SCCWRP #0698

Upper Santa Monica Bay

PHOTO: KAREN SETTY

Adapting and Adopting Rapid Molecular Methods for Beach Water Quality Monitoring

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Abstract

Protecting the public from potentially harmful pathogens requires periodic monitoring of recreational waters. For many years, scientists and managers have relied on culturing indicator bacteria from samples to count how many are present, leaving a significant time gap between when the sample is collected and when results become available to make decisions about issuing health warnings. Rapid molecular analytical methods offer a new paradigm in which more timely knowledge of water contamination issues would be available to help prevent exposure-related illness. The most advanced rapid method to date, quantitative polymerase chain reaction (qPCR), takes just a few hours and is on track for nationwide approval in 2012 (U.S. EPA 2007). In addition to research and development, efforts to transition potential new methods and ensure their real-world applicability are critical. Two pilot projects applying a rapid method at Los Angeles and Orange County beaches have demonstrated the feasibility of faster results.

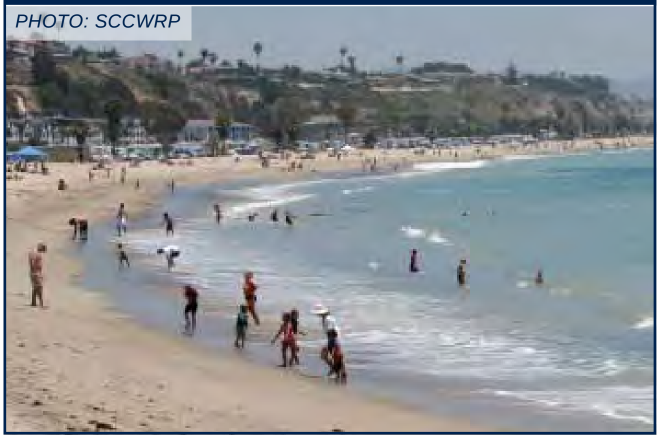
Introduction

The safety of swimming in America's rivers, lakes, and oceans was called into question as increasing urban and industrial development of the nineteenth and twentieth centuries led to more and more pollutant loading into waterways. As the environmental movement gained steam, the 1972 U.S. Clean Water Act set goals for rehabilitating waterways to recapture their once swimmable status. The same year, the newly formed U.S. Environmental Protection Agency (U.S. EPA) conducted a series of studies to improve national bathing water quality criteria. The criteria in place at the time had been proposed by the Department of the Interior in 1968, using an analytical method that dated to the turn of the century (U.S. EPA 1986; National Research Council [NRC] 2004; Dufour and Shaub 2007).

The prevailing trend was to isolate and culture "fecal indicator bacteria," strains of bacteria not necessarily implicated in causing illness, but well correlated with the presence of fecal pollution and the pathogens that

Figure 1. Popular Doheny State Beach

PHOTO: SCCWRP



do make people sick. Culturing means putting the sample in contact with a growth medium (bacterial food source) and waiting to see how many bacteria reach a growth endpoint, such as nutrient metabolism or reproduction to form visible colonies. Such tests typically require 18 to 96 hours for accurate results. Once the bacteria in the sample are quantified, their number can be related back to an observed relationship of how often people contract illness from that degree of exposure, based on epidemiology studies (Wade et al. 2003). The predominant fecal indicator bacteria used were total coliforms and fecal coliforms.

The 1968 federal guidelines and subsequent EPA guidance in 1976 advocated a similar fecal coliform enumeration approach. Based on new information, though, the focus shifted to *E. coli* for freshwater and enterococci for marine waters in 1986 (U.S. EPA 1976, 1986). States are allowed to adopt criteria more stringent than federal standards, and California did so in 1997 with Assembly Bill 411 (AB 411). Based in part on a Santa Monica Bay Restoration Project epidemiology study (SMBRP 1996), AB 411 requires at least weekly testing during the summer for multiple indicators at highly frequented beaches with nearby storm drains (Figure 1). This bill also closes beaches to swimmers when a known contamination source, such as a sewage spill, affects the area. Exceeding one of the California Department of Public Health's recommended water quality thresholds without a known or suspected sewage source leads to posting of a warning sign at the beach.

