays and NASBA for Water Quality Monitoring
Dr. Joan Rose - Michigan State University

4:20 p.m. The Challenges of Using Microtechnology-based Instrumentation on Macro-World Water Samples
Dr. Ray Mariella - Lawrence Livermore Laboratory

4:40 p.m. Closing Remarks
Dr. Stephen Weisberg - Southern California Coastal Water Research Project

5:00 p.m. Adjourn

APPENDIX B. WORKSHOP PARTICIPANTS

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APPENDIX B. WORKSHOP PARTICIPANTS (CONTINUED)

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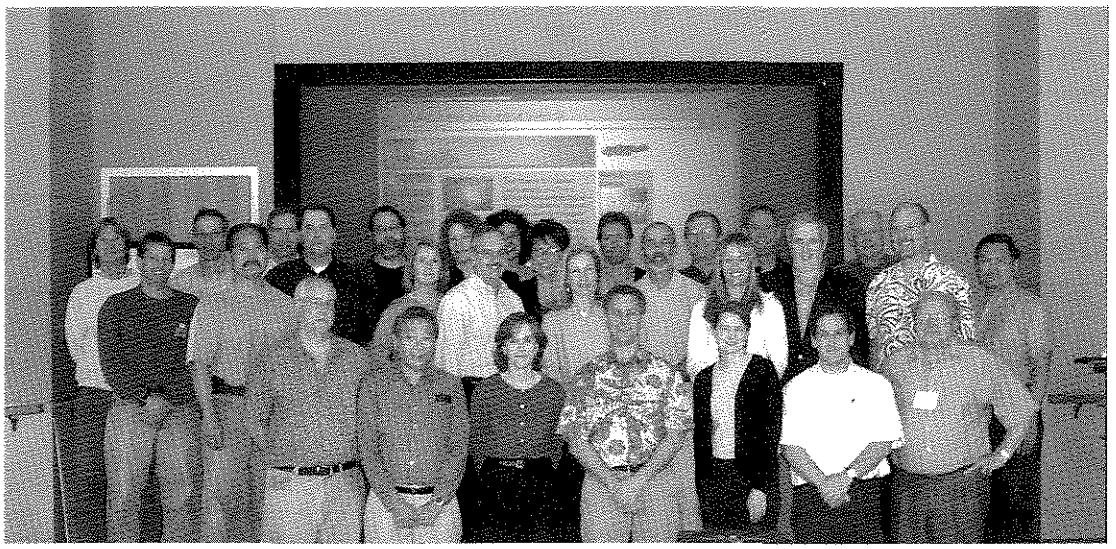
APPENDIX B. WORKSHOP PARTICIPANTS (CONTINUED)



WORKSHOP PARTICIPANTS
(TAKEN AT MONTEREY BAY AQUARIUM RESEARCH INSTITUTE - MAY 16, 2003)

APPENDIX C. ABSTRACTS FROM THE OVERVIEW SESSION

Rapid Microbial Detection: A Range of Possibilities and Challenges

Rachel T. Noble, Ph.D.
Assistant Professor, Institute of Marine Sciences
University of North Carolina at Chapel Hill

The preservation and restoration of sanitary water quality is of utmost importance for protecting public health. Billions of dollars in economic losses occur annually due to the closure of beaches and marine and freshwater recreational areas. Public perception and public health risk are important components of sanitary water quality.

The microbiological water quality of beaches and coastal zones is determined by measuring indicator bacteria, such as total and fecal coliforms (or *E. coli*) and *Enterococci*, to infer the presence of microbial pathogens. Bacterial indicators are used to infer the presence of human pathogens associated with fecal contamination, based upon the assumption that the bacteria are present in waters with fecal contamination and not in those without.

The methods for measuring bacterial indicator densities in recreational waters have changed little over the last few decades. These methods:

- Are largely based upon culturing the microorganism.
- Suffer from high variability.
- Take 24 to 48 hours for results.
- Can be labor intensive.

In recent years, however, a wealth of new molecular, biotechnological, and immunological advances has yielded methods that have potential for the rapid, possibly even "real-time" measurement of bacterial indicators. Examples of these approaches are nucleic acid detection-based methods like polymerase chain reaction (PCR), nucleic acid sequence based amplification (NASBA), and microarrays. Other advances have been made that pair immunoassay approaches (antibody-capture and antibody labeling-based methods) with a variety of techniques for detection, such as flow cytometry, fiber optics, and interferometry. In addition, a variety of permutations of fluorogenic and chromogenicsubstrate based techniques have been paired with highly advanced electronic and/or optical detection systems.

The basic premise behind these methods will be introduced in this presentation. In addition, the limitations, advantages, disadvantages, and applicability of several of these new methods will be discussed, as well as the issues relating to portability, cost, and training required for these methods. Methodological advancements for the real-time detection of bacterial indicators are

exciting and have the potential to both revolutionize management strategies for recreational waters and provide a means to better protect public health.

Rachel Noble is an Assistant Professor at the Institute of Marine Sciences at the University of North Carolina at Chapel Hill. Her research currently focuses on the quantification of enteric human pathogens in a variety of environments, including recreational areas, shellfish beds, and commercial fishing areas. She is interested in relating the presence of known human pathogens to levels of fecal coliforms, *E. coli*, and *Enterococci* in recreational waters to better protect human health.

Recently, she held a joint appointment between the University of Southern California's Wrigley Institute for Environmental Studies and the Southern California Coastal Water Research Project as postdoctoral scientist/research faculty, and focused her work there on regional assessment of water quality along the Southern California shoreline and the detection of enteroviruses in stormwater impacted areas of the coast. Noble received a B.S. in Molecular Biology from Carnegie Mellon University and a Ph.D. in Marine Biology from the University of Southern California.

