Department of Environmental Protection

DEP Pathogen Studies
on *Giardia* spp. *and Cryptosporidium* spp.

Protocol For Testing Equivalency Of Continuous Backwash, Upflow Dual Sand Filter With Microfiltration

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1. Background Information

1.1 Introduction

Preventing the discharge of pathogens from wastewater treatment plants (WWTP) is a key component to filtration avoidance. This is especially pertinent with regard to the parasitic protozoans *Giardia* and *Cryptosporidium*. Both these organisms have been identified as significant contributors to waterborne outbreaks of gastrointestinal disease. These organisms are also resistant to conventional disinfection through chlorination. With this in mind, NYCDEP with technical assistance from EPA and NYSDOH developed this protocol to evaluate the adequacy of various technologies in removing these organisms from wastewater.

Within the past three years, agreement on NYCDEP regulations for WWTPs has been finalized. Incorporated in the New York City watershed rules and regulations is the requirement for all wastewater treatment discharges to be treated with microfiltration or its equivalent. As such, it is important to establish a criteria by which to evaluate equivalent technologies. This criteria is dependent on generating information that is as precise and accurate as possible.

1.2 Previous DEP Studies of Wastewater Filtration

Prior to the development of this protocol, DEP performed three series of Giardia cyst and Cryptosporidium oocyst[1] challenge tests on two advance treatment technologies. The knowledge gained from these experiments improved our understanding of the limitations of the protocols used. This information was then used to improve the methods presented in this document. The objectives of these tests were to evaluate the performance of each individual technology and compare these performances to determine “equivalency” between technologies in removing (oo)cysts. More detailed descriptions of these studies and their results are presented In separate reports.

Two (oo)cyst spike challenge tests occurred on a pilot microfiltration plant in June 1993 at the Brewster WWTP. From March to May, 1995 ten (oo)cyst spike challenge tests occurred with a continuous backwash, upflow dual (CBUD) sand filter at the Delhi WWTP. The third series of tests occurred with two types of continuous backwashing dual sand filtration systems at the Stamford WWTP. These last tests were followed by ten weeks of intense monitoring to evaluate performance under routine (no spike) conditions.

Based on the results from these tests, EPA determined that more information was needed to adequately compare the “equivalency” between technologies. Concerns were raised that tests to determine microfiltration equivalency must be conducted under similar conditions, at the same time, with identical methods. In addition, there was consensus that “equivalency” needed to be defined in statistical terms. Accordingly, the protocol described in this document is for a study

1.*Giardia* cyst and *Cryptosporidium* oocyst will be referred together as (oo)cysts.

that compares the (oo)cyst removal capabilities of microfiltration and CBUD sand filtration by operating a pilot facility of each system, side by side, with the same influent, weather conditions and testing methods. The sampling and test design was worked out jointly by DEP, DOH and EPA based on statistical test requirements set forth by EPA's statistical consultant.

1.3 Objective

As indicated above, this protocol provides the methods needed to generate a data set to meet the statistical design criteria and test hypotheses set forth by EPA for testing log removal equivalency of CBUD sand filtration with microfiltration. The protocol focuses on the following specific objectives:

- Conduct enough experiments to generate a data set that provides the most significant differences between the two systems with a high degree of confidence.
- Measure the log removal capabilities for (oo)cysts by a CBUD sand filter and a microfilter under similar conditions.
- Measure the level of the (oo)cysts expelled by the two systems in backwash to confirm the effectiveness of each technology qualitatively.

1.4 Definition of Microfiltration Equivalency

The earliest discussions regarding the determination of equivalency involved agreement on its definition. The formulation of the definition began with using log removal as a measure of difference between the two systems. Using this measure is consistent with EPA's Enhanced Surface Water Treatment Rule (ESWTR) requirements for Giardia which are based on log removal capabilities. With the beginning of this study, it became clear that just comparing log removal was inadequate without defining how far apart the log removal differences need to be to consider them significantly different. Also, there was a need to set the level of confidence that such a difference was detectable.

With the beginning assumption (based on the review of the data generated from past studies),that the two systems would produce log removals in the range of 4 logs, EPA, NYSDOH and DEP agreed on the following criteria as a starting point:

(1) a significant difference in log-removal would be 0.5 or more (e.g., 3.5 vs. 4.0 log removal)
(2) 95% confidence that 0.5 log difference was detectable

These initial criteria provided the statistician with the information needed to determine the statistical tests that would be used in the study and number of tests that would be needed to meet the confidence level. All participants agreed that the criteria may change based on the evaluations of the data generated from this study.

With initial agreement on the statistical parameters, formulation of the hypotheses to be tested in the study could be presented. Accordingly, the hypotheses tested are:

Null hypothesis: The treatment systems are equivalent or the log removal of the CBUD sand filtration system is greater or equal to the microfiltration system.

Alternative hypothesis: The log removal of CBUD sand filtration system is less than that of the microfiltration system.

Since the study was designed so that the influent concentrations of each system were as equal as possible, the log removal differences were dependent only on effluent concentration differences . Therefor the equivalency test was a test of equal effluent concentrations.

More detailed information on the statistical basis of the study is provided in a separated report prepared for EPA by Research Triangle Institute.

