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Project Summary

Removal of *Cryptosporidium* and *Giardia* through Conventional Water Treatment and Direct Filtration

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Pilot- and full-scale evaluations of *Giardia* and *Cryptosporidium* cyst removals through direct filtration and conventional water treatment were conducted by the Utah Department of Environmental Quality. Cysts were seeded continuously in a step dose at a 0.5 gpm pilot plant, and in a spike at a 900 gpm full-scale plant; both plants were operated under conventional treatment and direct filtration regime. The results of 20 pilot-scale cyst seeding trials and 8 full-scale trials indicated that source water quality (turbidity and algal content), as well as treatment effectiveness in removing turbidity, controlled the removal of seeded *Giardia* and *Cryptosporidium.* Changes in source water quality influenced removal rates more than the mode of treatment. Higher removal rates were consistently observed for *Giardia* cysts (3.3-log) than for *Cryptosporidium* oocysts (3.0-log). A high correlation was found between cyst removal rates and removal of the respective size particles; poorer correlation existed between cysts and turbidity removal, while no significant correlation was established between the removals of cysts and heterotrophic bacteria.

To assure that the best available detection method was used in enumeration of the cysts in raw and treated water, two versions of the immunofluorescence staining method were evaluated for their efficiencies in detecting *Giardia* cysts and *Cryptosporidium* oocysts seeded at known concentrations in water: (1) the ASTM method for detection of *Giardia* cysts and *Cryptosporidium* oocysts in low-turbidity water and (2) a modified Sauch's procedure employing sampling by 2.0 μm membrane filters, Percoll/Percoll step gradient flotation, and immunofluorescence staining on 2.0 μm porosity polycarbonate membrane filters. The second method was selected, since it was characterized by higher recovery rates in all three types of waters tested: raw surface water, partially treated water from a flocculation basin, and filtered water. Cyst and oocyst recovery efficiencies decreased with increasing water turbidity regardless of the method used. Recoveries of seeded *Giardia* cysts exceeded those of *Cryptosporidium* oocysts in all types of water sampled.

This Project Summary was developed by EPA's National Risk Management Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at the back).

Introduction

Problem Statement and Study Objectives

The Surface Water Treatment Rule requires all public water system treating surface water to effectively remove enteric viruses and *Giardia* cysts. The removal of microbial contaminants by filtration is being re-evaluated by the U.S. Environmental Protection Agency in conjunction with the publication of the proposed Interim Enhanced Surface Water Treatment Rule. While the removal requirements for *Giardia* may be increased depending on cyst concentration in raw water, additional, more stringent regulations may be developed to control *Cryptosporidium* in response to health concerns surrounding this pathogen. As new rules are being developed, allowable filtration credits should be revisited and possibly revised. Also, as the analytical methods for detection of *Giardia* and *Cryptosporidium* cysts in water are being improved and new methods developed, surrogate water quality parameters should be established to allow for an accurate, economical, and practical evaluation of cyst removal effectiveness through treatment.

The project objectives were designed to address some of the questions associated with the development of the new regulations. The specific tasks were to examine the most critical relationships in removal of *Giardia* and *Cryptosporidium* by comparing the effectiveness of *Giardia* and *Cryptosporidium* removal through conventional treatment with that resulting from direct filtration, the effectiveness of *Giardia* removal with *Cryptosporidium* removal, and the effectiveness of *Giardia* and *Cryptosporidium* removals with the removals of turbidity, cyst-size particles, and heterotrophic bacteria.

To enable accurate evaluation of *Giardia* and *Cryptosporidium* removal efficiency in water treatment processes, a reliable method for measuring the concentration of these pathogens in water must be used.

Therefore, another objective of this project was to select an analytical method capable of measuring the concentration of *Giardia* cysts and *Cryptosporidium* oocysts that would be accurate, reliable, flexible, and verifiable, and could be applied to measuring the cyst/oocyst removal performance of water treatment processes.

Procedure

Evaluation of Analytical Methods

Two methods for finding *Giardia* cysts and *Cryptosporidium* oocysts in water were compared. Both methods follow flotation steps and immunofluorescence staining. These methods were 1) The American Society for Testing and Materials (ASTM) method and 2) another immunofluorescence antibody (IFA) method, referred to as the alternate method, and applied principally by Ongerth and Stibbs. The objective was to evaluate the two IFA methods using three factors as criteria for comparison. First, the applicability to cyst seeding experiments in full- or pilot-scale water treatment plant was evaluated. Second, the applicability of the methods to cyst detection in environmental water samples of varying water quality was assessed. The third criterion was the economics associated with the two methods. The superior method was then used in the cyst seeding experiments in the pilot- and in the full-scale treatment plant.

The ASTM method involves sampling 100 L or more of water through a 1.0 µm porosity polypropylene yarn cartridge filter, extracting the particulates from the cartridge filter, and concentrating the extracted particulates by centrifugation. The concentrated particulates are then processed to selectively concentrate cysts and oocysts by flotation in 50 mL tubes on a Percoll/sucrose gradient. The particulates recovered at the interface of the Percoll/sucrose are stained with fluorescent-tagged antibodies on 25 mm diameter, 0.2 µm pore size cellulose acetate filters. After mounting on slides, the membrane filters are scanned using a UV epifluorescent microscope for objects of the right size, shape, and fluorescence characteristic as *Giardia* cysts and *Cryptosporidium* oocysts. On finding such objects the microscope optics are switched to phase contrast to look for internal characteristics of the organisms.

