Use of Hydrophilic Polytetrafluoroethylene (HPTFE) Membrane in Detecting *Cryptosporidium* Oocysts from Source Water

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Abstract

A filtration and concentration method using hydrophilic polytetrafluoroethylene (HPTFE) membrane for detecting *Cryptosporidium* oocysts in source water is described.

Ten liters of sample water spiked with approximately 115 or 230 oocysts were filtered through 142 mm diameter HPTFE membrane with a pore size of 5.0 μ m. The oocysts captured on the filter were eluted by vortexing then concentrated by centrifugation. Subsequently, the oocysts in the suspension were purified by the immunomagnetic separation (IMS) method and stained with IFA. In addition, recovery efficiencies of the capsule filter filtration method using the IMS method were evaluated. The mean recovery efficiencies obtained were 48% for source water (n=37) and 20% for tap water (n=3) by the HPTFE membrane method, and 19% for source water (n=12) and 5% for tap water by the capsule filter filtration method (n=3). The total processing (filtration and concentration) time for the HPTFE membrane method was 30 min. These findings demonstrated that the HPTFE method was simple, easy, economic, and rapid, with considerably high recovery efficiency.

Key words: *Cryptosporidium*, oocyst, water, filtration, recovery efficiency, IMS method

INTRODUCTION

Many of the waterborne outbreaks of cryptosporidiosis that occurred in the past¹⁾ were attributed mainly to the resistance of the *Cryptosporidium* oocysts to disinfectants²⁻⁴⁾. Thus, the search for oocysts in the source water and treated water has become one of the most important objectives of waterworks facilities⁵⁾. Previous investi-

gators have employed various detection methods for the detection of *Cryptosporidium* oocysts in water, including cellulose acetate membrane dissolution⁶⁾, polycarbonate membrane filter⁷⁾, vortex-flow filtration and cross-flow filtration⁸⁾, calcium carbonate flocculation^{9, 10)}, capsule filter¹¹⁾, cellulose nitrate filter¹²⁾, and cotton filter¹³⁾ methods. Clancy *et al.* (1999)¹⁴⁾ as well as the Environmental Protection Agency (USA)¹⁵⁾

reported on the capsule filter method with immunomagnetic separation (IMS) technique. However, these methods are either time consuming or costly, which makes them of little use in waterworks. A rapid and cost effective method with high recovery efficiency is desirable for waterworks. In this paper, we describe a rapid and relatively more economical method for *Cryptosporidium* oocyst detection in source water using a hydrophilic polytetrafluoroethylene (HPTFE) membrane.

MATERIALS AND METHODS

Cryptosporidium oocyst sources

Cryptosporidium parvum oocysts recovered from naturally infected calves in Hyogo Prefecture, Japan, and the formalin-fixed *C. parvum* oocysts obtained from Waterborne Inc. (New Orleans, LA, USA) were used in this study. The spiking oocyst suspension was prepared by diluting the stock oocyst suspension with phosphate-buffered saline (PBS), then the suspension was stored at 4°C and used within a month after preparation.

Counting of oocysts in spiking suspension

The numbers of oocysts in the spiking suspension were counted by the vinyl-frame slide method (Inoue *et al.*, personal communication) and the well-slide method¹⁵⁾. In brief, for the vinyl-frame-slide method, 10 μ l of suspension and 5 μ l of fluorescein-labeled anti-*C. parvum* solution (Aqua G/C direct; Waterborne Inc.) were mixed at the center of the vinyl-frame-slide, covered with coverslip, incubated in a moist box at 37°C for 30 min then examined for *C. parvum* oocysts under a fluorescent microscope (Olympus BX50, Tokyo, Japan). For the well-slide method, 10 μ l of oocysts suspension was dried on a 14 mm diameter well-slide (Waterborne Inc.) at 50°C, fixed with methanol and stained by fluorescein-labeled antibody as mentioned above, then the slide was examined for oocysts. A modified well-slide method was also performed by adding 100 μ l of 0.1N-HCl and 10 μ l of 1N-NaOH to the added 10 μ l of suspension. The mean of twelve counts was taken as the numbers of oocysts in the spiking suspension.

HPTFE membrane filtration method

Filtration procedure: A ten-liter volume of

water spiked with *Cryptosporidium* oocysts was filtered through a 142 mm diameter HPTFE membrane of 5.0 μ m pore size (Millipore, Bedford, USA) housed in a stainless steel membrane filter apparatus (Advantec, Tokyo, Japan) at a rate of 2 l min⁻¹ using a peristaltic pump (Gelman Sciences, Ann Arbor, MI, USA). To complete filtration of oocysts added to the water sample, the container was rinsed with approximately 1 l of distilled water and the rinsed water was also filtered. Water without *Cryptosporidium* oocysts spiking was used as a negative control.