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Developing and Evaluating a Rapid (Less Than 4 Hour) Test for Enumerating *E. coli* and *Enterococcus* from Beach Water Samples

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Enterococcus spp. and generic *E. coli* have been identified as indicator organisms for fecal contamination of recreational waters. When *Enterococcus* spp. are detected at levels above the marine standard of 104 (instantaneous) or 35 (monthly average)/100 milliliter (mL) or when fecal coliforms are above 400 (instantaneous) or 200 (monthly average)/100 mL, the source water is considered potentially contaminated with fecal material and warnings can be issued. The current method involves a 24-hour incubation culture process during which people with access to the water risk exposure.

During the past several months, Advanced Analytical Technologies, Inc. has focused on three tasks in the development of rapid assays for detecting these organisms using rapid bacterial detector (RBD) cytometric technology for enumeration.

In Task 1, Advanced Analytical developed a method to concentrate bacteria from 100 mL of water into a final volume of 1 mL. Water samples were pre-filtered through a 3¼ micron filter, bovine serum albumin (BSA) was added, and the samples were centrifuged (3,100 × gravitational force [g], 45 minutes, 4°C). Recoveries were 85 percent ± 12 percent (n=23) of *Enterococcus* spiked water samples.

Task 2 defined the labeling methods and lower limits of detection for these organisms that would allow accurate low-level detection of *Enterococcus* and *E. coli* on the RBD system. Specific antibody or rRNA-specific probes labeled with Alexa 647 or Cy5 were used for the cytometric detection and enumeration of several *Enterococcus* species. *E. coli* was detected using rRNA-specific probes only. In serial dilution studies (101 to 106 colony forming units/mL), the correlation coefficients between RBD and plate counts were less than 0.9 for the detection of each organism in different labeling scenarios. Sensitivity testing demonstrated the labeling of several *Enterococcus* species or *E. coli* species tested, and specificity experiments showed no labeling of organisms closely related in phylogeny.

Task 3 is in progress as Advanced Analytical begins to verify the accuracy of these methods using real-world samples (clean and contaminated with varying numbers of indicator organisms) in comparison with traditional methods.

Co-authored by Angie Oppedahl, Advanced Analytical Technologies, Inc.

Kristi Harkins is the Director of Research & Development for Advanced Analytical Technologies, Inc. Prior to joining Advanced Analytical, she spent 19 years in academia with a focus on using flow cytometry as a research tool in a variety of areas including microbiology, plant science, and immunology. She has managed cytometry laboratories at University of Nebraska/Lincoln, University of Arizona, and Iowa State University. She has also published in journals such as Science, Proceedings of the National Academy of Sciences, *Plant Physiology*, and the *Journal of Veterinary Immunology*, and has recently authored three published reference book chapters on bacteria sorting, and antibody production and purification techniques. Harkins received a B.S. in Biology from Nebraska Wesleyan University, an M.S. in Molecular Biology from Northwestern University, and a Ph.D. in Plant Genetics (Cell Biology) from the University of Arizona. She is currently working on her M.B.A. at Iowa State University.

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Development of a Portable Instrument for the Rapid Enumeration of Bacterial Indicators

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California Assembly Bill No. 411, signed into law on October 7, 1997, requires the testing of waters adjacent to all public beaches for microbiological contamination. Such testing shall include, but is not limited to, assays for total coliform, fecal coliform, and Enterococci bacteria. The allowable bacterial concentrations, as defined by the California Department of Health Services and in keeping with recommendations by the United States Environmental Protection Agency, are stringent. In general, the average concentrations must not exceed:

- 10 total coliform bacterial per milliliter.
- 2 fecal coliform bacteria per milliliter.
- 0.35 *Enterococci* bacterium per milliliter.

At this time, no microbiological assay is able to directly measure bacteria at these concentrations. Current approved laboratory measurement methods require an 18- to 96 hour incubation period to raise the bacterial concentration to a detectable level. In general, no matter what the detection technique proposed, whether immunoassay-based or DNA-based, at least 100 to 1,000 bacterial equivalents will be required. Thus, any and all assay methodologies will require that the sample be "amplified" in some fashion.

This "amplification" may be concentration. If a large enough volume of water is collected and the bacteria present are concentrated, then any detection technique will measure them; however, the required sample volume may run to thousands of liters. In PCR-based DNA detection systems, the DNA is amplified enzymatically and then detected; however, the PCR reaction is sensitive to contaminants within and without the assay instrument. Immunoassays generally rely on incubating the bacteria in a growth medium and letting them amplify themselves.

To solve this problem, a preconcentrator, an incubator, and a fluoroimmunoassay detector were combined into a single package. This device will first preconcentrate indicator bacteria from the water source onto a suitable filter media using a pumped loop system. Culture media is added and, after a bacterial loosening step, the media in the cup (which may simply be buffered water if no incubation is desired) may be tested for the presence of bacteria. This initial test will be complete in about 10 to 15 minutes, but at any point after about 7 minutes of elapsed time, the cup may either be discarded or incubated at a pathogen-specific temperature for a user-selected time period. At multiple points during the incubation process, the media may be interrogated again to determine whether the target bacteria are growing in numbers. These additional assay measurements are essentially free - that is, no new detection elements or reagents are required to obtain these multiple data points during the culture process. This allows an accurate determination of the number of viable bacteria in the initial sample.

