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Immunomagnetic Separation of *Cryptosporidium parvum* from Source Water Samples of Various Turbidities

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Immunomagnetic separation (IMS) procedures which specifically capture *Cryptosporidium* oocysts and have the potential to isolate oocysts from debris have become commercially available. We compared two IMS kits (kit DB [Dynabeads anti-*Cryptosporidium*; product no. 730.01; Dynal A.S., Oslo, Norway] and kit IC1 [Crypto Scan IMS; product no. R10; Clearwater Diagnostics Company, LLC, Portland, Maine]) and a modification of kit IC1 (kit IC2 [Crypto Scan IMS; product no. R10; Clearwater Diagnostics Company, LLC]) at three turbidity levels (50, 500, and 5,000 nephelometric turbidity units [ntu]) by using water matrices obtained from different geographical locations. In deionized water, kit DB yielded recoveries between 68 and 83%, whereas the recoveries obtained with kits IC1 and IC2 were more variable and ranged from 0.2 to 74.5%. In water matrices with turbidity levels up to 500 ntu, the oocyst recoveries were more variable with kit DB; however, the recoveries were similar to those obtained in deionized water. In contrast, there were notable reductions in oocyst recoveries in the turbid matrices with kits IC1 and IC2, and the highest recovery (8.3%) was obtained with a 50-ntu sample. An examination of the effects of age on oocyst recovery with kit DB revealed that oocysts up to 16 weeks old yielded recoveries similar to the recoveries observed with fresh oocysts. These data indicate that all IMS kits do not perform equally well, and it is important to conduct in-house quality assurance work before a commercially available IMS kit is selected to replace flotation procedures for recovery of *Cryptosporidium* oocysts.

Cryptosporidium sp. oocysts are between 4 and 6 μm in diameter and usually occur in low numbers in environmental water samples. To increase the probability of detecting oocysts in such samples, large volumes of water are usually concentrated, and the retentate is eluted and subsequently concentrated further by using both large-scale centrifugation and small-scale centrifugation. The concentration process leads to accumulation of debris in the sample, and detection of oocysts requires clarification procedures to separate oocysts from debris. While flotation procedures may result in separation of oocysts from background debris, the degree of clarification can be highly variable. The material containing the oocysts 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 depend on a number of parameters, including the initial spike dose. For example, large numbers of oocysts ($>10^4$ oocysts) have been reported to yield recoveries between 40 and 65%, and the recoveries depend both on the numbers of oocysts originally present in the sample and on the viability of the oocysts (4).

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 water samples can result in recoveries of less than 10%. Furthermore, Fricker (9) demonstrated that recoveries obtained with sucrose flotation varied from 56 to 11% depending on the amount of time that oocysts had been in contact with raw water. High levels of background (nonspecific) fluorescence can lead to difficulties in oocyst detection by immunofluorescence microscopy and oocyst confirmation by Nomarski differential interference contrast (DIC) microscopy. In addition, compounds that inhibit molecular detection procedures may not be removed completely by flotation procedures and can compromise the sensitivity of molecular detection and characterization procedures.

Magnetic particles have been used previously to isolate target cells selectively from a heterogeneous mixture of cells and debris (3, 11, 12). For the genus *Cryptosporidium* this technique has been investigated both in an indirect immunoassay format (6, 10, 13) and in a direct immunoassay format (5, 7). The use of a direct immunomagnetic separation (IMS) procedure has been compared with sucrose flotation performed by both the United States Environmental Protection Agency Information Collection Rule method (15) and the United Kingdom Standing Committee of Analysts method (1). In clean water matrices, the IMS procedure has been reported to recover more than 90% of the oocysts added irrespective of the oocyst age. However, with both the Information Collection Rule and Standing Committee of Analysts flotation procedures, recoveries of less than 40% were observed, and the oocyst recoveries decreased further with increasing oocyst age (5).

In this paper, we describe the effects of sample turbidity on recovery of *Cryptosporidium parvum* oocysts with two commercially available IMS kits. Oocysts were added to three different water matrices that had previously been adjusted to three different turbidity levels. The first objective of this investigation was to identify an IMS kit that yielded highly purified oocyst suspensions and consistently high recoveries. The second objective was to determine turbidity levels beyond which there was a noticeable impact on oocyst recoveries, and the third objective was to use the IMS kit that yielded the most consistent oocyst recovery results to evaluate the effects of age on the recovery of *C. parvum* oocysts.

