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Concentration and Detection of *Cryptosporidium* Oocysts in Surface Water Samples by Method 1622 Using Ultrafiltration and Capsule Filtration

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The protozoan parasite *Cryptosporidium parvum* is known to occur widely in both source and drinking water and has caused waterborne outbreaks of gastroenteritis. To improve monitoring, the U.S. Environmental Protection Agency developed method 1622 for isolation and detection of *Cryptosporidium* oocysts in water. Method 1622 is performance based and involves filtration, concentration, immunomagnetic separation, fluorescent-antibody staining and 4′,6-diamidino-2-phenylindole (DAPI) counterstaining, and microscopic evaluation. The capsule filter system currently recommended for method 1622 was compared to a hollow-fiber ultrafilter system for primary concentration of *C. parvum* oocysts in seeded reagent water and untreated surface waters. Samples were otherwise processed according to method 1622. Rates of *C. parvum* oocyst recovery from seeded 10-liter volumes of reagent water in precision and recovery experiments with filter pairs were 42% (standard deviation [SD], 24%) and 46% (SD, 18%) for hollow-fiber ultrafilters and capsule filters, respectively. Mean oocyst recovery rates in experiments testing both filters on seeded surface water samples were 42% (SD, 27%) and 15% (SD, 12%) for hollow-fiber ultrafilters and capsule filters, respectively. Although *C. parvum* oocysts were recovered from surface waters by using the approved filter of method 1622, the recovery rates were significantly lower and more variable than those from reagent grade water. In contrast, the disposable hollow-fiber ultrafilter system was compatible with subsequent method 1622 processing steps, and it recovered *C. parvum* oocysts from seeded surface waters with significantly greater efficiency and reliability than the filter suggested for use in the version of method 1622 tested.

Cryptosporidium parvum, a coccidian protozoan parasite, remains a risk to drinking water consumers despite extensive efforts put forth by water providers and the U.S. Environmental Protection Agency (EPA) (7, 12, 14, 15, 20, 21, 22). Oocysts are present in many environmental waters because *Cryptosporidium* is not only a human pathogen but also a zoonotic pathogen infecting livestock, as well as feral animals, in many watersheds used as sources of drinking water. Oocysts persist in the environment and are resistant to the chlorine disinfection routinely used for drinking water (2, 11, 13, 17, 23). Therefore, physical removal by chemical pretreatment and filtration is the primary means for reducing oocysts in source waters. When deficiencies in chemical pretreatment and filtration processes occur, oocysts can breach the treatment system and cause disease outbreaks of the magnitude of the 1993 Milwaukee cryptosporidiosis outbreak (14).

Detection of *Cryptosporidium* oocysts in raw water sources is considered an important component in the management, prevention, and control of *Cryptosporidium* in drinking water supplies. Methods have been developed to detect *C. parvum* in both raw source waters and finished drinking waters. The EPA developed an Information Collection Rule requiring large municipal water supplies to use a specified method to recover and detect *Cryptosporidium* oocysts in source waters (22). Because that method was considered to be unreliable, giving low and variable recovery rates and often examining only small sample volumes, the EPA had a small working group develop an improved method for recovery and detection of *Cryptosporidium* oocysts in raw and finished water. This new method, called method 1622, includes four main steps: initial filtration to capture oocysts from a 10-liter sample of water, immunomagnetic separation (IMS) to concentrate and purify the oocysts washed from the filter, fluorescent-antibody staining and 4′,6-diamidino-2-phenylindole (DAPI) counterstaining of the IMS product, and microscopic examination and enumeration of the sample by epifluorescent and differential interference contrast (DIC) microscopy (21). The EPA's method 1622 is a performance-based method allowing the use of alternative filters if they are documented to meet performance characteristics specified by the agency. Because previous studies in our laboratory (19) indicated low rates of recovery of *Cryptosporidium* oocysts from seeded surface waters, the recommended EPA method 1622 filter system was compared to an alternative hollow-fiber ultrafiltration system in this study.