David McCrae is Vice-President of Research International, Inc. His work has focused on optical sensors of physical parameters, such as pressure and temperature, and chemical parameters, such as blood gases, sodium, potassium, and lead. This experience has led him beyond organic chemistry into polymer and analytical chemistry, micromachining, optics, and fluidics. Over the last 6 years, this background has been instrumental to the development of Research International's suite of instruments for the collection and optical detection of pathogens, particularly potential bioweapons. McCrae received a B.A. in Chemistry and Biochemistry from the University of California, San Diego, and a Ph.D. in Chemistry from the University of Oregon. He continued postdoctoral studies in drug syntheses at the University of Washington.

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Biosensor Studies at the Naval Research Laboratory for Environmental Monitoring

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The U.S. Navy is interested in developing detection methods for biological targets to provide rapid response in both medical diagnostics and environmental monitoring. For a number of years, the Naval Research Laboratory (NRL) has been instrumental in developing prototype biosensors and working to transition them to the commercial sector. The fiber optic biosensor, which started as a 100-pound instrument, has evolved into the Rapid, Automatic, and Portable Fluorometer Assay System (RAPTOR), a commercially available, portable (14 pound), automated biosensor for the rapid detection of biological threats. During this time, a number of systems were field-tested; one prototype was mounted in an unmanned aerial vehicle and demonstrated the ability to detect a cloud of aerosolized bacteria. Current work has focused on improving the RAPTOR's capabilities. The use of Alexa Fluor 647 to label tracer antibodies and the immobilization of capture antibodies via avidin-biotin interactions (rather than adsorption) have improved signal generation by a factor of 5. And, NRL has demonstrated the capability to test for eight targets simultaneously by combining multiple antibodies on each waveguide.

Another tack has been to optimize existing technology to meet Navy needs. Recently, NRL has determined the applicability of the Luminex 100 for environmental testing. The Luminex 100 is a specialized flow cytometer immunoassay system that can complete up to 100 tests on a single sample. This instrument performs multiple parallel immunoassays using up to 100 different types of polystyrene microbeads, each of which can be coated with a different antibody. It is able to discriminate these beads by determining the ratio of red and infrared dye, which is specific to each bead set. By performing the immunoassay on the surface of numerous microparticles free floating in solution, diffusion limitations are overcome. The detection of *E. faecalis* was found to be both rapid and sensitive enough to meet United States Environmental Protection Agency criteria.

George Anderson has been involved in biosensor research at the Naval Research Laboratory, Center for Bio/Molecular Science and Engineering for the last decade. As the principal investigator on the fiber optic biosensor project, he has overseen the transition of his patented technology from the laboratory prototype stage to that of a commercialized instrument. He has extensive experience in immunoassay development, including the purification and preparation of immunoreagents. Other interests include the development of novel recombinant binding ligands and proteins for use in materials research. His recent work has focused on developing novel bioconjugation methods for luminescent quantum dots and their use in multiplexed assays. Anderson received both a B.S and Ph.D. in Biochemistry from Ohio State University.

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Rapid Biosensor Detection of Microbial Pathogens

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Current methods for estimating microbial pathogen loads in beach water are based on the detection of indicator microorganisms, which provides a rough measure of potential fecal contamination. Such procedures are labor intensive and can take days to perform. Furthermore, they require trained scientific personnel and cannot be easily performed in the field. Evanescent wave, fiber-optic biosensors are capable of immediate, near real-time detection of indicator microorganisms as well as specific microbial pathogens, including *E. coli* O157:H7, *Salmonella typhimurium*, and *Cryptosporidium parvum*. These biosensors use robust antibody-based assays to capture and directly detect pathogens in complex matrix samples, such as food, water, and human clinical specimens, with little or no processing. Biosensor assays are automated and can be performed in the field by minimally trained personnel, and live organisms can be recovered after assay for viability determination and identification confirmation by nucleic acid analysis. When combined with filtration/concentration and/or selective enrichment procedures, biosensors can rapidly detect indicator microorganisms and specific pathogens in beach water samples.

Daniel Lim is Professor of Microbiology in the Department of Biology at the University of South Florida (USF). He also is associated with the USF Center for Biological Defense and directs the Advanced Biosensors Laboratory. His research focuses on pathogenic microbiology, with emphasis on virulence mechanisms of bacterial pathogens and the development of innovative rapid procedures to detect and identify microbial pathogens in food, water, and in infectious diseases. His research program is supported by over $3 million in annual funds from the Department of Defense, United States Environmental Protection Agency, and other agencies. Lim is a Fellow of the American Academy of Microbiology, Past President of the Southeastern Branch American Society for Microbiology, and a member of the American Society for Microbiology Council. He received a B.A. in Biology from Rice University, a Ph.D. in Microbiology from Texas A&M University, and completed a postdoctoral fellowship in the Department of Microbiology and Immunology at Baylor College of Medicine.

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Rapid Detection of Bacteria Using Immunomagnetic Separation (IMS) and Adenosine Tri-Phosphate (ATP) Bioluminescence

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It is well known that present tests for the presence of indicator bacteria in beach water are too slow and only lead to action days after a water sample was taken. The University of Michigan is working on a method that takes less then an hour and can be done at the beach with portable battery-powered equipment.

A beach water sample is pre-filtered to remove plant debris and algae. The University of Michigan has investigated several filters and has settled on one that removes the desired material, but lets most of the bacteria pass. After pre-filtration, the water sample is passed through a fine filter with a pore size of 0.45 microns. The filter is then backwashed with a solution that contains surfactants to recover the bacteria. Not all of the bacteria are recovered.

