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Recovery of *Cryptosporidium* oocysts and *Giardia* cysts from source water concentrates using immunomagnetic separation

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Abstract

Immunomagnetic separation (IMS) procedures for the simultaneous isolation of *Cryptosporidium* oocysts and *Giardia* cysts have recently become available. We validated Dynal's GC-Combo IMS kit using source water at three turbidity levels (5000, 500 and 50 nephelometric turbidity units [ntu]) obtained from different geographical locations and spiked with approximately 9–11 (oo)cysts per ml. Mean recoveries of *Cryptosporidium* oocysts and *Giardia* cysts in deionized water were 62% and 69%, respectively. In turbid water matrices, mean recoveries of *Cryptosporidium* oocysts were between 55.9% and 83.1% while mean recoveries of cysts were between 61.1% and 89.6%. Marginally higher recoveries of the heat inactivated (oo)cysts were observed (119.4% *Cryptosporidium* oocysts and 90.9% *Giardia* cysts) in deionized water when compared with recoveries of viable (oo)cysts (69.7% *Cryptosporidium* oocysts and 79% *Giardia* cysts). Age of (oo)cysts on recoveries using the GC-Combo IMS kit demonstrated no effects up to 20 months old. Recovery of *Giardia* cysts was consistent for isolates aged up to 8 months (81.4%), however, a significant reduction in recoveries was noted at 20 months age. Recoveries of low levels (5 and 10 (oo)cysts) of *Cryptosporidium* oocysts and *Giardia* cysts in deionized water using IMS ranged from 51.3% to 78% and from 47.6% to 90.0%, respectively. Results of this study indicate that Dynal's GC-Combo IMS kit is an efficient technique to separate *Cryptosporidium/Giardia* from turbid matrices and yields consistent, reproducible recoveries. The use of fresh (recently voided and purified) (oo)cysts, aged (oo)cysts, viable and heat-inactivated (oo)cysts indicated that these parameters do not influence IMS performance. © 2001 Published by Elsevier Science B.V.

Keywords: *Cryptosporidium*; *Giardia*; Immunomagnetic separation; Water

1. Introduction

The procedures for isolation of *Cryptosporidium* and *Giardia* from water samples have conventionally utilized filtration of large sample volumes through string wound filters, elution using several liters of weak detergent solution, clarification using density

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gradients and then detection by immunofluorescence assays. Until recently, these methods were used as standards in the US (Information Collection Rule Method, USEPA, 1996) and UK (Standing Committee of Analysts Method, 1990) for determining occurrence information for *Cryptosporidium* and *Giardia*; however, their laborious and inefficient nature has been well recognized. The concentration process leads to accumulation of debris in the sample and in turn, detection of (oo)cysts requires clarification processes to isolate (oo)cysts from debris. While flotation procedures may enable separation of oocysts from background debris, the degree of clarification can be highly variable. The material containing the (oo)cysts after flotation can contain large particulate matter and algal cells that interfere with oocyst detection by immunofluorescence and may limit the amount of material which can be examined microscopically. Flotation procedures also yield highly variable oocyst recoveries and data from seeding experiments indicate that recoveries can be dependent on a number of parameters, including the initial spike dose. For example, large numbers of oocysts (i.e. $>10^4$ oocysts) have been reported to yield between 20% and 74% recoveries (Bukhari and Smith, 1995), with recoveries being dependant both on oocyst numbers originally present in the sample and on their viability. For these reasons, a search for alternative, more user-friendly and efficient methods was initiated.

As part of the development of the new method for the USEPA, immunomagnetic separation (IMS) was investigated to specifically isolate *Cryptosporidium parvum* oocysts from environmental sample concentrates. Previous studies conducted by Bukhari et al. (1998) and Rochelle et al. (1999) compared performance of commercially available IMS kits in deionized water and source water of various turbidities and indicated that although some kits perform better than others, IMS was a promising technology for the isolation of oocysts form turbid matrices. In addition, although the manufacturers advocate the use of a packed pellet volume less than 0.5 ml, for some matrices, it was demonstrated that acceptable oocyst recoveries were obtainable with packed pellet volumes greater than 0.5 ml.

