Project Profile Information
(Please provide the following on a 3.5” disk)

Project Title: New Approaches for Isolation of Cryptosporidium and Giardia

Project Number: 364

Principal Investigators: Jennifer L. Clancy, Zia Bukhari, Randi M. McCuin, Thomas M. Hargy, Colin R. Fricker, Zoe Matheson, and Nelson Sykes

Objectives:
(State the relevant objectives of the project; 75 words or less.)

The objectives are to develop alternate methods for sample collection and separation to improve recovery of Giardia cysts and Cryptosporidium oocysts from water samples. Assessments of a variety of both new and established concentration techniques for recovering (oo)cysts from large and small sample volumes was done. Immunomagnetic separation techniques were optimized for high turbidity water samples. New methods were collaboratively tested to develop precision and bias data.

Background:
(Provide background information; 75 words or less.)

The problems surrounding the reliable measurement of Giardia cysts and Cryptosporidium oocysts in water samples has been well documented in several studies in the US and UK. The standard method using wound cartridges, density gradient flotation and IFA is highly variable both within and among laboratories. False negatives and false positives are common, the usefulness of any data generated with this method is suspect.

Highlights:
(Provide “at a glance” the main findings of the research [minimum of three]; 100 words or less.)

1. Low seeding levels (100 cysts and oocysts) were used successfully throughout the study, permitting significantly increased method sensitivity (ability to detect low levels of organisms).
2. Several filters (Gelman Envirochek™ capsule, Corning Separations polycarbonate track etch [PCTE] membranes) were tested and shown to provide 100% capture and highly reproducible (>75%) and effective elution of (oo)cysts. A new filter, the Corning Cryptest™ was developed and validated in this study.
3. Two commercially available IMS systems for Cryptosporidium (Dynal, Inc and ImmuCell, Inc.) were optimized for use with source waters of various turbidities. A combination IMS kit for Giardia and Cryptosporidium (Dynal, Inc.) was assessed using a variety of parameters (turbidity, (oo)cyst age and status, and spike dose). These kits are reliable and provide excellent recoveries of (oo)cysts, making detection of low levels of organisms from complex matrices possible.
4. The new method(s)/component(s) are commercially available. They are simple to use, perform well at both high and low seeding concentrations, are reproducible, and offer maximal cost-effectiveness.

Approach:
To address the problems of poor recovery, insensitivity, and lack of reproducibility in protozoan methods, the approach taken was to evaluate each step in the method - sampling, concentration, separation, and detection - for its ability to permit recovery of spiked (oo)cysts. Each individual step was first optimized in reagent water, and then assessed using actual samples or sample concentrates to determine matrix effects. To increase sensitivity (ability to recover low levels of organisms), seeding levels were reduced 100 (oo)cysts. Alternate filters were assessed for sample concentration, flow cytometry and IMS were optimized, and alternate staining techniques investigated. After optimization, the steps were combined into a full method for single laboratory validation in the two participating laboratories. Once developed and validated, a single method for simultaneous recovery of Giardia cysts and Cryptosporidium oocysts was collaboratively tested to determine robustness.

Results/Findings:
Low seeding levels (100 (oo)cysts) were used to significantly increase method sensitivity

Several filters (Gelman Envirochek™ capsule, Corning Separations polycarbonate track etch [PCTE] membranes) were tested and shown to provide 100% capture and highly reproducible (>75%) and effective elution of (oo)cysts. A new filter, the Corning Cryptest™ was developed and validated in this study.

Two commercially available IMS systems for Cryptosporidium (Dynal, Inc and ImmuCell, Inc.) were optimized for use with source waters of various turbidities. A combination IMS kit for Giardia and Cryptosporidium (Dynal, Inc.) was assessed using a variety of parameters (turbidity, (oo)cyst age and status, and spike dose). These kits are reliable and provide excellent recoveries of (oo)cysts, making detection of low levels of organisms from complex matrices possible.

Three of the successful components developed and/or optimized in this project were collaboratively tested following the procedures outlined by the USEPA for performance based measurements system (PBMS) validation. The Corning Cryptest cartridge, the Dynal Giardia/Cryptosporidium combo IMS kit, and Waterborne Aqua-Glo were collaboratively tested for inclusion as an alternate method for Method 1623: Giardia and Cryptosporidium in Water by Filtration/IMS/FA. The new method(s)/component(s) are commercially available and meet the criteria stated in the research objectives. They are simple to use, perform well at both high and low seeding concentrations, are reproducible, and offer maximal cost-effectiveness.

Impact:
These new methods will allow higher quality data to be generated for all protozoan measurements, whether routine monitoring, treatment effectiveness, or regulatory impact analysis. The methods have been developed for use with raw and finished water samples, but could be adapted for use with other types of samples, i.e. backwash recycle. These methods are significant improvements over previous methods and have now been adopted in many other countries for protozoan monitoring.

Participating Utilities (if applicable; maximum five):
Champlain Water District, South Burlington, Vt.
Regional Water Authority, New Haven, Conn.
Omaha Municipal Utility District, Omaha, Neb.
Philadelphia Water Department, Philadelphia, Pa.
City of Dallas, Texas