Thereafter, paramagnetic beads that are coated with antibodies are added, and the mixture is gently mixed. During this time, the beads attach themselves to the bacteria based on the chosen antibodies. The bead-bacteria complexes are then removed using a magnet. The procedure is called immunomagnetic separation (IMS). E. coli and Enterococci are successfully isolated. The complex is then re-suspended and concentrated further. The bacteria are then lysed (broken open) and their adenosine tri-phosphate (ATP) spills into the solution. Luciferne/luciferase (LL), an enzyme/substrate derived from the firefly, is added and produces light (relative light units [RLU]) that is measured with a luminometer. Comparing the light development to colony counts derived from the traditional plate method allows the development of correlation equations. These equations then allow an estimation of the colony forming units. The entire procedure can be accomplished in about 45 minutes on a picnic table at a beach with battery-powered equipment.

Co-authored by JiYoung Lee, Ph.D., University of Michigan

Rolf Deininger is Professor of Environmental Health Sciences at the School of Public Health at the University of Michigan. His current research interest is in the protection of drinking water systems. He has had support from the United States Environmental Protection Agency and the American Water Works Association to study early warning systems for water treatment plants against accidental and intentional contamination of the source water. He has worked on rapid methods for the detection of bacteria in the water distribution systems and, lately, on methods to detect indicator organisms in beach water. Deininger received a Dipl. Eng. in Civil Engineering from Stuttgart University and an MS. and Ph.D. in Sanitary Engineering and Environmental Engineering from Northwestern University.

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Optical Sensors for the Classification and Identification of Biological Material

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Echo Technologies, Inc. (ETI) is developing optical sensors and instrumentation for detecting and discriminating microorganisms and biological toxins. Sensors have been demonstrated to detect bacteria, bacterial spores, toxins, and viruses, and for the detection of ambient biological material, such as fungal spores. The sensors were originally designed for the direct detection of airborne material without the need for a liquid sample. Recently, these sensors have been adapted for use in natural and processed waters. As a critical compliment to this sensor array, ETI is also developing a new method for identifying specific waterborne bacterial pathogens, such as *E. coli* and *E. feacalis*. The biosensors are based on the use of Fluorescently Labeled Virus Probes (FLVPs), which are bacteriophage particles labeled with fluorescent reporters. The phage exhibit a rapid and highly selective interaction with their host bacteria. The probes are monitored by wavelengthspecific fluorescence spectroscopy, which indicates the presence of the phage/host complex.

The application of the FLVP technology to solid-state optical sensing represents a new approach to the real-time detection of bacterial pathogens. This approach will minimize the need for culturing to identify pathogens, and is an important departure from immunoassay or DNA-based sensing. A key advantage of using bacteriophage as the recognition element is that the phage can be readily isolated and selected for the degree of specificity required. Also, the FLVP/host interaction is very rapid, the FLVP probes are quite robust, and they can be produced at very low cost. The use of multiple optically encoded FLVPs will allow simultaneous detection of several waterborne pathogens and provide sensor redundancy, thereby improving measurement fidelity. ETI is currently developing prototype detection instrumentation for use with the FLVPs that can be configured for detection in pristine or complex water samples.

Mary Beth Tabacco has over 15 years experience in optical sensor development and applied sciences. She is co-founder, Vice President, and Director of Research at Echo Technologies, Inc., and is responsible for technology development and engineering activities. Prior to this, Tabacco started a research group in 1986 to develop fiber optic chemical sensors and measurement systems with funding from NASA and the Department of Defense. She has been a Visiting Scientist at Tufts University and also has acted as consultant in the area of sensor development to other young companies in the Boston area. She holds five patents in the area of optical sensing, and has received three NASA Technology Transfer Awards, as well as the AE50 Agricultural Engineering Award for innovation in product/system design for a multi-channel fiber optic pH measurement system. Tabacco received a B.S. in Chemistry from Wellesley College and a Ph.D. in Chemical Physics from Boston College.

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Industrial/Academic Synergy: The Journey from the Drawing Board to the Final Product

Mike Dziewatkoski, Ph.D.
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YSI Environmental

Future demands on the world's water resources are expected to increase the need for water-quality monitoring and evaluation technologies. Numerous institutions worldwide are involved in the basic research of developing such technologies. On the other hand, several industrial entities are focused on developing and applying these new discoveries and methodologies with the goal of supplying the end-user with a durable and reliable instrument platform.

This presentation will explore the synergistic relationship that can exist between research institutions and industry regarding product development. The basic concepts employed by industry for research and development activities will be discussed within the context of multi-group collaborations and business relationships. An overview will be given of:

- The roles of scientists and engineers.
- The steps involved with feasibility studies and the buffer zone concept.

This presentation will also explore a vision of new technologies required to meet future challenges encountered by our water resources.

Mike Dziewatkoski has been a Scientist with YSI Environmental, a division of YSI, Inc., since May 2002. His research interests include the application of techniques in electrochemistry, spectroscopy, chromatography, and electronics to continuous environmental monitoring systems. His position at YSI, Inc. involves the research and development of new sensor technologies for chemical measurements in the environment. He is also involved with the design and implementation of experiments related to product testing and competitive product analysis. Additionally, he serves as a science and technical resource for the company. Before joining YSI, Inc., he worked for several years in the areas of process analytical chemistry as well as taught at the university level. Dziewatkoski earned a B.S. in Chemistry from Ohio University and a Ph.D. in Analytical Chemistry from North Carolina State University.