The alternate method involves filtration of the water sample through either a 293 or 142 mm diameter, 2.0 µm pore-size polycarbonate membrane filter; recovery of particles from the filter by rinsing and scraping them from the surface; and concentration of the particulates by centrifugation. The cysts and oocysts are then selectively concentrated from other particulates by flotation in 15 mL tubes on a two-step Percoll/Percoll gradient, followed by IFA staining on 13 mm diameter, 2.0 µm pore-size polycarbonate membrane filters. After mounting on slides, the membrane filters are scanned using an UV epifluorescent microscope for objects of the right size, shape, and fluorescence as *Giardia* cysts and *Cryptosporidium* oocysts. Confirmation of internal structures is not performed in this method.

Cyst Seeding and Sampling Procedures

Monitoring of raw and filtered water quality was conducted throughout the seeding trials. In addition to monitoring the major water quality parameters, particle counting in four size ranges (2-4 µm, 4-7 µm, 7-14 µm, and 14-25 µm), was performed during the seeding trials. Raw water sources were sampled and analyzed for background count of *Giardia* and *Cryptosporidium*, naturally occurring in the two watersheds.

Inactivated, formalin-fixed *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts were used in seeding experiments in a pilot- and a full-scale treatment plant. A total of 20 trials were conducted in the pilot plant, and 8 trials in the full-scale plant, alternating between conventional treatment and direct filtration.

The first site for testing was a pilot plant residing at the 180 MGD Jordan Valley Water Treatment Plant in Bluffdale, UT. The pilot plant simulated the actual, conventional treatment plant, treating Provo River water downstream from Deer Creek Reservoir. Water flow rate was maintained at 0.5 gpm. Alum was used as a coagulant, at dosages established through jar testing. After each seeding trial using the conventional treatment train, the water was re-routed through the direct filtration train for seeding trials the following day. Alternating between the conventional treatment and direct filtration allowed for a comparison of treatment effectiveness of the water of comparable quality.

The second site was a 900 gpm Huntington Water Treatment Plant, situated near Price, UT. The plant was operated at 600 gpm for cyst seeding experiments. Polyaluminum chloride was used as a coagulant. The Huntington Plant was operated by conventional treatment during the first four seeding trials. After converting the plant to direct filtration mode, another four seeding trials were performed.

Detection of Giardia *and* Cryptosporidium *Cysts*

The alternate IFA method for sampling, processing, and detection of *Giardia* and *Cryptosporidium* cysts was chosen for the seeding trials, based on results of the method comparison. Samples were collected by membrane filtration through 2.0 µm porosity, 293 mm diameter polycarbonate membrane filters, processed on Percoll/Percoll step gradient in 15 mL centrifuge tubes, stained on 2.0 µm porosity, 13 mm diameter polycarbonate membrane filters, and enumerated under an epifluorescent microscope.

In calculations of cyst removal through treatment, a direct ratio of the difference between the cysts seeded and the cysts detected was calculated for each run in the pilot plant. Two cyst removal rates were determined based on two different initial cyst concentrations: concentration in the *seeding solution* prior to being pumped into the raw influent, and concentration in the *seeded influent* already mixed with the raw influent. The cyst removal rates, achieved in the full-scale plant, were also calculated as relative differences between the influent and effluent concentrations, but the influent concentrations were adjusted for dilution of cysts in respective basins.

Three conservative assumptions were made for data interpretation in calculations of the removal rates, both in the pilot- and full-scale plant. First, the removals based on the cyst concentrations found in the seeded influent were used in data analysis. Otherwise, the higher cyst concentrations found in the seeding solutions would result in higher removal rates reported. Secondly, cyst removal rates were calculated only for trials in which cysts were detected in *both* influent and effluent samples. Otherwise, calculations of removals during trials when cysts were not detected in the effluent would be based on very low detection limits, and therefore would result in higher removal rates. Finally, no adjustments were made for the differences in cyst recovery efficiencies in turbid raw influent samples versus clean filtered effluent samples. Adjusting for low recovery rates in influent samples would also result in higher removal rates being calculated.

Results and Discussion

Evaluation of Two IFA Methods for Detection of Giardia *and* Cryptosporidium

The effectiveness of cyst recovery from spiked water samples was impacted primarily by the number of analytical steps involved in the cyst detection. Both IFA methods were characterized by low recovery efficiency, when seeded raw water samples were filtered, then concentrated, transferred to gradients, stained, and enumerated. Higher recovery rates were observed in detecting *Giardia* cysts when the alternate method was employed (Figure 1). In spiked raw water samples, an average 12% of the seeded *Giardia* cysts were detected by the ASTM method, while the alternate method was characterized by an average 49% *Giardia* cysts recovery efficiency. Recovery rates for *Cryptosporidium* oocysts in spiked raw water averaged 8% detected by the ASTM method and 9% detected by the alternate IFA method. The recovery rates in filtered water were 14% and 52% for *Giardia* and 12% and 12% for *Cryptosporidium*, using the ASTM, and the alternate method, respectively. In flocculated water samples, an average 22% of *Giardia* cysts were detected using the ASTM method and 40% using the alternate method. The recoveries of *Cryptosporidium* in flocculated water were 7% with the ASTM method and 1% with the alternate method.

