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Evaluation of the ICR protozoan method and Method 1623 for detecting *Giardia* and *Cryptosporidium* in actual water samples

B.-M. Hsu*, C. Huang and H.-H. Yeh*****

* Department of Environmental Engineering and Health, Yuanpei Institute of Science and Technology, Hsinchu, Chinese Taiwan (E-mail: *bmhsu@pc.ymit.edu.tw*)

** Institute of Environmental Engineering, National Chiao Tung University, Hsinchu, Chinese Taiwan

*** Institute of Environmental Engineering, National Cheng Kung University, Tainan, Chinese Taiwan

Abstract *Giardia* and *Cryptosporidium* have emerged as waterborne pathogens of concern. This study examined both parasites in actual water samples, which were taken from Cheng-Ching Lake waterworks in southern Taiwan. The Method 1623 was characterized by a higher recovery rate and lower detection limit compared with the ICR protozoan method. It was discovered that water turbidity reduced the recovery efficiencies, and raised the detection limits for both parasites, regardless of the method used. The concentrations of both parasites showed inconsistency for different recovery rates and detection limits between the two methods. A significant correlation between water turbidity and *Cryptosporidium* oocysts in raw water samples were found in this study.

Keywords *Cryptosporidium*; *Giardia*; ICR protozoan method; Method 1623

Introduction

Protozoan parasites, *Giardia* and *Cryptosporidium*, have been recognized as common pathogenic protozoa of the gastrointestinal tract (Cook, 1995). Water is perhaps the major route for massive outbreaks of pathogen infection, as a result of contamination of either raw or treated water. The occurrence of *Giardia* and *Cryptosporidium* in drinking water has aroused attention on the detection of the protozoa at levels concerning human health (Teunis *et al*., 1997; Eisenberg *et al*., 1998; Perz *et al*., 1998).

The Information Collection Requirement (ICR) Rule proposed a standard method for detecting *Giardia* and *Cryptosporidium* in water samples by the fluorescent antibody procedure (USEPA, 1995), which, however, has been heavily scrutinized by many researchers (Clancy *et al*., 1994; LeChevallier *et al*., 1995). Method 1623, with an improved concentration procedure and adoption of immunomagnetic separation (IMS), are expected to have a higher recovery rate and lower detection limit (USEPA, 1999). However, most water utilities still rely on the ICR method regardless of its high variability and low sensitivity, because of the high equipment and materials costs of Method 1623.

In this study, the ICR protozoan method and Method 1623 were evaluated for their recovery efficiencies and detection limits for *Giardia* and *Cryptosporidium*. We assessed the performances of the two analysis methods for raw and treated water samples collected from Cheng-Ching Lake waterworks in Taiwan.

Materials and methods

The cysts and oocysts prepared as the stock solution were obtained from Waterborne, Inc. (Louisiana, USA) and the Pleasant Hill Farms (Idaho, USA), respectively, and were diluted to desired concentrations by 0.1% PBS. To investigate the recovery efficiency of the ICR protozoan method and Method 1623, we visually counted the numbers of cysts and oocysts

of the seeded water samples before filtration and after clarification through epifluorescent microscope. To obtain the (oo)cyst concentrations in the water samples, the numbers of cysts and oocysts observed under the microscope were recorded, multiplied by the detection limit and divided by the recovery efficiency.

Two protozoan analyzing methods: ICR protozoan method and Method 1623, were used in this study. The procedure of Method 1623 was modified as detailed in this study. Two types of filtration apparatuses: Gelman Envirochek capsules (Ann Arbor, MI) and polycarbonate membrane filters, were chosen for raw water and treated water, respectively. After the water samples were filtered and eluted, the eluting fluid was centrifuged at 1,050 × g for 10 minutes. The concentrated suspension, aliquots of Dynabeads, 10 × SL™ Buffer A/B (Dynal A.S.) and deionized water were added into the flat-sided sample tube (Dynal® L10 tubes) and incubated on a rotary shaker. The bead-protozoa complexes were captured using a magnetic particle concentrator (MPC®-1, Dynal A.S.), the supernatant was discarded, and the magnet was removed. The bead-protozoa complexes were resuspended in the 1 × SL™ Buffer A, and the suspension was transferred to the eppendorf. The complexes were recaptured by a magnetic particle concentrator of smaller size (MPC®-M, Dynal A.S.). After the supernatant was discarded, the magnet was removed. Aliquots of HCl (50 µl, 0.1 M) were added into the Eppendorf and the suspension was incubated for 10 minutes to separate the protozoa from the beads. After neutralization with NaOH (5 µl, 1 M), the protozoa-contained suspension was thoroughly mixed and immediately transferred onto the glass slide (Dynal® Spot-On). After drying at 38°C, the sample was stained with the fluorescent-labeled antibodies (Hydrofluor™ Combo *Giardia/Cryptosporidium*) and enumerated with a epifluorescent microscope.