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Rapid Detection of Indicator Bacteria with Dual Wavelength Fluorimetry

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The dual wavelength method is based on a recent discovery made and patented by scientists at the Coastal Environmental Laboratories at the University of Connecticut Marine Science and Technology Center. The test introduces a new way to achieve rapid results (4 to 5 hours) from modified versions of standard and approved bacterial monitoring methods using fluorescent enzyme products. The dual wavelength method simultaneously monitors the emission of both the uncleaved fluorogenic substrate and the released fluorophore. The ratio of the intensities of these two wavelengths is monitored. The rate of ratio change allows for the rapid and sensitive estimation of bacteria concentration in the sample.

Presently, the ratiometric method can indicate the presence of *E. faecalis*, total coliforms, and *E. coli* in seawater at the concentration limits set by the state of Connecticut within 4 to 5 hours from the time of sampling. Experiments testing the influence of recognized non-target positive and negative controls demonstrated that these bacteria have no effect on the results within the short assay period. Based upon ongoing test results, further optimization of both the fluorescence detection system and the assay conditions will shorten the detection time by a minimum of 1 hour.

Split samples with the local and state health departments have demonstrated the accuracy of this method, determined by comparison with standard most probable number and membrane filtration methods. The method is simple; all measurements can be performed in the container used to collect the sample. Sample preparation is minimal, and the skill level required of the operator is low. The method is suitable for conducting rapid field tests using a highly portable, dedicated fluorimetry detection system that is currently under development.

Co-authored by Robert T. Vinopal, Ph.D., University of Connecticut; Joseph R. Jadamec, University of Connecticut; Stephen A. Jakubielski, University of Connecticut; Christine G. Green, University of Connecticut, and Carol P. Anderson, Ph.D., University of Connecticut

Claudia Koerting is Assistant Research Professor with the Marine Sciences Department at the University of Connecticut. She is also Director of the Coastal Environmental Laboratory at the University. Recent projects include the development of a rapid assay for Lyme disease, bacterial diagnostics of lobsters in a Long Island Sound die-off, and the characterization of stress tolerance responses of foodborne pathogens. Her current interests include exploring applications for immunomagnetic separation (IMS) in pathogen detection, seafood safety, and the microbial ecology and detection of toxins from harmful algal blooms. Koerting received both a B.S. in Microbiology and a B.A. in Chemistry from the University of Rhode Island, followed by a year as a visiting graduate student in Chemistry at the

Ludwig Maximillian University in Munich, Germany. She also received an M.S. in Oceanography from the University of Connecticut for studies done on petroleum biodegradation and a Ph.D. from the University of Rhode Island, specializing in the microbial ecology and detection of toxins from algae.

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Rapid *In Situ* Coastal Monitoring of Indicator Bacteria Based on a Fluorescent Enzymatic Assay

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Two promising rapid methods are being developed for seawater applications. The first approach is to develop an automated *in situ* sensor system for use on a buoy or pier that measures microbiological water quality in coastal waters. Existing enzymatic assays (i.e., Colilert and Enterolert assays, IDEXX Laboratories, Inc., Westbrook, Maine) are being modified for indicator bacteria, and detection is being automated so that bacterial indicators can be combined with existing real-time sensors of standard oceanographic instruments that measure temperature, salinity, dissolved oxygen, and chlorophyll concentrations. This approach should yield both rapid analysis (under 8 hours) of *E. coli* and *Enterococcus* and ancillary data for regulators to better assess health risks. Fluorescence assays are being carried out in solution to maximize sensitivity and minimize the cost and complexity of detection. The strategy takes advantage of recent advances in biotechnology (the development of fluorescent probes) and the inherent sensitivity of fluorescence detection in solution. By automating these methods and by deploying them on an *in situ* platform, dependence on human sampling protocols and biases, lengthy transport times, and storage effects are eliminated. By automatically reporting analyses via the Internet, regulators can more effectively respond to threats to human health in recreational waters, eventually with "same-day" information. Initial challenges to bringing the proposed method to practical use are, first, to increase the sensitivity of the assay, both by increasing the ability to detect low extant numbers of indicator bacteria in seawater, and by decreasing the incubation time required to achieve an adequate fluorescent signal. The second major hurdle is to reduce fluorescence from enzyme activities of some common seawater bacteria (false positives). Third, the issue of false negatives, which lead to underestimating indicator bacteria abundances, must be addressed. Currently, experiments with bacterial concentration approaches and media formulation are in progress to optimize the assay.

The second method is a "rapid" DNA-based method for strain differentiation, or microbial source tracking, of bacteria in natural waters. The initial focus was on *E. coli.* To significantly reduce sample preparation and the time required for analysis, an E. coli-specific thermal gradient gel electrophoresis (TGGE) method based on the 16S-23S rRNA intergenic spacer region from a single operon was developed. The entire *E. coli* population of a water sample can be analyzed in one TGGE gel lane loaded with polymerase chain reaction (PCR) amplification products from a single extract of community DNA. To test the method, *E. coli* was enumerated and a TGGE analysis of both community DNA and randomly isolated *E. coli* strains was conducted on 10 water samples collected along a stream running through residential areas originating from a nature reservation. It was conclude that a single 16S-23S rRNA intergenic spacer (IGS) operon analysis by TGGE is a rapid and useful technique for microbial source tracking. The method can be adapted to other bacterial species. The challenge for broad use of this method will be to standardize and automate it.