1.5 Studies on (oo)cyst filtration in the literature

Several studies have been reported in the literature which involve spiking a treatment system with a known concentration of *Cryptosporidium* oocysts and/or *Giardia* cysts to test its removal effectiveness. Table 1.1 provides a review of these published reports. Most of the studies involve sand filtration of drinking water. However, there have also been spiking studies on the effects of soil and pool filter sand on reducing *Cryptosporidium* oocysts and/or *Giardia* cysts. Wastewater was evaluated in one report. Removal performance for sand filtration of drinking water was evaluated in one study using particle counts as a surrogate for *Cryptosporidium* oocysts and *Giardia* cysts.

The concentration of spike material ranged from 50 to 1.0×10^9 (oo)cysts/L with an arithmetic mean concentration of 2.3×10^8 (oo)cysts/L. The reports did not provide information on the duration of the spikes. There was an implied assumption that the spike material traveled as a uniform slug. Most studies did not report on whether the (oo)cysts were live or inactivated with a preservative. The three reports that did report on (oo)cyst preservation indicated formalin as the preservative used. One study used heat to inactivate the *Cryptosporidium* oocysts.

Table 1.1. Review and summary of studies evaluating treatment plant performance in removing *Cryptosporidium* oocysts and *Giardia* cysts.

Reference	Filter type/ Water sampled	Pathogen Exam-ined+	Spike Concentration	Preservation	Sampling points	Analysis method	Recovery	%detect or Log removal
Al-Ani et.al. (1986)	Raw water	G[a]	500 cysts/L to 5000 cysts/L	n/a	Inf.,Eff.	non ICR	80 % to 99.9 %	99.9%
Bellamy et.al. (1985)	Finished water	G	50cysts/L to 5075cysts/L	n/a	Eff.	non ICR	n/a	99.9%
Bellamy et.al. (1993)	Finished water	Particles	n/a	n/a	Eff.	Particle counter	n/a	G size 2.0 log C size 2.0 log
Chapman & Rush (1990)	Pool filter sand	C[b]	10^6 oocysts/mL	n/a	Eff.	IFA[g]	assumed 100 %	top layer of sand in filter
Fogel et.al. (1993)	Raw and finish water	G & C	n/a	n/a	Inf., Eff	ICR[f]	G mean 93 % C mean 48%	G: 89% to 96% C: 27% to 68 %
Hansen & Ongerth (1991)	Raw water	C	n/a	n/a	raw water	ICR	18.6 % - 34.3%	21.6-34.3%
Horn et.al (1988)	Raw and finish water	G	46,000 to 1,500,000	n/a	Inf.,Eff	non ICR	n/a	99%
Lechev. et.al. (1995)	Raw water	C & G	n/a	Formalin	n/a	IFA	42 - 89% 9 - 95 %	G 68.6 % C 25.3%
Lechev.& Norton (1995)	Raw and fin-ished water	C & G non spike	n/a	n/a	Inf., Eff.	IFA	G 42.4 % C 23.6 %	G: R[e]50.1 F[h]42.4 C: R40.9 F23.6
Mawdsley et.al. (1994)	Soil	C	$5x10^8$ oocysts g^{-1}	n/a	soil core	non ICR	61.6 %	most in top two cm of soil
Nieminski & Ongerth (1995)	Finished water	C & G	$5x10^6$ to 4L of filtered water	G: F[c] C: H[d]	Inf., Eff.	IFA	20 %	G 3.4 & 3.26 C 2.98 &2.25
Ongerth & Pecoraro (1995)	Raw and finish water	C	10^7 in 1.5 L	n/a	Inf., Eff	ICR	C 47 % G 60 %	C2.7&3.1 log G3.1&3.5 log
Patania et.al. (Undated)	Raw and finish water	G & C	n/a	F	Inf., Eff	ICR	n/a	50%
Villacorta - Martinez et.al. (1992)	Waste water (activated sludge)	C	$2.5x10^8$/L $4.0x10^8$/L	n/a	lab test	non ICR	n/a	82 to 99% reduction in infection

Notes: a - C represents Giardia.; b - G represents Cryptosporidium.; c - F represents preservation with Formalin.; d - H represents fixed with heat.; e - R represents results from raw water sample.; f - ICR represents the use of methods similar to those described in EPA's ICR (including ASTM P229 method).; g - IFA represents the use of an Immunofluorescent assay without information to determine whether this method was similar to the ICR method.; h - F represents results from finished water sample.

The most often used methodologies for detection of *Cryptosporidium* oocysts and *Giardia* cysts in the studies were the techniques described in EPA's Information Collection Rule (ICR) such as proposed standard method P229. Part of ICR method uses an Immunofluorescent dye assay (IFA)

Most of the reports evaluated the efficiency of pathogen removal (one possible test of equivalence) by the percent detection of *Cryptosporidium* oocysts and *Giardia* cysts seen at the effluent of the system tested. Three studies evaluated performance by calculating the log removal between the influent and effluent of the facility (a second possible test for equivalency). The eight studies that evaluated performance by percent detection indicated a range of detections from 21.6% to 99.9%. Average detection in the effluent of the facilities tested in these studies was 59.1% for *Cryptosporidium* oocysts and 72.5% for *Giardia* cysts. The three studies that evaluated performance with log removal indicated a range of log removals from 2.0 to 3.5. Average log removals were 2.6 for *Cryptosporidium* oocysts and 2.7 for *Giardia* cysts.

2. Evaluation of Problems With Past Studies and Solutions

Table 2.1 provides a review of the problems and their causes as identified during past DEP studies. The table also offers the solutions for these problems which were implemented in this protocol.