To analyze water samples by by ICR protozoan method, the samples were filtered through cartridge filters. The filters were removed from the cartridge, cut off the supporting core, and divided into three parts. The filter fibers were eluted with 0.7–1.2 L eluting fluid in a mechanical stomacher, and then the eluate was centrifuged at 1,050 × g for 10 minutes in a swinging-bucket rotor. The volume of the packed pellet was recorded. After the supernatant was aspirated, the pellet was resuspended in an equal volume of 10% formalin and the eluting fluid was added to a total volume of 20 mL in the same centrifuge tube. The mixture was underlaid with 30 mL of Percoll-sucrose gradient (sp. gravity = 1.10) followed by centrifuging. The top 20 mL and 5 mL below the interface were collected, diluted with eluting fluid to 50 mL, and centrifuged. The upper-layer liquid was then aspirated until only 5 mL of concentrate was left. The resuspended sediment samples were applied to each 25 mm diameter cellulose-acetate membrane, stained with fluorescent-labeled antibodies, and examined with epifluorescent microscope.

Results and discussion

Recovery efficiency of two analyzing methods in raw and treated water

The average protozoa recoveries of two analyzing methods in raw and treated water samples of Cheng-Ching Lake waterworks are shown in Figure 1. For the ICR protozoan method, the recovery efficiencies of raw and treated water were 5.9% and 11.0% for cysts, 7.9% and 10.7% for oocysts, respectively. The average recoveries of Method 1623 of treated water were 63.0% for cysts and 52.7% for oocysts: those of raw water samples were 51.0% for cysts and 42.4% for oocysts, respectively.

Our analysis showed higher protozoa recovery for treated water samples than for raw water samples, which was similar to that reported in the literature (LeChevallier and Norton, 1995). Our results also showed that the protozoan recovery efficiencies of Method 1623 are about five times higher than those of the ICR protozoan method, regardless of the types of water samples.

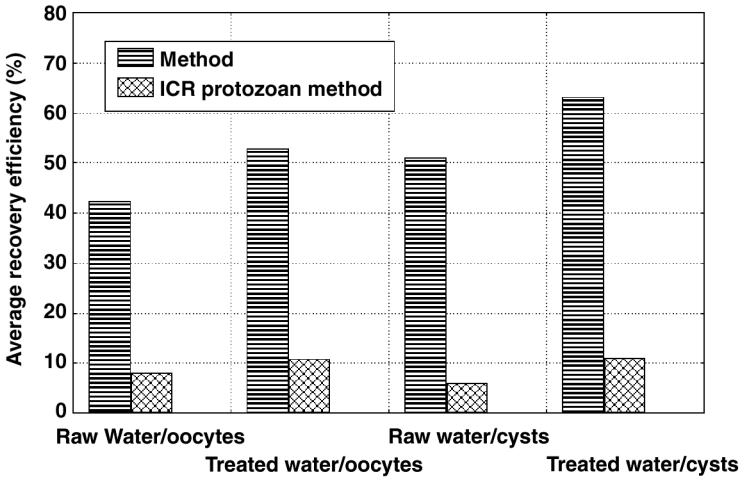

Figure 1 Average protozoa recoveries of two analyzing methods in raw and treated water samples of Cheng-Chin Lake waterworks

Concentrations of protozoa and their detection limits in water samples

The count via the microscope, detection limit and the concentration of protozoan parasites of the two analyzing methods in the raw and treated water samples are shown in Table 1. The treated water samples analyzed with Method 1623 displayed the lowest mean detection limit (4.0 cysts/100 L for Giardia and 4.7 oocysts/100 L for *Cryptosporidium*) while raw water samples collaborating with the ICR protozoan method showed the highest mean detection limit (196.6–416.5 cysts/100 L for *Giardia* and 146.8–311.4 oocysts/100 L for *Cryptosporidium*). The detection limits of Method 1623 for all samples were quite consistent while the ICR protozoan method exhibited much greater variation in detection limits.

Table 1 The count via the microscope, detection limit and the concentration of protozoan parasites of two analyzing methods in the water samples of Cheng-Chin Lake waterworks

