



## **WATER RESOURCES RESEARCH GRANT PROPOSAL**

**Title:** Ultrafiltration based concentration of viruses and *Cryptosporidium* oocysts from environmental water samples

**Priority Problem Area:** Water quality Focus Category: Groundwater, Surface water, Water Quality,

**Key Words:** Water-borne pathogens, ultrafiltration, viral contamination, enteroviruses, *Cryptosporidium*, water quality

**Duration:** 12 months starting July 1999- June 2000.

**1999 WRRI Award:** Total: \$35,500

**Federal:** \$25,000

**Non-Federal:** \$10,500

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**Congressional District Number:** New Mexico II

### **Water Problem**

In terms of drinking water safety, very little is known about the extent of viral and parasitic contaminants in source (influent) and product (finished) drinking water and their relationship to disease. Critical to both identifying and quantitating water-borne pathogens is the development and use of methods which reliably concentrate pathogens from drinking, surface and groundwater.

The Environmental Protection Agency (EPA) has mandated that large water utilities in the U.S. test their source and product water for viral pathogens from surface or groundwater systems (EPA 1996). Although current methods for concentrating pathogens from these sources have allowed for the filtration of large volumes of water, there are difficulties in terms of 1) procedural complexity (time and expense), 2) variable efficiency and consistency of virus and parasite recovery and 3) use of different methods for detecting viruses, bacteria and protozoan organisms from water which increases the complexity and expense.

There is growing concern for the potential health risks associated with the presence of pathogens in surface, ground and drinking water however, little data is available to determine how significant these risks are. Improvement in the ability to document the

prevalence and concentration of these pathogens in water from the standpoint of detection sensitivity, reproducibility and consistency of results will make it more feasible to obtain results that are more analyzable and cost effective.

### **Statement of the Results, Benefits Expected**

By the end of the funding period the following objectives should be completed: 1) optimized method(s) to recover viruses under field size (100-1000 L) samples along with documentation to indicate the potential detection sensitivity and reproducibility with different viruses and water qualities for both a hollow fiber and if necessary for the tangential flow ultrafiltration system; 2) determine the correlation between small and field scale testing for virus recovery; 3) determine the recovery efficiency of *Cryptosporidium parvum* oocysts from environmental water using the small scale hollow fiber ultrafiltration system, 4) develop and optimize a downstream sample processing procedure leading to the concentration of the sample to < 1ml for the field scale samples. As a result of these experiments, the feasibility of ultrafiltration to concentrate waterborne viruses under field conditions will be well characterized. An integrated prototype apparatus (containing the filter, pumping system) that is able to process field size volumes of water will be developed and tested. The feasibility of the ultrafiltration system will be determined in terms of the major variables for developing a concentration method for viruses in water (efficiency of recovery, reproducibility, speed, ease of use and cost). In addition, 5) detailed information on the feasibility of PCR methods using concentrated samples processed through ultrafiltration will be determined for enteroviruses and *Cryptosporidium*. Ultrafiltration may offer advantages in reducing the level of PCR inhibitors, compared to the adsorption/elution method. Thus ultrafiltration will be examined not only from the standpoint of recovery of infectious virus particles but also for its possible advantages for improving the efficiency of PCR amplification of target viral nucleic acid.

With this data, long-term support from sources such as the EPA, American Water Works Association Research Foundation, World Health Organization and filter manufacturers would be more feasible. These agencies have supported research to determine the level of risk to the human population from waterborne viruses or in developing methods to provide needed information. In addition, there may be regional interest from the Southwest Center for Environmental Research and Policy (SCERP) and by the Waste-Management Education and Research Consortium (WERC). The long-term objective is to use this system to develop a series of studies to determine the relationship between the level of viral and protozoan contamination and documented cases of disease from these organisms as well as the integration of these systems for routine monitoring.

In addition to this proposal, I have a project underway to develop membrane supported detection of viral agents by nucleic acid amplification which is currently supported in part by a major membrane manufacturer and the development of biosensors for the detection of PCR product amplified from viral nucleic acid. Both of these projects have potential as downstream sample detection methods for waterborne pathogens

concentrated by ultrafiltration and provide further utility for the methods developed in this proposal.

### **Nature, Scope and Objectives**

The contamination of surface, ground and drinking water from viral, bacterial and protozoan organisms is a growing concern in developed and developing countries. The risk of waterborne viral and protozoan agents to public health remains largely unknown due in large part to the difficulty in concentrating and detecting these organisms from these environments both from a technical and practical standpoint and the associated high monetary costs involved in generating this information. Current methods to concentrate viral and protozoan agents have tended to be technically cumbersome (require a large number of steps) and have documented variable results based on differences in water quality and target viruses (see related research).

In response in part to the lack of information on the levels of viral pathogens in source and drinking water, The Information Collection Rule was initiated in 1997 by the EPA to mandate large utilities to collect and analyze water samples from their intake and finished product for viruses (EPA, 1996). In the future, there will continue to be a need for technologies to reliably concentrate and detect water-borne viruses as water supplies continue to be threatened by human activity.

This project is designed to improve the sensitivity and consistency of the recovery of waterborne viruses from surface, ground and drinking water as well as simplify the process of concentrating viral particles in the sample compared to the existing methods. Part of the objective is also to optimize PCR detection of viral nucleic acids from the concentrate in terms of sensitivity, reproducibility, simplicity and cost. Efficient and cost effective methods for concentration and recovery of these organisms can then be used to monitor the levels of contamination and also to document the level of inactivation by the water treatment processes implemented by utilities.

The overall objective is to apply results from small scale (2 L) optimization experiments as the bases for scale-up to field size (100-1000 L) ultrafiltration systems. Optimized procedures for different types of water will be developed along with a single procedure that will efficiently recover viruses from all types of water. Because these filters concentrate organisms based on size, the recovery of non-viral organisms are also feasible. This proposal will also optimize the recovery efficiency for *Cryptosporidium* in the small scale filtration system to demonstrate the utility of using a single filtration step to concentrate viral and larger organisms simultaneously.