Losses were demonstrated to occur due to incomplete yarn cartridge filtration. In sampling raw water, 5% *Giardia* cysts and 6% *Cryptosporidium* oocysts were captured from the yarn cartridge filtrate by passing it through a 293 mm diameter 2.0 µm pore size Nuclepore membrane filter. An average 7% of *Giardia* and 8% of *Cryptosporidium* were recovered from membrane filters after cartridge filtration.

The sampling step resulted in a high loss of seeded *Giardia* cysts and *Cryptosporidium* oocysts. When the sampling step was eliminated and cysts were seeded directly onto flotation gradients, the resulting recovery rates increased dramatically (Figure 2). An average 53% of *Giardia* cysts and 27% of *Cryptosporidium* oocysts was detected from seeded Percoll/sucrose gradients used in the ASTM method. The alternate method employing Percoll/Percoll flotation, yielded recoveries of 82% for seeded *Giardia* cysts and 69% for *Cryptosporidium* oocysts.

The highest cyst recovery rates were reported when both sampling and flotation steps were avoided and spiked with *Giardia* cysts and *Cryptosporidium* oocysts samples were stained directly onto the membrane filters used for IFA assay. The ASTM method resulted in recoveries of 72% and 56% for *Giardia* cysts and *Crypto-*

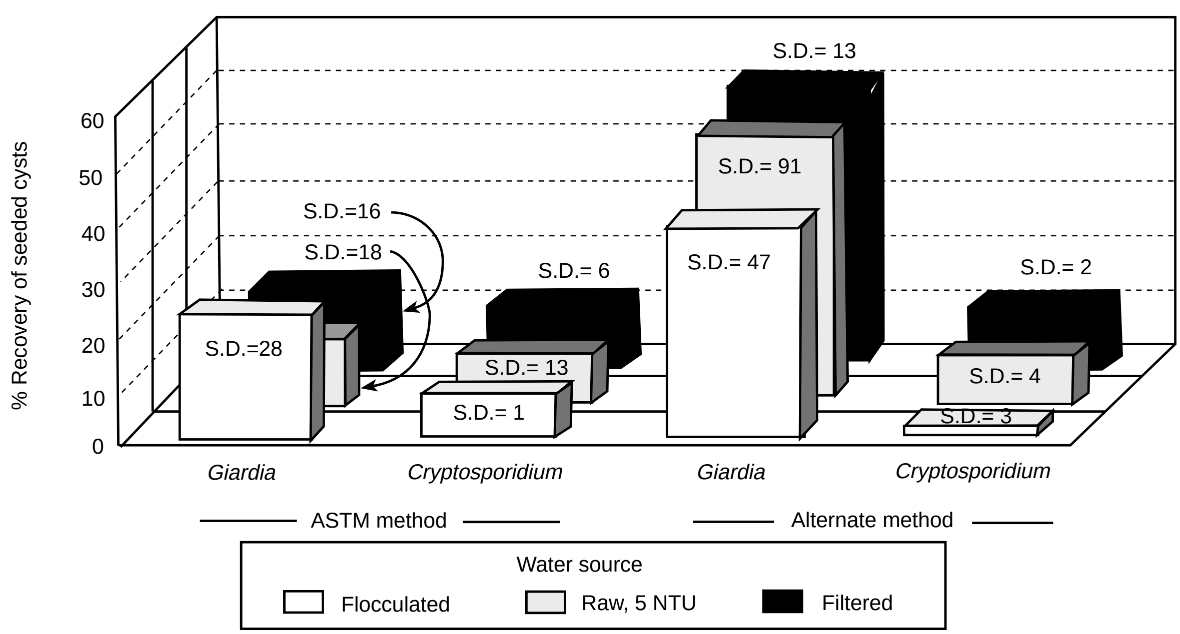

Figure 1. Water quality vs. recovery of cysts seeded into water.

sporidium oocysts, respectively. The alternate method was characterized by an average recovery of 86% for *Giardia* cysts and 78% for *Cryptosporidium* oocysts.

Testing of cyst recovery efficiencies from raw, flocculated, and filtered water indicated that any increase in water turbidity, whether due to presence of algae or to that of chemical floc, resulted in a significant decrease in parasite recoveries. The results also indicate both methods were more effective in detecting seeded *Giardia* cysts than *Cryptosporidium* oocysts.

The results of testing the processing and detection methods indicated that recoveries of cysts were substantially and consistently higher with the alternate method, employing Percoll/Percoll step gradient combined with IFA on 13 mm polycarbonate filters, than with the ASTM method, in all three types of water. Consequently, the alternate method was selected as the method of choice for seeding experiments. Added support for this choice was provided by a comparison of the qualitative advantages and disadvantages of the two methods. In summary, the main advantage of the ASTM method was its ability to confirm presumptive cysts and oocysts. The most serious disadvantages of this method were its relatively high cost and the amount of time required to complete it. The alternate method, on the other hand, was found to be less expensive and required less time to complete than the ASTM method. Attractive features of the membrane filter sampling method include relatively small sample volumes, flexibility, and compatibility with frequent seeded controls. The major limitation of the alternate method was its lack of a confirmation step.

