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ORIGINAL ARTICLE

Portable continuous flow centrifugation and method 1623 for monitoring of waterborne protozoa from large volumes of various water matrices

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Abstract

Aims: The aims of this study were to validate a portable continuous flow centrifuge (PCFC) as an alternative concentration step of US-EPA Method 1623 and to demonstrate it's efficacy for recovery of low numbers of protozoa from large volumes of various water matrices.
Methods and Results: Recoveries of *Cryptosporidium parvum* oocysts, *Giardia intestinalis* cysts and *Encephalitozoon intestinalis* spores spiked into 10–1000 l volumes of various water matrices were evaluated during in-house and collaborative trials. Spiked protozoa were either approved standards or diluted stock samples enumerated according to USEPA Method 1623. *Cryptosporidium* recoveries exceeded method 1623 criteria and substantially high recoveries were observed for *Giardia* and *E. intestinalis*.
Conclusions: Portable continuous flow centrifuge methodology exceeded method 1623 acceptance criteria for *Cryptosporidium* and could be easily adopted for other protozoa.
Significance and Impact of the Study: The PCFC could be adopted as an alternative user-friendly concentration method for *Cryptosporidium* and for monitoring of large volumes of source and tap water for accidental or deliberate contamination with protozoa and potentially with other enteric pathogens. It is anticipated that PCFC would also be equal or superior to filtration for protozoa monitoring in wastewater and effluents.

Introduction

Cryptosporidium parvum was responsible for the largest recorded waterborne outbreak of diarrheal illness in the US (MacKenzie *et al.* 1994). The median infective dose in healthy volunteers was reported to be as low as 132 (DuPont *et al.* 1995), and despite considerable effort, currently there is no effective treatment. The oocysts are also highly resistant to disinfectants and other methods of water treatment (Dickinson and Renner 1999), all of which makes this pathogen a potential candidate for deliberate contamination of water supplies as a means of bioterrorism (Rose 2002).

Two additional groups of enteric protozoa have been linked to waterborne diarrheal illness in humans, and monitoring for their presence in source water is highly desirable. They include *Giardia intestinalis* and several Microsporidian parasites (Dowd *et al.* 1998; Cotte *et al.* 1999). The concentration of intestinal Microsporidia spp. has been problematic due to the small size of the spore (<2 μm in diameter). Currently approved protozoa filtration components were evaluated for recovering intestinal microsporidian spores from water and it was reported that continuous flow centrifugation is by far more efficient (Battigelli *et al.* 2000).

Cryptosporidium monitoring in treatment works with significant risk of having oocysts in drinking water is mandatory in the UK, while in the US source water monitoring will become mandatory in 2006 for up to 6 years, under EPA long term two Enhanced Surface Water Treatment

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Rule (LT2ESWTR). The EPA proposes to rely on methods 1622/1623 (USEPA 2001), for the concentration and detection of *Cryptosporidium* and *Giardia*. These methods are based on performance based measurement system (PBMS), and allow modifications, provided that quality control acceptance criteria are met (Clancy *et al.* 2003).

Continuous flow centrifugation has been previously used for concentrating coccidial cysts/oocysts from stools (Vetterling 1969). The same principle was used to concentrate parasites and bacteria from water, with reported recoveries of 4–100% (Whitmore and Carrington 1993; Swales and Wright 2000; Borchardt and Spencer 2002). However, these reports described large stationary centrifuges, operating at low flow rates with a cumbersome elution process and requiring reusable and expensive elements. These devices are impractical for field use, for the concentration of large numbers of samples, and for continuous monitoring.

We have previously shown the applicability of an earlier prototype of a Haemonetics modified blood separation apparatus to efficiently concentrate *Cryptosporidium* oocysts and *Giardia* cysts from tap and source water samples (Zuckerman *et al.* 1999). The present study describes the in-house evaluation of a portable continuous flow centrifuge (PCFC) as an alternative concentration methodology to filtration of *Cryptosporidium* oocysts, *Giardia* cysts and Microsporidian spores (*Encephalitozoon intestinalis*) from spiked 50–1000 l of tap water, in conjunction with method 1623. This method allow laboratories to demonstrate improved performance, increased laboratory efficiency or reduced costs through a standardized, quantitative process – Tier 1 for a single laboratory and Tier 2 which requires minimum of three laboratories. Tier 2 validation studies require in addition both USEPA involvements throughout the process and approval before the modification become part of the standard method.

