**Title**
Developing New Techniques for Evaluating Human Fecal Water Contamination



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# Developing New Techniques for Evaluating Human Fecal Water Contamination

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Coastal communities trying to clean up their beaches face many challenges, a significant one being the technical difficulty of accurately measuring human fecal bacteria in seawater. This problem arises because standard water tests are based on the abundance of “indicator” bacteria—not on direct measurements of the potentially most dangerous human pathogens.

Indicator counts have many fundamental limitations. For instance, they are not specific to a particular species, making it impossible to identify the source of contamination. Birds, cats, dogs, horses, seals and people all excrete fecal coliform bacteria, the most commonly used indicator bacteria. Another drawback, fecal coliform bacteria are rarely a major component of bacterial contamination.

The main advantage of using indicator bacteria is simply that these bacteria grow quickly and easily on culture plates, and they are associated with mammalian or avian feces. Many pathogens, however, do not grow in culture.

*Bacteroides fragilis*, for instance, constitutes as much as 30 percent of the total bacteria in human feces. But because these bacteria do not grow in culture, they are not monitored by public health officials.

## Method

Biology professor Dr. Clifford F. Brunk at the University of California, Los Angeles, and colleagues isolated DNA from bacteria in water samples. They then used a polymerase chain reaction (PCR) analysis to amplify the genetic information of several small subunits of ribosomal RNA genes. From these, the scientists identified a set of nine genetic markers that made it possible to "fingerprint" the bacteria and so identify its source.

The researchers, for instance, found genetic markers that distinguish storm drain runoff from sewage effluent and human from animal feces.

PCR amplification, however, has its own limitations. It does not amplify all gene sequences equally, meaning that it may overestimate the abundance of some bacteria while underestimating the abundance of others. There are, however, ways to circumvent this limitation. One is to co-amplify nearly identical gene sequences. Another is to use DNA fingerprinting to search for the presence of certain bacteria, as opposed to taking a snapshot of the full complement of bacterial flora.

## Applications

Dr. Brunk's work expands the spectrum of bacterial species that can be detected and monitored in coastal waters. Improved detection methods will make it possible to identify sources of bacterial pollution, linking contamination events to upstream sources.

## Collaborations

The Orange County Water District is using Dr. Brunk's techniques to identify the organisms that

When bacterial counts reach dangerous levels, health officials post warning signs, such as the one pictured above on a beach at Scripps Institution of Oceanography, La Jolla. Photo: Georgia Ratcliffe, California Sea Grant.

are creating bothersome films on the city's water filters. Dr. Jed Fuhrman of University of Southern California is also using DNA fingerprinting to compare the assortment of bacterial flora in coastal waters around the world.

## Publications


Brunk, C.G., and J. Li. 2000. Quantitative polymerase chain reaction assay. In: Environmental molecular microbiology: Protocols and applications, ed. P. Rochelle, 115–124. Wymondham, UK: Horizon Scientific Press.

Fogel, G.B., C.R. Collins, J. Li, and C.F. Brunk. 1999. Prokaryotic genome size and SSU rDNA copy number: Estimation of microbial relative abundance from a mixed population. *Microb. Ecol.* 38:93–113.


## Trainee

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