Experience with detecting *Giardia* cysts and *Cryptosporidium* oocysts in the waters tested during this study and the results generated during this part of the study, indicate that the alternate method be recommended to evaluate water treatment processes that use high concentrations of seeded parasites in which algae, occurring in concentrations much lower than the seeded parasites, are not of concern. The alternate method can be considered for analysis of environmental samples, particularly for low-turbidity waters. When high water turbidity requires higher-volume samples to be collected and examined, and when cross-reacting algae should be differentiated from the organisms of interest, the ASTM method should be used with environmental samples.

The results of this stage of the study have indicated *Giardia* cysts and especially, *Cryptosporidium* oocysts are lost during the gradient flotation steps of both methods. Therefore, it is recommended that the flotation step should be avoided, whenever possible when processing treated (filtered) water samples.

A hybrid method, combining the most efficient steps from the two methods, should be investigated. Such a hybrid method should include sampling by membrane filtration only for low-turbidity waters. High-turbidity waters should be sampled by the ASTM cartridge sampling method. Since the Percoll/Percoll step gradient in 15 mL tubes is more economical and had higher cyst recovery than the Percoll/sucrose gradient, it should be used. Staining on cellulose acetate membranes, as opposed to polycarbonate membranes, allows the demonstration of the internal morphological characteristics of the organisms. Consequently, staining on cellulose acetate membranes should be incorporated into a hybrid method. Elvanol mounting medium should not be incorporated into a hybrid method. As a water-based medium, it is not compatible with the dehydrated cellulose acetate membrane and does not allow the membrane to be cleared so that the cyst's internal structure can be visualized by contrast microscopy.

Removal of Giardia *and* Cryptosporidium *through Conventional Water Treatment and Direct Filtration*

A general observation about removal of seeded *Giardia* and *Cryptosporidium* was made that was valid in both pilot- and full-scale plant throughout the entire seeding studies. Consistent removal rates of *Giardia* and *Cryptosporidium* were achieved, when the treatment plant was producing water of consistently low turbidity (0.1-0.2

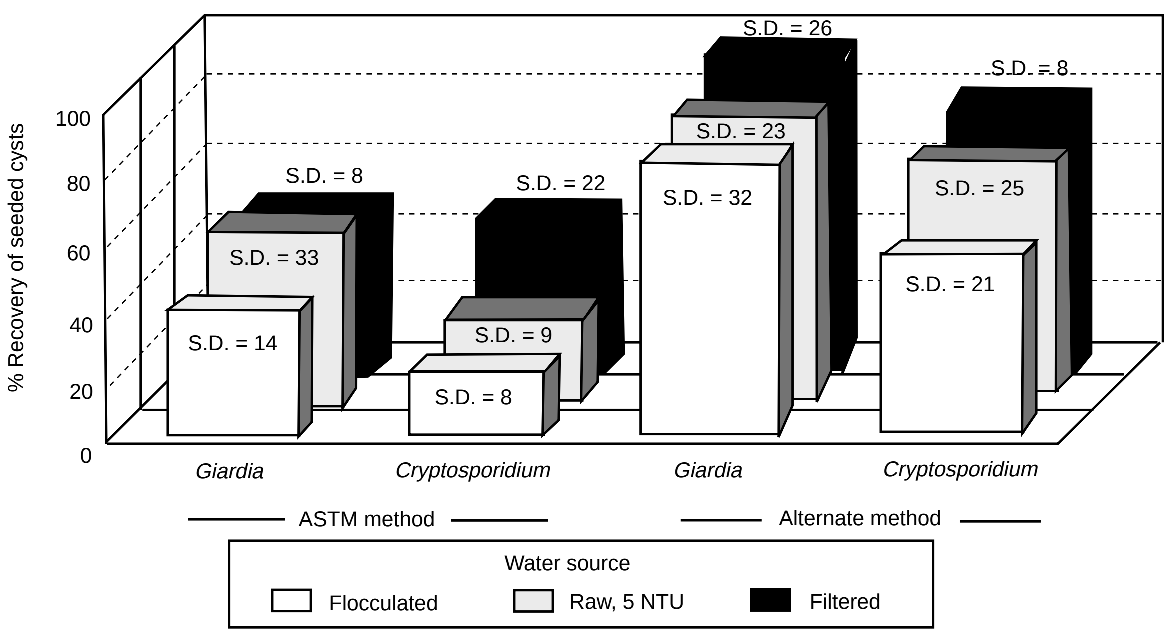

Figure 2. Water quality vs. recovery of cysts seeded into flotation gradients.

NTU). As soon as the plant's performance changed, and resulting filtered water turbidity fluctuated, a high variability in cyst concentrations was detected in collected samples.