The objective of the present study was to determine recoveries of *C. parvum* oocysts as well as *Giardia intestinalis* (synonyms *G. lamblia* or *G. duodenalis*) cysts using the GC-Combo IMS kit manufactured by Dynal. Trials conducted in our laboratory-assessed performance of these kits in deionized water and turbid water matrices. Fresh (recently voided and purified) (oo)cysts, aged (oo)cysts, viable (oo)cysts and non-viable (oo)cysts were also examined to determine whether these parameters influenced IMS recoveries.

2. Materials and methods

2.1. Sources of oocyst and cyst suspensions

C. parvum oocysts were obtained from the Sterling Parasitology Research Laboratory, University of Arizona. This isolate was originally isolated from a calf and is referred to as the Iowa strain (Moon and Bemrick, 1981). It has been maintained by passage in neonatal calves. The feces of experimentally infected calves was collected and clarified by using cesium chloride density gradient centrifugation (Arrowood and Donaldson, 1996). Purified oocysts were stored in deionized water and antibiotics (gentamicin, 0.1 mg/ml; streptomicin, 0.1 mg/ml; and penicillin, 100 U/ml) at 4°C (DIA). The age of oocysts used in these trials was less than 1 month unless otherwise specified. Stock suspensions were diluted to a concentration of 1×10^3 ml^{-1} with deionized water and stored at 4°C. *G. intestinalis* cysts were obtained from the USEPA National Exposure Research Laboratory (NERL), Cincinnati, OH and Waterborne, New Orleans, LA. Both strains of *G. intestinalis* cysts were originally isolated from humans. The CDC0284:1 cyst strain from EPA-NERL was obtained from experimentally infected Mongolian gerbils. The cysts were purified using sucrose density flotation followed by Percoll gradient purification and velocity sedimentation ($600\times g$; 10 min). Purified cysts were placed in deionized water containing antibiotics (gentamicin, 0.1 mg/ml; streptomicin, 0.1 mg/ml; and penicillin, 100 U/ml) at 4°C (DIA). The H3 cyst strain was also obtained from experimentally infected Mongolian gerbils. Cysts were purified by sucrose density gradient centrifugation ($950\times g$; 10 min) followed by Percoll gradient purification ($950\times g$; 10 min) and stored in

DIA. The age of cysts used in this study was less than 1 month old unless specified otherwise. Stock suspensions of cysts were diluted to a concentration of 1×10^3 ml^{-1}.

2.2. Preparation / enumeration of stock suspensions of (oo)cysts

Separate suspensions of *G. intestinalis* cysts and *C. parvum* oocysts were enumerated by placing ten 100 μl replicate aliquots on glass well microscope slides as described in EPA Method 1 622 (USEPA, 1998).

2.3. Water matrices selected for assessing C. parvum oocyst recoveries following IMS

Concentrates from three raw water matrices, derived from different geographical locations in the US, were used for the spiking studies. These samples were collected from a river in California, a river in Pennsylvania and a river in Nebraska. Sample collection and concentration was as described in the ICR Microbial Laboratory Manual (EPA/600/R-95/178). Source water matrices for spiking were prepared as described previously (Bukhari et al., 1998). Briefly, samples were collected by filtering 100–120 l of water through a spiral wound cartridge filter and elution of trapped particulates in a phosphate buffer solution with detergents (PBS). The eluant was centrifuged at $1050 \times g$ and the supernatant discarded. The pellet was rinsed twice with deionized water to remove the PBS and detergents. The final pellet was resuspended in deionized water, to a total volume of 125 ml. A HACH 2100P turbidimeter, which was capable of measuring turbidity levels between 0 and 1000 ntu, was used to determine turbidity of each raw water concentrate. Aliquots to each water matrix concentrate were diluted in deionized water for turbidity measurements. The appropriate dilution factor of the concentrate was used to estimate turbidity of the original concentrate. For each concentrate, the dilution factors necessary to yield samples with target turbidities of 5000, 500 and 50 ntu were calculated and these working solutions were prepared by appropriate dilution with deionized water. Turbidity of diluted samples was measured directly for 500 and 50 ntu samples. Aliquots of 5000 ntu samples were used to prepare 10% solutions in deionized water and their turbidity was also determined. Where necessary, minor turbidity adjustments were made accordingly, by addition of either the respective raw water concentrate or deionized water.