Table 2.1. Review of problems and proposed solutions to meet objectives of project.

Problems	Causes	Solutions
1. Recovery	Standard Method has low recovery. Even lower recover for high concentration samples.	Use materials that (oo)cysts do not adhere to. For high concentration samples, use direct count method.
	Sampling only portion of flow. (oo)cysts may not be well mixed.	Collect aliquot from well mixed injection vessel instead of influent stream.
	Collecting filter leakage	Use absolute pore filters.
2. Lack of breakthrough	Spike concentration not large enough to overcome treatment.	Spike with maximum doses of (oo)cysts (i.e., 10^8). Perform dye and (oo)cyst test to determine time of travel. Challenge systems continuously until steady state is reached. Monitor during study state. Monitor log removal of particles and turbidity as surrogates
3. Variable detection limit	Varying volumes collected and portion of packed pellet examined	Collect similar volumes and examine similar portions of pellets.
4. Lack of hydraulic balance with Dual Sand filters	Recycling of backwash from unit 2 to unit 1 extends time of travel through system resulting in larger volumes to be sampled at the effluent.	No recycling of backwash. Both units will discharge backwash.
5. Data can't be compared between studies	Different location Different times Different methods	Conduct study at same location, with similar conditions and identical methods.

3. Protocol

3.1 Location For Protocol Testing

The most appropriate location for testing the equivalency of the CBUDSF with microfiltration was a facility with the following attributes:

(1) located within the New York City water supply watersheds,
(2) with treatment technology that is typically found in the watershed,
(3) a facility where DEP has already been monitoring the effluent (especially for *Cryptosporidium* oocysts and *Giardia* cysts),
(4) where pilot CBUDSF and microfiltration facilities are nearby with trained operators, and
(5)associated with minimal delay to set-up the pilots for testing.

Of the facilities available for testing, the Stamford WWTP offered the best opportunities in conducting challenge testing without delay. This facility has been used in past evaluation studies. The plant operators had a record of providing outstanding support in setting-up, operating and reporting on the previous pilot tests.

Figure 3.1 Photograph of Stamford WWTP with CBUD sand filtration and microfiltration pilot facilities

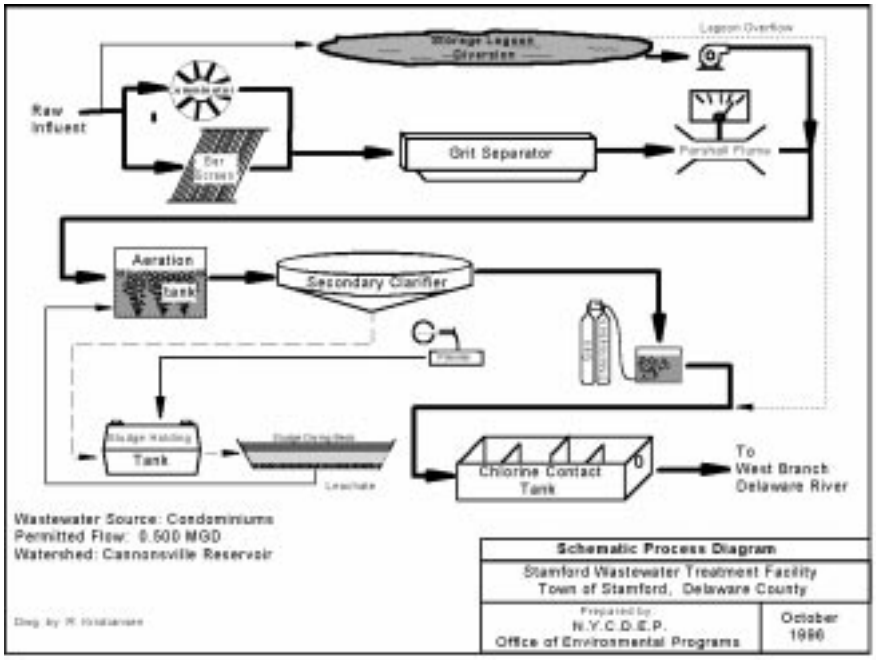


Figure 3.2 Schematic of Stamford WWTP.

3.2 Schedule

The length of the testing was determined by several factors:

1. Number of tests needed to achieve statistical criteria.
2. The frequency of sampling could not be so great as to over-utilize staff.
3. Acquire enough results to meet the statistical requirements in time to comply with the WWTP upgrade schedule as laid out in the FAD and MOA.

Based on DEP's previous studies, there was an initial decision among the three agencies that ten spike tests might be adequate in meeting the statistical test for equivalency. Six spike tests was adequate to indicate a difference between the Parkson and Andritz systems. During the study however, statistical analysis of the first five spikes indicated that more tests were needed. It was determined that the maximum number of additional spike tests that could occur (and still meet schedule requirements and budget constraints) was two.

3.3 Description and Operation of Systems Evaluated

Each of the two filtering systems were fed independently from the same general area of the secondary clarifier. The two supply lines were approximately 12" apart and extended approximately 30" below the water surface (Figure 3.3). The sections to follow describe the general operation of each system. A summary of the operating history of the two systems during the study is provided in appendix C.

