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IMS Method Performance Analyses for *Giardia* in Water Under Differing Conditions

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Abstract Immunomagnetic separation (IMS) has been specified as a standard method for the measurement of *Giardia* under USEPA Method 1623. In this study, IMS was evaluated on the basis of recovery efficiencies for *Giardia* cysts under various IMS operation conditions. Significant change in recovery was observed by altering the debris ratio of water samples. Notably, cyst recovery efficiencies utilizing IMS dropped with increased turbidity, and results for varying dosages of magnetic beads and cysts indicate that 1/100 immunomagnetic beads is sufficient to conjugate large numbers of cysts. Changing vessel volume and replacing the sample buffer had no significant effect on cyst recovery efficiencies.

Keywords *Giardia* · Immunomagnetic separation (IMS) · Method 1623

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1 Introduction

Giardia is a common human parasite causing nonbacterial diarrhea. Contamination of drinking water with *Giardia* spp. has been increasingly recognized over the past decades as a cause of outbreaks in waterborne diseases (Ahmad, Lee, Tan, & Mohamad-Kamel, 1997; Frost, Craun, & Calderon, 1996). Infection is frequent among infants, children, travelers, campers, and immunocompromised patients (Backer, 2002; Lane & Lloyd, 2002; Nimri, 1994; Thompson, 1994).

Fluorescent antibody procedures specified in the Information Collection Rule (ICR) of the USA was the first standard method implemented for detecting *Giardia* in water samples (USEPA, 1995). The ICR method, however, has been heavily scrutinized in the recent past (Hsu, 2003; Hsu, Huang, Hsu, Jiang, & Hsu, 2001; Hsu, Huang, Lai, Tai, & Chung, 2001; Hsu et al., 2005). Presently, USEPA Method 1623, which offers improved filtration procedures and the adoption of immunomagnetic separation (IMS), is expected to have a higher recovery and lower detection limit (USEPA, 1999). IMS is conducted in two stages. Firstly, the capture stage uses para-magnetic beads coated with antibodies targeted against *Giardia* cysts that react with epitopes on the cysts' outer walls. In the separation stage, the conjugated magnetic cysts are separated from the debris using a magnet. Currently, evaluation and improvement of IMS is necessary to increase recovery efficiency and improve the detection

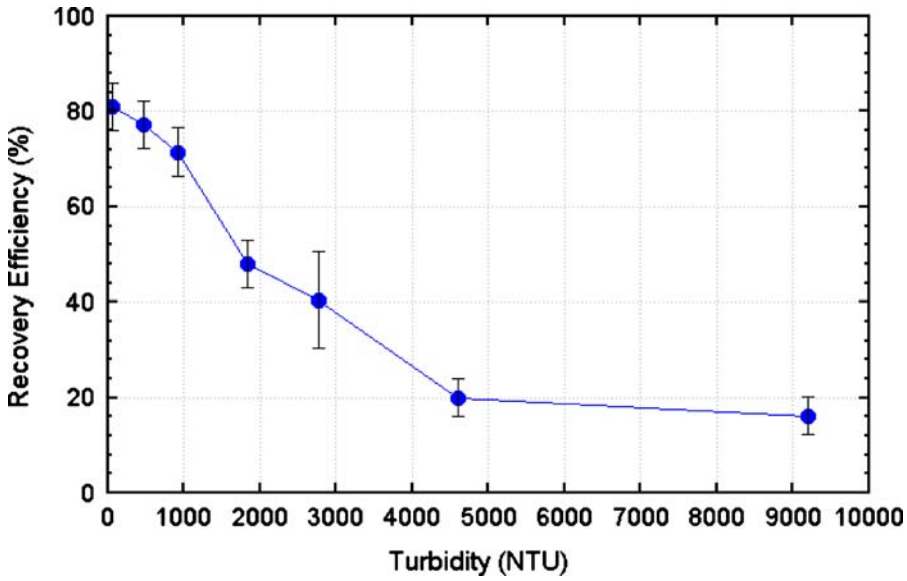

Figure 1 Average recovery efficiencies and their standard deviations of IMS for deionized water and turbid water samples containing 700 cysts/ml.

limit. Consequently, this study aims to evaluate the IMS method and compare recovery efficiencies by applying the method under a variety of operation conditions leading to recommendations for improved recovery with the IMS method.

