

Category III - Water Quality

Simultaneous Detection of Fecal Waste Sources and a Major Pathogen Associated with Waste, *E. coli* O157:H7 in Irrigation and Recreational Waters using a Low-Density Microarray.

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Executive Summary:

Total Maximum Daily Load (TMDL) requirements for a variety of contaminants are required under the Clean Water Act for states to develop when a body of water is impaired. In California alone, there are currently 685 impaired water bodies listed (SWRCB, 2003). Water quality managers throughout the state of California are challenged to meet TMDLs for fecal coliforms because most sources of fecal contamination in waterways are of unknown origin. Further, testing methods give no indication of the type of fecal waste entering the water body. By identifying major non-point sources of fecal pollution, TMDLs can be better implemented to protect water quality and public health from diseases associated with fecal coliforms. To aid water managers in identifying waste quickly and inexpensively, we propose to develop an oligonucleotide microarray that will identify four fecal waste sources (human, cow, pig and bird) and a major bacterial pathogen in California, *E. coli* O157:H7 simultaneously and with results available within 48 hours of the sample arriving at a laboratory. Although microarray technology could hypothetically examine thousands of genes, we have limited ours to five in order maximize focus on validity testing and because the budget limits have been considered to insure the success of the project.

Contamination of drinking and recreational waters by pathogenic bacteria associated with fecal waste has significant impacts on public health. The Centers for Disease Control and Prevention have reported several waterborne outbreaks of *E. coli* O157:H7 that resulted in severe illness and some fatalities (CDC, 1999). In California in the month of December 2003, 15 of the 61 counties reported 34 cases of *E. coli* O157:H7 (CDHS, 2003). *Cryptosporidium* is another pathogen of concern associated with many fecal wastes, most notably harbored by cattle and dairy herds. For the same month, the California Department of Health Services indicated that 10 of the 61 counties had 24 cases of Cryptosporidiosis. For both *Cryptosporidium* and *E. coli* O157:H7, the average number of cases per year in California during 2001, 2002 and 2003 was approximately 250. Although these pathogens are also transmitted by food, the source of contamination is often linked to water involved in agriculture, such as the recent outbreak in San Diego, where lettuce grown in Ventura County was the suspected vehicle.

The Santa Ana River in southern California originates in the San Bernardino and San Gabriel Mountains and flows through the Chino area into the Pacific Ocean. This river is exposed to a

wide spectrum of potential contaminants as a result of the many recreational uses and the close proximity of the agricultural industry. Although dairy farms and feed lot acreage has decreased over the past decade, the number of cows has increased. These factors elevate the risk of accidental release and elevate concern of disease agents entering the Santa Ana River, making it an excellent testing ground for pathogens and our biomarker method. The developed DNA microarray will be applicable to all areas of California, because the biomarker method has been tested in Fresno, Visalia, Monterey County and various locations in Southern California as well as in states across the nation.

Although there are no approved methods for microbial source tracking of fecal contamination, a variety of molecular methods have been developed to identify the sources of fecal waste in environmental waters. These methods include DNA fingerprinting (Simmons et al., 1998), ribotyping (Hoi et al. 1997), male specific phage genotyping (Hsu et al., 1995), antibiotic resistance (Whitlock et al. 2002), culture independent Bacteroidetes (Walters et al. 2000) and toxin biomarkers (Oshiro and Olson 1997; Khatib et al., 2002; Khatib et al., 2003). Some of these methods, such as ribotyping, DNA fingerprinting, and antibiotic resistance require a database and have been shown to have low predictability (Guan et al., 2002), although antibiotic resistance had an excellent ability to predict fecal sources from wildlife. To date, these methods are very location specific requiring the development of a new database for each geographical region. Our approach is designed with broad geographic distribution and genetic stability of *E. coli* toxin genes associated with cows, pigs, humans, or birds. Previous research in our laboratory has demonstrated the detection sensitivity (one cell per sample) and specificity of these toxin genes (false-positive (<1%) in the environment. In the early 1990s, the State Water Resources Control Board tested the method, but the detection level was insufficient (10^{-14} g). Since then, the detection limit has been improved by several orders of magnitude (10^{-18} g). Additionally, the biomarker method screens all the fecal coliforms in a water sample; while data base methods rely on subsamples containing 2 (ribotyping) to 37 (antibiotic resistance) isolates per sample based on the assumption that the occurrence of the source and its distribution in the sample is the same. Our data indicate that source distribution can change in a 12 hour period (Laguna Niguel Report). While the false-positive values of the biomarkers are determined, ribotyping has the problem of strains being found in a different host from which it has been isolated (20% of isolates). In the biomarker method, sequencing can be done at a cost of \$25/sample to differentiate hosts if false-positives are suspected. We propose to expand the biomarker method to DNA microarrays to rapidly and simultaneously identify multiple sources of fecal waste and one major pathogen, *E. coli* O157:H7, associated with fecal waste. We anticipate DNA microarrays will be a successful tool to use to analyze water samples from the Santa Ana River for the presence of cow, pig, human or bird waste or *E. coli* O157:H7. The availability of a microarray method would be beneficial to public health officials, due to decreased cost and time to complete as well as identification of three sources that can be controlled (cow, pig and human) and one that cannot (bird), so they can develop appropriate management strategies to reduce the risk of disease to the public.