Ultrafiltration is used to remove, separate, or recover particulate and colloidal components from a liquid stream, typically using hydraulic pressure to increase the rate at which the liquid moves through the filter (1, 4, 5, 6, 10, 16). The particle size retained by the ultrafilter is determined by the pore size and molecular configuration of the filter and is typically in the range of thousands to hundreds of thousands as a molecular

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weight cutoff (MWCO). The ultrafilter used in this study is in the form of a series of polysulfone hollow fibers contained within a polycarbonate housing. Particulate matter is retained within the recirculating water sample, and the particulate-free permeate water is discharged as the filtrate. Recirculating a water sample through the hollow-fiber ultrafilter efficiently retains and concentrates all particles larger than the MWCO of the filter in the hold-up volume of the ultrafilter assembly (200 to 250 ml). The ultrafilters used for these experiments are self-contained, single-use, inexpensive (approximately $35) units. Because the original intended use of these ultrafilters is for hemodialysis and hemofiltration, they meet rigorous quality standards, which is extremely important for any sampling apparatus used by the drinking water industry for recovery and detection of *Cryptosporidium* or other pathogens.

MATERIALS AND METHODS

Water samples. Ten-liter surface water samples were collected in disposable, collapsible containers. Sample sites were selected on the basis of land use (urban versus agricultural), historical turbidity measurements scored as high (above 30 nephelometric turbidity units [NTU]) versus low (below 30 NTU), and potential use as sources of drinking water. Land use assessments for this study were qualitative and were based on characterizations of prevailing activities (e.g., urban development or agricultural activities) as the basis for descriptions of the watersheds. All surface water samples were collected as single grab samples. The water quality parameters, which were measured either at the time of collection or in the laboratory after overnight storage at 4°C, included turbidity (measured as NTU), total dissolved solids, and pH. All samples were processed within 48 h of collection, as outlined in EPA method 1622. For precision and recovery experiments and method blank experiments, distilled, filtered, and UV-irradiated ultrapure reagent water produced in the laboratory was collected as 10-liter volumes drawn from a reagent water tap into sterile, 10-liter disposable collapsible containers and was used immediately.

***C. parvum* oocysts.** *C. parvum* oocysts (Iowa strain) were produced in calves by Pat Mason at Pleasant Hill Farms, Troy, Idaho. Shed oocysts were collected from the host and purified by ether extraction and sucrose gradient flotation (18). Approximately 10^7 oocysts per ml were resuspended in a solution containing phosphate-buffered saline (PBS) supplemented with 1,000 U of penicillin and 1,000 μg of streptomycin per ml; the oocysts were not exposed to dichromate or bleach. Upon arrival in our laboratory, oocysts were enumerated microscopically in a hemacytometer and observed by DIC microscopy for quality. The oocyst stock suspensions were stored at 4°C until they were needed for experiments. *C. parvum* stocks were used only for a period of 3 months, after which they were discarded and a new stock was obtained. All oocyst dilutions were made in 0.01% Tween 20 in reagent water using the protocol of 2 min of vortexing, 2 min of sonication, and 2 min of vortexing to insure adequate mixing and dispersion of the oocysts. Target oocyst concentrations for all experiments were 100 to 150 oocysts per 10-liter sample volume.

Filters for primary concentration of *C. parvum* oocysts from water. The Envirochek capsule filter (Gelman Sciences, Ann Arbor, Mich.), a 1-μm nominal pore size pleated polyethersulfone filter in a polycarbonate housing, was compared to the Hemoflow F80A ultrafilter (80,000 MWCO; Fresenius USA, Lexington, Mass.), a polysulfone hollow-fiber single-use unit contained within a polycarbonate filter housing. Ten-liter water samples, as previously described, were filtered at a flow rate of 2 liters/min through the capsule filter. To filter the samples through the ultrafilter system, a variable-speed peristaltic pump was used to recirculate the water at a pressure of 25 lb/in^2 within the closed recirculation system. When the volume of the sample was reduced to the hold-up volume of the ultrafilter system (200 to 250 ml), oocysts were eluted from the ultrafilter by recirculating an eluting solution (100 mM PBS with 1% Laureth-12) at low pressure (5 to 10 lb/in^2) through the system. The hold-up volume was then collected using pump pressure, and any additional liquid was purged with air pressure (<25 lb/in^2). Oocysts were eluted from the capsule filter with elution buffer and wrist action agitation, as specified in method 1622, during the first half of the experiments (through 27 October 1999). In order to increase recovery rates with the capsule filters, the elution procedure was modified during the remaining experiments to use a horizontal shaker platform with two elution periods of 15 min each rather than the prescribed series of two elution periods of 5 min each.