3.3.1 Microfiltration

Figure 3.4 depicts the layout of the Microfiltration system (MFS). The system consisted of a self-cleaning 500-micron strainer prior to the Memcor 6M10C Microfiltration unit shown in Figure 3.5. The membrane filtration unit itself consists of a series of six filtration modules which contain numerous polypropylene hollow fiber filter membranes rated at 0.2 micron nominal pore size (Figure 3.6).

Figure 3.3 Demonstration influent drawn from secondary clarifier.

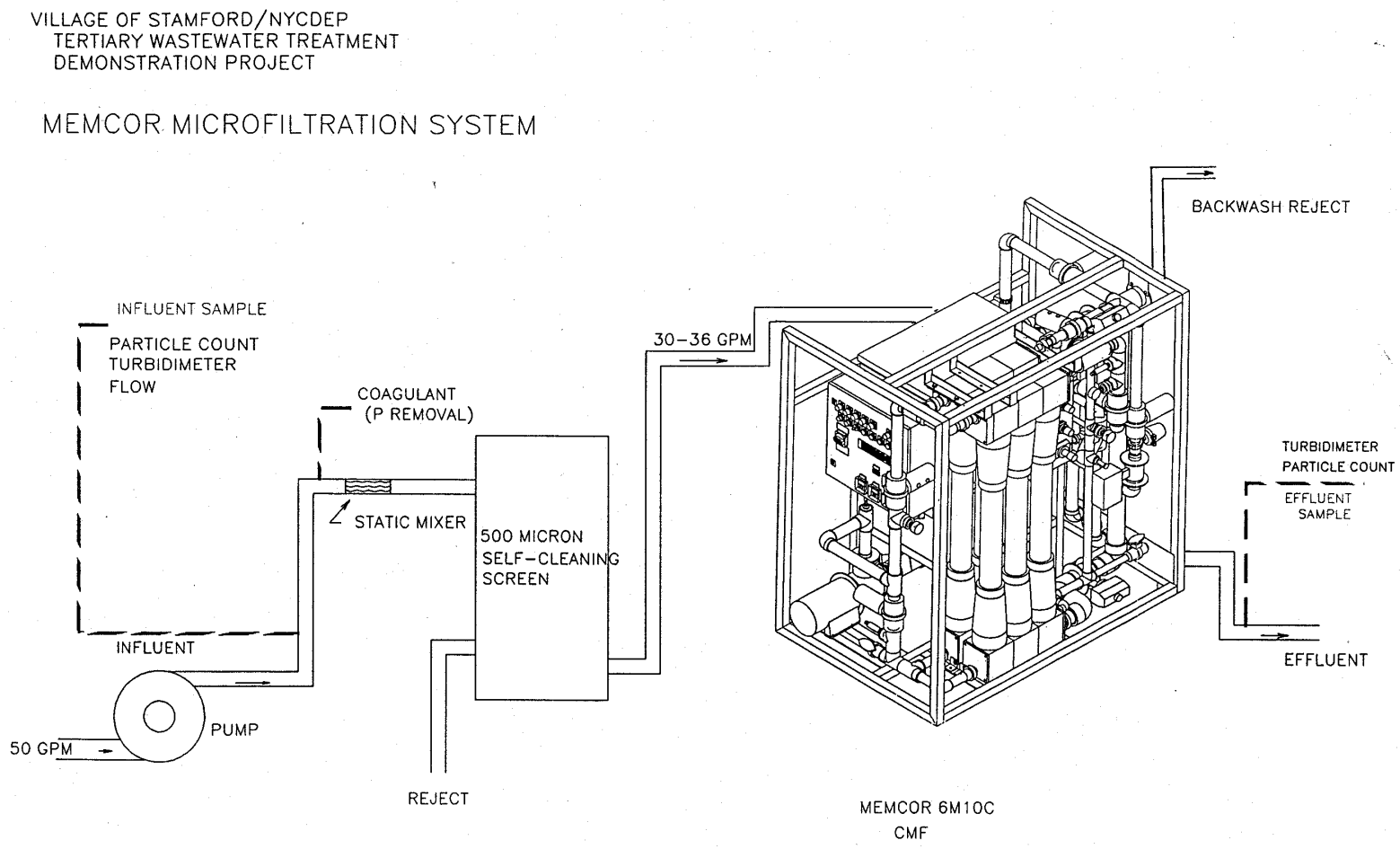


Figure 3.4 **Microfiltration system (MFS).**

Figure 3.5 Microfiltration facility.

Figure 3.6 Microfiltration membranes.

The system was fed by a separate feed pump, delivering in excess of 50 gpm, required to supply both the strainer and the 6M10C's break tank. The MF unit itself was fed from the break tank at between 36 gpm and 30 gpm, depending on operating conditions (e.g. during protozoan spike events, the flow was consistently 36 gpm; at other times the flow varied depending upon

coagulant usage and chemical cleaning cycles). Coagulant addition for phosphorus removal was begun on July 8; the results of the chemical addition program are discussed in a separate document prepared by the Village of Stamford's consultant engineer Delaware Engineering.

Secondary effluent was pumped from the Stamford WWTP's secondary clarifier through the 500-micron screen and into the MFS's break tank, the level of which was controlled by three level switches (e.g. low level: shut unit down; mid level: open influent valve to break tank; and high level: close influent to break tank). The flow was fed to the MFS unit at a constant pressure of 30 psi. The system operates initially at a transmembrane pressure (TMP) of 5 psi. During continual operation, the TMP increases to 15 psi, at which point a Clean in Place (CIP) is manually done. For this study, TMP's up to 18 psi were allowed, with a CIP cycle of 14 days. After the initiation of coagulant addition, the CIP was increased to every 7 days.