2.4. Spiking of source water matrices

An appropriate volume of the *C. parvum* oocysts and *G. intestinalis* cysts suspensions, known to contain between 89.1 and 98.7 oocysts and 97.9 and 107.0 cysts, were added to 10 ml aliquots of each source water matrix for evaluation of the IMS kit. For each turbidity level (5000, 500 and 50 ntu) three replicates were analyzed. For each matrix, one spiked 10 ml positive control (deionized water only) and duplicate 10 ml unspiked controls (source water matrix without addition of oocysts) were analyzed at both 5000 and 50 ntu.

2.5. Immunomagnetic separation using Dynabeads® GC-Combo IMS kit

The Dynabeads® GC-Combo IMS kit (Prod. No. 730.02; Dynal A.S. PO Box 158 Sk∅yen, N-0212 Oslo, Norway) was used to recover *G. intestinalis* cysts and *C. parvum* oocysts from deionized water or environmental matrices as described by the manufacturer. Briefly, 1 ml of SL-A 10 × and SL-B 10 × buffers were added to Leighton tubes containing 10 ml volumes of deionized water or source water samples spiked with known number of *C. parvum* oocysts and *G. intestinalis* cysts. To each tube, 100 μl of the Dynabeads® conjugated anti-*Cryptosporidium* monoclonal antibody (mAb) and 100 μl of Dynabeads® conjugated anti-*Giardia* mAb were added. Samples were incubated for one hour at room temperature while rotating on the sample mixer. At the end of the incubation period, each Leighton tube was placed in the MPC-1 magnetic particle concentrator with the flat side of the tube adjacent to the magnet. The tube was gently rocked through a 90° angle, tilting it from top to bottom with the magnet side facing up, for 2 min. The supernatant was decanted, the tube removed from the MPC-1 and 1 ml of 1 × SL-A buffer was added to the tube. The tube was gently rocked to re-suspend the bead com-

plex and using a Pasteur pipette, the suspension was transferred to a 1.5 ml eppendorf tube. Eppendorf tubes were then placed in the MPC-M magnetic particle concentrator, the magnet inserted into place and the MPC-M was rocked through a 90° motion for 1 min. The bead-(oo)cyst complex collected as a dot on the back of the eppendorf tube and the supernatant was aspirated. The magnet was removed from the MPC-M and 50 μl of 0.1 N HCl was added to each tube to resuspend the bead-oocyst complex and each sample was vortexed for a minimum of 10 s. The samples were allowed to stand for 10 min in an upright position, vortexed again for 10 s and the magnet of the MPC-M was inserted into place. The tubes were allowed to stand undisturbed for 2 min. The resulting supernatant was placed in the center of a well of a three-welled microscope slide containing 5 μl of 1 N NaOH. Slides were allowed to dry at 42°C for 30 min, fixed with 25 μl absolute methanol, air-dried and stained with fluorescein isothiocynate conjugated anti-*Cryptosporidium* and anti-*Giardia* mAbs (FITC-mAb). Slides were examined using epifluorescence microscopy.

2.6. Heat treatment of Giardia cysts and Cryptosporidium oocysts

Heat treatment to inactivate *C. parvum* oocysts and *G. intestinalis* cysts was performed on separate stock suspensions of each organism by their incubation in a 93–97°C water bath for 5 min. After the suspensions cooled, sub-samples of heat-treated organisms were FITC-mAb stained on glass well slides and enumerated using epifluorescence microscopy.