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MATERIALS AND METHODS

Enumeration of oocyst stock suspensions. The *C. parvum* isolate used in this study was obtained from the Sterling Parasitology Laboratory, University of Arizona. This isolate is referred to as the Harley Moon or Iowa strain. This strain was originally isolated from a calf and has been maintained by passage in neonatal calves. The oocysts voided in the feces of experimentally infected calves were clarified by using cesium chloride density gradient (2) and were stored at 4°C in phosphate-buffered saline (PBS). Stock suspensions of *C. parvum* oocysts were enumerated with a hemocytometer and diluted in deionized water, and 10 100-μl aliquots of the diluted oocyst suspension were placed into individual wells of three-well microscope slides. The samples were dried (42°C, 1 to 2 h), fixed in methanol, and air dried, and a 50-μl aliquot of fluorescein isothiocynate-conjugated anti-*Cryptosporidium* sp. monoclonal antibody (FITC-mAb; Waterborne Inc.) was placed into each well. The slides were placed in a humid chamber and incubated (at 37°C for 30 min), and the excess FITC-mAb was aspirated. Any remaining FITC-mAb was removed by adding 50 μl of 150 mM PBS (pH 7.2) to each well, allowing the slides to stand for 1 min, and aspirating the excess PBS. This washing step was repeated three times, and 50 μl of a weak 4′6-diamidino-2-phenylindole (DAPI) solution (0.4 μg/ml in PBS) was placed into each well. The slides were allowed to stand at room temperature for 2 min, and the excess DAPI solution was removed by washing the slides twice in PBS and once in deionized water. The slides were placed in the dark until they were dry, and then 10 μl of mounting medium (2% DABCO in 60% glycerol–40% PBS) was placed into each well, a coverslip was applied, and the slides were examined by using epifluorescence microscopy.

Water matrices used to assess *C. parvum* oocyst recoveries following IMS. Concentrates from three raw water matrices derived from different geographical locations in the United States were used for the spiking studies. The geographical locations of the samples were a river in California, a reservoir in Connecticut, and a river in Nebraska. Samples were collected and concentrated as described in the *ICR Microbial Laboratory Manual* (15). Briefly, samples were collected by filtering 100 to 120 liters of water through a spiral-wound cartridge filter and eluting trapped particulates in PBS containing detergents. The eluant was centrifuged at 1,050 × *g*, and the supernatant was 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 1,000 nephelometric turbidity units (ntu), was used to determine the turbidity of each raw water concentrate. Aliquots of each water matrix concentrate were diluted in deionized water in order to obtain turbidity measurements. The appropriate dilution factor for the concentrate was used to estimate the turbidity of the original concentrate. For each concentrate, the dilution factors necessary to yield samples with target turbidities of 5,000, 500, and 50 ntu were calculated, and working solutions were prepared by diluting the concentrates with deionized water. The turbidities of diluted samples were measured directly for the 500- and 50-ntu samples. Aliquots of the 5,000-ntu samples were used to prepare 10% solutions in deionized water, and their turbidities were also determined. When necessary, minor turbidity adjustments were made by adding either the appropriate raw water concentrate or deionized water.

Spiking of test samples. An appropriate volume of the *C. parvum* oocyst suspension, which was known to contain between 525 and 870 oocysts, was added in order to evaluate the performance of the two IMS kits used. For each matrix, duplicate spiked 10-ml positive controls (containing only deionized water) and duplicate 10-ml unspiked controls (containing source water matrix but no oocysts) were used at both 5,000 and 50 ntu.