Co-authored by Julie Carruthers, Ph.D., University of Massachusetts Boston; Michie Yasuda, Ph.D., University of Massachusetts Boston; and Robert Chen, Ph.D., University of Massachusetts Boston

Michael Shiaris is a Professor and the Chairman of the Department of Biology at the University of Massachusetts Boston, where he teaches general microbiology and graduate courses in microbial ecology and physiology. His research interests include environmental microbiology/microbial ecology, and he is developing methods for the rapid detection and differentiation of indicator bacteria in natural waters. He is also studying bacterial population ecology in coastal environments and the biodegradation of organic pollutants and polycyclic aromatic hydrocarbons in coastal ecosystems. His laboratory group uses DNA fingerprinting, DNA sequencing, and routine microbiological techniques to study bacteria in coastal environments. Shiaris received a B.S. in Microbiology from the University of Maryland, an M.S. in Microbiology from Colorado State University, and a Ph.D. in Microbiology and Ecology from the University of Tennessee.

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Development of an Instrumental Technology for the *In Situ* Detection and Rapid Near Real Time Reporting of Fecal Contamination in Marine Waters

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Because current methods for assessing water quality for fecal contamination require both the manual sampling of waters and lengthy incubation times, a new submersible instrument designed to detect indicator bacteria in less time would offer substantial benefits. The goal is to develop and demonstrate a suitable procedural methodology or protocol to rapidly monitor selected bacterial indicators of fecal contamination in fresh and marine waters. One approach is to adapt a commercially available, defined substrate technology that is approved by the United States Environmental Protection Agency for use in an autonomous instrument. The defined substrate media enhances the growth and enzyme production of target organisms (total coliforms, *E.coli*, and/or *Enterococci*) while suppressing other bacteria. Upon hydrolysis by bacterial enzymes, these substrates release fluorescent (4-methylumbelliferone [MU]) or colored (o-nitrophenol [ONP]) compounds that can be quantified with optical detection systems. The submersible instrument would ultimately have the hand-held and/or autonomous capability to rapidly quantify and report enteric bacterial contamination in aquatic waters in near real-time.

As the first phase of instrument development, a proof of concept evaluation was conducted with a bench-top system. An investigation of the optical properties of the hydrolysis products revealed the following:

- Linear correlations for concentrations of MU and ONP with fluorescence and absorbance, respectively.
- Maximum fluorescence of MU at a pH greater than 10.
- Only slight interference by ONP on fluorescence that can be corrected for.

Growth curves using optical density (600 nanometers) and plate counts of the indicator bacteria were established to independently quantify bacterial densities. Initial experiments show the detection of E.coli in concentration levels of 13 to 1,300 cells/milliliter within 4 to 6 hours and of Enterococci as low as 100 cells/milliliter in less than 4.5 hours. These results support the feasibility of the technical approach and aid in determining the specifications required to further develop the instrumentation.

Co-authored by Alfred Hanson, Ph.D., SubChem Systems, Inc., and David Smith, Ph.D., University of Rhode Island

Heather Saffert attended Wesleyan University in Middletown, Connecticut, and received a B.A. in Biology with honors in 1996. After graduating and publishing her research on salt marsh foraminiferal ecology, she moved to Newport, Rhode Island, and worked in environment consulting at Science Applications International Corporation. She entered the University of Rhode Island Graduate School of Oceanography as a Ph.D. candidate in Fall 2000. She works under the guidance of chemical oceanographer, Dr. Alfred Hanson. Together, they have formed an interdisciplinary team to develop a new submersible instrument, the BioAnalyzer, to detect indicator bacteria and monitor water quality for shell fishing and recreational purposes.

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Rapid Measurement of Bacterial Fecal Indicators in Surface Waters by Quantitative Polymerase Chain Reaction (QPCR) Analysis

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Current methods for determining fecal contamination of recreational waters rely on the culture of bacterial indicators and require at least 24 hours to determine whether the water is unsafe for use. By the time monitoring results are available, exposures have already occurred. New methods are needed that will allow near real-time determination of water quality, such that public notifications can be made and hazardous exposures avoided. With assistance from the United States Geological Survey Laboratory in Porter, Indiana, the National Exposure Research Laboratory of the United States Environmental Protection Agency has conducted a 2-year pilot study to evaluate the use of quantitative polymerase chain reaction (QPCR) analysis for measuring waterborne fecal indicator microorganisms at two recreational beaches on Lake Michigan.

A rapid, simple, and generally applicable method for the recovery of total DNA from various microorganisms in water samples has been developed. The method involves the filtration of water samples on polycarbonate membrane filters and disruption of the collected cells directly on the membranes by glass bead milling. Recovered DNAs are subjected to QPCR analysis using the TaqMan™ polymerase chain reaction (PCR) product detection system in a real-time PCR product detection instrument. Procedures have also been developed for the use of cycle threshold (CT) values generated by the instrument to enumerate cells in the water samples. The approach is based on the comparative CT method, which employs an arithmetic formula to determine target sequence quantities in DNA extracts from test samples relative to those in similarly-prepared DNA extracts from calibrator samples containing a known quantity of target organism cells. Assay CT values for a DNA sequence from an exogenous reference organism, added in equal quantities to both the test and calibrator samples before extraction, are used to normalize results for differences in the amount of total DNA added to each reaction (e.g., caused by differences in DNA extraction efficiency between samples) or to signal potential PCR inhibition in test samples. The entire analysis process can be performed in approximately 2 to 3 hours.