2.7. Recovery efficiency of the IMS kit using low spike doses of (oo) cysts

Pre-enumerated stock suspensions of *C. parvum* oocysts and *G. intestinalis* cysts were diluted accordingly to yield suspensions containing 5 or 10 (oo)cysts per 100 μl. Suspensions were enumerated using the well slide technique and slides were examined using epifluorescence microscopy. Spiking studies with low (oo)cyst numbers (viable or heat inactivated) were conducted in triplicate 10 ml volumes of deionized water. The samples were subjected to IMS according to the procedure described earlier and (oo)cyst recoveries were determined.

2.8. Effects of oocyst / cyst age on recovery by IMS

Predetermined volumes of (oo)cysts at varying ages were spiked at approximately 100 (oo)cysts per sample, in triplicate 10 ml volumes of deionized water. The samples were subjected to IMS according to the procedure described earlier and (oo)cyst recoveries were determined.

2.9. Epifluorescence microscopy

A Zeiss Axioskop fluorescence microscope, equipped with a blue filter block (excitation 490 nm; emission 510 nm) was used for detection of FITC-mAb labeled oocysts at 200× magnification. Confirmation of oocysts was achieved at ×400 magnification and by using a UV filter block (excitation 400

Table 1
Microscopic particulate analysis of source water matrices

Biological characteristics	Analysis per 100 μl of 500 ntu sample concentrate		
	Matrix **A**	Matrix **B**	Matrix **C**
Algae	5×10^2	1.3×10^4	1.4×10^2
Crustaceans	ND	10	5
Diatoms	3.1×10^2	90	2.3×10^2
Free-living protozoa	ND	5	5
Rotifers	ND	5	ND
Spores	ND	ND	5
Vegetative debris	ND	5	15
Other comments	Very fine mineral debris	Very little inorganic debris	Moderate levels of inorganic debris

ND—none detected.

nm; emission 420 nm) for visualization of DAPI. Internal morphology of oocysts was observed by using Nomarski-DIC microscopy.

3. Results

3.1. Characteristics of selected water matrices

Characteristics of the three water matrices used in this comparative study were determined by the Consensus Method for Determining Groundwater Under the Direct Influence of Surface Water Using Microscopic Particulate Analysis (EPA 910/9-92-029). This entailed examination of a 20 μl aliquot from each concentrate, without flotation, at 200× magnification and by using bright-field microscopy. General observations on the nature of the inorganic constituents were recorded and for spores, observations were limited to fungi and plants. These data have been summarized in Table 1.

3.2. Recoveries of (oo)cysts in deionized water and source water matrices

Triplicate deionized water and source water samples were spiked with predetermined numbers of *C. parvum* oocysts and *G. intestinalis* cysts (Tables 2, 3 and 4). Recoveries of oocysts in deionized water were between 54.7% and 67.0%. *G. intestinalis* cyst recoveries were marginally higher and ranged from 63.9% to 72.3%.

Mean recoveries of *C. parvum* oocysts in matrix **A**, regardless of turbidity, were similar to those obtained in deionized water. Mean recoveries of oocysts in matrices **B** and **C** were slightly higher than in matrix **A** or deionized water (71.5% and 72.6%, respectively). While mean oocyst recoveries

Table 3
Recovery of *Cryptosporidium* oocysts and *Giardia* cysts in source water B at three turbidities and deionized water using immunomagnetic separation
Spike Dose—89.1 ± 8.4 *Cryptosporidium* oocysts; 97.9 ± 9.2 *Giardia* cysts ($n = 10$).