IMS with Dynabeads anti-*Cryptosporidium* kit DB. The Dynal IMS (Dynabeads anti-*Cryptosporidium*; product no. 730.01; Dynal A.S., Oslo, Norway) procedure was performed as recommended by the manufacturer. Briefly, 10 ml of the test sample was placed in a screw-cap Leighton tube, and 1 ml of 10× SL buffer A, 1 ml of 10× SL buffer B, and 100 μl of the bead conjugate were added. Each sample was rotated through 360° for 1 h at room temperature, and the tube was placed in a magnetic particle concentrator (MPC-1) to separate the bead-oocyst complex from the contaminating debris. The beads were resuspended in 1 ml of 1× SL buffer A, transferred into an Eppendorf tube, and separated by using a magnetic particle concentrator (MPC-M), and the supernatant was removed and discarded. While the manufacturer recommended using 50 μl of 0.1 N HCl in the oocyst dissociation step, in our investigation 100 μl of 0.1 N HCl was used to dissociate the bead-oocyst complex. The neutralization procedure was performed on a microscope slide with 10 μl of 1 N NaOH. Each sample concentrate (100%) was placed in an individual well of a three-well microscope slide, dried at 42°C, labeled with anti-*Cryptosporidium* FITC-mAb, and examined by epifluorescence microscopy.

IMS with Immucell Crypto Scan kit IC1. An initial preclearing step, in which only immunomagnetic beads were used, was suggested in the protocol recommended by the manufacturer of the Crypto Scan IMS system (product no. R10; Clearwater Diagnostics Company, LLC, Portland, Maine) in order to remove magnetic material from water sample concentrates; however, our preliminary investigations indicated that the preclearing step could nonspecifically capture 50% of the spike dose, and as a result, the preclearing step was omitted and the following modifications were included in the Immucell IMS protocol. A 9.5-ml portion of the test sample was placed in a 50-ml centrifuge tube, 0.5 ml of 20× PBS and 100 μl of a suspension of WDX reagent A were added, and the tube was rotated through 360° for 15 min at room temperature. Anti-*Cryptosporidium* immunomagnetic beads were vortexed, 50 μl of a 5-mg/ml stock suspension of beads was added to each sample, and the sample tube was rotated through 360° for an additional 30 min at room temperature. A magnetic panning device containing a 100-ml petri dish was placed on an orbital shaker, and the shaker was switched on. A 20-ml portion of WDX reagent B was added to each 50-ml tube containing a test sample, and the entire 30 ml was transferred into the petri dish and agitated for 2 min. This resulted in separation of the bead-oocyst complex from the remainder of the sample. The supernatant, which contained debris, was removed with a 10-ml pipette, and the beads were resuspended in 1 ml of WDX reagent B and quantitatively transferred into an Eppendorf tube. The tube was placed in a slot that was provided on the pan magnet for the separation process, and the supernatant was removed and discarded. The beads were resuspended in 100 μl of deionized water, and the entire sample concentrate (100%) was placed in two wells of a three-well microscope slide, dried at 42°C, labeled with anti-*Cryptosporidium* FITC-mAb, and examined by epifluorescence microscopy.

IMS with Immucell Crypto Scan kit IC2. A modified IMS kit, in which releasable immunomagnetic beads were used, was purchased from the manufacturer of kit IC2 (Crypto Scan IMS; product no. R10; Clearwater Diagnostics Company) and evaluated for its ability to assess oocyst recoveries from both deionized water samples and water samples of various turbidities. The protocol used for these studies was the protocol recommended by the manufacturer and included the use of 150 μl of preclearing magnetic beads in samples that had turbidities of 500 ntu or more. Addition of the preclearing beads was followed by end-over-end rotation for 15 min, and then the sample was poured into a 100-mm petri dish. The sample was swirled manually, and the petri dish was placed on a magnetic panning device, which was then placed on an orbital shaker. The orbital shaker was switched on at a predetermined speed for 2 min, and then a pipette was used to remove and transfer the supernatant (10 ml) into a 50-ml conical tube. Anti-*Cryptosporidium* magnetic beads (50 μl) were added to the 10-ml sample, and then the sample was rotated end-over-end for 60 min at room temperature.