Removal of *Cryptosporidium* was compared with removal of *Giardia* by both methods of treatment, conventional and direct filtration, at both the full-scale plant, and the pilot plant. Trials at the full-scale plant were impacted by the change in seasonal water temperature and algal content. Because of the need for a construction to bypass the sedimentation basin, the first four trials by conventional treatment were conducted from June through September, while the four trials using direct filtration were conducted in November and December. Greater flexibility of the pilot plant allowed trials by both treatment methods to be conducted within one day of each other and enhanced not only the comparison between removal of *Cryptosporidium* with *Giardia* removal, but also the comparison between conventional treatment and direct filtration, as well as the comparison of cyst removal with removal of other water quality indicators.

Removal of Seeded *Giardia* and *Cryptosporidium* During Pilot-Scale Seeding Trials

Table 1 summarizes cyst removal rates calculated based on cyst concentration in seeded influent (after mixing the cysts with the incoming raw water). These removal rates, based on seeded influent concentration, were consistently lower than the removal rates based on cyst concentrations in seeding solution (indicating 99.99% or 4 log removal for both *Giardia and Cryptosporidium* regardless of the treatment mode). Calculations of cyst removals, observed during seeding experiments, can also be highly impacted by measurements of cyst concentration in filter effluent samples. When cysts were not detected in filter effluent samples, their concentration could be estimated based on analytical detection limits, determined for each sample batch. Such estimates lead to underestimation of cyst concentration in finished water samples and in turn, result in overestimation of calculated cyst removal rates.

Table 1. Removal of *Giardia* and *Cryptosporidium* Through Conventional Treatment and Direct Filtration at Jordan Valley

		Giardia removal		*Cryptosporidium* removal	
Trial No.	Date	Percent removal	Log removal	Percent removal	Log removal
Conventional treatment					
1-C	4/27/93	ND	ND	99.65	2.81
2-C	5/11/93	99.16	2.20	98.66	1.94
3-C	5/25/93	ND	ND	99.87	2.94
4-C	6/8/93	99.98	3.90	99.95	3.98
5-C	6/22/93	ND	ND	ND	ND
6-C	7/6/93	99.95	3.69	99.88	2.94
7-C	7/20/93	99.95	3.69	99.45	2.64
8-C	8/4/93	ND	ND	ND	ND
9-C	8/17/93	99.91	3.03	99.69	2.84
10-C	8/31/93	99.98	3.90	99.96	3.78
Average log removal			3.40		2.98
Standard deviation			0.67		0.64
Direct Filtration					
1-D	4/29/93	ND	ND	99.95	3.60
2-D	5/13/93	ND	ND	ND	ND
3-D	5/28/93	99.78	2.90	92.06	1.31
4-D	6/15/93	ND	ND	99.96	3.78
5-D	6/23/93	ND	ND	ND	ND
6-D	7/8/93	ND	ND	ND	ND
7-D	7/22/93	99.90	3.00	99.80	2.90
8-D	8/5/93	ND	ND	ND	ND
9-D	8/19/93	ND	ND	99.92	3.31
10-D	9/2/93	99.99	4.00	99.84	2.93
Average log removal			3.30		2.97
Standard deviation			0.77		0.89

ND indicates that cysts were not detected in filter effluent.

Removal of Seeded *Giardia and Cryptosporidium* During Full-Scale Seeding Trials

Table 2 presents a summary of results and removal rates calculated only from the trials where cysts were detected both in influent and effluent in the full-scale plant. Similar to the pilot-scale experiments, the removal of *Giardia* and *Cryptosporidium* can be overestimated when calculations are based on estimated effluent concentrations. Removal rates based on cyst concentrations detected and enumerated both in influent and effluent can be considered conservative.

Several factors impacted the results of the full-scale seeding trials, which made the comparison between conventional treatment and direct filtration more dependent on uncontrolled variables. Changes in raw water quality, observed from the time the plant was in operation by the conventional mode, compared to raw water quality during operation by the direct filtration mode, influenced removal rates more than the mode of treatment. The water was treated in the conventional plant during summer, when treatability was more difficult, while direct filtration was used in late fall, when the water was easier to treat. The presence of prolific algal blooms in samples collected during the first four trials, and the lack of algal content in samples from the last four trials, was another variable making the comparison of removal data problematic.

The results of the pilot-plant experiments indicate that *Giardia* cysts were removed more effectively than were *Cryptosporidium* oocysts. This observation was valid regardless of the treatment mode. The difference between log removals of *Giardia* and *Cryptosporidium* ranged from 0.1

Table 2. Removal of *Giardia* and *Cryptosporidium* Through Conventional Treatment and Direct Filtration at Huntington

		Giardia removal		*Cryptosporidium* removal	
Trial No.	Date	Percent removal	Log removal	Percent removal	Log removal
Conventional treatment					
1-C	6/11/92	99.95	3.7	99.60	2.78
2-C	7/7/92	ND	ND	99.05	2.07
3-C	8/5/92	ND	ND	97.87	1.89
4-C	10/6/92	99.66	2.82	ND	ND
Average log removal			3.26		2.25
Standard deviation			0.67		0.47
Direct Filtration					
1-D	11/10/92	99.97	3.87	99.75	2.88
2-D	11/20/92	ND	ND	99.82	2.92
3-D	12/8/92	99.97	3.87	99.37	2.57
4-D	12/22/92	ND	ND	ND	ND
Average log removal			3.87		2.79
Standard deviation			0.00		0.19

ND indicates that cysts were not detected in filter effluent.

to 1.1 log and averaged 0.3 log, as calculated across all seeding runs. The difference between cysts and oocysts removals were even more pronounced in the full-scale plant than those observed in the pilot plant.