Accordingly, the PCFC was further evaluated during collaborative trials for the recovery of low densities of *Cryptosporidium* oocysts and *Giardia* cysts from spiked 10–50 l of reagent and source water and compared with an approved filtration device.

Materials and methods

Source and preparation of protozoa

For the experiments conducted at Tufts University, stock samples of 10^6 ml^{-1} organisms were purchased from Waterborne, Inc. (New Orleans, LA, USA). *Cryptosporidium parvum* oocysts (Iowa), *G. intestinalis* cysts (human isolate H-3) and *E. intestinalis* (harvested from RK–13 cultured cells) were stored in a solution containing PBS with penicillin, streptomycin, and gentamicin, at 4°C. For the experiments conducted at clancy environmental consultants (CEC), stock sample of *C. parvum* (Iowa isolate) was obtained from the Sterling Parasitology Laboratory, Tucson, AZ, USA.

For each stock of (oo)cysts and spores, the spike dose was initially enumerated with a hemocytometer and then diluted accordingly to yield approx. 100 organisms per 100 μl inoculum. Each diluted working suspension was enumerated by using USEPA method 1623, utilizing counts of ten replicate wells by immunofluorescence microscopy.

For all other experiments, flow cytometry sorted standards of 150 ± 1·5 (oo)cysts were purchased from Wisconsin State Laboratory Hygiene, WI, USA. These method 1623-approved standards were prepared using *C. parvum* oocysts (Sterling Parasitology, Harley Moon <3-month old) and *G. intestinalis* cysts (waterborne, H3, <2-week old).

Antibodies

Oocysts and cysts were reacted with AquaGlo G/C (fluorescein Isothiocyanate-conjugated anti-*Giardia* and anti-*Cryptosporidium* monoclonal antibodies; Waterborne Inc.). *Encephalitozoon intestinalis* spores were stained with a Fluorescein Isothiocyanate-conjugated polyclonal antibody (A 700 FL, Waterborne Inc.). Slides were incubated at 37°C for 30 min.

Immunomagnetic separation

The Dynal IMS (Dynabeads G/C combo of anti–*Giardia* and anti *Cryptosporidiu*m, Dynal A.S., Oslo, Norway) procedure was employed for all experiments (excluding the simultaneous spiking experiments) followed according to the manufacturer's instructions.

Sample matrices

Tufts tap water (North-Grafton ground water supplied to Tufts University) was used during the in-house trials. For all other trials and validations, each participating laboratory provided its own reagent water and raw surface water. Raw surface water samples were collected as grab samples from a local surface water body serving as a source for drinking water. Secondary effluent was obtained from a local sewage treatment plant.

Spiking procedures

For 1000 l tap water samples, approx. 100 oocysts were spiked into a beaker, which contained 1 l of distilled

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water and the suspension was continuously stirred. The oocyst suspension was then injected continuously over the sampling period into a 50 l carboy, positioned on a magnetic stirring plate, by using a peristaltic pump at a flow rate of 0·6 ml min^{-1}. Simultaneously, tap water was continuously added into the carboy at a flow rate of 0·75 l min^{-1}, while continuously mixing with a magnetic stir bar. The tap water background was found negative for the presence of *Cryptosporidium* oocysts, *Giardia* cysts and *E. intestinalis* spores. For all other volumes, (oo)cysts and spores were seeded directly into the carboy. Oocyst densities ranged between 0·1 and 30 l^{-1}, cyst densities ranged between 3 and 50 l^{-1} and spore density was 10 l^{-1}.

