Report to the Legislature

Development of Reliable, Rapid, and Affordable Diagnostic Tests for Measuring Bacterial Indicators in Coastal Waters

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STATE WATER RESOURCES CONTROL BOARD
CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

EXECUTIVE SUMMARY

California's coastline is one of its most important natural features. In 1999, 33 million persons visited California's beaches, generating $17 billion in direct revenue. In recent years, the public has become increasingly concerned about beach water quality and its effect on the health of swimmers. A 1995 Santa Monica Bay epidemiological study found a correlation between increased incidence of gastrointestinal illnesses and increased levels of bacterial indicator organisms in storm drain runoff. A direct result of this study was the passage of Assembly Bill (AB) 411 (Chapter 765, Statutes of 1997), increasing the monitoring required for heavily used ocean beaches.

A complicating factor of this required monitoring is the limitation of the current analytical techniques for bacterial indicator organisms. Current techniques for indicator bacteria take between 18 and 96 hours for completion. This lag time means that a beach with bacterial levels exceeding water quality standards on the day the sample is collected is not posted or closed until at least the following day. What is needed is a more rapid analytical method, ideally one that can be completed within an eight-hour workday. As part of Governor Gray Davis' 2001 budget, the State Water Resources Control Board (SWRCB) received $1.5 million to fund the development of a rapid analytical method for bacterial indicator organisms in coastal waters. In conjunction with this funding, the Legislature enacted AB 639 (Chapter 502, Statutes of 2001) requiring SWRCB to develop reliable, rapid, and affordable diagnostic tests for measuring bacterial indicators in coastal waters. The law also requires SWRCB to report to the Legislature on or before July 1, 2003, on the progress of the research.

SWRCB contracted with the Southern California Coastal Water Research Project (SCCWRP) to coordinate this project. Because of a number of unforeseen events, the contract for this work was not finalized until July 2002. Once the contract was finalized, work began immediately. The project was designed to be conducted in two phases. The first phase involved soliciting proposals from researchers actively working on rapid microbiological measurement methods for other industries. Nine proposals were submitted, and five were selected for funding. A sub-group of the five researchers will be selected for further funding during the second phase, which involves six months of continued method development followed by a three-month period of comparative testing.

A workshop was conducted May 14–16, 2003, in Monterey. The workshop brought together all researchers active in the field. Researchers discussed their individual methods on the first day. The next two days focused on defining technical, administrative and financial obstacles to these new technologies and on identifying the best approaches to overcome these obstacles. Among the 30 invited attendees for the final two days were representatives from the California Department of Health Services (DHS), Heal the Bay, various universities, and private firms.

Background

California's coastline is one of its most important natural features. It extends over 1,000 miles from the rocky cliffs of the north coast to the sandy, sun-drenched beaches in the south. The coastal areas represent a desirable place to live, with approximately 80 percent of California's 33 million residents living within a 30-mile drive of the coastline. In 1999, 33 million persons visited California's beaches, generating $17 billion in direct revenue.

Increasingly, the public is becoming concerned about beach closures and the safety of public beaches. When a beach is closed due to contamination, the economic effect can be devastating to local business owners.

Beach water quality is routinely monitored by local county health agencies, publicly-owned water treatment works, and environmental groups. These water samples are analyzed for the indicator organisms total and fecal coliform, and enterococcus bacteria. Because routine monitoring for all possible human disease-causing agents is impractical, these indicator bacteria are used as an alternative to the measurement of pathogens with the assumption that high levels of the indicators imply the presence of fecal contamination. These indicators are not human specific: total coliform bacteria can exist on soil particles and plant surfaces, and fecal coliform and enterococci bacteria are normally found in the gastrointestinal tracts of warm-blooded animals. The ocean is the final deposition site for most land-based pollutants in California's coastal watersheds. Runoff from creeks, rivers, and storm drains provides a significant source of bacteria to California's beaches. Increased recreational water monitoring along with more stringent standards as the result of recent state legislation has heightened public awareness and focused attention on water quality at our beaches. Epidemiological studies have demonstrated that gastrointestinal illnesses associated with swimming in both salt and fresh waters can be linked to levels of bacteria that indicate sewage contamination. Further, a 1995 study conducted at Santa Monica Bay found that the incidence of gastrointestinal illnesses increased with increased concentrations of indicator bacteria in storm drains flowing to ocean beaches. California law (Health and Safety Code Section 115880 et. seq.) requires weekly indicator bacteria testing between April and October, along with public notification of monitoring results for ocean beaches having 50,000 or more visitors annually and that are located near a flowing storm drain. These requirements have resulted in an increased number of beach postings and closures, particularly in Southern California.