A pan magnet containing a 100-ml petri dish was placed onto an orbital shaker, and the shaker was switched on. A 20-ml portion of 1× WDX reagent B was added to each 50-ml tube containing a sample, and the entire 30 ml was transferred into the petri dish and agitated for 2 min. This resulted in separation of the bead-oocyst complex from the remainder of the sample. The supernatant, which contained debris, was removed with a 10-ml pipette, and the beads were resuspended in 1 ml of 1× WDX reagent B and quantitatively transferred into an Eppendorf tube. The tube was placed in a slot that was provided on the pan magnet for the separation process, and the supernatant was removed and discarded. While the manufacturer recommended using 50 μl of 0.1 N HCl for the oocyst dissociation step, in our investigation 100 μl of 0.1 N HCl was used to dissociate the bead-oocyst complex. The neutralization procedure was performed on the microscope slide with 10 μl of 1 N NaOH. The beads were subjected to a second acid dissociation step, as described above, and the concentrate (100%) from each dissociation step was placed in an individual well of a three-well microscope slide, dried at 42°C, labeled with anti-*Cryptosporidium* FITC-mAb, and examined by fluorescence microscopy.

Effects of oocyst age on recovery by IMS. Predetermined volumes of oocysts that were different ages and contained approximately 850 oocysts were spiked in triplicate 10-ml portions of deionized water. The samples were subjected to IMS with kit DB by using the procedure described above, and oocyst recoveries were determined.

Epifluorescence microscopy. A Zeiss Axioskop fluorescence microscope equipped with a blue filter block (excitation wavelength, 490 nm; emission wavelength, 510 nm) was used to detect FITC-mAb-labeled oocysts at a magnification of ×200. The presence of oocysts was confirmed at a magnification of ×400 by using a UV filter block (excitation wavelength, 400 nm; emission wavelength, 420 nm) for visualization of DAPI, and the internal morphology of oocysts was determined by using Nomarski DIC microscopy.

RESULTS

Characteristics of the three water matrices investigated. Characteristics of the three water matrices that were 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 of each concentrate without flotation at a magnification of ×200 by bright-field microscopy. General observations of the nature of the inorganic constituents were recorded, and the observations for spores were limited to fungi and plants. The data are summarized in Table 1.

TABLE 1. Characteristics of the three raw water matrices evaluated, as determined by direct microscopic examination

Matrix	Origin	No. of particles/100 μl of sample concentrate						Comments
		Algae	Crustaceans (parts, eggs)	Diatoms	Insect parts	Rotifers (eggs)	Spores	
A	California river	3.2×10^5	2.8×10^2	ND[a]	20	ND	ND	Algae (primarily blue-green algae), little inorganic debris
B	Connecticut reservoir	2.4×10^4	ND	5.8×10^4	10	10	ND	Primarily algae, some inorganic debris
C	Nebraska river	4.6×10^4	ND	1.4×10^4	ND	ND	10	Large amounts of inorganic silt and clay

[a] ND, not detected.

Oocyst recoveries in deionized water and source waters of various turbidities. Three duplicate trials were conducted in deionized water in order to assess oocyst recoveries with three IMS procedures. Kit DB yielded oocyst recoveries ranging from 68 to 83%; however, the oocyst recoveries with kits IC1 and IC2 were more variable and ranged from 0.2 to 74.5% (Tables 2 through 4).

With kit DB, at turbidity levels up to 500 ntu, the oocyst recoveries in matrix A were similar to the recoveries in deionized water (Table 2). With kit IC1, increasing the turbidity to 50 ntu resulted in a noticeable reduction in oocyst recovery compared to the recoveries in deionized water (Table 2). In contrast, with kit IC2 the recoveries were less than 1% in deionized water, and increasing the turbidity to 50 ntu in matrix A resulted in marginal increases in the recoveries (3.2 to 8.3%). Increasing the turbidity to 500 ntu reduced the oocyst recoveries substantially with both kit IC1 and kit IC2.

In matrices B and C, while turbidity levels up to 500 ntu did not have a significant deleterious effect on oocyst recoveries with IMS kit DB, the recoveries with kit IC1 decreased substantially as the turbidity increased. With kit IC2, the oocyst recoveries in deionized water were between 53 and 58%, and, with the exception of an oocyst recovery of 0.6% at a turbidity level of 50 ntu, no oocysts were recovered from spiked matrix B source water at different turbidities (Table 3).

At a turbidity of 5,000 ntu, oocyst recoveries greater than 35% were obtained with kit DB, whereas kits IC1 and IC2 recovered less than 6% of the spike dose of oocysts in all three matrices (Tables 2 through 4).