Turbidity (ntu)	Packed pellet (ml)	Mean percent recovery ± SD ($n = 3$)	
		Cryptosporidium	Giardia
5000	0.8	63.2 ± 5.6	80.0 ± 2.1
500	0.1	62.5 ± 7.3	83.1 ± 7.9
50	Trace	89.6 ± 14.4	79.7 ± 9.7
DI Water	N/A	62.2 ± 6.5	69.2 ± 4.6
Negative Controls ($n = 2$)			
5000	0.8	0	0
50	Trace	0	0

Table 2
Recovery of *Cryptosporidium* oocysts and *Giardia* cysts in source water A at three turbidities and deionized water using immunomagnetic separation
Spike Dose—98.7 ± 9.7 *Cryptosporidium* oocysts; 107.9 ± 9.8 *Giardia* cysts ($n = 10$).

Turbidity (ntu)	Packed pellet (ml)	Mean percent recovery ± SD ($n = 3$)	
		Cryptosporidium	Giardia
5000	0.2	63.2 ± 5.2	59.9 ± 7.4
500	0.02	61.1 ± 5.0	58.1 ± 10.8
50	Trace	71.6 ± 3.6	57.5 ± 4.9
DI Water	N/A	62.2 ± 6.5	69.2 ± 4.6
Negative Controls ($n = 2$)			
5000	0.2	0	0
50	Trace	0	0

Table 4
Recovery of *Cryptosporidium* oocysts and *Giardia* cysts in source water C at three turbidities and deionized water using immunomagnetic separation
Spike Dose—98.7 ± 9.7 *Cryptosporidium* oocysts; 107.9 ± 9.7 *Giardia* cysts ($n = 10$).

Turbidity (ntu)	Packed pellet (ml)	Mean percent recovery ± SD ($n = 3$)	
		Cryptosporidium	Giardia
5000	0.5	70.6 ± 10.0	70.7 ± 6.3
500	0.05	78.3 ± 25.1	62.7 ± 6.8
50	Trace	68.9 ± 7.3	55.9 ± 4.6
DI Water	N/A	62.2 ± 6.5	69.2 ± 4.6
Negative Controls ($n = 2$)			
5000	0.5	0	0
50	Trace	0	0

Table 5
A comparison of recoveries of heat inactivated and viable *Cryptosporidium* oocysts and *Giardia* cysts by IMS

(Oo)cyst state	*C. parvum* oocyst spike dose ± SD ($n = 10$)	Mean percent recovery ± SD($n = 3$)	*G. intestinalis* cyst spike dose ± SD ($n = 10$)	Mean percent recovery ± SD($n = 3$)
Viable	89.1 ± 7.9	69.7 ± 2.5	97.9 ± 8.7	79.0 ± 2.0
Heat inactivated	94.1 ± 8.3	119.4 ± 10.4	93.1 ± 9.4	90.9 ± 5.5

decreased slightly as turbidity increased from 50 to 500 ntu in matrices **A** and **B**, an increase in mean recoveries was noted in matrix **C**.

Recovery of *G. intestinalis* cysts was 58.5% in matrix **A**, which was the lowest values obtained among the three matrices examined. The highest cyst recoveries were observed in matrix **B**, with a mean recovery of all turbidities tested at 80.9%. Mean cyst recoveries increased as turbidity increased in matrix **C** (55.9 ± 4.6% at 50 ntu, 62.7 ± 6.8% at 500 ntu and 70.7 ± 6.3% at 5000 ntu).

3.3. The effect of heat treatment on (oo)cyst recovery by IMS

Organisms subjected to heat treatment were assumed to be non-viable and no viability determination were performed. Recovery comparisons between viable and heat inactivated oocysts demonstrated dramatic differences, increasing from 69.7% to 119.4%. These data are presented in Table 5.

3.4. Recovery of low spike doses of (oo)cysts in deionized water

Three separate trials were conducted with triplicate 10 ml volumes of deionized water spiked with approximately five (oo)cysts (Table 6). Using viable (oo)cysts, recoveries were 51.3% and 69.4% for *C. parvum* and 47.6–90.9% for *G. intestinalis* in Trials 1 and 2, respectively. In the third trial, heat-inactivated organisms were used and similar recoveries to those in Trial 1 were obtained. A fourth trial using 10 viable (oo)cysts, yielded mean recoveries of 78.0% and 70.4% for oocysts and cysts, respectively.