Complicating this required monitoring are the limitations of current analytical techniques. Current analytical techniques for indicator bacteria take from 18 to 96 hours for completion. This means that a beach with bacterial levels exceeding water quality standards on the day the sample is collected is not posted or closed until the next day. What is needed is a more rapid analytical method, ideally one that can be completed within an eight-hour workday. The 2001 Budget Act provided funding for projects that

would increase the public's access to clean beaches and reduce health risks. As part of this funding, SWRCB received $1.5 million to fund the development of a rapid analytical method for bacterial indicator organisms in coastal waters.

In conjunction with this funding, the Legislature enacted AB 639, which requires SWRCB to work with DHS to develop reliable, rapid, and affordable diagnostic tests for measuring bacterial indicators in coastal waters. Methods that can be completed within six hours without the benefit of laboratory facilities have priority. SWRCB is required to prepare a report to the Legislature, on or before July 1, 2003, on the progress of the funded research. The following is a progress report on the work to date.

Project Description

SWRCB contracted with SCCWRP to coordinate this effort. Because of a number of unforeseen circumstances, the contract was not finalized until the end of July 2002. Once the contract was finalized, work began immediately.

The development of rapid analytical methods was designed to be conducted in two phases. During phase I, multiple contractors have been selected to refine their existing methods. After approximately eight months, contractors presented their work at a public workshop in May 2003. The contractors will be rated on their progress; a sub-group of these contractors demonstrating the best progress will be selected to participate in phase II. Phase II consists of a six-month period of continued development followed by a three-month period of comparative testing.

Phase I

SCCWRP prepared and distributed a Request for Proposals (RFP) to solicit proposals from researchers throughout the United States who are actively working on rapid microbiological measurement methods for other industries, such as drinking water, food service, counter-terrorism or freshwater ambient monitoring. The RFP was sent to several University of California researchers; however, none of these researchers submitted proposals. Nine proposals were submitted. SCCWRP convened a technical advisory committee to review the proposals, using the minimum criteria for ranking listed below:

1) The method would be ready for laboratory testing before April 2003.

2) The method will detect viable indicator organisms or a molecular substructure of the organism that can be related to the viability of the indicator bacteria. This distinction is important because the current DHS regulations and California Ocean Plan standards are based on enumeration of viable organisms (living organisms capable of reproducing). If the method detects an agent or molecular substructure of an agent other than viable indicator bacteria, the relationship between the detected agent and the current water quality standards must be established and accepted by peers.

3) The method detection limit will allow measurement of bacterial concentrations at or below DHS and the California Ocean Plan bacterial standards.

4) The analysis can be completed within a normal workday.

5) The method will be practical and simple to use without extensive training.

6) The cost of the method will be approximately the same as current analytical costs ($25 - $50 per sample).

Five proposals were selected and funded. These proposed methods are summarized below.

Research International

Research International (RI) is developing an immunoassay-based biosensor system, modifying instruments they have helped develop for the detection of pathogens such as *Escherichia coli* 0157:H7*, Salmonella typhimurium*, *Bacillus anthracis* spores, and *Cryptosporiduim parvum* oocysts. A battery-operated pump will draw a water sample through a filter mounted at the pump inlet. The filter is then loaded into a cup containing a culture medium, and the cup is vibrated to loosen the bacteria from the filter. The sample cup is then mounted on a motorized rotation stage, and a waveguide is immersed in the cup. A waveguide is a hollow circular metallic tube that allows an electromagnetic field to travel into the culture media. The waveguide's baseline signal is then measured. This initial measurement can be completed in 10 to 15 minutes from the time the sample was collected. The culture media is then transferred to a reagent cup containing an antibody tagged with florescent molecules. The antibody/culture media mixture is incubated for three to six minutes. Then the waveguide is returned to the cup, and the signal is measured again. The signal level above the baseline measurement is proportional to the number of bacteria in the sample.

At this point, the waveguide is removed and a heater jacket is placed around the sample cup to allow for growth of enterococci. Ideally, this growth period will be less than six hours, allowing sample analyses to be completed within an eight-hour workday.

Advanced Analytical Technologies, Inc.

