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ORIGINAL PAPER

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Evaluation of immunomagnetic separation method for detection of *Giardia* for different reaction times and reaction volumes

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Abstract Immunomagnetic separation (IMS) has been specified as a standard method for the measurement of *Giardia*. In this study, Dynal IMS was evaluated on the basis of recovery efficiencies of *Giardia* cysts for various IMS operational conditions. The average recoveries for *Giardia* in deionized, treated and raw water samples were $82.6 \pm 12.2\%$ ($n = 6$), $75.6 \pm 15.2\%$ ($n = 3$), and $70.6 \pm 18.2\%$ ($n = 3$), respectively. Significant changes in recovery were observed by altering the debris ratio and the debris components of water samples. Changing the reaction volume within the same vessel had no significant effect on cyst recovery efficiencies. However, prolonging the reaction time did increase recovery efficiencies.

Introduction

Giardia infections occur worldwide and the widespread occurrence of this kind of protozoa indicates their great adaptability to the environment. In Taiwan, the first case of giardiasis was diagnosed in 1975 on an offshore island. Of the children residing on the island, 32% tested positive for *Giardia* in their stool specimens (Chung and Cross 1975). In the United States, about half of the outbreaks of disease have been caused by *Giardia*. Attention has been drawn to *Giardia* because of its frequent outbreaks and its resistance to inactivation by chlorine (USEPA 1997).

The fluorescent antibody procedure specified in the Information Collection Rule (ICR) in the United States was the first standard method for detecting *Giardia* in water samples (USEPA 1995). This ICR method, however, has been heavily criticized. USEPA method 1623, which recommends the Gelman capsule filtration procedure, immunomagnetic separation (IMS) procedure and Meridian sample staining procedure, is expected to have higher recovery and lower detection limits (USEPA 1999).

The IMS method for the separation and concentration of protozoa has been used increasingly in recent years. Bifulco and Schaefer (1993) were the first to use IMS technology to concentrate *Giardia* cysts from water samples. The IMS technique employs a mouse immunoglobulin G anti-*Giardia* antibody as a primary antibody and an anti-mouse immunoglobulin G antibody-coated magnetite particle as a secondary labeling reagent. A novel, stable and simple *Giardia* direct IMS method, Dynal IMS, was recommended in method 1623 (USEPA 1999). Dynal IMS consists of two stages. In the capture stage, the para-magnetic beads (Dynalbeads anti-*Giardia*) coated with antibodies against *Giardia* cysts react to epitopes on the outer wall of cysts. In the separation stage, the conjugated magnetic cysts are separated from debris using a magnet. In order to increase the recovery efficiency and decrease the level of detection limit, it is necessary to improve and evaluate the IMS method.. The aim of this study was to evaluate *Giardia* recovery efficiencies of the Dynal IMS method in a variety of water samples as well as using various reaction times and reaction volumes.

Materials and methods

The cysts used in this study were obtained from Waterborne (Louisiana, USA). The numbers of cysts for seeding were recorded using the immunofluorescence assay technique. To do this, samples were mixed thoroughly, pipetted directly from the stock preparation vial onto the glass slides (Dynal Spot-On; Dynal, Oslo,

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Norway), stained with fluorescent-labeled antibodies (Hydrofluor Combo *Giardia*/*Cryptosporidium*; Ensys, North Carolina, USA) and counted. Deionized water and concentrated raw and treated water samples were seeded with known numbers of cysts. The raw and treated water samples were taken from Cheng-Ching Lake located in southern Taiwan. Particle pellets were collected by filtering the water samples through an Envirochek (Pall Gelman Sciences, Michigan, USA), followed by elution from the Envirochek using a shaker (PN 7822; Pall Gelman Sciences). The eluate was concentrated by centrifugation. The debris was washed and mixed with deionized water. The original quantity of the cysts in the debris was less than 1/0.5 ml debris.