TABLE 1. Reagent water initial and ongoing precision and recovery experiments and method blank recovery rates

Sample[a]	Date (mo/day/yr)	Recovery (%)[b]			
		Fresenius F80A ultrafilter		Gelman capsule filter	
		Spike	Blank	Spike	Blank
IPR1	5/21/99	40	0	42	0
IPR2	5/21/99	45	0	33	0
IPR3	5/21/99	47	0	ND	0
IPR4	5/28/99	ND	ND	46	0
IPR5	5/28/99	ND	ND	53	0
IPR6	5/28/99	ND	ND	45	0
OPR4	6/10/99	27	0	37	0
OPR5	6/10/99	13	0	35	0
OPR6	6/10/99	24	0	21	0
OPR7[c]	11/10/99	54	0	42	0
OPR8[c]	12/2/99	42	0	70	0
OPR9[c,d]	1/11/00	86	0	70	0
OPR10[c,d]	1/18/00	76	0	78	0
OPR11[c,d]	2/8/00	11	0	22	0
Avg		42		46	
SD		24		18	

[a] IPR, initial precision and recovery: OPR, ongoing precision and recovery.
[b] 10-liter volumes were examined for all reagent water samples processed. ND, not determined.
[c] Modified elution procedure for Gelman filter.
[d] Defective Gelman filter lot (no. 13810).

Concentration, IMS, and staining of eluted *C. parvum* oocysts. Elution solutions from filters were collected in 250-ml conical-bottom centrifuge tubes, and the oocysts were concentrated by centrifugation at 1,164 × *g* and 4°C for 20 min. The supernatants from each tube were aspirated to the 5-ml mark on the 250-ml conical tubes. Reagent water was added to the pellet-eluting solution volume so that the packed pellet volumes were 5% or less within the 10-ml samples subjected to IMS. Anti-*Cryptosporidium* IMS kits (Dynal Inc., Lake Success, N.Y.) were utilized for separating the oocysts within the samples from other, interfering particulate matter using the IMS protocol described in method 1622 (3). Samples were transferred to well slides (Meridian Diagnostics, Cincinnati, Ohio) and dried for 2 h in a desiccant chamber. The slides were stained with the Crypt-a-Glo fluorescent-antibody kit (Waterborne, Inc., New Orleans, La.) and DAPI counterstained (0.002 mg/ml), as described in method 1622 with slight modification (2, 8). DAPI counterstaining of the surface water samples facilitates visualization of internal morphological features in oocysts. After the slides were dried in a desiccating chamber for 1 h, a glycerol-DABCO mounting medium was added to each well, and a coverslip was applied. The slides remained in the desiccant chamber until they were microscopically observed, which was done within 72 h of the staining procedure.

***C. parvum* oocyst spiking solution and modified staining procedure.** Stock oocysts received from the supplier were enumerated by DIC microscopy with a hemacytometer. Oocysts were diluted for experiments and enumerated by the Meridian well slide method, as described in method 1622 with slight modifications. The well slide immunofluorescent-staining method using Crypt-a-Glo was modified to include one reagent water rinse of the slides during staining instead of the three washes specified. With this modification, the standard deviations (SD) of oocyst counts were lower and background fluorescence did not interfere with microscopic evaluation of the slides (data not shown).

Statistical analysis of *Cryptosporidium* oocyst recoveries. Paired nonparametric statistical analysis to compare filter recoveries from surface water samples and reagent grade water samples was performed using a computer-based statistical software package (Instat; GraphPad Software, San Diego, Calif.).

RESULTS

Table 1 summarizes the results of the *Cryptosporidium* precision and recovery experiments and method blank recoveries from seeded reagent water with each of the two filter systems. Ten-liter volumes were spiked with 100 to 150 *Cryptosporidium*

TABLE 2. Water quality parameters of surface water samples

Sample site	Date (mo/day/yr)	pH	Turbidity (NTU)	TDS[a] (mg/liter)
New Hope Creek	6/11/99	6.6	45	190.6
New Hope Creek	6/11/99	6.6	45	190.6
New Hope Creek	6/11/99	6.6	45	190.6
Cane Creek	6/15/99	6.3	4.0	35.1
Cane Creek	6/15/99	6.3	4.0	35.1
Cane Creek	6/15/99	6.3	4.0	35.1
Goldsboro	10/7/99	6.4	8.2	32.2
New Hope Creek	10/27/99	6.6	11.6	98.1
Haw River	12/2/99	6.1	17.7	77.8
Jefferson Bridge	12/8/99	8.0	2.9	291
Leeper Park	12/8/99	7.5	2.5	294
Raleigh	12/16/99	6.0	11.6	32.6
Brown	1/25/00	6.3	4.1	35.9
Williams	1/25/00	6.4	12.5	35.6
Burlington	2/1/00	6.5	5.3	52.9
Avg		6.6	14.9	108.5