The gas backwash cycle was set for 18-minute intervals. Once every 18 minutes, collected feed contaminants on the membrane surface were removed by the automatic gas backwash sequence. During this backwash, the filtrate is drained from the modules, compressed air at 90 psi is injected into the center of the fibers. The air is then released, explosively, when the discharge valve is abruptly opened. The high pressure air causes the hollow membrane fibers to expand and then abruptly collapse, which shakes loose any built-up contaminants. The outside surface of the membranes is then flushed with influent water. The total backwash cycle is approximately two (2) minutes, although this time sequence can be adjusted. The backwash volume ranges between 6% and 16% depending upon the MF's settings.

The integrity of the membranes is verified by the standard "membrane integrity test", which was manually activated daily during the pilot program. The membranes are pressurized to between 15 and 20 psi, and the pressure drop, if any, is recorded for four (4) minutes. A slight drop of 0.1 to 0.2 psi per minute is within the allowable range; a drop of 1.2 psi/minute verifies membrane integrity of 4 log removal, of 0.12 psi/minute of 5 log removal, etc. This is the test which is part of the standard operating procedures of a full scale microfiltration facility.

Another, more sensitive, membrane integrity test is the diffusive air flow (DAF) test. This test is not routinely performed on full scale microfiltration facilities. The DAF test is a measure of the air flow through the wetted membrane at a known air pressure below the bubble point of the membrane. Any defects in the membrane or through leaky O-rings results in higher than calculated air flow through the unit. Comparing a DAF measurement of a fully integral membrane to a field unit will indicate a log removal value for the field unit. The DAF testing apparatus was installed on the pilot unit on June 25 and was used to more accurately assess the integrity of the membrane modules in the pilot unit. Results of these tests during the study are provided in Appendix B.

3.3.2 CBUD Sand Filtration

The CBUD sand filtration facility evaluated consists of two Parkson Corporation Dyna-sand filters in-series identical to the facilities evaluated in the Delhi and Stamford studies (Figure 3.7). Figure 3.8 provides a schematic of the CBUD sand filtration system. The filtering system employs two filtering units: first stage a standard 80" deep bed with 1.4 mm sand; second stage a 40" shallow bed with 0.9 mm sand. Coagulant and sodium hypochlorite are injected prior to the first stage. Both filters have a surface area of 10.7 sq. ft., with influent flows averaging 36 gpm for a filter application rate of 3.36 gpm/ft.2.

From the treatment plant's secondary clarifier, effluent is pumped to the top of Filter #1, open to the atmosphere. The flow passes down through a center tube to a distribution spreader, from where the flow is forced up through the continuously down-moving sand bed to an overflow weir and into Filter #2. The flow through the second stage filter is driven by the head of the first stage, so that no additional pumping is required. Final effluent travels over the overflow weir; effluent samples are collected from a tube just below the second filter's overflow weir.

Continuous sand washing is accomplished by the downward flow of the sand bed, with the dirtiest sand drawn into the airlift pipe Figure 3.9. Compressed air is injected into the bottom of the airlift; the air rises, draws the sand and dirt into the airlift and scours the dirt from the sand as it rises in the airlift. Once the sand and dirt slurry reaches the top of the airlift and spills into the washing compartment, a small amount of filtered water passes upward through the washing compartment, washing the dirt away and allowing the sand to fall onto the downward-moving sand bed. The filtrate weir is set at a higher elevation than the reject weir, thus assuring a positive hydraulic gradient safeguarding the integrity of the filtrate. The air lift controls the circulation time of the sand column, with turnover rates of approximately 4 hours.

Figure 3.7 CBUD sand filtration facility.

