Water turbidity measurements were used to determine guidelines for optimizing sample volumes for the detection of *Cryptosporidium* oocysts in surface water by method 1622 (USEPA, 2001). The optimum sample volumes for turbidities of < 2, 2–10, and >10 ntu were determined to be 50, 35, and 10 L, respectively. Use of the optimum-volumes strategy suggested in this study would save sample filtration time, enhance filter performance, and offset the poor recovery efficiency of oocysts that typically results from higher volumes of water with high turbidity.

Technical Note: A guide for optimizing sample volume for the detection of *Cryptosporidium* oocysts by USEPA method 1622

BY HODON RYU

AND MORTEZA ABBASZADEGAN

Cryptosporidium, a protozoan parasite, was listed as a potential drinking water contaminant under the 1986 Amendments to the Safe Drinking Water Act. The general public became concerned about *Cryptosporidium* in drinking water following the largest recorded outbreak in Milwaukee, Wis., in 1993, with an estimated 403,000 people affected and more than 100 deaths. Recently, the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR), which requires drinking water source monitoring for *Cryptosporidium* oocysts, was promulgated by the US Environmental Protection Agency (USEPA, 2006).

According to the rule, the standard detection method for *Cryptosporidium* oocysts in water is method 1622 (USEPA, 2001), which requires sample filtration, sample purification using immunomagnetic separation (IMS), and an immunofluorescence assay. Method 1622 was developed for source water sample volumes of 10 L; however, a sample volume of 50 L is allowed for low-turbidity source water. For the LT2ESWTR, a minimum 10-L sample volume is required. For samples with more than 0.5 mL of packed pellet, <10 L of equivalent sample volume can be analyzed, but such analyses usually result in a high incidence of nondetectable samples because of low concentrations of oocysts and

TABLE 1 Ratios of packed pellet volumes of ≤0.5 or >0.5 for different sample volumes collected

Turbidity ntu	n†	Sample Volume Filtered—L*																	
		10		15		20		30		35		40		50		60		100	
		≤0.5	>0.5	≤0.5	>0.5	≤0.5	>0.5	≤0.5	>0.5	≤0.5	>0.5	≤0.5	>0.5	≤0.5	>0.5	≤0.5	>0.5	≤0.5	>0.5
< 2	14	100	0	93	7	93	7	93	7	86	14	86	14	**86**‡	**14**	43	57	14	86
2–10	50	100	0	96	4	94	6	90	10	**76**	**24**	72	28	46	54	24	76	8	92
> 10	7	**100**	**0**	57	43	29	71	14	86	0	100	0	100	0	100	0	100	0	100

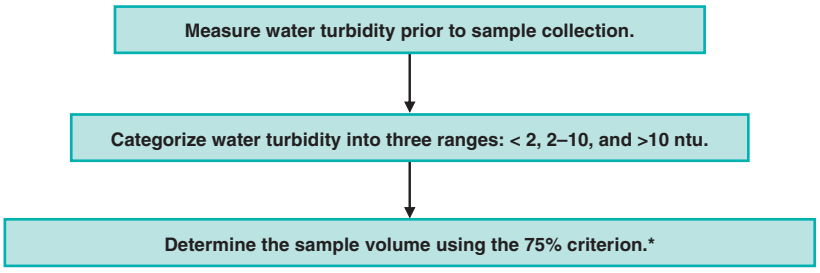
*Volumes of packed pellet for each sample volume filtered were interpolated or extrapolated.
†The number of samples for each turbidity category.
‡Bold numbers represent a reasonable selection criterion for optimum sample volumes.

low recovery efficiencies in environmental water using method 1622 (Ryu & Abbaszadegan, 2007; Ryu et al, 2005; LeChevallier et al, 2003; Simmons et al, 2001; Connell et al, 2000). Therefore, greater volumes need to be assayed to obtain reliable, representative results (Erickson, 1998).

In practice, there are limitations in analyzing high sample volumes, including reduced filter performance (loss of oocysts may increase during high-volume filtration and filter elution) and limited volume-processing capabilities of the IMS procedure. The maximum volume of packed pellet allowed for each IMS assay is only 0.5 mL, which does not increase the effective sample volume, even if a higher volume is filtered. Therefore, balancing the volume of packed pellet and sample volume can minimize the method's limitations. In addition, high-sample-volume filtration in the field is time-consuming and difficult to carry out, especially during summer and winter. The objective of this study was to provide guidelines for using water turbidity measurements to determine optimum sample volumes for the detection of *Cryptosporidium* oocysts using method 1622.

In practice, there are limitations in analyzing high sample volumes, including reduced filter performance and limited volume-processing capabilities of the immunomagnetic separation procedure.

FIGURE 1 A summary for selecting an optimum sample volume based on water turbidity



**For turbidities of <2, 2–10, or >10 ntu, collect 50, 35, or 10 L, respectively.*

MATERIALS AND METHODS

Seventy-one surface water samples from central Arizona source waters were analyzed. Sample filtration and filter elution were performed as described in method 1622. Accordingly, each water sample was filtered through a sampling capsule[1] at flow rates of no more than 2 L/min and shipped at 4°C to a university-run environmental microbiology laboratory.[2] The filters were eluted, and the eluate was transferred into two 175-mL polystyrene conical tubes[3] and concentrated

by centrifugation at 1,800 × *g* for 10 min. The concentrate was transferred into a 15-mL polypropylene conical tube with 0.1-mL scale graduations[4] and concentrated by centrifugation at 1,050 × *g* for 10 min. The volume of packed pellet was measured using the 0.1-mL scale graduations. Water turbidity and sample volume were measured during sample collection.

Using an assumed linear relationship between sample volume and the volume of packed pellet, estimated volumes of packed pellet were both interpolated and extrapolated by changing the volume of the water samples. Water turbidity was correlated with the estimated volume of packed pellet (R^2 = 0.5432; $p \leq 0.05$). Therefore, the turbidities of the surface water samples were categorized into three ranges (< 2, 2–10, and > 10 ntu), and then the number of samples for each category was determined to be less than or more than the designated 0.5 mL of packed pellet (Table 1). A reasonable selection criterion for optimum sample volumes is that the ratio of samples with less than 0.5 mL of packed pellet be greater than 75% (Table 1). A summary of the procedure for the optimization of sample volume is shown in Figure 1.

RESULTS AND DISCUSSION

Sample volumes ranged from 15 to 100 L, and water turbidities ranged from 0.6 to 42.4 ntu. The sample volume variation was primarily caused by the differences in water turbidity. For central Arizona source water, 50, 35, and 10 L were determined to be the optimum sample volumes for turbidities of <2, 2–10, and >10 ntu, respectively. These volumes are within the range of 10–50-L sample volumes of source water allowed in method 1622. Use of the optimum-volumes strategy suggested in this study would save sample filtration time, enhance filter performance, and offset the poor recovery efficiency of oocysts that typically results from higher volumes of water with high turbidity.

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FOOTNOTES

[1]Envirochek-HV, Gelman Sciences, Ann Arbor, Mich.
[2]The Environmental Microbiology Laboratory, Arizona State University, Tempe, Ariz.
[3]Catalog number 3144–0175, Nalge Nunc Intl., Rochester, N.Y.
[4]Catalog number 89004, VWR, Plainfield, N.J.

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