2 Materials and Methods

The cysts used in this study were obtained from Waterborne, Inc. (Louisiana, USA). The numbers of cysts for seeding were examined using immunofluorescence assay technique. To do this, samples were mixed thoroughly, pipetted directly from the stock preparation vial onto glass slides (Dynal® Spot-On; Dynal A.S, Oslo, Norway), stained with fluorescent-labeled antibodies (Hydrofluor™ Combo *Giardia/Cryptosporidium*; Ensys, Inc., North Carolina, USA), and counted. Water samples were taken from the Hsinchu Waterworks located in northern Taiwan. Particle pellets were collected by filtering the water samples through Envirochek (Pall Gelman Sciences, Michigan, USA), followed by elution from Envirochek using a shaker (PN 7822; Pall Gelman Sciences, Michigan, USA). The eluate was then concentrated by centrifugation and the debris washed and mixed with deionized water. The original quantity of cysts in the debris was less than one cyst per 0.5 ml of debris.

Dynal IMS was conducted according to the instruction provided by the Dynal company (Dynalbeads® GC-Combo Kit; Dynal A.S, Oslo, Norway). Aliquots of Dynabeads® and 10× SL™ Buffer A/B were added into the water sample and then incubated on a rotary shaker at 15 rpm. The bead-cyst complexes were first captured by a magnetic particle concentrator (MPC®-1 or MPC®-M). The supernatant was discarded and the magnet removed. The bead-cyst complexes were re-suspended in a solution of 1× SL™ Buffer A, transferred to 2 ml-centrifugation tubes (eppendorf tubes), and then re-captured by a smaller sized magnetic particle concentrator (MPC®-M). Aliquots of HCl (0.01 N) were added to dissociate 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, Inc., North Carolina, USA) and enumerated by an epifluorescent microscope (Olympus, Japan).

To examine IMS under various operating conditions, we designed an experiment that manipulated several parameters relating to existing IMS procedures. These parameters include sample turbidity, concentrations of cysts and immunomagnetic beads, usage of buffer and reaction volume. Turbidity effects were tested on deionized water and concentrated raw water samples of different turbidities, namely 460, 920, 2,760, 4,600, and 9,200 NTU. Seeding concentrations were varied from 1.8×10^2 to 1.4×10^3 cysts/ml of deionized water to evaluate their effect on recovery efficiency. In order to realize the effect of sample volume on the determination of cyst recovery, we