Target DNA sequences for QPCR detection in the pilot study included the large subunit ribosomal RNA gene of *Enterococcus* spp. and the small subunit ribosomal RNA gene of *Bacteroides* spp. Tests with pure cultures of representative species within these two genera gave extrapolated detection limits of approximately two cells per sample for the *Enterococcus* assay and 25 cells per sample for the *Bacteroides* assay. Tests on a subset of the Lake Michigan water samples spiked with ~1,000 cells of *Enterococcus* cells gave an overall mean value of 0.96 for the ratio of measured to added cells and a 95-percent occurrence range for individual sample ratios of ~0.3 to 3, based on analyses of three replicates of each sample. Mean QPCR-measured quantities of native *Enterococci* in the 100-millimeter water samples ranged from less than 10 to ~1,000 cells, whereas meanquantities of *Bacteroides* ranged from less than 100 to ~100,000 cells. DNA

extracts of the water samples were routinely diluted tenfold prior to analysis to eliminate the effects of PCR inhibitors. The distributions of QPCR-measured cell quantities of both *Enterococcus* and *Bacteroides* in the water samples paralleled those of culturable *Enterococci* measured in corresponding water samples by the currently accepted mEI filter plating method.

These results indicate that the QPCR method has the potential to detect a broad range of fecal indicator densities in recreational water samples. Past findings of a correlation between the quantities of culturable *Enterococci* in water samples and illness rates among bathers have provided the basis for establishing recreational water quality guidelines. New studies will be initiated this summer to establish whether similar correlations exist between fecal indicator measurements by QPCR and other rapid methods and rates of illness among bathers.

Richard Haugland is a microbiologist for the National Exposure Research Laboratory of the United States Environmental Protection Agency. His early research included studies on the genetic mechanisms by which new catabolic pathways for hazardous chemicals are created in bacteria and the development of hybridization probes for identifying these bacteria in wastewater treatment processes. More recent studies have focused on the development and application of PCR-based methods for the detection and quantification of allergenic and toxigenic fungal species in the indoor environment, as well as the application of PCR technology in other areas such as drinking and recreational water quality and the detection of bioterrorism agents. In addition, he has authored or co-authored over 30 peer-reviewed publications and holds one patent. Haugland received a BS in Biology from Muskingum College and a Ph.D. in Developmental Biology from Ohio State University. He completed post-doctoral and industrial research at McGill University, Oregon State University, and Allied Corporation on the molecular characterization and genetic improvement of microbial symbionts in biological nitrogen fixation.

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Rapid, Automated Polymerase Chain Reaction (PCR) Methods for Microbial Detection

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Cepheid

DNA is specific enough to indicate not only the species of a pathogen, but also the presence of a significant mutation, such as drug resistance. Known DNA sequences canbe correlated with specific diseases (genetic diseases, cancer) and can be detected with high sensitivity using DNA amplification methods, such as the polymerase chain reaction (PCR). Amplification methods have significantly increased the power of DNA-based analysis, but in their current state, they have drawbacks, including:

- The complexity of sample preparation.
- The time required for amplification.
- A cumbersome and separate detection methodology.

To ready DNA for amplification, it must be extracted, concentrated, and (to some degree) purified. The DNA may come from a complex sample such as blood, biological tissues, food, or soil. The sample preparation procedures are multi-step, time consuming, and require a great deal of skill and training. Although faster than culture, amplificationmethods may be slow (90 minutes or more), especially when using traditional equipment. The operator is usually required to carry out a time-consuming protocol for reagent preparation and addition, requiring both training and practical expertise. Many amplification methods involve a separate, time-consuming gel electrophoresis detection step. Amplified DNA target is transferred to a separate piece of equipment, where additional reagents are prepared and added, and a time consuming procedure is carried out.

Cepheid's technologies are designed to significantly simplify and reduce the time for all of these steps. Its patented I-CORE module enables rapid amplification and DNA detection to be carried out in a single reaction tube. Cepheid has developed fluidic systems designed to carry out the preparation and processing of raw specimens and reagents in a rapid, automatic, hands-off manner. Cepheid's I-CORE and fluidic systems are designed as modules to be integrated into a wide range of system configurations for rapid, automatic DNA based analyses in a wide range of settings (both labatory and field) where time is critical.

Environmental quality management is becoming crucial to a wide range of industries. And, with greater pressure to recycle water, minimize the use of antibacterial agents, and maintain quality discharges, manufacturers in a wide variety of industries are seeking technologies to rapidly identify contamination problems at the source. For example, Cryptosporidium parvum is a waterborne pathogen infective at a dose of a single organism. It is responsible for frequent widespread outbreaks of intestinal disease that can be life-threatening for individuals with compromised immune systems. Cepheid believes the rapid, on-site testing systems it is

developing will play an important role in further enabling the detection of environmental pathogens. This effort may be of use to groups engaged in recreational waters management and beach monitoring.

Jeffrey R. Ryan is a retired Army Lieutenant Colonel whose last military assignment was with the Walter Reed Army Institute of Research in Silver Spring, Maryland, where he served as the Research Coordinator for the Common Diagnostics Program and was the Chief of the Entomology Department. He now works as a Strategic Business Developer for Cepheid of Sunnyvale, California. Cepheid develops novel platforms and assays that utilize real-time PCR for nucleic acid detection. Ryan received a B.S. in Entomology from both the State University of New York and Syracuse University, an M.S. in Biochemistry from Hood College in Maryland, and a Ph.D. in Medical Entomology from North Carolina State University.

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Optical Sensor Microarrays for Rapid Detection

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Coherent imaging fibers can be used to make fiber-optic chemical sensors. Sensors can be made with spatially discrete sensing sites for multianalyte determinations. Currently, there is an investigation on the limits of the ability to create high-density sensing arrays containing thousands of microsensors and nanosensors. Micrometer- and nanometersized sensors have been fabricated by etching the cores of the optical imaging fiber to create wells and by loading them with micro- and nanospheres. Such arrays can be employed for making genosensors with high sensitivity and reproducibility.