Advanced Analytical Technologies, Inc. (AATI) is developing a laser-based optical system, expanding work they began under contract with the U.S. Environmental Protection Agency (U.S. EPA) for the rapid detection and enumeration of enterococcus. Water samples will be passed through a filter in order to concentrate the organisms present in the sample. The bacteria are then back flushed from the filter into a collection tube, with a goal of recovering greater than 90 percent of the organisms from the filter. A technique called immunomagnetic separation will be used to isolate enterococcus and *E. coli* from other bacteria in the collection tube. Enterococcus and

E. coli are then combined with fluorescent material. Once the target bacteria are tagged, they can be counted using a specialized cytometer. This instrument focuses a laser beam on the tagged bacteria. Because these bacteria are fluorescently tagged, each individual bacterium emits light, which is collected into a detector tube and processed to give a numeric value.

AATI hopes that this method will produce reliable results within four hours of sample collection.

Dr. Rolf Deininger and Dr. JiYoung Lee, University of Michigan

Drs. Deininger and Lee have developed a rapid analytical method for *E. coli* in fresh water and are adapting this method for use in marine waters. This procedure begins with filtration of the sample. The filtration step takes about five minutes. After filtration, the bacteria captured on the filter are resuspended into solution in test tube containers. Antibody-coated beads are added to the suspension. During this time, target bacteria (E.coli) attach themselves to the beads, tagging the E.coli. The test tubes containing the tagged bacteria are inserted into magnetic separators, which separate the tagged bacteria from the liquid. The liquid is poured off, and the tagged bacteria are treated with an agent that ruptures cell walls and releases adenosine triphosphate (ATP) from each cell. ATP is the major energy source within cells that drives a number of biological processes. Two chemicals are added, which react with the ATP and result in the formation of bioluminescence. The resulting light development is read. The amount of light is proportional to the concentration of bacteria present in the sample. The entire procedure should be completed within an hour.

Sub Chem Systems, Inc.

Sub Chem Systems has developed a prototype instrument that uses a modification of a technology approved by U.S. EPA for microbiological analyses. Both U.S. EPA and the National Oceanic and Atmospheric Administrative Sea Grant Program have provided funding to Sub Chem for external evaluations of the prototype instruments. The researchers are adapting a currently used analytical method which uses fluorescence to quantify indicator bacteria. The submersible BioAnalyzer will take a water sample, incubate it at the correct temperature, and read and transmit the results to a computer. The goal of Sub Chem Systems is to develop an instrument that can be remotely deployed for near real-time measurement of bacterial indicator organisms, possibly in less than 30 minutes. The instrument could either be attached to a fixed position in the water or allowed to travel with ocean currents.

University of Connecticut

The University of Connecticut is developing an analytical method using fluorescence. The method is based on recent work that several University scientists have patented. Under appropriate conditions, certain enzyme substrates exhibit fluorescence in their unmetabolized state. When the substrate is metabolized, the fluorescent spectrum

shifts to a lower frequency. In this method, a substrate capable of fluorescence is added to the sample to be tested. As the target bacteria break down the substrate, the amount of intact substrate decreases as fluorescent emission occurs. By simultaneously monitoring the fluorescent intensity at both emissions bands (the original and the lower frequency), the concentration of bacteria present in the water sample can be determined. Sample analysis should be in the range of two to four hours using this method.

Progress to Date

A workshop was conducted May 14–16, 2003, in Monterey. During the first day of the workshop, 15 researchers, including the five that received SWRCB funding, made presentations on their individual methods. The first day of the workshop was open to the public. During the next two days, a group of thirty invited attendees met with the five researchers under contract. The invited attendees included representatives from DHS, SWRCB, the environmental group Heal the Bay, county health agencies, universities, industry, and New Jersey and Hawaii water quality agencies. The focus of this group was to define the technical, administrative, and financial obstacles to these new technologies, and the best approach(es) to overcome these obstacles. The outcome of this workshop is an assessment of the status of rapid analytical method development. Based on what was discussed over the last two days of the workshop, SWRCB, SCCWRP, and the technical advisory committee will select the contractors who will receive Phase II funding and will be able to design the studies necessary to utilize the developed method or methods. SWRCB staff will again invite DHS to participate in the Phase II decision. We anticipate that this project will be completed by December 2004. At this time, SWRCB staff will submit a final report to the legislature, along with the final report from SCCWRP.