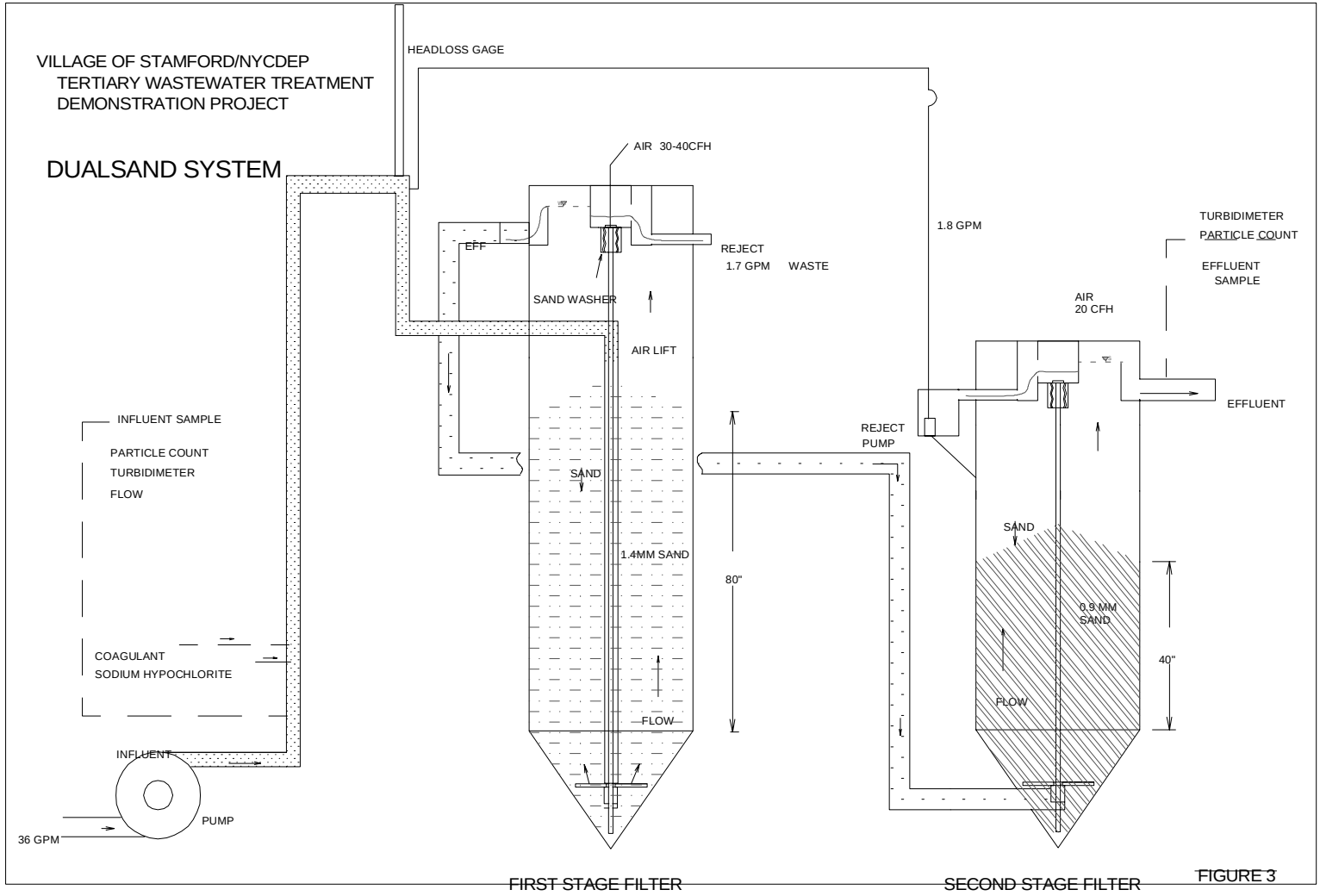


Figure 3.8 Schematic for CBUD sand filtration system.