[a] TDS, total dissolved solids.

oocysts and processed as previously described. Spike recovery rates ranged from 11 to 86% (average, 42%) with the ultrafilters and 21 to 78% (average, 46%) with the capsule filters. When a Mann-Whitney two-tailed test was used to compare the median recovery values for the ultrafilter and capsule filters, the differences were not significant ($P = 0.7762$). Decontamination of the filtration hardware between experiments was successful, as no oocysts were ever detected in the method blanks.

The physical parameters of the surface water samples processed for *Cryptosporidium* oocysts are summarized in Table 2. The pH, turbidity, and total dissolved solids measurements for each sample indicate a wide range of water quality. The pH ranged from 6.0 to 8.0 and averaged 6.6, and total dissolved solids ranged from 32.2 to 294 mg/liter and averaged 108.5 mg/liter. Sample turbidity was highly variable and ranged from 2.5 to 45 NTU, with an average of 14.9 NTU.

Table 3 summarizes the *Cryptosporidium* oocyst recovery rates from the spiked surface water samples with both filter systems tested. The 10-liter volumes were spiked with 100 to 150 oocysts and processed as previously described. In two samples with the capsule filter, high turbidity precluded the processing of the entire 10-liter volume. Table 3 shows the sample volumes that were filtered and the pellet volumes after concentration. The pellet volumes from the ultrafilter and capsule filter were 0.8 and 0.6 ml, respectively, and these volumes were significantly different ($P = 0.0195$ by Wilcoxon signed rank test). The values for filtered sample volume and pellet volume were used to calculate the volume of the original sample that was examined by the method, and recovery efficiencies were based on only this "volume examined." The average oocyst recovery rate using the ultrafilters was 42% (SD, ±27%); using the capsule filter, it was 15% (SD, ±12%). When a Mann-Whitney two-tailed test was used to compare the median values for the ultrafilters and capsule filters, the differences were significant ($P = 0.0017$). Table 4 summarizes the oocyst recovery rates from unspiked surface water samples. The volume filtered, pellet volume, and overall volume examined are listed for each sample. Few unspiked surface water samples were positive for oocysts. There was only one positive sample with the ultrafilter system (Raleigh, with 36 oocysts) and two positive samples using the capsule filter (Raleigh, with 8 oocysts; Brown, with 3 oocysts).

Positive and negative IMS control samples were processed, and the rates of *Cryptosporidium* oocyst recovery from positive control samples ranged from 31 to 117%, with an average recovery of 83% (SD, ±21%) from the 17 trials; no oocysts were found in the corresponding negative control blank samples (data not shown).

TABLE 3. *Cryptosporidium* detection in spiked surface water samples processed by ultrafilters versus capsule filters

Sample site	Ultrafilter				Capsule filter			
	Vol filtered (liters)	Pellet vol (ml)	Vol examined (liters)	Oocyst recovery (%)	Vol filtered (liters)	Pellet vol (ml)	Vol examined (liters)	Oocyst recovery (%)
New Hope Creek 1	10.0	0.5	10.0	19	10.0	0.7	7.1	10
New Hope Creek 2	10.0	1.0	5.0	39	10.0	0.7	7.1	9
New Hope Creek 3	10.0	1.0	5.0	50	10.0	0.5	10.0	4
Cane Creek 1	10.0	2.0	2.5	16	10.0	1.0	5.0	18
Cane Creek 2	10.0	1.5	3.3	15	10.0	0.9	5.5	16
Cane Creek 3	10.0	1.5	3.3	12	10.0	0.9	5.5	14
Goldsboro	10.0	0.5	10.0	25	10.0	0.4	10.0	8
New Hope Creek 4	10.0	0.5	10.0	17	10.0	0.5	10.0	7
Haw River[a]	10.0	0.5	10.0	65	8.5	0.5	8.5	17
Jefferson Bridge[a]	10.0	0.4	10.0	59	10.0	0.5	10.0	44
Leeper Park[a]	10.0	0.5	10.0	93	10.0	0.5	10.0	25
Raleigh[a]	10.0	0.7	7.1	78	9.5	0.5	9.5	39
Brown[a,b]	10.0	0.3	10.0	55	10.0	0.3	10.0	6
Williams[a,b]	10.0	0.5	10.0	14	10.0	0.5	10.0	7
Burlington[a,b]	10.0	0.4	10.0	73	10.0	0.2	10.0	7
Avg	10.0	0.8	7.7	42	9.9	0.6	9.9	15
SD				27				12