Effects of oocyst age on recovery by IMS. Oocysts of a single isolate of *C. parvum* were used to evaluate the recoveries of oocysts of various ages (Table 5). In the first trial, in which fresh oocysts (age, 16 days) and aged oocysts (age, 6 weeks) were used, the mean recoveries were 104.1 and 81.4%, respectively. Analysis of these data with Student's *t* test indicated that the recoveries of fresh oocysts were significantly higher than the recoveries of aged oocysts ($P = 0.01$). Trial 2 revealed that the recoveries of aged oocysts were marginally higher (Table 5) than those of fresh oocysts, but no significant differences were detectable ($P = 0.13$). In trial 3 we utilized recently voided oocysts (age, 10 days) and 12-week-old oocysts, and again, the recoveries of the fresh oocysts were more than 100%, whereas the mean recovery of the aged (12-week-old) oocysts was significantly lower ($P = 0.003$), 94.6% (Table 5). In trial 4, fresh oocysts (age, 35 days) were compared to aged oocysts (age, 16 weeks), and higher recoveries of the aged oocysts were obtained (Table 5); however, the oocyst recoveries were not significantly different ($P = 0.36$). The recoveries of the fresh oocysts ($n = 12$) were pooled, and the recoveries of the aged oocysts were pooled, and then the data were analyzed by using a two-sample *t* test. Based on the assumption that the data for both populations were normally distributed and the assumption that the population standard deviations for fresh and aged oocysts were equal, no significant differences were detected in the oocyst recoveries for these two populations ($P = 0.45$).

DISCUSSION

Over the last decade, waterborne transmission of *Cryptosporidium* spp. has become a significant concern primarily because of the robust nature of the organisms, their ability to withstand normal water disinfection processes, their low infectious doses, and the absence of chemotherapeutic drugs for treatment of infected individuals. Increasing numbers of immunocompromised individuals have increased the need for accurate detection of *C. parvum* oocysts in various water types in order to determine the occurrence of the organisms and to obtain information on the risk of waterborne transmission of infection. The methods currently utilized for isolation, concentration, and detection of oocysts are time-consuming, tedious, and inefficient and result in underestimates of the occurrence of *Cryptosporidium* oocysts (14). For example, clarification of small numbers of oocysts from contaminating debris has conventionally been achieved by flotation procedures. These procedures yield highly variable recoveries that depend on the original numbers of oocysts in a sample, as well as oocyst viability, sample turbidity, and the length of time that oocysts are in contact with particulates in the water sample (4, 9). In

TABLE 2. Comparison of three IMS kits when matrix A source water was used

Prepn	Turbidity (ntu)	Packed pellet vol from 10-ml sample (ml)	% Oocyst recovery with kit DB		% Oocyst recovery with kit IC1		% Oocyst recovery with kit IC2	
			Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Matrix A water	5,000	2.5	43.2	58.3	0	0.13	ND[a]	ND
	500	0.25	75	78.7	1.5	1.7	0.25	0.25
	50	0.025	62.2	80.1	3.2	7.0	8.3	3.2
Deionized water		NA[b]	76	83.2	66.5	74.5	0.25	0.74
Unspiked control	5,000	2.5	0	0	0	0		
	50	0.025	0	0	0	0	0	0

[a] ND, not done.
[b] NA, not applicable.

TABLE 3. Comparison of three IMS kits when matrix B source water was used

Prepn	Turbidity (ntu)	Packed pellet vol from 10-ml sample (ml)	% Oocyst recovery with kit DB		% Oocyst recovery with kit IC1		% Oocyst recovery with kit IC2	
			Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Matrix B water	5,000	0.8	68	69	0	0	0	0
	500	0.1	74	85	0	0.23	0.12	0.12
	50	Tr[b]	70	81	0.12	4.3	0.6	0
Deionized water		NA[a]	75	81	13	52	58	53.1
Unspiked control	5,000	0.8	0.1	0.2	0	0	0	0
	50	Tr	0	0	0	0.23	0	0

[a] NA, not applicable.
[b] Tr, trace.

