USGS

WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: Molecular Probes for Human Pathogens in Water and Soils

Duration:September 1, 1996 to August 31, 1998

Federal Funds Requested: $57,130

Non-Federal Funds Pledged: $119,783

Principal Investigators:

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Center, University of Wisconsin-Madison

Congressional District: Wisconsin 2nd

Statement of Critical Regional and State Water Problems Addressed:

Human pathogens (mainly enteric bacteria, protozoa and viruses) introduced into soil through septic system effluent and land-applied sewage sludge can be conveyed to surface and groundwater used as sources of drinking water and therefore pose significant public health risks on a nationwide scale. but especially in rural and agricultural areas such as the North Central States. Traditional techniques used to examine water for the presence of pathogens rely mainly on the culturing of non-pathogenic indicator organisms for detection by inference. The methods used are slow, are unable to distinguish between closely related pernicious or benign strains, and fail to detect viable but non-culturable bacteria. Thus, when fecal contamination is suspected based on indicator-organism test, the presence of pathogens and the source of contamination (i.e., human v. animal excreta) remain uncertain. Alternatively, fecal contamination may go undetected if indicator bacteria are in a viable but non-culturable condition.

To resolve these inadequacies of existing tests, this project focuses on developing a rapid molecular method using the polymerase chain reaction (PCR) coupled to an enzyme immunoassay (EIA) to test soils for the presence of specific sewage-borne pathogens. The new protocol would obviate the need to culture organisms for detection, and could remedy shortcomings of traditional techniques by allowing rapid, sensitive, and specific identification of the pathogens of concern rather than indicator organisms. To establish the validity and efficacy of the approach, a model PCR-EIA protocol will be developed to detect *Escherichia coil* and *Shigella dysenteriae* based on unique ribosomal RNA (rRNA) sequences, and will be used to examine questions regarding relationships between survival/occurrence of indicator organisms and pathogens in soil.

Statement of Results and Benefits:

Successful demonstration of the utility of the proposed PCR-EIA test would bring biological testing of water quality up to the state-of-the art in modern microbiology, allowing inexpensive, rapid, direct, specific detection of harmful micro-organisms via identification of specific genetic markers. These and additional tests based on this new technology would be expected to replace the archaic indirect-inference tests employing, laboratory culturing of indicator bacteria, and replace tests that require days with ones that can provide measurements within hours and therefore be used more routinely for real-time monitoring of water supplies and surface and groundwater quality.

Many laboratories nationwide conduct a thriving business testing water samples from private wells and public water supplies and recreational facilities, e.g. swimming pools and beaches, using *Standard methods for the Examination of Water and Wastewater* (Part 9000 Microbiological Examination). Turnaround time for detailed analyses to identify causes of epidemic illness outbreaks is sometimes weeks! Microbiological analyses are conducted in-house daily at major sewage treatment plants and municipal water works. A enormous market exits for faster, easier and more reliable tests using molecular probes specific for fecal pathogens or *Cryptospiridium* spp. in water supply sources. In another market sector, utilization of sites for home construction and commercial development in suburban and rural areas is also often hampered by unavailability of soils deemed suitable for on-site wastewater disposal: here concern for spread of fecal pathogens is a motivator for *de facto* zoning restrictions, preventing beneficial development. Both areas of application would contribute substantially to economic development, environmental protection, and improved public health, both within the region and nationwide. Long-term, the technology will also be useful for water testing in third world countries and in areas where good sanitation is disrupted by military. conflicts and natural disasters such as earthquakes and floods. Short-term, the new test proposed here would most benefit researchers and regulators by allowing, the fate and source of pathogenic organisms in soils to be determined and traced to identifiable sources, and public health officials by providing approaches that can be used to improve pathogen screening protocols for both public and private water supplies.