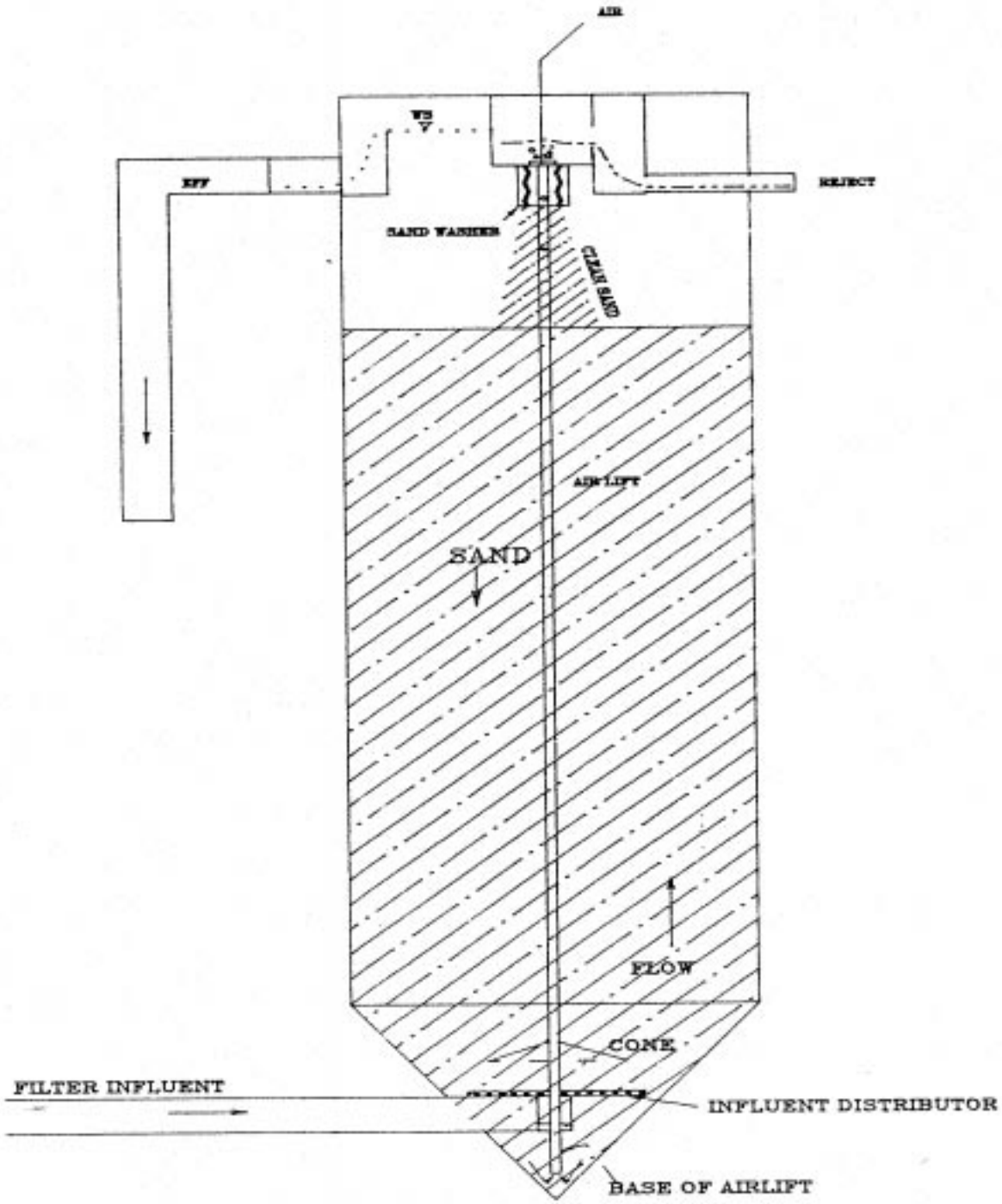


Figure 3.9 Cross-section of Parkson Continuous Backwash, Upflow Sand "Dynasand" filter.

3.4 Preliminary Experiments

The spike challenge tests were preceded by a number of preliminary experiments designed to address several of the shortcomings of the previous spike challenges.

3.4.1 Influent sampling

A series of experiments were undertaken designed to duplicate pathogen injection into the filtration systems to determine whether the direct count method will improve: (1) (oo)cyst recovery for highly concentrated influent samples and; (2) the variability of the influent (oo)cyst concentration over the 100 minute injection time. Using full strength seed material identical to that used in the actual spike tests and using the identical injection protocol, 2 milliliter samples were collected from each glass spiking solution vessel at variable intervals. Due to the high concentration of (oo)cysts in the spike solution, the samples were analyzed using the direct count technique. The results from these experiments were used to determine the actual recovery efficiency and sample variability we could expect when the actual spiking experiments were performed. (Figure 3.10) presents the results of the preliminary experiment (using the direct count method) along with the results from previous studies (using a modified P229 method). The use of the direct count method significantly improved both the recovery and variability of the results. Based upon the data gathered, it was agreed that two 2 milliliter samples collected at 33 minute intervals would be sufficient to determine influent pathogen concentrations.

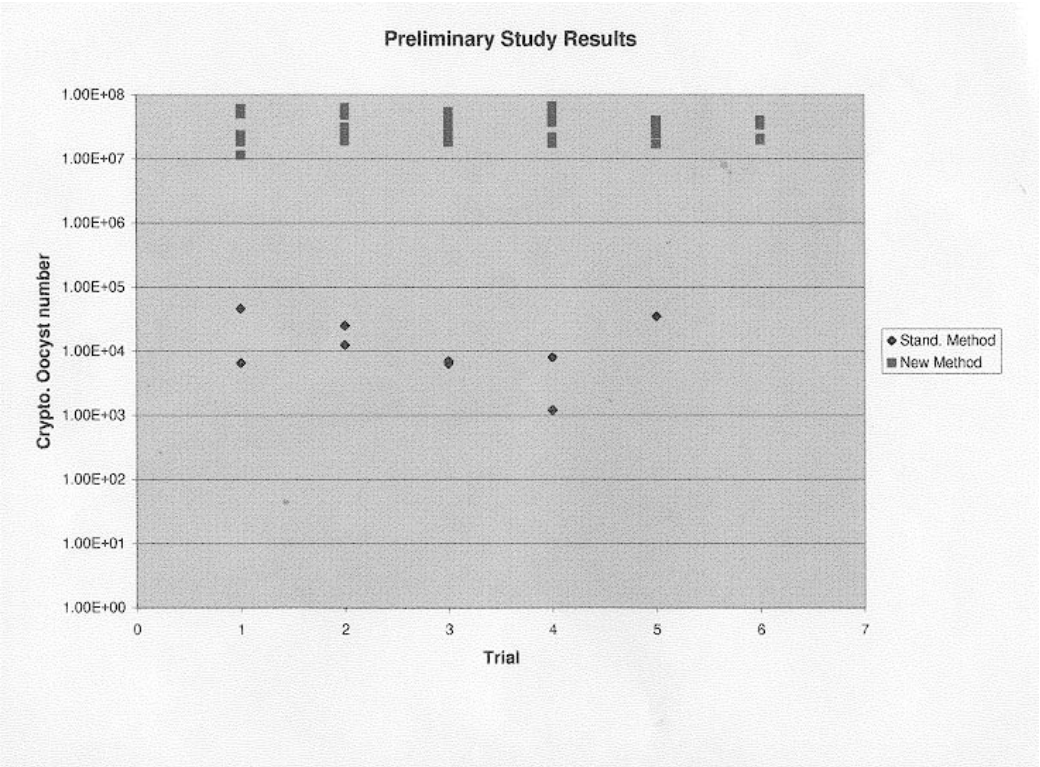


Figure 3.10 Results of preliminary study showing improvements to influent sample recovery and variability.

3.4.2 Time of travel

In order to determine the duration of pathogen sampling at each systems filtered output, a dye study was initiated to accurately measure travel time of a conservative substance through both the CBUD sand filtration and microfiltration systems. Fluorescene dye was injected into each system while system flowrates were identical to the flowrates which were to be used during the spike experiments. Samples were collected once per minute at strategic points in each filtration system and analyzed using a flourometer. A second set of experiments was also performed after the first spike challenge to validate the travel time data for the CBUD sand filtration system collected during the dye study. Since the fluorescene dye injected theoretically behaved as a dissolved solution and not as a set of particles as it travelled through the sand filtration system, it was not known whether or not the (oo)cysts would have the same travel time as the fluorescene dye.