California's current beach monitoring program is one of the strongest in the nation, but the program still relies on methods based on bacterial growth endpoints. Research has found most sources of beach water contamination are intermittent and last less than one day (Leecaster and Weisberg 2001). This means results that lead to posting or removal of warnings are usually received one or two days after the contamination was present. Such delayed results set up decision-makers for failure. Public health managers could potentially miss contamination events completely or cut visitation and revenues to beaches that no longer pose a threat. Faster alternative methods for beach water quality monitoring have not yet been widely used outside research laboratories, but steps have been taken to build a foundation for better public health protection.

Speeding up Microbiological Tests

Recent advances in molecular biochemistry, genetics, and imaging technology have set the stage for newer, faster methods to complement or replace the growth-based approaches of the last century. The basic goal—identifying when a sample may contain potentially harmful pathogens—can be addressed in many ways. Thus, a number of methods and method permutations have been proposed by scientists for water quality and food testing, such as detecting tiny viruses called phages that infect human fecal bacteria; multiplying characteristic genetic sequences in bacterial DNA millions of times to allow easier detection; and attracting target bacteria with tiny antibody-coated magnetic beads, and then separating and rupturing them to detect the concentration of an essential cellular component.

On the West Coast, the Southern California Coastal Water Research Project (SCCWRP) has spearheaded rapid method testing for marine beaches. SCCWRP began investigating beach water quality issues and testing molecular methods in the late 1990s. Initial studies aimed to sort through the various rapid methods proposed for recreational water monitoring applications and determine which held the greatest promise. As a third-party evaluator, SCCWRP compared and contrasted a number of rapid methods performed by research personnel and end users at public laboratories. SCCWRP also ran samples in conjunction with epidemiology investigations and compared the methods' aptitudes for predicting swimmer illness at Southern California's nonpoint-source influenced beaches (Figure 2). SCCWRP and the EPA concluded one technology was a clear leader and was ready to move ahead to the application stage.

Figure 2. Popular Swimming Beach

PHOTO: JASON GREGORY

Figure 3. Children Playing at Doheny

PHOTO: SCCWRP

Quantitative polymerase chain reaction (qPCR) uses a bioengineered enzyme and temperature cycling to exponentially amplify the specified bacterial DNA present in a water sample. As the targeted genetic sequence becomes more abundant, it is detected using a probe, which fluoresces only in the presence of its specific DNA target. The pattern of rising fluorescence can be tracked with imaging software and compared to known controls to determine the number of "cell equivalents" in the sample. Quantification is an important component of the test because presence/absence information alone does not translate to known thresholds of swimmer illness risk and cannot be compared to historical or side-by-side data from growth-based cell enumeration. Detecting cell components, rather than waiting for cell growth, means the test can be performed quickly, allowing for same-day results. Depending on the method permutation, a sample can be run in just two to four hours. Since most beach contamination issues in California stem from the state's threshold for enterococci, and enterococci have the strongest association with adverse health risks, scientists have focused the qPCR method on this type of bacteria.

First Run: An Orange County Demonstration Project

In 2009, SCCWRP's Commission formed a Rapid Methods Task Force (Task Force; see insert, page 53) to effectively and efficiently translate qPCR technology development into an applicable method. The Task Force consisted of eight representatives from federal, state, and local regulatory agencies—a county health department, wastewater treatment agencies, city government, and an environmental nonprofit. Although a few kinks in the technology had to be worked out, the Task Force thought conducting a pilot demonstration the following year was feasible and began prepping for a 2010 pilot rollout. The U.S. EPA plans to approve a new, national method in 2012, but the Task Force agreed that locally-relevant trials under California's specific environmental conditions were needed to inform the national criteria development and improve local public health agencies' ability to protect swimmers (Figure 3).