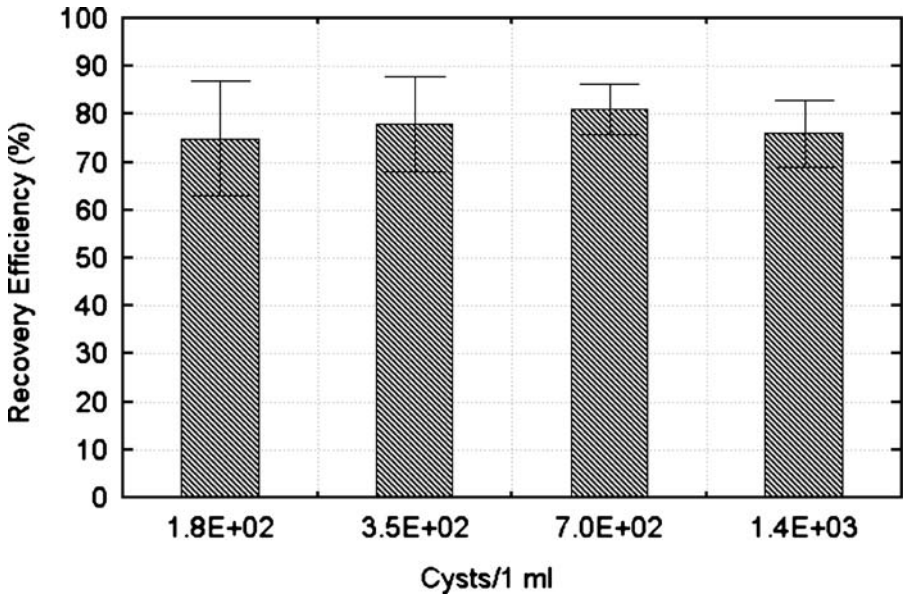

Figure 2 The average recovery efficiencies and their standard deviations for deionized water containing various concentrations of cysts.

compared the recovery efficiencies between the eppendorf tubes containing 1.2 ml of samples and the glass tubes (Dynal® L10 tubes) containing 12 ml of samples. Different dosage of magnetic beads (water sample containing 3/1,000, 6/1,000, 8/1,000, 1/100, 15/1,000, and 2/100 immunomagnetic beads, respectively) and different buffers (10 × PBS and 10 × SL™ Buffer A/B) were also explored for their influence on IMS performances. The number of cysts observed on each slide was taken as the number of cysts per milliliter of sample. Recovery efficiency was calculated from the numbers of cysts seeded in the sample and those remaining after the IMS procedure.

3 Results

3.1 Effects of sample turbidity

In order to understand the influence of turbidity on recovery efficiencies, deionized water (0 NTU) and water samples containing turbidities of 460, 920, 1,840, 2,760, 4,600, and 9,200 NTU were seeded with

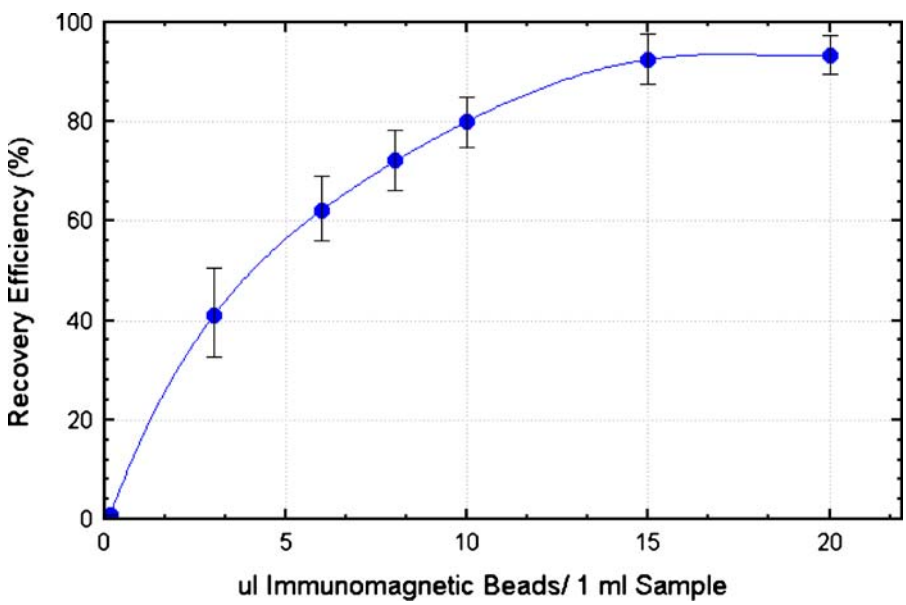

Figure 3 Relationship between dosages of Dynabeads® anti-*Giardia* and the corresponding recovery efficiencies of IMS in deionized water containing 700 cysts/ml.