Among the seeding trials, where the cysts were detected both in influent and effluent, the following average removals were calculated for the pilot plant runs:

- average removal of *Giardia* through conventional treatment: 3.40 log; S.D. = 0.67
- average removal of *Cryptosporidium* in conventional treatment: 2.98 log; S.D. = 0.64
- average removal of *Giardia* through direct filtration: 3.30 log; S.D. = 0.77
- average removal of *Cryptosporidium* through direct filtration: 2.97 log; S.D. = 0.89

Similarly, the following average removals were reported from the full-scale seeding trials:

- average removal of *Giardia* through conventional treatment: 3.26 log; S.D. = 0.67
- average removal of *Cryptosporidium* in conventional treatment: 2.25 log; S.D. = 0.47
- average removal of *Giardia* through direct filtration; 3.87 log
- average removal of *Cryptosporidium* through direct filtration: 2.79 log; S.D. = 0.19

Taking into consideration that the reported removal rates of *Giardia* and *Cryptosporidium* were calculated very conservatively, the values presented above can be interpreted as expected removals, resulting from a consistent performance and steady operation of the treatment plants.

Surrogate Parameters for Evaluation of Giardia *and* Cryptosporidium *Removal*

Both surface water sources treated in the treatment plants were characterized by a high quality water, with low levels of inorganic, organic, and microbial contamination. Both sources, however, were tested positive for both *Giardia* and *Cryptosporidium* cysts, confirming previous hypothesis and observations about these pathogens being ubiquitous in surface waters.

Both effectiveness and consistency of removal of seeded *Giardia* and *Cryptosporidium* cysts depended on the effectiveness and consistency of the removal of turbidity. When raw water turbidity was high and it could not be removed by direct filtration, resulting removals of seeded cysts were low and inconsistent. On the other hand, if treatment by direct filtration consistently produced low turbidity effluent, resulting cyst removals were comparable to those achieved from conventional treatment.

The results of seeded cyst removals, generated throughout the study regardless of treatment mode, were compared with the respective results from particle counting, turbidity measurements, and heterotrophic bacteria counts. Correlation between *Giardia* and *Cryptosporidium* cyst removal and removal of these potential surrogates are presented in Figures 3 through 5.

The analysis of correlation between cyst removal and particle removal was performed separately for *Giardia* cyst and cyst-size particles, and for *Cryptosporidium* oocysts and oocyst-size particles (Figure 3). High correlation was reported between both sets of data. A correlation coefficient of 0.82 was calculated ($p<0.1$) for the relationship between *Giardia* cyst removal and removal of particles ranging between 7 µm and 11 µm. Similarly, a correlation coefficient for the relationship between *Cryptosporidium* oocyst removal and removal of 4 µm to 7 µm particles was 0.79.

The results indicated that particle counting could serve as a reliable indicator of cysts and oocysts removal. Particle counters, even though capital intensive, are cheap to operate and are more sensitive than the assays used in *Giardia* and *Cryptosporidium* analyses.

Much lower correlation was established between removals of *Giardia* and *Cryptosporidium* and removal of turbidity (correlation coefficients of 0.64 and 0.55, respectively). As presented in Figure 4, log removal of turbidity can be used as an indicator of cyst and oocyst removals, but with lower accuracy than particle counting. The most pronounced differences between removal of *Giardia* and *Cryptosporidium* and the expected removals of turbidity were observed, when very high cysts removals (4-log) were reported.

Heterotrophic plate count was not shown to be a surrogate in evaluation of cyst removals (Figure 5). No correlation was found between log removal of seeded cyst and log removal of HPC. Despite the fact that the filters in both pilot- and full-scale plants were backwashed with chlorinated water, a growth of heterotrophic bacteria was reported in the filters during the seeding experiment and plant run.

Plant performance evaluation using particle counting and turbidity measurement can be an effective tool in evaluating expected removals of *Giardia* and *Cryptosporidium*. The search for a biological surrogate for *Giardia* and *Cryptosporidium* should continue and result in identification of a parameter that defines both occurrence and removal of *Giardia* and *Cryptosporidium*. The results of the study coincide with previously reported relationships between cysts and particulates. Effective removal of *Giardia* and *Cryptosporidium* from the water would, however, require treatment plants to consistently produce very low turbidity (0.1-0.2 NTU)—much lower than the levels currently required.