Between July 6 and August 31, 2010, Orange County Sanitation District, Orange County Public Health Laboratory, and South Orange County Wastewater Authority, the three local laboratories that regularly monitor Orange County beaches, processed water samples from nine locations using both their regular, growth-based methods and qPCR (Griffith and Weisberg 2011). The goal was not only for the labs to process the samples for both methods, but also for the labs to process the samples quickly and efficiently enough for public health officials to access the information by noon, allowing them to immediately transmit warnings if poor water quality presented a risk to swimmers. SCCWRP provided training and quality assurance during the project, and used the data to evaluate method performance and differences between the two types of methods.

Lessons Learned

The demonstration project was a success in terms of a number of aspects. The project put theory into practice and resulted in greater insight about the challenges facing rapid water monitoring. The project showed that qPCR technology is easily transferable to typical end users, even those who have never performed this type of molecular testing. The project also revealed that logistical considerations were just as important as technical issues. Even a perfectly tuned method falls short if it is too difficult to execute or costs too much, and the demonstration project identified several small method modifications that will enable even better success in the future.

One of the main logistical challenges was achieving same-day warnings in time for swimmers to make an informed decision before entering the water. The Task Force made public notifications of beach water quality conditions before noon a priority (Figure 4). Although the qPCR method takes only about two hours, numerous

Figure 4. Sample of Notification Screen

IMAGE: MIOCEAN

Figure 5. Electronic Sign above Beach Parking Entrance Booth

PHOTO: JOHN GRIFFITH

other steps take place before and after the test is run, such as collecting samples and delivering the resulting information to beachgoers. Several modifications were made to help participants achieve the noon deadline. First, sampling began at 7:00 AM, earlier than usual but still respectful of daylight hours for worker safety. Second, sampling was limited to a subset of beaches, and samples from priority beaches were returned first to the laboratory, substantially reducing the typical sample collection time of four hours. Finally, the County developed new electronic means of providing timely updates to the public, including a Twitter feed. In addition, the County partnered with the Orange County–based nonprofit Miocean to use electronic signage at the beach (Figure 5), allowing them to share results with the public almost instantaneously.

