EPA/600/F-10/021

www.epa.gov

science BRIEF

BUILDING A SCIENTIFIC FOUNDATION FOR SOUND ENVIRONMENTAL DECISIONS

SOIL AND WATER

WHAT IS DETECTABLE THROUGH MICROBIOLOGICAL SAMPLE PREPARATION TECHNIQUES

Issue:

The accidental or intentional contamination of water and soil by biological agents are real and growing concerns with respect to human health and the environment. These are not unwarranted in light of the anthrax attacks in the fall of 2001. In addition to air and food borne biological pathogens, the vulnerability of our water resources to bioterrorism is a salient problem because induced waterborne outbreaks can involve sizable populations. Surface water resources can be susceptible to post treatment contaminations, as evidenced by the 13,000 cases of cryptosporidiosis that occurred in a surface water supply in Carrollton, Georgia, following coagulation, sedimentation, filtration, and chlorination. Another example is the largest waterborne outbreak in United States history where cryptosporidiosis infected an estimated 400,000 people served by the City of Milwaukee. Ground-water contamination by biological agents introduced directly or through infiltration, also presents a formidable challenge since ground water serves as the source of drinking water for roughly half of the domestic needs of the country. To meet this challenge, the U.S. Environmental Protection Agency (EPA) National Homeland Security Research Center (NHSRC), in collaboration with experts from this Center, which houses the Ground Water and Ecosystems Restoration Division (GWERD) of the National Risk Management Research Laboratory (NRMRL), and other federal agencies, initiated an effort to validate, modify, or develop sampling activities to be used by all laboratories in the event of a homeland security incident.

Scientific Objectives:

The preparation of environmental samples that could potentially contain biological pathogens is an area in need of standardized methods. Efforts in sample preparation development may include, but are not limited to: appropriate and optimal sample collection; transport media; matrix preparation; and validation of analyte-specific filtration, elution, concentration, purification, and staining procedures. Unfortunately, most available sampling techniques have been developed for clinical samples. As there are significant distinctions between environmental samples and clinical specimens, in terms of concentrations of pathogens and matrix effects, there is a need to develop sample preparation procedures that specifically address the isolation and concentration of pathogens from complex environmental matrices. To that end, molecular and culture based-techniques will be used to accurately demonstrate when soil or water samples harbor potential pathogens. It should be noted that biomass and biodiversity levels in subsurface samples are low relative to a typical soil ecosystem. Soil extraction and analytical protocols need to be substantially modified in order to reliably detect and identify pathogens in subsurface samples. Therefore, the approach requires the use of high sensitivity methods, such as those based on PCR. It is also important to conduct thorough validation of these methods, and the techniques for the collection, preservation, and preparation of the environmental samples. The outcome of the present research efforts will provide an effective interface between sample preparation, molecular assays, and culture techniques to provide accuracy, precision, and reproducibility, necessary precursors to method standardization and adoption.

U.S. Environmental Protection Agency
Office of Research and Development




Molecular techniques will be used to demonstrate that environmental samples (i.e., soil, water) harbor potential pathogens. Initially, a subset of analytes of concern were selected from EPA's *Standardized Analytical Methods for Environmental Restoration following Homeland Security Events Revision (SAM) 3rd Edition*. This document suggests analytical methods for use by laboratories tasked with performing confirmatory analysis of environmental samples following a homeland security event. Parasites and bacteria will be used to demonstrate sample preparation techniques from complex matrices that also contain other interfering non-target biological and chemical analytes.

The overarching goal of this project is to develop analytical methods for pathogens *Salmonella enterica* serovar Typhi, *Cryptosporidium parvum*, and *Giardia lamblia* in environmental water and soil samples using real time PCR.

Research:

The ongoing efforts focused on method development, modification, and optimization with environmental samples seeded with *S. enterica* serovar Typhi, *C. parvum*, and *G. lamblia* to address the objectives. Compared with traditional PCR, as proposed in the original plan, real time PCR is more rapid, precise, and sensitive. Therefore, real time, TaqMan® based PCR was used in place of traditional PCR, although traditional PCR was still used to test primers for specificity. Considerable effort has been taken to design and test primers/probes and find optimal conditions instead of using available primers and running conditions.

Immunomagnetic separation (IMS) is used in EPA Method 1623 to separate oocysts of *C. parvum* and cysts of *G. lamblia* from water samples. There are no methods described for DNA extraction of *C. parvum* and cysts of *G. lamblia* in environmental soils in this method. Disadvantages of IMS include variation of (oo)cyst recovery rates, high cost, and the time-consuming nature of this process. Extraction of high-quality DNA is a critical step in real time PCR detection of pathogens. Reduction or removal of PCR inhibitors is also an essential component in the molecular detection of microorganisms in environmental samples. DNA yields extracted from environmental soils are influenced by soil properties (e.g., pH, contents, size, and type). Therefore, various genomic extraction techniques were evaluated using five different soils as well as the ground-water samples from the Arbuckle Simpson Aquifer seeded with *S. enterica* serovar Typhi, *C. parvum*, and *G. lamblia*.

Findings:

- When tested against *S. enterica* serovar Typhi, in environmental water and *C. parvum* in reagent water the Instagene Matrix (BioRad, Herculese CA) a buffered chelex matrix, yielded the greatest recovery. This was measured by the lowest threshold cycle (Ct) value, when compared to boiling, alkaline lysis, and QiAmp DNA mini kit
- The PowerSoil® DNA isolation kit (MoBio Laboratories Inc. Carlsbad, CA) was more effective in DNA extraction for soil samples seeded with *S. enterica* serovar Typhi and *C. parvum* than other kits tested (UltraClean, Mega UltraClean, and Regular UltraClean Soil DNA isolation kits; all from MoBio Laboratories). Incubation of samples at 70°C was found to help lyse the cells. Concentrating multiple DNA samples onto a single spin filter did not improve DNA yield.
- Using real time PCR, *S. enterica* serovar Typhi was detected at 400 cells per ml in water and and 400 cells per gram of soil. The nested real time PCR assay for *C. parvum* detected 5 oocysts seeded in 0.5 g of a soil matrix and 3 oocysts seeded into a 1 ml water sample. The nested real time assay for *G. lamblia* was able to detect 10 cysts seeded into 1 g of soil and 1 ml of water respectively. We intend to verify these results and publish the final protocols for use.

Current Milestones:

- 2010 Presentation - Keeley, A. and Q. Wu. "Microbiological Detection Systems for Molecular Analysis of Environmental Water and Soil Samples." Society for Industrial Microbiology and Biotechnology Annual Meeting. San Francisco, CA.
- 2011 Internal Report - Development, application, and verification of microbiological detection limit guidelines.
- 2011 EPA Report - Completion of the project final report.

REFERENCES:

USEPA. "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA" *December 2005*, US EPA, Office of Water (4607), EPA/815/R-05/002.

USEPA. "Standardized Analytical Methods for Environmental Restoration following Homeland Security Events" (Revision 3.1) *November 15, 2007*, National Homeland Security Research Center, USEPA, Office of Research and Development, Cincinnati, OH, EPA/600/R-07/136.

CONTACTS:

Ann Keeley, Ph.D.
USEPA/ORD/NRMRL
Ground Water & Ecosystems Restoration Division
(580) 436-8890 keeley.ann@epa.gov

Qingzhong Wu, Ph.D.
National Research Council
(580) 436-8500
wu.qingzhong@epa.gov