order to address the limitations of existing methods, we investigated the use of IMS procedures to isolate oocysts that were suspended in three water matrices at three different turbidity levels. Recovery trials performed in deionized water indicated that on three separate occasions, kit DB yielded consistent recoveries (range, 68 to 83%), whereas greater variabilities were observed with kit IC1 (range, 13 to 75%) and kit IC2 (range, 0.25 to 58%). The great variations observed with kits IC1 and IC2 in deionized water were probably due to a combination of several factors, including a poor separation step. Kit IC1 did not require a dissociation step, which meant that oocyst-bead conjugates were placed onto glass slides and subjected to a microscopic analysis. This probably resulted in increased oocyst loss during the sample-staining steps, as well as occlusion of recovered organisms during microscopy.

The three water matrices selected for this study had either high algal contents or low algal contents or contained large amounts of inorganic debris (Table 1). Despite the differences in the physical characteristics of the three water matrices, little variability in oocyst recoveries was observed with kit DB at turbidity levels up to 500 ntu. In contrast, with kit IC1 there were substantial reductions in oocyst recoveries when the sample turbidity was increased to 50 ntu (recovery range, 0 to 7%). Increasing the turbidity to 500 ntu reduced the oocyst recoveries further (recovery range, 0 to 1.7%). The manufacturers of these IMS kits advocate using packed pellet volumes of less than 0.5 ml; however, in our experiments, 5,000-ntu samples from matrices A, B, and C yielded packed pellet volumes of 2.5, 0.8, and 0.2 ml, respectively (Tables 2 through 4). Although packed pellet volumes of 2.5 ml reduced oocyst recoveries to less than 60%, good oocyst separation and high recoveries were obtained with kit DB. In the second matrix, in which the packed pellet volume was 0.8 ml, the oocyst recoveries increased marginally (Table 3); however, when the packed pellet volume was 0.2 ml, there was no significant increase in recoveries (Table 4). These data indicate that kit DB may yield acceptable oocyst recoveries from samples with packed pellet volumes greater than 0.5 ml.

While kit DB utilized approximately 5-μm-diameter beads that were conjugated to an immunoglobulin M (IgM) anti-*Cryptosporidium* monoclonal antibody, kits IC1 and IC2 utilized approximately 0.8-μm-diameter beads that were conjugated to IgG3 and IgG anti-*Cryptosporidium* monoclonal antibodies, respectively. The beads were mixed with the samples, and then the oocyst separation procedure was performed as recommended by the manufacturer. We anticipated that kit IC1, which utilized the IgG3 isotype, would improve both the specificity and the sensitivity for oocysts and in turn would improve oocyst recoveries during the IMS process. Despite this expectation, higher recoveries were obtained with kit DB than with kit IC1, and this was probably a reflection of differences in the separation process. With kit DB, the capture and separation procedure was performed in a Leighton tube, and the bead-oocyst complexes were magnetically captured approximately one-third of the distance from the bottom of the tube. This resulted in magnetic attachment of the bead-oocyst complexes to the side of the tube, whereas the contaminating debris remained either in suspension or settled in the bottom of the tube. In contrast, kits IC1 and IC2 utilized a pan magnet containing a petri dish that was placed horizontally on an orbital shaker. Theoretically, the bead-oocyst complexes should have collected in the center of the petri dish, whereas the debris should have remained in suspension; however, during our experiments we found that large-particulate matter was usually trapped in the collection area for the bead-oocyst complexes. Consequently, the use of kits IC1 and IC2 did not result in good separation of oocysts from the debris. The final concentrates obtained with kit DB were highly purified suspensions of oocysts which stained brightly with FITC-mAb, allowing easy detection of oocysts at a magnification of ×200 and

TABLE 4. Comparison of three IMS kits when matrix C source water was used

Prepn	Turbidity (ntu)	Packed pellet vol from 10-ml sample (ml)	% Oocyst recovery with kit DB		% Oocyst recovery with kit IC1		% Oocyst recovery with kit IC2	
			Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Matrix C water	5,000	0.2	35	70	0	0	2.1	5.3
	500	0.02	77	99	0	0	0	0.4
	50	Tr[b]	76	79	0	0	1.0	6.8
Deionized water		NA[a]	68	81	24	50	0.6	0.2
Unspiked control	5,000	0.2	0	0	0	0		
	50	Tr	0	0	0	0	0	0

[a] NA, not applicable.
[b] Tr, trace.