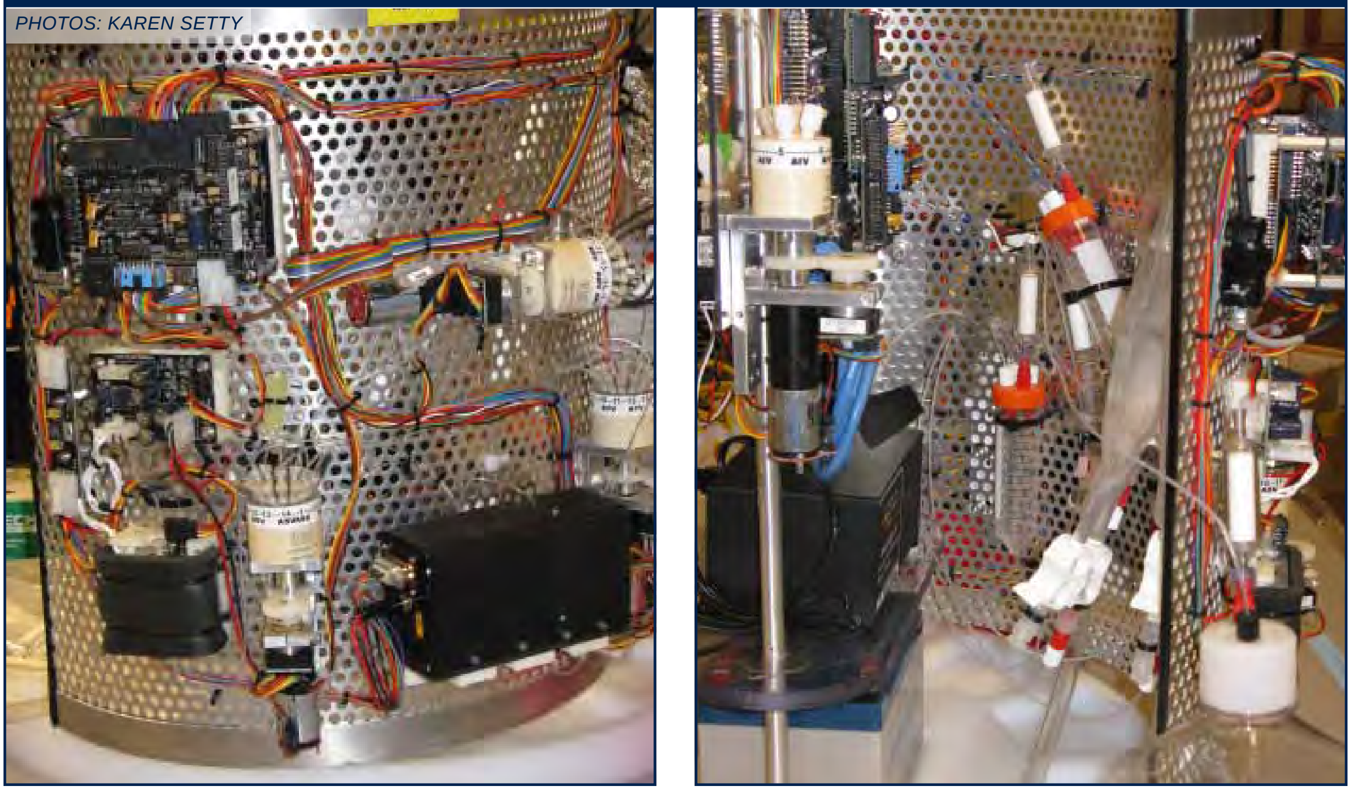
Owing to such timing issues, the groups participating in the demonstration learned qPCR use at select sites is preferable to blanket application at all sites, leading the groups to target only a handful of the regularly monitored beaches. Sparsely visited beaches or those with consistently favorable water quality, such as open ocean beaches with no consistent pollution source, may not warrant the extra driving distance and time to collect a sample. Meanwhile, those beaches with poor cleanliness history or throngs of visitors offer greater return (in terms of health protection) when rapid method samples are prioritized. Likewise, beaches monitored five days per week derive greater public benefit from more accurate, same-day warnings than those sampled only once a week. In addition, rapid methods are not intended to replace existing water quality monitoring methods in all applications. In a beach monitoring application, rapid methods may offer the substantial benefit of timely public health warnings. In contrast, ongoing permit compliance monitoring for wastewater and stormwater dischargers, which is used to inform periodic regulatory review and identify problem beaches for cleanup, does not require the same urgency.

Relatively few technical issues were encountered during the demonstration, but opportunities remain for short- and long-term improvements. The main concern confronting researchers was avoiding inhibition of the normally predictable rapid reaction that multiplies genetic material. Substances naturally present in environmental samples, such as tannins, can slow or stop DNA replication and cause odd results. Using controls, researchers found that such inhibitory substances affected qPCR results in about 14% of the Orange County pilot samples (Griffith and Weisberg 2011). Investigators continue to explore multiple solutions to address inhibition without sacrificing time. Another technical issue related to qPCR, which detects genetic material, is differentiating live, viable bacteria from dead, nonviable bacteria such as those found in disinfected wastewater.