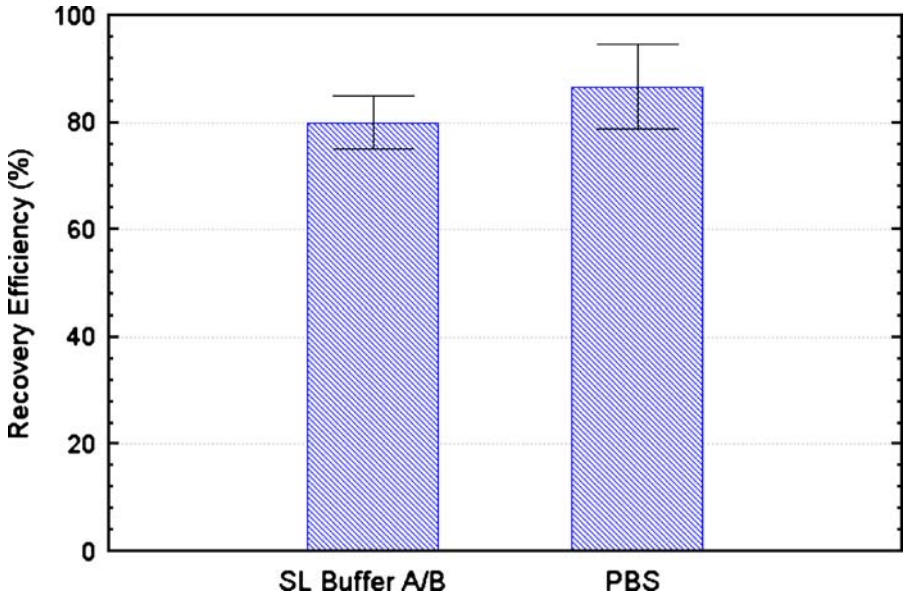

Figure 4 Recovery efficiencies of IMS and their standard deviations for deionized water containing 700 cysts/ml prepared in 1×SL™ Buffer A/B or 1×PBS.

700 cysts/ml of *Giardia* cysts. As shown in Figure 1, there is significant difference in recovery among various turbid samples. Cyst recovery efficiencies of IMS dropped from 81.0 to 20.0% when the turbidity was increased from 0 NTU to 4,600 NTU. From 4,600 NTU to 9,200 NTU, the recovery efficiencies remained low and didn't change significantly.

3.2 Effects of cysts concentration

In this study, four levels of cyst concentration ranging from 1.8×10^2 to 1.4×10^3 per milliliter were seeded into deionized water, and their recovery efficiencies are shown in Figure 2. There is no significant difference in average recoveries among various seeding concentrations. The average recovery for these samples was 82.6±18.2% (n=52). The result indicates that the dosage recommended by Method 1623, 1/100 immunomagnetic beads, is sufficient to conjugate large numbers of cysts. The highest and the most consistent recovery efficiency was found at a seeding concentration of 7.0×10^2 cysts/ml. Large deviations occurred at lower seeding concentrations (e.g., 1.8×10^2 and 3.5×10^2 cysts/ml). Inaccuracy in cysts counting, deficiency in

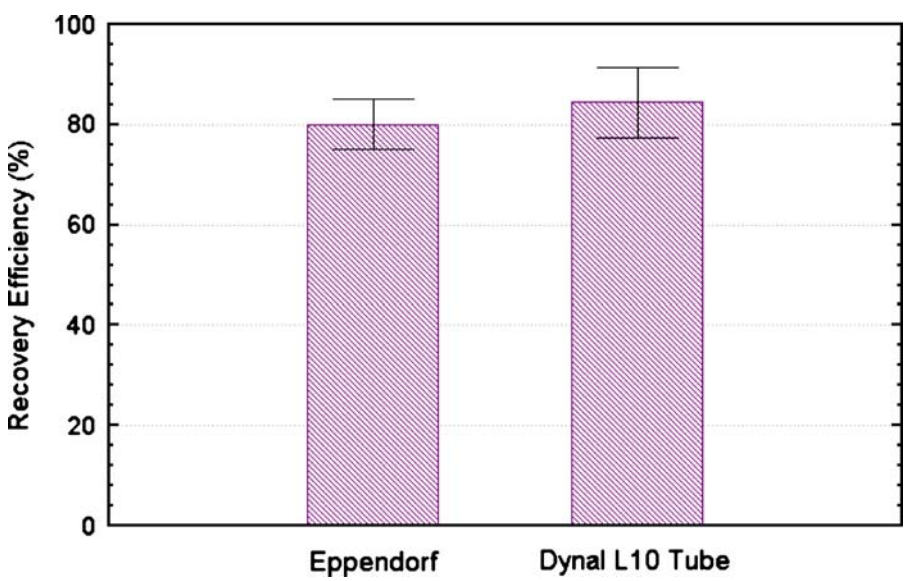

Figure 5 Recovery efficiencies of IMS of deionized water samples in two reaction vessels.

experimental procedures and flocculation of cysts in the stock solution may account for these large deviations.