3.5. Effects of (oo)cyst age on recovery by IMS

A single isolate of *C. parvum* was used to evaluate recoveries. Fresh and 4-month-old *G. intestinalis* isolate was the CDC0284:1 strain obtained from EPA-NERL. The 8-month and 20-month-old *G. intestinalis* isolate was the H3 strain obtained from Waterborne (Table 7). Isolates were stored at 3–5°C in deionized water for the up to 20 months. Approximately 100 fresh or aged (< 1, 4, 8 and 20 months), (oo)cysts were seeded into deionized water in triplicate. Mean oocyst recoveries declined slowly with increasing age (81.4% for fresh [< 1 month] oocysts to 68.6% for aged [20 months] oocysts), whereas for *Giardia* cysts mean recoveries remained consistent for up to 8 months age (81.4%); however, a signifi-

Table 6
Recovery efficiencies of low levels of *G. intestinalis* cysts and *C. parvum* oocysts in deionized water using the dynabeads® GC combo IMS kit

Trial number	Mean *G. intestinalis* cysts spike dose ± SD ($n = 10$)	Mean *G. intestinalis* cysts recovered ± SD ($n = 3$)	Mean % recovery ± SD ($n = 3$)	Mean *C. parvum* oocysts Spike dose ± SD ($n = 10$)	Mean *C. parvum* oocysts recovered ± SD ($n = 3$)	Mean % recovery ± SD ($n = 3$)
1	4.2 ± 1.6	2.0 ± 2.0	47.6 ± 38.9	3.9 ± 2.0	2.0 ± 0	51.3 ± 0
2	4.4 ± 1.8	4.0 ± 2.2	90.9 ± 49.1	4.8 ± 1.7	3.3 ± 0.5	69.4 ± 9.8
3[a]	4.9 ± 2.1	2.33 ± 0.5	47.6 ± 9.6	4.6 ± 2.0	2.7 ± 0.5	58.0 ± 10.2
4	9.0 ± 3.2	6.3 ± 1.9	70.4 ± 20.9	9.4 ± 4.0	7.3 ± 3.8	78 ± 41.0

[a] *G. intestinalis* cysts and *C. parvum* oocysts were heat inactivated.

Table 7
Effects of *G. intestinalis* cyst and *C. parvum* oocyst age on recovery efficiencies by IMS

(Oo)cyst age	*C. parvum* oocyst Spike dose ± SD ($n = 10$)	% Recovery ± SD ($n = 3$)	*G. intestinalis* cyst spike dose ± SD ($n = 10$)	% Recovery ± SD ($n = 3$)
Fresh	99.9 ± 9.7	81.4 ± 8.2	99.6 ± 16.6	73.0 ± 7.0
4 months	99.9 ± 15.6	84.8 ± 6.9	100 ± 13.4	81.0 ± 4.5
8 months	99.7 ± 11.9	73.9 ± 3.9	102.0 ± 18.7	81.0 ± 6.1
20 months	99.5 ± 9.7	68.6 ± 3.3	99.8 ± 11.6	49.1 ± 7.9

cant reduction in recoveries (49%) did occur at 20 months age.

4. Discussion

C. parvum and *G. intestinalis* are gastrointestinal parasites that have been responsible for several waterborne outbreaks in the US and UK. Methods for the collection, isolation and detection of these organisms from the environment have been reported to produce low and highly variable recoveries (Clancy et al., 1996). This study indicates that the GC-Combo IMS kit for the simultaneous isolation of *Cryptosporidium* and *Giardia* is a promising alternative to the cumbersome, inefficient flotation procedure used in conventional methods. With the Dynal GC combo IMS, recoveries of the target organisms were consistent, reproducible and significantly higher than those obtained using the ICR or SCA method. Our unpublished observations indicate that, following sucrose flotation, high spike doses of heat inactivated oocysts or low spike doses of oocysts that have been suspended in turbid sample concentrates can result in recoveries below 10% (Bukhari, unpublished). Recoveries with sucrose flotation vary from 56% down to 11% depending on the increased exposure time that the oocysts have been in contact with the raw water (Fricker, 1995). Additionally, due to specific capture of target organisms, the background fluorescence produced by samples purified by IMS is reduced drastically when compared with the background debris present with samples clarified by the non-specific flotation procedure.