[a] Gelman modified elution procedure.
[b] Defective Gelman filter lot (no. 13810).

TABLE 4. *Cryptosporidium* detection in unspiked surface water samples processed by ultrafilters versus capsule filters

Sample site	Ultrafilter				Capsule filter			
	Vol filtered (liters)	Pellet vol (ml)	Vol examined (liters)	No. of oocysts recovered	Vol Filtered (liters)	Pellet vol (ml)	Vol examined (liters)	No. of oocysts recovered
New Hope Creek 1	10.0	0.7	7.1	0	10.0	0.8	6.3	0
Cane Creek 1	10.0	2.5	2.0	0	10.0	1.0	5.0	0
Goldsboro	10.0	0.8	6.3	0	10.0	0.4	10.0	0
New Hope Creek 4	10.0	0.5	10.0	0	10.0	0.5	10.0	0
Haw River[a]	10.0	0.5	10.0	0	8.5	0.5	8.5	0
Jefferson Bridge[a]	10.0	0.4	10.0	0	10.0	0.5	10.0	0
Leeper Park[a]	10.0	0.5	10.0	0	10.0	0.5	10.0	0
Raleigh[a]	10.0	0.7	7.1	36	10.0	0.4	10.0	8
Brown[a,b]	10.0	0.3	10.0	0	10.0	0.3	10.0	3
Williams[a,b]	10.0	0.5	10.0	0	10.0	0.6	8.3	0
Burlington[a,b]	10.0	0.4	10.0	0	10.0	0.2	10.0	0
Avg	10.0	0.7	8.4		9.9	0.5	8.9	

[a] Gelman modified elution procedure.
[b] Defective Gelman filter lot (no. 13810).

DISCUSSION

Over the past several years, considerable data have been gathered on the presence and concentrations of *Cryptosporidium* oocysts in waters with the potential to be used for drinking water supplies by the method described in the Information Collection Rule (7, 13). EPA method 1622 was developed to improve the recovery and detection of *Cryptosporidium* oocysts in water. We tested the accepted pleated capsule filter and an alternative disposable hollow-fiber ultrafilter system for *C. parvum* oocyst recovery from seeded reagent and surface water samples. The disposable hollow-fiber ultrafilter showed promise in earlier *C. parvum* recovery trials in our laboratory (9), but the compatibility of this system with the subsequent processing steps of method 1622 needed to be demonstrated.

Overall, the capsule filter gave slightly higher recovery rates (46%) than the ultrafilter (42%) in reagent water samples, but the differences between the oocyst recovery rates were not significant (Mann-Whitney *P* value, 0.7762). However, when tested with seeded surface waters, the recovery efficiencies of the capsule filter were only 15%, which is much lower than those from seeded reagent water (46%). For surface waters, the average recovery efficiency of the ultrafilter remained relatively high at 42%. These nearly threefold recovery rate differences between the capsule filter and ultrafilter were statistically significant (Mann-Whitney *P* value, 0.0017).

There were few problems in filtering 10-liter surface water sample volumes through either the ultrafilter or the capsule filter, although recirculation through the ultrafilter to reduce the sample volume and concentrate the oocysts was relatively time-consuming. The average time to reduce the 10-liter sample volume to the hold-up volume of the ultrafilter system (200 to 250 ml) was approximately 1.5 h (data not shown). Once filtration was started, however, the analyst could allow the peristaltic pump to run unattended, because the hold-up volume in the ultrafilter allows the oocysts to remain suspended in the retentate without possibility of desiccation. The hollow-fiber ultrafilter system would be amenable to automation for collecting and processing composite water samples over time. Because the ultrafilter system is very efficient at concentrating particulates in the sample, it results in comparatively large pellet volumes when processing surface water samples (pellet volumes averaged 0.8 ml with the ultrafilter and 0.6 ml with the capsule filters). However, these large pellet volumes did not limit subsequent IMS processing steps because either pellets larger than 0.5 ml can be processed in a single IMS or multiple 0.5-ml pellets can be separately processed by IMS.