TABLE 5. Effects of *C. parvum* oocyst age on recovery by IMS

Trial	Oocyst category (age)	Spike dose[a] (n = 10)	% Recovery[a] (n = 3)
1	Fresh (16 days)	885.3 ± 111.1	104.1 ± 7.6
	Aged (6 weeks)	886.7 ± 128.5	81.4 ± 3.4
2	Fresh (28 days)	843.6 ± 142.1	66.4 ± 2.3
	Aged (11 weeks)	898.8 ± 46.0	71.2 ± 3.8
3	Fresh (10 days)	858 ± 196.1	115.5 ± 4.1
	Aged (12 weeks)	861.2 ± 117.0	94.6 ± 3.8
4	Fresh (35 days)	876.6 ± 160.5	83.8 ± 24.7
	Aged (16 weeks)	877 ± 122.7	99.6 ± 10.1

[a] Mean ± standard deviation.

confirmation by Nomarski DIC optics. The final concentrates obtained with kits IC1 and IC2 contained oocyst-bead complexes or oocysts suspended along with different levels of contaminating debris and algal cells. The amount of contaminating debris and algal cells increased with increasing sample turbidity, and at a turbidity level of 5,000 ntu, the packed pellet volumes of samples concentrated by IMS kits IC1 and IC2 exceeded 0.5 ml on occasion. In such samples, FITC-mAb-stained oocysts displayed poor or patchy surface fluorescence, and in order to reduce the likelihood of stained oocysts not being detected, this necessitated microscopic examination at a magnification of ×400. When oocysts were detected by FITC-mAb, confirmation by Nomarski DIC optics was difficult due to the presence of occluding immunomagnetic beads and/or debris. Although the manufacturer of kits IC1 and IC2 recommended using preclearing immunomagnetic beads to extract magnetizable material and nonspecifically binding debris from sample concentrates before the anti-*Cryptosporidium* monoclonal antibody-conjugated beads are used to specifically capture oocysts, the preclearing step was omitted from our investigations with kit IC1. This decision was based on the results of preliminary spiking studies (data not shown), which indicated that the preclearing beads could nonspecifically capture up to 50% of the spike dose. With kit IC2, the oocyst recoveries were assessed by following the manufacturer's instructions, which included using preclearing beads for samples that had turbidity levels of 500 ntu or more. As the oocyst recoveries were lower with kit IC2 (which used releasable beads) than with kit IC1 (which used nonreleasable beads), the data supported our original finding that preclearing beads were responsible for nonspecifically extracting oocysts from spiked samples.

Using oocysts of various ages to evaluate recoveries revealed that age did not appear to have a noticeable impact on the recoveries obtained with kit DB. On several occasions, the oocyst recoveries exceeded 100%; however, this was not regarded as an aberration, especially when the inherent variability in enumerating oocyst spike doses was taken into consideration. Furthermore, recoveries greater than 100% have been obtained previously with the Dynal IMS procedure (5). At this time there is not sufficient information concerning the biology of *Cryptosporidium* spp. to identify factors that promote uneven oocyst distribution. Some investigators have proposed that dead oocysts may be more likely than live oocysts to adhere to each other and to debris (4); however, in this study, recoveries greater than 100% were obtained with fresh oocyst populations with high viabilities. This suggests that isolation of oocysts from feces of donor calves by using cesium chloride (highly alkaline) purification may have influenced the surface chemistry and/or surface charges on oocysts and may have affected their distribution in suspension. There is evidence which indicates that hydrophobicity and zeta potential for *C. parvum* change with increasing pH and/or ionic strength of the suspending medium (8).