Optical sensors have been created based on principles derived from the olfactory system. A cross-reactive array of sensors is created such that specificity is distributed across the array's entire reactivity pattern rather than contained in a single recognition element. The ability to use such information-rich assemblies for broad-based diagnostic sensing will be discussed.

David Walt, the Robinson Professor of Chemistry at Tufts University, serves on many government advisory panels and boards. He chaired a National Research Council (NRC) panel on New Measurement Technologies for the Oceans and is a member of the NRC Committee on Waterborne Pathogens. He is also Executive Editor of Applied Biochemistry and Biotechnology and serves on the editorial advisory board for numerous journals. Walt has received numerous national and international awards and honors, including a National Science Foundation Special Creativity Award, The Biosensors and Bioelectronics Award, and the Samuel R. Scholes Award in Glass Science. In 2000, he was elected a fellow of the American Association for the Advancement of Science. In addition, he has published over 150 papers, holds over 30 patents, and has given hundreds of invited scientific presentations. Walt received a B.S. in Chemistry from the University of Michigan and a Ph.D. in Chemical Biology from the State University of New York at Stony Brook. He completed postdoctoral studies at the Massachusetts Institute of Technology.

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The Future of Microarrays and Nucleic Acid Sequence Based Amplification (NASBA) for Water Quality Monitoring

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New contaminants with the potential for waterborne transmission are constantly being identified. In many cases, conventional cultivation technologies will not be applicable for their detection. In addition, it would be of great interest to be able to evaluate water samples for numerous microbial contaminants at one time. Nucleic acid sequence based amplification (NASBA) has been shown to have several advantages over polymerase chain reaction (PCR) technologies: It targets RNA and, thus, is quite applicable to RNA viruses and functional targets (mRNA); is isothermal; and provides more rapid and sensitive detection. It has been used for *Cryptosporiium* and Noroviruses. Current work is ongoing, focusing on enterovirus targets and the development of *in situ* instrumentation. Improvements in specificity and primer design will be key to the success of this technology. Collaborations between the University of Michigan and Michigan State University are focusing on the use of light-directed synthesis of microarray platforms, which can be used to address an array of waterborne pathogens. The microarray can harbor 30,000 different targets up to 1,600 unique oligonucleotides derived from up to 100 distinct genomes. For example, gene sets have been developed to detect both genogroup I (GGI) and GGII strains of Noroviruses. GGI Noroviruses are currently divided into at least seven clusters that differ by ~30-percent amino acid variability in the major capsid gene. GGII Noroviruses have at least nine clusters that also encode ~30-percent amino acid heterogeneity in the capsid gene. These differences can be captured. Various approaches for preprocessing the samples and experimentation on hybridization will be needed.

Co-authored by John Paul, Ph.D., University of South Florida; Erdogan Gulari, Ph.D., University of Michigan, and Syed Hashsham, Ph.D., Michigan State University

Joan Rose has made groundbreaking advances in understanding water quality and protecting public health for more than 20 years. She is widely regarded as the world's foremost authority on the microorganism *Cryptosporidium* and was the first person to present a method for detecting this pathogen in water supplies. Among her honors, Rose was named as one of the 21 most influential people in water in the twenty-first century by *Water Technology Magazine* (2000) and received the 2001 Clarke Prize from the National Water Research Institute for her advances in microbial water quality issues. Rose joined Michigan State University in 2003 as the Homer Nowlin Chair For Water Research. Currently, she is one of only a handful of scientists around the world today who are examining the relationship between climate, water quality, and public health. Rose received a B.S. in Microbiology from the University of Arizona, an M.S. in Microbiology from the University of Wyoming, and a Ph.D. in Microbiology from the University of Arizona.

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Challenges of Using Microtechnology-Based Instrumentation on Macro-World Water Samples

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Although significant progress has been made in creating microtechnology-based instruments to perform bioassays, each of these instruments has in common the need to deliver a sample that has been cleaned up and (probably) concentrated, along with the necessary reagents, into an analysis chamber that has dimensions of millimeters. When faced with millions of liters of coastal water, wherein there is no reason to expect a uniform distribution of potential hazards to humans, the problem of sufficient sampling is daunting. This problem is exacerbated by the fact that there may be reservoirs for the hazards that are, in some sense, hidden from easy sampling - viruses inside of algae or shellfish, for example. Certainly, while researchers have developed advanced diagnostic assays using RNA and DNA to identify and/or characterize ocean-borne pathogens, sample collection and preparation remain difficult. If the reservoir of a pathogen is the water itself or a free-floating microbe in seawater, we can be optimistic that a hybrid system that combines existing, macro-scale commercial filters followed by millimeter-scale microfluidic-based instrumentation for sample preparation may serve some of the needs for monitoring coastal water. If the preponderance of the threat is contained inside of shellfish, the collection and processing of samples will require new technologies. This work was performed under the auspices of the United States Department of Energy by the University of California Lawrence Livermore National Laboratory under Contract Number W-7405-Eng-48.

Ray Mariella is Director of the Center for Micro- and Nano Technology at Lawrence Livermore National Laboratory. He began his career by teaching physical chemistry at Harvard University for 1 year, was a Visiting Scientist in the physics department at the Massachusetts Institute of Technology for 2 years, and was a research fellow at the IBM research laboratory in San Jose, California, for 1 year. He spent 10 years at the Allied-Signal Corporate Research Center before joining Lawrence Livermore National Laboratory, where he has been for the past 15 years. Mariella received a B.A. in Mathematics, Chemistry, and Chemical Engineering from Rice University, and an A.M. and Ph.D. in Physical Chemistry from Harvard University.

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