Portable continuous flow centrifugation

The dimensions of the PCFC are 18″ × 14″ × 20″ and its weight is 22 lb (Fig. 1). It consists of three components: a three phases, single direction, variable speed, brushless DC motor, a speed controller which enables maximum centrifugal force of 4000 ***g***, and an integrated peristaltic pump which enables sample feeding at a graded flow up to 3 l min^{-1}; a second integrated pump designated for seeding studies enables flow rates of up to 100 ml min^{-1} (Easy Load, Cole Parmer, IL, USA). The PCFC operates at 110 V AC and may be adjusted to 12 V DC. The centrifuge head accommodates the concentration component – a disposable Latham bowl [(Fig. 2), volume 275 ml, 6″ height and 3″ diameter, Haemonetics, MA, USA]. The basic principle underlying the capture of organisms by this methodology has been described previously (Zuckerman *et al.* 1999). Briefly, in all trials the PCFC power source was set to 110 V AC. Operation for the 1000 l samples was set to a centrifugal force of 3000 ***g*** and a flow rate of 0·75 l min^{-1}.

Due to the fact that blood separation devices were originally developed to operate for restricted length of time, it contradicted our application to continuously concentrate *C. parvum* oocysts from large volumes. This procedure involved continuous operation of the PCFC for 2 months except for short pauses each 24 h for replacing the bowl. Thus, no overheating of the motor was experienced.

For all other spiking runs the flow rate through the PCFC remained 0·7 l min^{-1} and the centrifugal force varied between 3000 and 4000 ***g***. The desired flow rate and force were preset using the designated controllers. The inlet tubing (20″ long 3/8″ Tygon) was mounted into the peristaltic pump's head and connected to the bowl's inlet port (Fig. 2) and the water source – the outlet valve of a 50 l carboy. A second piece of tubing was connected to the bowl's outlet port and to the designated drain. Pumping started after the centrifuge attained the desired centrifugal force. After the desired sample volume had passed through the PCFC, flow from the inlet port was terminated; The PCFC was allowed to operate for a short additional period to ensure that the residual sample volume within the bowl had been reduced to below 250 ml. Once the centrifuge stopped, the bowl was removed from the centrifuge head, the tubing was disconnected and both ports were capped. For each experiment, new set of tub-

Figure 1 The portable continuous flow centrifuge.

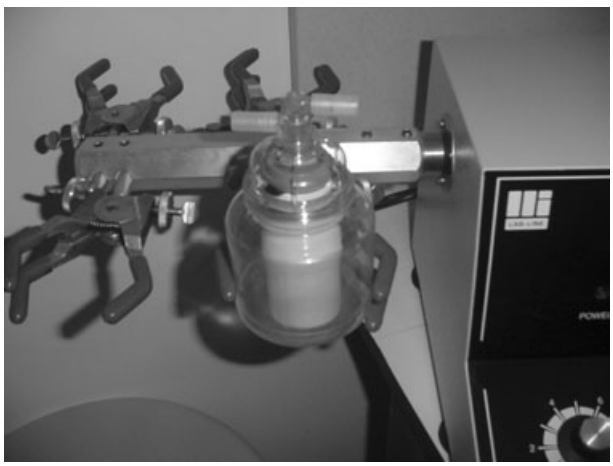

Figure 2 The disposable bowl.

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ing was used and the carboy was disinfected and cleaned to prevent carryover contamination.

Elution and second concentration/detection of (oo)cysts

A volume of 1 l of an elution buffer [1% sodium dodecyl sulphate, 0·01% Tween-80 and 0·001% antifoam A] was prepared and 5 ml from this stock was injected after each run via the inlet port of the bowl. The bowl was clamped in an upright position in a wrist shaker (Lab-line, Multi wrist shaker, Model 3589) and shaken vigorously (600 rev m^{-1}) for 10 min. The bowl orientation was changed to 90° and shaking was continued for additional 5 min. Finally, the bowl orientation was changed to 270° and shaken for 5 min more. The bowl was inverted and the eluate was decanted into a 250 ml conical centrifuge tube. The following steps were conducted according to EPA method 1623.

Second concentration and detection for simultaneous recovery of (oo)cysts and spores

Following the elution process, the 250 ml conical centrifuge tube was centrifuged at 1050 ***g*** for 15 min, and the supernatant was reduced to 2·8 ml. The tube was vortexed and the entire concentrate containing the pellet was transferred to two 1·5 ml Eppendorf tubes. The tubes were spun at 12 000 ***g*** for 12 min, the supernatant was reduced to 100 μl which was vortexed and split into two aliquots of 50 μl. Each aliquot was placed on an individual well slide and dried at 42°C. One well was labelled with an anti–G/C FITC mAb, the second with an anti–*E. intestinalis* FITC polyclonal antibody, and slides were examined by employing epifluorescence microscopy.