Round Two: Bringing Rapid Testing to Los Angeles County Beaches

As 2010 ended, members of the Rapid Methods Task Force mulled over next steps. Promulgation of the new EPA criteria was still two years away, and it seemed an opportune time to continue building on what was learned in Orange County. A few agencies responsible for beach water quality testing in Los Angeles (LA) County decided to try qPCR in their neighborhoods

Figure 6. Front and Rear Internal Components of In-Situ Sample Processor



PHOTOS: KAREN SETTY

in 2011. SCCWRP again provided training and quality assurance during the summer trial. Three laboratories, the City of LA Bureau of Sanitation's Environmental Monitoring Division, the LA County Department of Public Health, and the LA County Department of Public Works, collected samples four days a week at eight sites between Malibu and Long Beach, and the City of LA ran qPCR tests side-by-side with their usual methods from July 11 to August 31. This time, the results were not used to make warning and closure decisions because, as was learned in Orange County, this requires significant logistical adjustments. Depending on the outcome of the demonstration, the agencies may repeat the process again in 2012 with public notification in mind.

Future Prospects

The Southern California trials were the first in the nation to apply rapid methods to routine beach monitoring. These demonstrations produced a great deal of information to aid the U.S. EPA as the agency works concurrently to approve a rapid method for nationwide use by October 2012 (U.S. EPA 2007, 2011). The trials modified the EPA's trajectory by showing the practicality of applying rapid methods only at select sites for select monitoring applications, as well as the utility of offering some flexibility in the method permutation. The qPCR method used in the trial, for instance, used newer reagents than the EPA currently uses, cutting a significant 45 minutes from the processing time.

Approving qPCR as a nationally-acceptable alternative method will require further attention to regulatory and management issues, such as:

1. Balancing initial capital and ongoing operational costs with steady or shrinking budgets.

2. Ensuring suppliers are ready and willing to provide test materials at a reasonable rate.

3. Developing standard protocols to train and certify laboratory personnel.

4. Providing guidance on which beaches or sites should be prioritized for rapid testing.

5. Adjusting workforce hours as needed to adapt to sampling schedules.

As evident in the demonstrations, the qPCR technology itself is realistic and becoming simpler. Reagent suppliers are working to develop products that could reduce the number of steps needed to conduct the method, thereby reducing room for error. Quality assurance and quality control checks have already been automated to save time. Over the longer term, researchers and manufacturers will pursue further method automation. Some potential directions include mobile laboratories that process samples at the beach, or ultimately a portable hand-held device that lifeguards could use for instantaneous results.

More tangibly, several marine laboratories along the West Coast, including the Monterey Bay Aquarium Research Institute, SCCWRP, Stanford University, and the National Oceanic and Atmospheric Association, are partnering to develop an automated in-situ environmental sample processor for rapid microbiological testing (Figure 6). The device, which autonomously runs a qPCR analysis and sends data by satellite to researchers' computers every two hours, can be mounted underwater to any pier or mooring. This technology could resolve timing issues and eliminate many of the existing obstacles to rapid method adoption. These devices would also fill in the data gaps between daily samples, providing a greater understanding of how water quality fluctuates throughout the day. Within the next year, researchers plan to pilot this technology by deploying it on a Southern California pier.

Investing in the basic technologies behind rapid qPCR also allows laboratories to conduct other types of molecular analyses without a sharp learning curve. Adapting the test protocol to a different type of target organism, for example, might involve only switching a few reagents. Another potential advantage of rapid monitoring is the ability to quickly respond to contamination events with follow-up upstream sampling to find the contamination source. Microbial source tracking and identification methods that would allow municipalities to detect and potentially resolve chronic pollution inputs are currently being developed as part of the California Clean Beaches Initiative Task Force's Source Identification Protocol Project.

In summary, the field of rapid water monitoring technology has experienced accelerated progress in recent decades, and managers will soon have more options for providing timely water quality notifications at high-risk beaches. The pilot demonstrations in Orange County and LA County set a helpful precedent for other areas looking to try out rapid methods. Subsequent early adopters can learn from these experiences and explore new possibilities for refining the testing and notification process. As the science of waterborne illness prevention continues to advance, the future holds promise of safer and more swimmable rivers, lakes, and oceans.

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