Water type	Analyzing method	Cyst count	Oocyst count	Detection limit of cysts	Detection limit of oocysts	Cyst conc. (cysts/100l)	Ooyst conc. (oocysts/100l)	Sampling date
Raw	ICR method	7	18	196.6	146.8	1,376.2	2,643.0	14/01/1998
Treated	ICR method	1	0	33.6	34.6	33.6	<34.6	14/01/1998
Raw	ICR method	1	0	196.6	146.8	196.6	<146.8	07/01/1999
Treated	ICR method	0	0	23.6	24.3	<23.6	<24.3	07/01/1999
Raw	ICR method	3	5	416.5	311.4	1,250.8	1,557.0	07/05/1999
Treated	ICR method	1	15	42.7	43.9	42.7	658.5	07/05/1999
Raw	Method 1623	0	6	9.8	11.8	<9.8	70.8	01/12/1999
Treated	Method 1623	0	1	4.0	4.7	<4.0	4.7	01/12/1999
Raw	Method 1623	1	3	9.8	11.8	9.8	35.4	12/01/2000
Treated	Method 1623	0	1	4.0	4.7	<4.0	4.7	12/01/2000
Raw	Method 1623	1	5	19.6	11.8	23.6	118.0	23/02/2000
Treated	Method 1623	0	1	4.0	4.7	<4.0	4.7	23/02/2000
Raw	Method 1623	1	4	9.8	11.8	9.8	47.2	15/03/2000
Treated	Method 1623	0	0	4.0	4.7	<4.0	<4.7	15/03/2000
Raw	Method 1623	0	4	9.8	11.8	<9.8	47.2	12/04/2000
Treated	Method 1623	0	0	4.0	4.7	<4.0	<4.7	12/04/2000
Raw	Method 1623	1	2	9.8	11.8	9.8	23.6	10/05/2000
Treated	Method 1623	0	0	4.0	4.7	<4.0	<4.7	10/05/2000
Raw	Method 1623	1	6	9.8	11.8	9.8	70.8	30/08/2000
Raw	Method 1623	1	3	9.8	11.8	9.8	35.4	20/09/2000
Treated	Method 1623	0	1	4.0	4.7	<4.0	4.7	20/09/2000

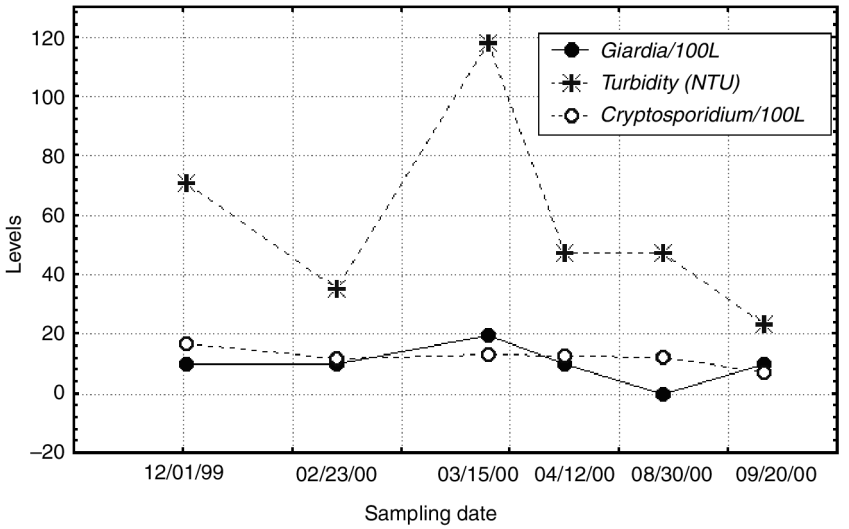

Figure 2 The trends of (oo)cyst concentration and turbidity for raw water samples at different sampling times

The concentrations of *Giardia* and *Cryptosporidium* listed in Table 2 also indicated that the detection result of parasites varied significantly. The results may be caused due to the differences in detection limits and variation in water quality for different sampling time. The occurrences of *Giardia* and *Cryptosporidium* in this water treatment plant are 81.8% and 90.9%, respectively for raw water samples, and the occurrences of treated water samples are 20% for cysts and 60% for oocysts.

The influence of turbidity on two analyzing methods

Figure 2 presents the trends of (oo)cyst concentration and turbidity for raw water samples at different sampling time. Results showed similar trends and significant correlation between turbidity and oocyst concentration (Spearman $R = 0.715$, $p < 0.05$). However, no significant correlation was found between water turbidity level and cyst concentration (Spearman $R = 0.389$, $p = 0.341$), as well as that between cyst and oocyst concentration (Spearman $R = 0.218$, $p = 0.604$).

Conclusion

Seeded water samples analyzed by Method 1623 achieved higher recovery efficiency than the ICR protozoan method. The detection limits of water samples analyzed by the ICR protozoan method were higher than Method 1623. Results of recoveries and detection limit suggested that Method 1623 is suitable for various turbid water samples. The detecting results of both parasite concentrations suggest that the detection of parasites by Method 1623 is quite different from that of ICR protozoan method. Although Method 1623 has been validated for its high recovery rate and low detection limit, it is too expensive to adopt in most countries. Therefore, optimizing the ICR method is very important. The significant correlation was found between oocyst concentrations and turbidity suggested that turbidity level would be a suitable indicator to predict the existence of *Cryptosporisium* oocysts in the raw water samples.

Acknowledgements

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