Evaluation of portable continuous flow centrifuge recoveries compared to filtration

Following the initial training one of the participant laboratories conducted an evaluation of the PCFC recoveries compared to standard filtration. The whole procedure was conducted in accordance with method 1623. Volumes of 10–50 l source water samples of maximum turbidity of two NTU were spiked and recoveries were assessed employing PCFC methodology and Envirochek HV capsule (Pall Gelman Sciences Inc., Montreal, Cananda Ann Arbor, Mich.). The total of 6 tests per each method was performed, PCFC was operated at a centrifugal force of 4000 ***g*** and a flow rate of 0·7 l min^{-1} and elution was performed as previously mentioned following method 1623. Filtration was conducted at a flow rate of 2 l min^{-1} and following method 1623.

Results

Since continuous flow centrifugation enables the concentration of a wide range of particles, we optimized the assay to enable the simultaneous recovery of *C. parvum*, *G. intestinalis* and *E. intestinalis*.

Simultaneous recovery of (oo) cysts and spores from 50 l tap water samples

Cryptosporidium parvum, *G. intestinalis* and *E. intestinalis* were recovered from 50 l tap water samples at a flow rate of 0·75 l min^{-1} and 3000 ***g***. For the *C. parvum* oocysts, recovery ranged between 37·4 and 84% with a trend for higher recovery with higher oocysts densities, averaging 66·85%. For *G. intestinalis* cysts, recoveries ranged between 10·6 and 82·6%, averaging 47·25%. For the *E. intestinalis* spores recoveries ranged from 54–82·4%, averaging 71·6% mean recovery of cysts was the lowest, while recoveries of oocysts and spores was similar (Table 1).

Recoveries of *Cryptosporidium* from 1000 l tap water samples

Low numbers of oocysts (90 ± 57·5) were spiked and recovered from 1000 l of tap water (turbidity = 0·5 NTU), at a centrifugation force of 3000 ***g*** and a flow rate of 0·75 l min^{-1}.

For the 40 independent runs that were conducted, recoveries ranged from 11 to 72% with a mean of 35%. Recovery efficiencies for the independent runs did not show any correlation with the spike dose (Table 2).

Recoveries of *Cryptosporidium* from source water samples

During an earlier evaluation of the PCFC at CEC, data for the recoveries of *C. parvum* from 10 l source water samples

Table 1 Portable continuous flow centrifugation* simultaneous recoveries of *Cryptosporidium parvum* oocysts *Giardia intestinalis cysts*, and *Encephalitozoon intestinalis* spores from 50 l tap water† samples

Oocysts spiked	Recovery (%)	Cysts spiked	Recovery (%)	Spores spiked	Recovery (%)
1500 ± 180	75	2500 ± 210	38	500 ± 93	7·4
1500 ± 180	71	2500 ± 210	83	500 ± 93	82·4
500 ± 75	84	2500 ± 210	58	500 ± 93	54
500 ± 75	37	2500 ± 210	11	500 ± 93	N.A.
67 ± 18		47 ± 26		72 ± 13	

*PCFC was operated at centrifugal force of 3000 ***g*** and a flow rate of 0·75 l min^{-1}.
†Turbidity = 0·5 NTU.

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Table 2 Portable continuous flow centrifugation* recoveries for *Cryptosporidium* from1000 l of tap water† samples

Number of independent tests (*n*)	Oocysts spiked/test	Recovery (%)
2	24 ± 15	54 ± 35
1	26	15·4
8	44 ± 28	65 ± 41·5
2	58 ± 37	19 ± 12
1	60	20
8	66 ± 42	72 ± 47
11	80 ± 51	63·5 ± 41
2	120 ± 77	41·25 ± 27
2	129 ± 82	14 ± 9
2	190 ± 121	14·5 ± 9
1	194	11·3
	Mean ± SD = 90 ± 57·5	Mean ± SD = 35·4 ± 23

*PCFC was operated at centrifugal force of 3000 ***g*** and a flow rate of 0·75 l min^{-1}.
†Turbidity = 0·5 NTU.