Various commercial vendors have developed IMS for the isolation of *Cryptosporidium* oocysts from environmental samples. Bukhari et al. (1998) and Rochelle et al. (1999) have evaluated three of these kits independently and noted the Dynal kits performed consistently better than the other two kits studied, with recoveries ranging from 35% to 100% depending on sample turbidity.

In the current study, the three water matrices selected were similar to the characteristics of those chosen in our previous study (Bukhari et al., 1998). Despite differences in the characteristics of these three matrices, IMS recoveries for *C. parvum* oocysts were similar. In matrices **A** and **B**, a slight decrease in mean oocyst recovery was observed between 50 and 500 ntu. The mean oocyst recoveries of these two matrices at the 500 and 5000 ntu turbidity levels were not significantly different (Tables 2 and 3). This would indicate that the increased levels of debris were not a factor in the IMS kit's ability to capture and isolate the oocysts. In Matrix **C**, mean oocyst recoveries remained constant with increased turbidities (Table 4). *G. intestinalis* cyst recoveries were consistent over turbidity levels tested for Matrix **A** and **B**, however, in Matrix **C** mean cyst recoveries escalated with increased turbidities (55.9% at 50 ntu to 70.7% at 5000 ntu) (Table4). While the manufacturer of the GC-Combo IMS kit suggests use of packed pellets less than 0.5 ml, mean recoveries for *Giardia* cysts in Matrix **B** were 80% (at 5000 ntu) with a pellet volume of 0.8 ml. This matrix contained the least amount of inorganic debris when examined for microscopic particulates. Lowest cyst recoveries were observed in Matrix **A** where the packed pellet volume was 0.2 ml at 5000 ntu level. While the packed pellet volume of this matrix was considerably lower than the manufacturers recommended maximum, the composition of the material was high in clays and silts. These data indicate the sample matrix characteristics to be important in (oo)cyst recoveries with IMS.

The ability of the IMS kit to recover low spike doses of (oo)cyst in deionized water indicated high capture efficiency for the target organisms. This is an important performance criterion for any IMS kit

since relatively low numbers of numbers of *Cryptosporidium* and *Giardia* are usually present in environmental water samples. Another objective of this study was to determine the effects of age on the recovery of (oo)cysts, which demonstrated no significant differences in recoveries of *C. parvum* oocysts at less than 1, 4, 8 or 20 months age. These results are similar to those obtained by Bukhari et al. (1998). In contrast, a marked decrease in recoveries for *Giardia* cysts aged 20 months was noted. Perhaps this was due to reduced IMS capture efficiency for aged cysts, which may have occurred due to declining concentrations of surface expressed antigens on the surface of *Giardia* cysts with age.

(Oo)cysts present in the environment are generally subjected to a variety of stresses such as freezing, desiccation, or water treatment processes. These conditions will render a portion of the population non-viable. The ability of IMS to capture non-viable organisms is just as important as recovering viable organisms in order to ascertain organism occurrence information in source waters. The G/C combo IMS kit was able to recover oocysts and cysts regardless of their viability status. Furthermore, others (Rochelle et al., 1999; DiGiovanni et al., 1998) have shown that with minor modifications the IMS protocol can be used to recover oocysts without affecting their viability, which should allow the incorporation of viability/infectivity assays for determination of the significance of environmentally isolated (oo)cysts.

In conclusion, data in this study indicated that regardless of their condition or age, the Dynal GC combo IMS procedure can effectively capture (oo)cysts from deionized and source water. In the latter, a packed pellet volume greater than 0.5 ml did not appear to adversely effect recoveries.

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