The Dynal IMS was operated following the instructions provided by the Dynal company (Dynalbeads GC-Combo Kit; Dynal). Aliquots of Dynabeads and 10 × SL Buffer A/B were added to the water sample and then incubated on a rotary shaker at 15 rpm. The bead-oocyst complexes were first captured by a magnetic particle concentrator (MPC-1 or MPC-M). The supernatant was discarded and the magnet was removed. The bead-oocyst complexes were re-suspended in the solution of 1 × SL Buffer A, transferred to 2-ml centrifugation tubes (Eppendorf tubes), and then re-captured by a smaller magnetic particle concentrator (MPC-M). Aliquots of HCl (0.01 N) were added to separate the protozoa from the beads. The protozoa-containing solution was neutralized with NaOH (0.1 N), transferred to a glass slide (Dynal Spot-On), stained with fluorescent-labeled antibodies (Hydrofluor Combo *Giardia*/*Cryptosporidium*; Ensys) and enumerated by an epifluorescent microscope (Olympus, Japan).

To investigate the applicability of IMS under various operational conditions, we carried out the evaluation by manipulating several parameters related to the existing IMS procedures. These parameters included reaction time, reaction volume and sample turbidity. Firstly, we compared the effect of turbidity of water, using samples of deionized water and concentrated raw and treated water. Secondly, to discover the influence of reaction time on recovery efficiency, six different reaction times (0.25, 0.5, 1.0, 1.5, 2.0, 3.0 h) were tried. Finally, to determine the cyst recovery efficiency at different reaction volumes, various volumes of the same water samples (1.2, 0.8 and 0.4 ml) were used. The number of cysts counted on each slide was equivalent to the number of cysts per milliliter of the water sample. Recovery efficiency was calculated from the number of cysts seeded in the sample and the number that remained after the IMS procedure.

Results and discussion

Effect of water quality

The IMS instructions advocate the use of water samples containing debris ratios of less than 1:20. In order to understand the influence of water quality and turbidity on recovery efficiency, deionized water (0 nephelometric turbidity units) as well as concentrated treated and raw water samples containing 1:20 debris (5,000 nephelometric turbidity units) were seeded with 1,000 *Giardia* cysts per milliliter. As shown in Fig. 1, there were significant differences in recovery among the three water samples. The average recovery efficiency for turbid-free samples was $82.6 \pm 12.2\%$ ($n = 6$) and for turbid water samples was $73.8 \pm 17.7\%$ ($n = 6$). It is evident that water turbidity lowers parasite recovery, which may be due to the interference from the particles. Bifulco and Schaefer (1993)used the indirect IMS method to evaluate cyst recovery from water samples. As in this study, cyst recovery was reduced when the water turbidity was over 600 nephelometric turbidity units. It is noted that their recovery (82%) was higher than ours. This might be attributed to them labeling the cysts by magnetite reagent after removing most of sample turbidity by flotation. In our study, the concentrated raw and treated water samples were diluted to contain the same turbidity value, but different debris content. The average recovery efficiency for the concentrated treated water was $75.6 \pm 15.2\%$ ($n = 3$) and that for the concentrated raw water was $70.6 \pm 18.2\%$ ($n = 3$). The fact that the concentrated raw water samples had lower cyst recovery efficiency than the concentrated treated water samples also indicates that parasite recovery is related to the content of the debris, such as the algae, bacteria and other micro-organisms.

Effect of reaction volume

The recovery efficiencies of cysts at various reaction volumes are shown in Fig. 2. Various volumes of deionized water samples with the same cyst concentra-

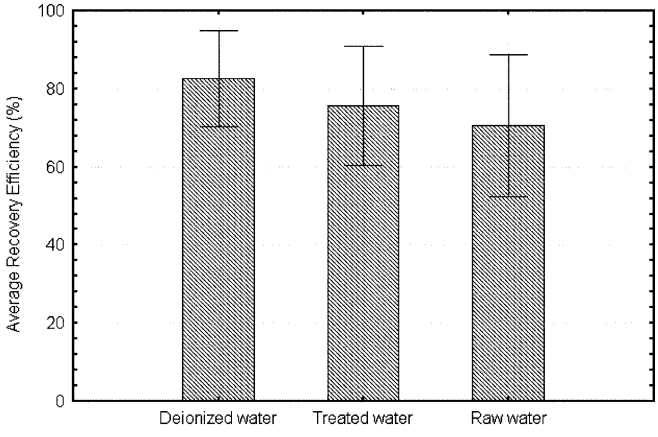

Fig. 1 Average cyst recovery efficiencies and standard deviations for deionized, treated and raw water samples