3.3 Effects of amount of immunomagnetic beads

The effect of differing dosages of immunomagnetic beads on cyst recovery was evaluated on deionized water containing 700 cysts/ml. The results given in Figure 3 show that recovery efficiency was increased rapidly when the ratio of Dynabeads® anti-*Giardia* in water samples was raised from 3/1,000 to 1/100. When the ratio exceeded 1/100, recovery efficiency increased mildly. Recovery efficiency plateaus at a ratio of 1.5/100 Dynabeads® anti-*Giardia* in water samples.

3.4 Effects of sample buffer

To investigate the effect of buffer on conjugation, two buffer reagents, namely PBS and SL™ Buffer A/B, were added in separate test samples seeded with 700 cysts/ml of *Giardia*. The results in Figure 4 show that the average recovery of cysts in SL™ Buffer A/B was 81.0±5.2% (n=7) and that in PBS was 86.7±10.6% (n=3). The deviation of *Giardia* recovery efficiency in PBS was higher than that in SL™ Buffer A/B. However, there was no evident difference observed after evaluating these data using Student t-test (P>0.1).

3.5 Effects of vessel volume

Two reaction vessels, eppendorf tube and Dynal L10 tube, were compared. The reaction volumes were 12 and 1.2 ml for the Dynal L10 tube and the eppendorf tube, respectively. Seven hundred cysts per milliliter were seeded in both trials. The results are shown in Figure 5. The average recovery for the eppendorf tube was 81.0±5.2% (n=7) and 85.5±8.3% (n=3) for the Dynal L10 tubes. There is no evident difference, however, observed after evaluating these data using Student t-test (P>0.1). Although higher recovery was achieved by the Dynal L10 tube, eppendorf tube, because of its smaller volume, was generally adopted to reduce the cost for evaluating unknown samples.

4 Discussion

IMS procedure has higher and more stable recovery efficiency than previously utilized purification methods for *Giardia* cysts. However, this study has shown that efficiencies are affected by the turbidity of samples and the dosage of immunomagnetic beads. Cyst recovery efficiency of IMS dropped with increased turbidity and reduced dosage of Dynabeads®. Debris was concentrated from collected water at a ratio of 1:10,000 in the IMS study (USEPA, 1999). While the collected water volume is 10 l and turbidity is 0.5 NTU, concentrated debris should be 1 ml and 5,000 NTU. In this study, recovery efficiency of IMS dropped significantly when turbidity reached 4,600 NTU. This suggests that if the collected water volume is 10 l, water turbidity should be less than 0.46 NTU when 100 µl Dynal beads are added to the concentrated debris. These results can be used to estimate suitable dosages of Dynabeads® for differing collected water volumes and turbidities. Consistent average recoveries were achieved between seeded concentrations of 1.8×10^2 to 1.4×10^3 cysts/ml. Results for different dosages of magnetic beads and cysts indicate that a plateau occurs at a ratio of 1.5/100, which is 50% higher than the supplier's recommended dosage. Recognition of the plateau is essential for people wanting to minimize operational errors and thus increase recovery consistency. With consideration given to cost, a ratio of 1/100 immunomagnetic beads is sufficient to conjugate cysts. Samples in PBS achieved higher recovery efficiency with no evident difference indicating that PBS is suitable for this procedure. A glass tube, Dynal L10 tube, with a large reaction volume displayed similar recovery to the eppendorf tube of smaller reaction volume. When evaluating unknown samples, the use of a smaller reaction volume and vessels can reduce the cost of the kit.

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