In conclusion, our investigations showed that IMS appears to be a promising alternative to flotation procedures for recovering oocysts from turbid water matrices but that not all IMS procedures yield the same results. For example, at a turbidity of 5,000 ntu, IMS kit DB yielded recoveries ranging from 35 to 70%, whereas at a similar turbidity, kits IC1 and IC2 failed to recover more than 6% of the spike dose in all tests. Thus, all IMS kits do not perform equally well, and caution must be exercised when a commercially available IMS kit is selected to replace flotation procedures for recovery of *Cryptosporidium* oocysts. Furthermore, our data indicated that it may be possible to use IMS with samples having packed pellet volumes greater than 0.5 ml. Further work performed with a single raw water matrix and different packed pellet volumes should help identify packed pellet volumes beyond which oocyst recoveries decline significantly with kit DB.

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REFERENCES

1. **Anonymous.** 1990. Methods for the examination of waters and associated materials. Isolation and identification of *Giardia* cysts, *Cryptosporidium* oocysts and free living pathogenic amoebae in water etc., 1989. Department of Environment, Standing Committee of Analysts, Her Majesty's Stationery Office, London, United Kingdom.
2. **Arrowood, M. J., and K. Donaldson.** 1996. Improved purification methods for calf derived *Cryptosporidium parvum* oocysts using discontinuous sucrose and cesium chloride gradients. J. Eukaryot. Microbiol. **43:**S89.
3. **Bifulco, J. M., and F. W. Schaefer.** 1993. Antibody-magnetite method for selective concentration of *Giardia lamblia* cysts from water samples. Appl. Environ. Microbiol. **59:**772–776.
4. **Bukhari, Z., and H. V. Smith.** 1995. Effect of three concentration techniques on viability of *Cryptosporidium parvum* oocysts recovered from bovine feces. J. Clin. Microbiol. **33:**2592–2595.
5. **Campbell, A. T., B. Grøn, and S. E. Johnsen.** 1997. Immunomagnetic separation of *Cryptosporidium* oocysts from high turbidity water sample concentrates, p. 91–96. *In* C. R. Fricker, J. L. Clancy, and P. A. Rochelle (ed.), Proceedings of the 1997 AWWA International Symposium on Waterborne *Cryptosporidium*, p. 91–96.
6. **Deng, M. Q., D. O. Cliver, and T. W. Mariam.** 1997. Immunomagnetic capture PCR to detect viable *Cryptosporidium parvum* oocysts from environmental samples. Appl. Environ. Microbiol. **63:**3134–3138.
7. **Di Giovanni, G. D., M. LeChevallier, D. Battigelli, A. Campbell, and M. Abbaszadegan.** 1997. Detection of *Cryptosporidium parvum* by enzyme immunoassay and the polymerase chain reaction. *In* Proceedings of the AWWA Water Quality Technology Conference (on CD-ROM).
8. **Drozd, C., and J. Schwartzbrod.** 1996. Hydrophobic and electrostatic cell surface properties of *Cryptosporidium parvum*. Appl. Environ. Microbiol. **62:** 1227–1232.
9. **Fricker, C. R.** 1995. Detection of *Cryptosporidium* and *Giardia* in water, p. 91–96. *In* W. B. Betts, D. P. Casemore, C. R. Fricker, H. V. Smith, and J. Watkins (ed.), Protozoan parasites and water. The Royal Society of Chemistry, Cambridge, England.
10. **Johnson, D. W., N. J. Pieniazek, D. W. Griffin, L. Misener, and J. B. Rose.** 1995. Development of a PCR protocol for sensitive detection of *Cryptosporidium* oocysts in water samples. Appl. Environ. Microbiol. **61:**3849–3855.
11. **Kronick, P., and R. W. Gilpin.** 1986. Use of superparamagnetic particles for isolation of cells. J. Biochem. Biophys. Methods **12:**73–80.
12. **Owens, C. S., and N. L. Sykes.** 1984. Magnetic labelling and cell sorting. J. Immunol. Methods **73:**41–48.
13. **Parker, J. F. W.** 1993. *Cryptosporidium* sp. oocysts in the aquatic environment: occurrence, removal and destruction. Ph.D. thesis. University of Glasgow, Glasgow, Scotland.
14. **Smith, H. V., and J. B. Rose.** 1990. Waterborne cryptosporidiosis. Parasitol. Today **6:**8–12.
15. **U.S. Environmental Protection Agency.** 1996. ICR microbial laboratory manual. Publication EPA/600/R-95/178. Office of Research and Development, Government Printing Office, Washington, D.C.