Eluting procedure: The membranes removed from the holder were placed in a 50 ml centrifuge tube containing 10 ml PBS-Tween (PBST) (PBS containing 0.1% of Tween 80) and a rugby-ball-shaped stirring bar (35×16 mm). The tube was screw-capped tightly, then subjected to vigorous vortexing for two minutes, after which the filters were squeezed with forceps and transferred to a petri dish. The eluant was transferred into another 50 ml centrifuge tube. The membranes were placed back into the original tube, and then another 10 ml of PBST was added. The tube was vortexed again and the eluant was collected into the tube containing the first eluant. This vortex procedure was repeated twice. Finally, the eluant was centrifuged at 4°C in a swinging bucket rotor, at 1050 × g for 10 min with the brake off. The deposit in the tube was suspended in 2 ml of PBST.

Separation procedure using immunomagnetic separation method: The immunomagnetic separation method (IMS) was performed following Method 1622¹⁵⁾.

Fluorescent staining procedure: The samples were stained by the well-slide method described previously¹⁵⁾.

Capsule filtration method The filtration procedure using the capsule filter (Gelman sciences), separation procedure using with IMS, and fluorescent staining procedure by well-slide were performed as described in the Method 1622¹⁵⁾.

Comparison of membrane types for filterable volume Several 142 mm diameter membrane filters, including cellulose acetate (pore size 1.2 μ m) (Millipore),

HPTFE (pore size 5.0 μm) (Millipore), polycarbonate track etch (pore size 3.0 μm) (Whatman), and the capsule filter (pore size 1.0 μm), (Gelman sciences), were evaluated to determine the filterable volume of the source water.

Quality control of HPTFE membrane A 10 l volume of distilled water and the same volume of source water, each spiked with 10,000 oocysts, were filtered with size selective continuous flow filtration apparatus¹⁶. The HPTFE (pore size 5.0 μm) membrane filter was fitted into the upper 142mm diameter holder and a polycarbonate track-etch (PCTE) membrane filter (pore size 2.0 μm) (Whatman, Springfield Mill, UK) was placed into the lower 47 mm diameter holder respectively, and were joined with a stainless steel tube. After filtration the PCTE membrane filter was removed from the holder and subjected to elution, staining, and examination according to the well-slide method described by the size selective continuous filtration method¹⁶. This procedure was repeated five times.

RESULTS AND DISCUSSION

In the present study, we first evaluated our well-slide method with and without modification for loss of *Cryptosporidium* oocysts during staining steps, for considering the oocyst counts obtained by the vinyl-frame slide method to be one hundred percent. The vinyl-frame slide method was employed for counting the oocysts in spiking suspension because of its relatively high recovery rate

(Inoue *et al.*, personal communication). Compared with the counts obtained by the vinyl-frame method, the loss of oocysts in staining steps by the present HPTFE method was 4 to 7% (Table 1). In other words, the recovery efficiency rate was 93 to 96%. Other researchers^{11, 17} have counted oocysts in spiking suspension by the well-slide method¹⁵. Had the well-slide method been applied in the spiking oocysts counting, the recovery rate obtained by the present method would have been much higher.

Various types of filters employed for the detection of *Cryptosporidium* in water include polycarbonate filter^{18, 7}, cellulose nitrate filter¹², and cellulose acetate filter^{6, 10}.

However, these methods are not fully satisfactory in terms of ease of application, efficiency, costs-effectiveness, and time consumption.

In this study, the average recovery efficiencies using the HPTFE membrane in all trials varied from 41% to 73% (Table 2). The mean recovery efficiencies of oocysts in spiked water samples from various sources by HPTFE membrane and capsule filter filtration method were 42% (20% to 54%) and 20% (5% to 36%), respectively (Table 3). Recovery efficiency of the capsule filter method is not necessarily high, but the method has been recommended by EPA and used world wide as the standard method for detecting oocysts from environmental water. We therefore, selected this method to compare with our HPTFE method. The recovery efficiencies of the HPTFE membrane

Table 1 Comparison of three different techniques for counting *Cryptosporidium* oocysts in spiking suspension

Counting technique	Mean*	SD**	CV***(%)	Ratio****(%)
Waterborne <i>Inc.</i>				
vinyl-frame-slide	115	13	11	100
well-slide	107	9	9	93
well-slide with added 0.1N HCl 100 μl and 1N NaOH 10 μl	107	15	14	93
Purified from calves				
vinyl-frame-slide	124	14	11	100
well-slide	119	12	9	96
well-slide with added 0.1N HCl 100 μl and 1N NaOH 10 μl	115	16	14	93

*Mean of twelve counts.