were very encouraging (data not shown). Since little success has been achieved at this laboratory when using the conventional filtration methods to recover *C. parvum* from wastewater matrices, it was suggested to challenge the PCFC with a similar matrix. *Cryptosporidium parvum* oocysts (250) were spiked into 10 l of secondary effluent samples and recovered at a flow rate of 0·75 l min^{-1} and 3000 ***g***. Recovery rates ranged from 40·4 to 66·8%, with a mean recovery of 57·9% for this matrix. For the last four runs the spiking method was slightly modified to include a 1 l rinse of the carboy with PBS/Tween–20, which further increased oocyst recovery (Table 3).

Initial recoveries of *Cryptosporidium* and *Giardia* at the collaborating laboratories

Flow cytometry calibrated spike standards of 150 (oo)cysts were recovered from 10 l of reagent and source water samples. Operating at a flow rate of 0·7 l min^{-1} and 4000 ***g***, all recoveries passed method 1623 criteria (Table 4). *Cryptosporidium parvum* oocysts recoveries from reagent water ranged from 19 to 65% with a mean of 47%. For source water samples, recoveries ranged from 39 to 58% with a mean of 49%. *Giardia intestinalis* cysts recoveries from reagent water samples ranged between 33 and 76% with a mean of 51%. Source water recoveries ranged between 69 and 72% with a mean of 71%. For both organisms, greater recoveries were therefore achieved using source water samples.

Table 3 Portable continuous flow centrifugation* recoveries for *Cryptosporidium*† from 10 of secondary effluent

Turbidity (NTU)	Spiking procedure	Recovery (%)	Mean ± SD
4·6	Without rinse	47	58 ± 10
2·8	Without rinse	68·0	
2·8	Without rinse	40·4	
4·6	Rinse; PBS/T20	60·4	
4·6	Rinse; PBS/T20	67	
2·8	Rinse; PBS/T20	64·0	
2·8	Rinse; PBS/T20	59·2	

*PCFC was operated at centrifugal force of 3000 ***g*** and a flow rate of 0·75 l min^{-1}.
†Spikedose of 250 oocysts.

Table 4 Portable continuous flow centrifugation* recoveries for *Cryptosporidium* and *Giardia*† from 10 l source water‡ samples during the initial on-site training of the collaborative laboratories

Matrix/organism	Lab 1 Recovery (%)	Lab 2 Recovery (%)	Lab 3 Recovery (%)	Mean (%) Recovery (%)
Reagent water				
Cryptosporidium	65	57	19	47 ± 20
Giardia	76	43	33	51 ± 18·4
Source water				
Cryptosporidium	58	51	39	49 ± 8
Giardia	72	72	69	71 ± 1·4

*PCFC operated at a centrifugal force of 4000 ***g*** and a flow rate of 0·7 l min^{-1}.
†Spike dose of flow cytometry sorted standards of 150 ± 1·5 (oo)cysts.
‡Turbidity range was 0·5–2 NTU.

Evaluation of portable continuous flow centrifuge recoveries compared to standard approved filtration component

Recoveries for the two methods exceeded method 1623 criteria for both *Cryptosporidium* and *Giardia*, however those of PCFC were superior. Mean percent recoveries of *Cryptosporidium* and *Giardia* employing PCFC were 60·1 and 66·8, respectively. Mean percent recoveries of *Cryptosporidium* and *Giardia* employing capsule filtration were 18·2 and 42·7, respectively (Table 5).

Summary of portable continuous flow centrifuge recoveries during the validation of 50 l source water samples

Portable continuous flow centrifuge recoveries exceeded method 1623 criteria. For spiked reagent water *Cryptosporidium* and *Giardia* mean percent recoveries were 43·3 and 48·98, respectively. For spiked source water samples *Cryptosporidium* and *Giardia* mean percent recoveries were 48·33 and 40·37, respectively (Table 6).