Two modifications were made to the protocol specified in method 1622 during these experiments. The first modification, made at the start of the experiments and carried through for all of them, was to include only one PBS rinse during the oocyst-staining procedure instead of the three specified by the protocol. This modification resulted in smaller SD for the microscopic oocyst counts (data not shown). The second modification, made midway through the capsule filter trials (after 27 October 1999), was to use a horizontal shaker platform instead of the wrist action shaker and to increase the eluting times from 5 to 15 min for elution of the oocysts trapped in the capsule filter. This modification resulted in a statistically significant increase (Mann-Whitney *P* value, 0.0081) in the rates of oocyst recovery from the surface water using the capsule filters (from 11% [SD, ±5%] to 31% [SD, ±12%]; data from a faulty filter lot were excluded).

There are advantages and disadvantages associated with both the capsule filters and ultrafilters. The capsule filters are easy to use, field portable, and capable of processing relatively large volumes of environmental water samples. However, a major disadvantage associated with the capsule filter is the cost (approximately $100 per filter). The ultrafilters have the same advantages as the capsule filters with regard to ease of use, field portability, and ability to process large volumes of water, but the cost of the ultrafilters is much less (approximately $35 per filter). A disadvantage of the ultrafilter is the time it takes to process a water sample by recirculation (average time, 1.5 h; data not shown). Because the ultimate goal is to detect infectious oocysts in water, the gentle laminar flow of the ultrafilter should be compatible with infectivity assays, such as cell culture. The vigorous shaking necessary for consistent elution of oocysts from the capsule filters may damage the oocysts and

cause a loss of infectivity. However, further studies are needed to determine the effects of these filtration methods on oocyst infectivity.

Another disadvantage of the capsule filters is the potential for using filters of variable quality. For example, some experimental trials, specifically, the last three reagent water samples (OPR9, OPR10, and OPR11) and the last three surface water samples (Brown, Williams, and Burlington), were unknowingly performed with a defective lot of capsule filters (Gelman lot 13810). With only three water samples processed with the faulty filter lot, we were unable to statistically compare these recovery data with previous recovery data using other lots of filters. We included these data to calculate the overall recovery rates by the capsule filter method because these are conditions (defective filter lots, etc.) that could be encountered when using this filter system. Because the disposable hollow-fiber ultrafilter system is designed for medical uses, it is produced under very strict quality assurance and quality control standards.

The surface water samples examined in this study were generally from North Carolina streams and reservoirs that are affected by a wide range of land uses and could be used as sources of drinking water. Because the present study also included reservoir samples, the turbidities were not as high as those found in the previous study using only stream waters (the turbidities averaged 35.3 NTU in the previous study versus 14.9 NTU in the present study) (19). Only a few surface water samples were positive for naturally occurring *Cryptosporidium* oocysts. Of the samples that were positive, the majority of the oocysts were found to lack internal structure, and therefore it is possible that they were not viable or infectious. Because internal morphology is not a reliable predictor of infectivity, however, a simple, rapid, and reliable method is needed for detection of infectious *Cryptosporidium* oocysts concentrated from environmental water samples.

In summary, a disposable hollow-fiber ultrafilter was more efficient than the recommended pleated capsule filter at recovering *C. parvum* oocysts from surface waters and was compatible with the subsequent sample-processing steps of EPA method 1622. With the hollow-fiber ultrafilter, the rates of recovery of seeded oocysts from the reagent water of precision and recovery experiments and from the surface waters tested were relatively high. The hollow-fiber ultrafilter is capable of processing sample volumes larger than 10 liters (9). Therefore, if the present volume limitations of the subsequent IMS processing steps are overcome by using multiple IMS separations for environmental water samples, the ultrafiltration system has the potential to examine water volumes larger than 10 liters. Furthermore, because the hollow-fiber ultrafilter system is a very gentle method for primary concentration and is used to process blood without damage to red or white blood cells, it is likely that recovered environmental oocysts can be reliably examined for infectivity by tissue culture assay systems. However, further studies are needed to verify this point.

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