Conclusions and Recommendations

Analytical Procedures for Detection of Cysts in Water

Based on the results generated during the evaluation of the effectiveness of the IFA methods in enumeration of *Giardia* cysts and *Cryptosporidium* oocysts, it was concluded that the alternate IFA method was more suitable for meeting the project objectives. This method employed sam-

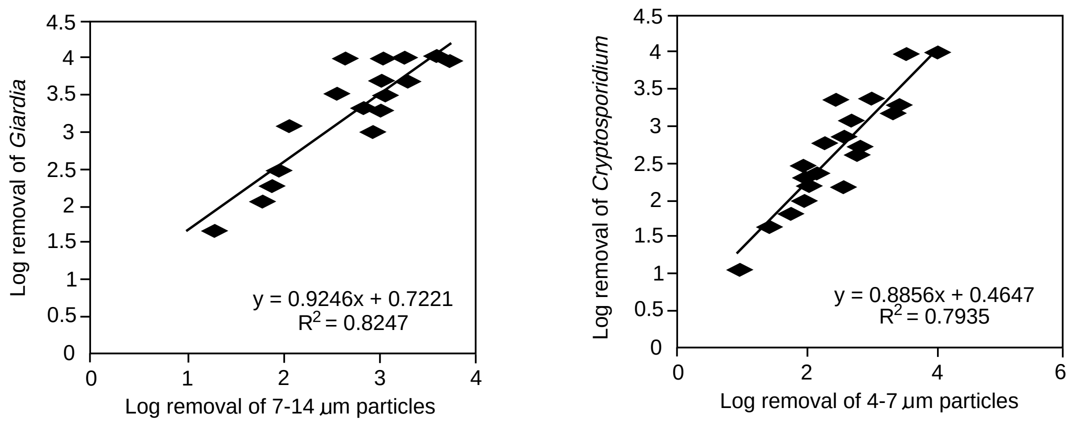

Figure 3. Relationship between removal of cysts and particles.

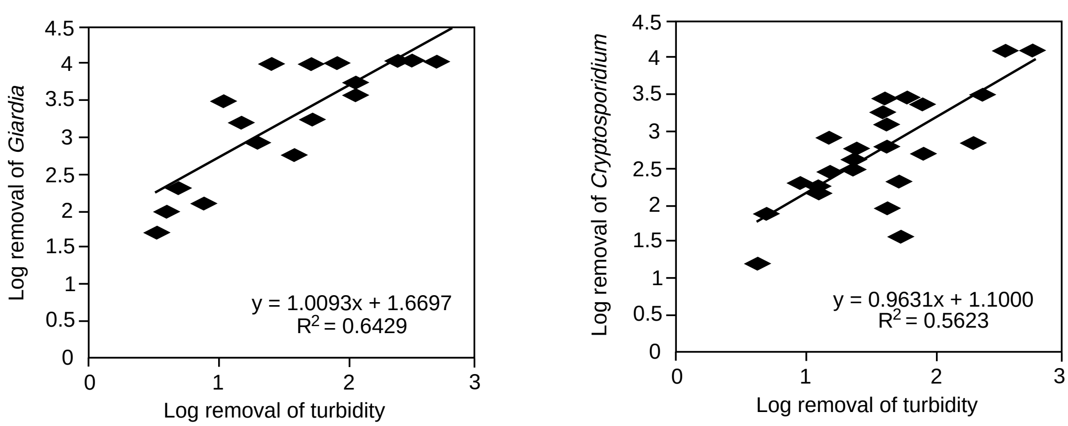

Figure 4. Relationship between removal of cysts and turbidity.

pling through a 2.0 µm polycarbonate membrane filter, centrifugation in 15 mL tubes, flotation on a two-step Percoll/ Percoll gradient, IFA staining on 13 mm diameter, 2.0 µm pore-size polycarbonate membrane filters, and enumeration under an UV epifluorescent microscope. The following conclusions were formed:

- The membrane filtration for low turbidity samples outperformed the polypropylene yarn sampling method in terms of recovery efficiency of seeded organisms.
- The Percoll/Percoll step gradient had better recoveries of seeded organisms then the Percoll/sucrose gradient. Since 15 mL tubes were used in place of 50 mL tubes, the procedure of the step gradient flotation was more economical.
- The alternate method has proven more effective in recovering seeded cysts, and therefore, was considered more suitable in parasite seeding experiments, where evaluation of water treatment process efficiencies was conducted using high concentrations of seeded cysts.

The ASTM method, employing staining on cellulose acetate membranes, had the advantage since the gradients could be cleared and the internal structure of the organisms could be visualized under the phase- or differential-interference contrast microscopy. The ASTM method, due to its ability to confirm presumptive cysts and distinguish between algal cells and the cysts by contrast microscopy, was found very applicable in testing of the environmental water samples.

Based on the above results, it is recommended that the alternate method should be used in evaluating water treatment process efficiencies using high concentrations of seeded parasites. The ASTM method is recommended in analyses of environmental samples where the confirmation step is essential.

A hybrid method, combining the most efficient steps from the two methods, should be investigated. The membrane filtration for low turbidity samples, which far outperformed the polypropylene yarn sampling method in terms of recovery efficiency of seeded organisms, shows promise in sample collection. Similarly, Percoll/Percoll step gradient, used in the alternate method, had better recoveries of seeded organisms then the Percoll/sucrose gradient. Since 15 mL tubes were used in place of 50 mL tubes used in the ASTM method, the procedure of the step gradient flotation was more economical. On the other hand, staining on cellulose acetate membranes used in the ASTM method, had the advantage since the gradients could be cleared and the internal structure of the organisms could be visualized under the phase- or differential- interference contrast microscopy.