**Standard deviation

***The coefficient variation, defined as the ratio of standard deviation to the average.

****Ratio of each of counting techniques counts to the vinyl-frame-slide counts.

Table 2 Recovery efficiencies of *Cryptosporidium* oocysts from 10 l of source water using HPTFE membrane with IMS technique

Sample No.	Water source	Turbidity (NTU)	Oocysts spiked	No. of each trials	Recovery efficiency (%) (SD*)
1	reservoir	4.7	230	8	41 (10)
2	reservoir	3.8	115	2	43 (29)
3	reservoir	5.0	115	2	63 (22)
4	reservoir	4.3	115	3	73 (11)
5	river	4.5	115	10	45 (12)

Oocysts (Waterborne Inc.) were counted by vinyl-frame-slide counting method.

No oocysts were found in negative controls.

*Standard deviation

Table 3 Recovery efficiency of *Cryptosporidium* oocysts from 10 l of various water sources using HPTFE membrane and capsule filter with IMS technique

	Turbidity (NTU)	Water source	No. of filters	Pellet volume (ml)		Recovery efficiency (%)	
				HPTFE filter	Capsule filter	PTFE filter	Capsule filter
						Mean (SD*)	Mean (SD*)
1	0.6	subsoil	1	0.15	0.05	54 (13)	27 (3)
2	7.2	reservoir	2	0.4	0.2	40 (10)	17 (5)
3	7.5	river	2	0.6	0.3	35 (6)	20 (1)
4	7.2	reservoir	3	1.0	0.5	54 (2)	12 (3)
5	0.03	tap water	1	0.01	<0.01	20 (2)	5 (1)
6	0.02	finished water	1	<0.01	<0.01	42 (6)	23 (3)
7	0.00	distilled water	1	<0.01	<0.01	51 (4)	36 (6)

n = 3 for each trial

Oocyst (purified from calves) = 124 were enumerated by vinyl-frame-slide counting method.

No oocysts were found in negative controls.

*Standard deviation

were almost twice the recovery rates obtained by capsule filter. One explanation for this difference could be insufficient elution of oocysts from the capsule filter, given that the pellet volume compared with that obtained by HPTFE method, was smaller.

The oocyst recovery rates from spiked tap water were lower than those from spiked source water using the HPTFE membrane. Similar results were obtained by the capsule filter methods used in this study. Hoyle *et al.*¹⁹⁾ also found low recoveries with capsule filtration followed by IMS from spiked treated water. This finding could be explained by the loss of oocysts during centrifugation¹⁷⁾ or by the presence of interfering material in tap water. With use of environmental water, Clancy *et al.* examined the recovery efficiency of oocysts by the capsule filter method combined with IMS separation, and reported 38.9% recovery.

Our result (48%) was superior in recovery rate to this method.

In this study, the oocysts in the spiked distilled and source water did not pass through the 5.0 μ m pore size HPTFE filter. This was confirmed by the absence of oocysts in filtrate water filtered through a 5.0 μ m pore size HPTFE filter then through a polycarbonate track etch membrane filter having a pore size of 2.0 μ m. This indicated the effectiveness of the HPTFE membrane for trapping the *Cryptosporidium* oocysts, despite the fact that the pores are larger than the oocysts. This could be explained by the irregular mesh structure of the HPTFE membrane.

The filterable volumes of the HPTFE membrane were 6.3 l and 4.5 l for water having a turbidity of 6.8 NTU and 8.3 NTU, respectively (Table 4). These volumes were larger than those of polycarbonate mem-

Table 4 Comparison of membrane types for filterable volume

Membrane	Pore size (μ m)	cost (\$)	Filterable volume (l)		Method	References
			River (6.8NTU)	Reservoir (8.3NTU)		
Hydrophilic polytetrafluoroethylene	5.0	4	6.3	4.5	HPTFE method	This study
Cellulose acetate	1.2	8	9.5	7.5	Membrane dissolution	Aldom and Chagla, 1995
Polycarbonate	3.0	10	3.8	2.3	Method 1622 Draft (1997)	USEPA, 1997
Polyethersulphone (Envirocheck)	1.0	100	ND	35	Method 1622 (1999)	USEPA, 1999

ND= not determined

brane²⁰), and smaller than those of capsule filter¹⁵ or cellulose membrane⁹. Though the filterable water volume of the HPTFE membrane was approximately 5 l at a time, vortexing of up to three membranes in a 50-ml centrifuge tube for the elution of oocysts was time-saving and cost-effective, i.e. filtration and concentration time for the HPTFE membrane method was 30 min., while the capsule filtration method takes 45min. and further, cost needed for this method is one third than that of other method¹⁵ reported. Because of these advantages, we can test more water samples, and subsequently, we can obtain more precise information on the contamination of source water with *C. parvum* oocysts.

CONCLUSIONS

The present method using the HPTFE membrane, was found to have various advantages over the capsule filter. Furthermore, it is easy to use, as well as cost-effective, and can be applied to accurately detect *Cryptosporidium* in source water samples.

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