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Table 5 Summary of Portable continuous flow centrifuge* recoveries compared to filtration† for spiked‡ source water samples§

	Cryptosporidium		*Giardia*	
Method	Mean (%)	RSD	Mean (%)	RSD
Filtration	18·2	40·2	43	37
PCFC	60·1	27·5	67	22

*Centrifugation at 4000 ***g*** and a flow rate of 0·75 l min^{-1}.
†Filtration employing Envirochek HV capsule at a flow rate of 2 l min^{-1}.
‡Spike dose of flow cytometry sorted standards of 150 ± 1·5 o(o)cysts.
§Data was calculated for six samples (per each method) of 10 and 50 l, turbidity range was 0·5–2 NTU.
PCFC, portable continuous flow centrifuge.

Discussion

The objectives of this study were to assess and validate the performance of a PCFC to recover waterborne protozoa of a broad size spectrum (1–12 ±m) from large volumes of various water matrices.

Several filters for concentration of protozoa from water have been developed and used worldwide (Wholsen *et al.* 2004), while currently just the Envirochek capsule and the Filta-Max depth filter are approved by the USEPA and the UK Drinking Water Inspectorate (DWI). These filters comprise of nominal 1 ±m pores and thus are inefficient in retaining particles of a size range <2 ±m. In addition, they are costly, involve cumbersome elution processes and readily clog during filtration of large volumes of turbid matrix.

In contrast, the PCFC protocol is rapid, user-friendly, and it is likely to be less expensive. A key advantage of the PCFC is the reduced effect of suspended particles, which are common in surface water, in contrast to a clogging and recovery reduction effect encountered with currently used filtration components (DiGiorgio *et al.* 2002; Feng *et al.* 2003).

We believe the PCFC method offers several advantages. They include the capability to concentrate simultaneously a large spectrum of microorganisms, including those within the size range of of bacteria (1 μm), demonstrated by our preliminary data of substantially high recovery (>70%) of low numbers of *E. intestinalis* spores from tap water samples, an efficiency which has not been demonstrated with the currently approved filters. Other studies reported the efficiency of filtration for recovery of microsporidia spores from water; Dowd *et al.* (1998) employed 1 μm cartridge filtration but did not quantify the precise recovery efficiency. Additional study evaluated filtration and continuous flow centrifugation of microsporidia from water and concluded that continuous flow centrifugation is the most efficient methodology for this purpose (Battigelli *et al.* 2000, Proceedings of the Water Quality Technology Conference, American Water Works Association, Salt Lake City, Utah).

The PCFC recoveries for *Cryptosporidium* and *Giardia* presented here are either equal or higher than those obtained by filtration for all water matrices. Portable continuous flow centrifuge recoveries for both protozoa in various water matrices were consistent and ranged from 35 to 67% for oocysts and 40 to 71% for cysts. Except for the 1000 l results, all standard deviation of PCFC recoveries for both protozoa in all matrices met method 1623 criteria and was less or equall compared to those of filtration. The reason for this discrepancy is the significantly high standard deviation of the 1000 l spike dose (90 ± 57·5 oocysts). Our side by side comparison of PCFC to filtration employing Envirochek HV resulted in substantial higher recoveries for both *Cryptosporidium* and *Giardia* for source water samples.

Portable continuous flow centrifuge percent recovery of *Cryptosporidium* from secondary effluent indicates that it is a good alternative to conventional filtration methods for monitoring the presence of protozoa in treated sewage samples. Thus, in a study by Quintero-Betancourt *et al.* (2003), the mean percent recovery of 32 ± 97 for *Cryptosporidium* was achieved for the same matrix when concen-

Table 6 Summary of results of the portable continuous flow centrifuge Tier 2 validation* compared to method 1623 criteria

		Method 1623		PCFC	
Matrix	Organism	Acceptable recovery range (%)	Acceptable RSD/RPD (%)	Mean recovery (%)	Mean RSD/RPD (%)
Reagent	*Cryptosporidium*	21–100	40	43·3	17·9
Water	*Giardia*	17–100	41	49	16·1
Source	*Cryptosporidium*	13–111	61	48·3	12·5
Water	*Giardia*	15–118	30	40·4	9·6

*Tier 2 validation (method 1623) of the PCFC as an alternative concentration method for *Cryptosporidium* for 50 l of source water was approved by USEPA.
PCFC, portable continuous flow centrifuge.

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trating with the Envirochek HV and processing in accordance with method 1623.