The results of the testing of IFA method effectiveness have indicated that *Giardia* cysts and especially *Cryptosporidium* oocysts are lost during the gradient flotation steps of both methods. Recovery efficiencies increased dramatically in samples, did not contain much debris, and could be processed without the flotation steps and stained directly on membranes. Therefore, it is recommended that the flotation step should be avoided when processing treated (filtered) water samples whenever possible.

Removal of Giardia *and* Cryptosporidium *through Conventional Treatment and Direct Filtration*

The following conclusions were formed from the pilot- and full-scale study on *Giardia* and *Cryptosporidium* cysts removal through conventional treatment and direct filtration:

- In a properly operated treatment plant effectively removing turbidity to 0.1-0.2 NTU, either conventional treatment or direct filtration can result in a 3-log removal of *Giardia*.
- *Cryptosporidium* oocysts are more difficult to remove than *Giardia* cysts,

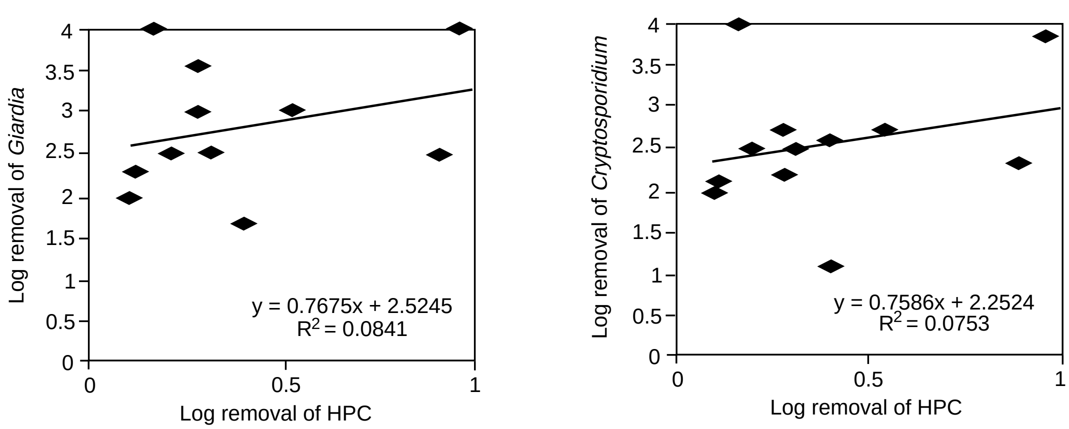

Figure 5. Relationship between removal of cysts and heterotrophic bacteria.

both in a conventional plant and through direct filtration (up to 1.0-log difference).

- Removals of cyst-size particles and removal of turbidity can be used as indicators of cyst removal effectiveness.

A general observation about removal of seeded *Giardia* and *Cryptosporidium* cysts was made that was valid in both pilot- and full-scale plant throughout the entire seeding studies. Both effectiveness and consistency of removal of seeded *Giardia* and *Cryptosporidium* cysts depended primarily on the effectiveness and consistency of the removal of turbidity. When treatment by direct filtration consistently produced low turbidity effluent (0.1-0.2 NTU), the resulting *Giardia* and *Cryptosporidium* cyst removals were consistent and comparable to these achieved from conventional treatment. As soon as the plant's performance changed, and resulting filtered water turbidity fluctuated, a high variability in cyst concentrations was detected in collected samples. When raw water turbidity was high, and it could not be removed by direct filtration, resulting removals of seeded cysts were low and inconsistent.

A high correlation coefficient was calculated for the relationship between *Giardia* cyst removal and removal of particles ranging between 7 µm and 14 µm, and similarly, for the relationship between *Cryptosporidium* oocyst removal and removal of particles of 4 µm to 7 µm in size. Much lower correlation was established between removals of *Giardia* and *Cryptosporidium* and removal of turbidity. Heterotrophic plate count was not shown to be a surrogate in evaluation of cyst removals, with no correlation found between log removal of seeded cyst and log removal of HPC.

A combination of particle counting and turbidity measurement was shown to be an effective tool in water treatment plant performance evaluation in terms of predicting removals of *Giardia* and *Cryptosporidium*. Effective removal of *Giardia* and *Cryptosporidium* from the water would, however, require treatment plants to consistently produce very low turbidity (0.1-0.2 NTU)—much lower than the levels currently required. The results of the study indicate that the removal of particulates, measured through particle counting and turbidity monitoring, should be a critical factor used in the evaluation of plant performance in *Giardia* and *Cryptosporidium* removal. Continuous and consistent removal of particulates should be monitored by continuous particle counting and turbidity monitoring.

The results of the project imply that the credits given for *Giardia* cyst removal in direct filtration plants, may be similar to credits obtained in conventional treatment plants, and also may be higher than the credits applicable under the current regulations. Since *Cryptosporidium* is more difficult to remove than *Giardia,* and it is also more resistant to disinfection than *Giardia*, new requirements need to be developed to control this pathogen. Finally, due to the need of further defining the credits given to treatment plants for physical removal of *Giardia* and *Cryptosporidium*, a study on evaluation of removal of these pathogens through pre-sedimentation should be conducted.

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The complete report, entitled "Removal of Cryptosporidium *and* Giardia *through Conventional Water Treatment and Direct Filtration," (Order No. PB97-162507; Cost: $35.00, subject to change) will be available only from:*
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