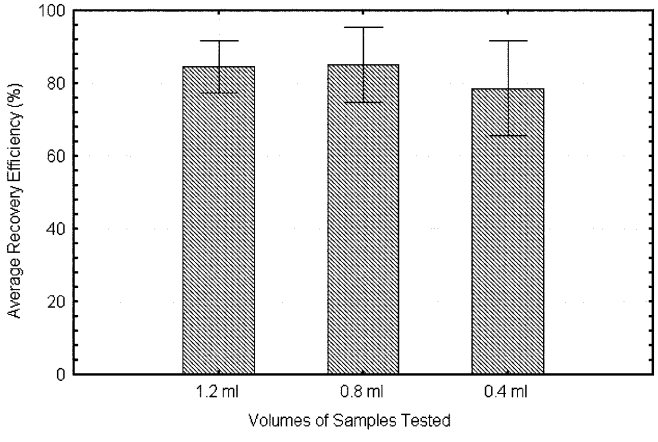

Fig. 2 Average cyst recovery efficiencies and standard deviations for deionized water samples in three reaction volumes

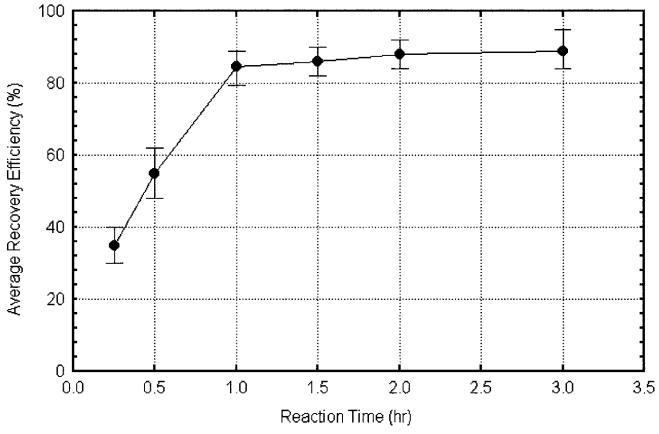

Fig. 3 Trends of cyst recovery efficiencies for deionized water at six reaction times

tion (1.2, 0.8 and 0.4 ml) were processed in the Eppendorf tubes (2.0 ml). The average recovery was $84.5 \pm 7.2\%$ ($n = 3$) for reaction volume of 1.2 ml, $85.1 \pm 10.2\%$ ($n = 3$) for 0.8 ml, and $78.7 \pm 13.1\%$ ($n = 3$) for 0.4 ml. The results indicate that changing the reaction volume had no significant influence on the recovery efficiency of the cysts.

Effect of reaction time

The influence of reaction time on the recovery efficiency of cysts for deionized water samples is presented in Fig.3. It is obvious that recovery efficiencies were increased by lengthening the reaction time, although the increases over the five time intervals were different. The recovery efficiency of 1 h reaction time is twice that of the 0.25 h reaction. When the reaction time is over 1 h, the difference is not as significant. From 1.0 h to 3.0 h, the increase was only between 3.0% and 3.9%.

Conclusion

The IMS procedure has higher and more stable recovery efficiencies than previously recognized. The average recovery efficiency of Dynalbeads anti-*Giardia* was highest when cysts were in deionized water, followed by those for concentrated treated water and concentrated raw water samples, respectively. Variations in the content of debris are the major factors to influence the recovery efficiencies. It is necessary, therefore, to evaluate the IMS recovery for different sampling conditions. Changing reaction volume within the same vessel would not significantly influence the recovery efficiencies of cysts. When evaluating unknown samples, the use of smaller reaction volumes and vessels can reduce the cost of the equipment. The reaction time is a key factor in improving the recovery efficiency of IMS, with the critical reaction time about 1 h.

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