Gennaccaro *et al.* (2003) reported a mean percent recovery of 15·3 ± 2·9 when employing the Envirochek HV for the concentration of *Cryptosporidium* from secondary effluents. In comparison, our mean percent recovery for *C. parvum* was (57·9 ± 9·6).

Portable continuous flow centrifuge recoveries of *C. parvum* oocysts and *G. intestinalis* cysts for small volumes (10 l) of source water during the initial method evaluation at the collaborative laboratories passed and exceeded method 1623 criteria. These high recoveries show the simplicity of this methodology, which enables laboratories to switch from their traditional concentration methods without compromising recoveries which is a common experience. DiGiorgio *et al.* (2002) reported for *Cryptosporidium* and *Giardia* overall mean recoveries of 54 and 25%, respectively, when concentrating from a similar matrix and volume with the Envirochek HV. Wholsen *et al.* (2004) reported mean percent recovery of 51·1 ± 4 for *Cryptosporidium* oocysts and 37·4 ± 3·8 for *Giardia* cysts for the Envirochek HV and with the Filta-Max 19·4 ± 2·2 for oocysts and 23·8 ± 3·5 for cysts. Ferguson *et al.* (2004) reported mean percent recoveries for *Cryptosporidium* and *Giardia* of 24·9 ± 18·3 and 40·6 ± 18·7, respectively when employing the Envirochek HV and 20·4 ± 9 and 41·4 ± 18·6, respectively when employing the Filta-Max, where our recoveries for *Cryptosporidium* and *Giardia* during the on-site training were equal or higher, 49 ± 7·8 and 71 ± 1·4, respectively.

Portable continuous flow centrifuge mean percent recoveries during the Tier 2 validation for 50 l source water samples for *Cryptosporidium* and *Giardia* – 48·33 ± 12·53 and 40 ± 9·63, respectively, correlates well with other studies which evaluated the efficiency of currently USEPA approved filters for this matrix; Clancy *et al.* (2003) reported for *Cryptosporidium and Giardia* a percent recovery range of 29–72 and 20·3–44·1, respectively and a percent RSD range of 9·5–18·8 and 4·7–69·7 for the Envirochek HV Tier 2 validation for 50 l source water samples.

McCuin and Clancy (2003) reported mean percent recoveries of 40·7 ± 37·8 and 59·3 ± 34·8, respectively for the Filta-Max.

In an effort to evaluate the potential of the PCFC to operate as a continuous monitoring tool, we passed through it 1000 l tap water samples. This involved also evaluation of the technical capacity of the motor and the bowl to operate for extended period of time.

Portable continuous flow centrifuge mean percent recoveries for *Cryptosporidium* from 1000 l tap water samples (35 ± 22·9) correlate well with a previously reported mean percent recovery (38·6 ± 17·3) for the Filta-Max (McCuin and Clancy 2003), when tested in accordance with method 1623. Sartory *et al.* (1998) reported for the Filta-max much higher mean percent recoveries (90·2) for 100–2000 l spiked tap water samples, however, experiments were performed employing nonvalidated protocols. Lee *et al.* (2004) reported for smaller volumes (200 l) and ten fold greater oocyst spike dose higher mean percent recoveries for *Cryptosporidium* when employing the Filta-Max and the Envirochek HV; 57·2 ± 11·5 and 46·2 ± 18·5, respectively.

Based on these reports and a recently published low mean percent recoveries for *Cryptosporidium* and *Giardia* of 4·4 ± 2·27 and 0·85 ± 0·7, respectively when concentrated by use of a compact continuous flow centrifuge (Higgins *et al.* 2003) it is assumed that currently, none of other commercially available continuous flow centrifugation methodologies can be validated or approved under USEPA methods 1622/1623 and most of them appear to only have a limited laboratory use.

Based on our results and those of a method 1623 nationwide Tier 2 validation (Zuckerman *et al.* 2004) the PCFC was approved by USEPA as an alternative concentration method under method 1623 and our preliminary unpublished data shows that the methodology is also applicable for bacteria. The PCFC will help water utilities monitor the presence of protozoa in effluent, source water and drinking water. Moreover, it will assist in continuous monitoring of the safety of watersheds and reservoirs for accidental or deliberate contamination with waterborne pathogens.

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