Of particular interest was whether or not (oo)cysts were shortcircuiting through the CBUD system prior to sampling. To address this issue, spike solution was injected as per the normal protocol and effluent samples were collected at 10 and 15 minute intervals 15 minutes into the spike (Figure 3.11). Backwash samples were also collected. Figure 3.12 provides the graph of the results of these tests. The resultant data collected here indicated that the *Cryptosporidium* oocysts broke through the system earlier than the dye. *Giardia* cysts were able to break through the filter. Based upon all of the data collected, spike injection time was set at 100 minutes and effluent sample collection time was set at 80 minutes to ensure the effluent was tested under study state conditions (Figure 3.13).

Following this series of pre-experiments designed to address the various shortcomings of the previous spike experiments, the final series of twelve spike challenge experiments and subsequent intensive baseline sampling was initiated.

Figure 3.11 Collection of discrete samples to determine (oo)cyst time of travel through CBUD sand filtration system.

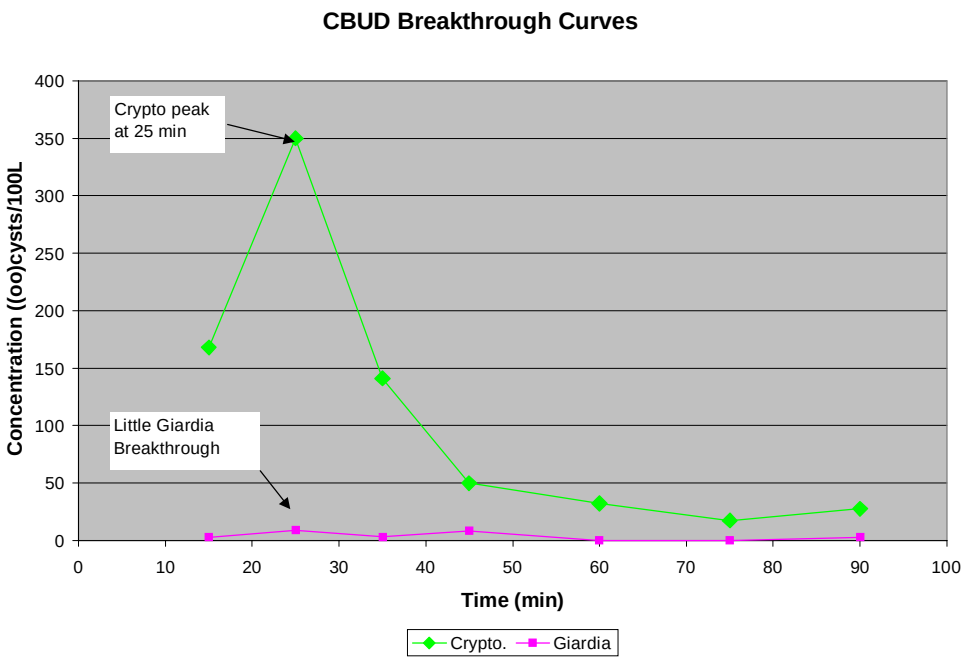


Figure 3.12 CBUD breakthrough curves.

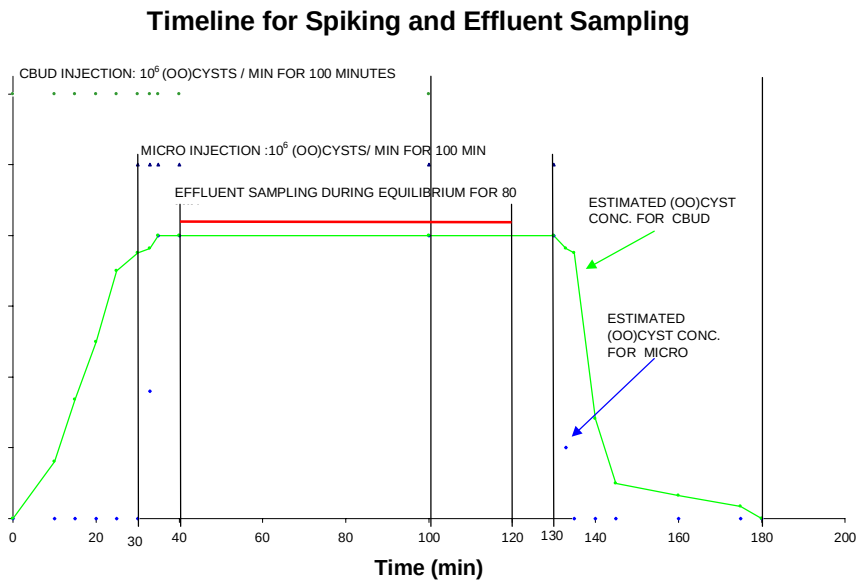


Figure 3.13 Timeline for spiking and effluent sampling.

3.5 Testing Approach

3.5.1 Efforts to reduce (oo)cyst loss

Improvements were made to the (oo)cyst injection system and tested. Based upon suggestions from NYSDOH staff stemming from pathogen research being conducted by that department, the composition of the vessel containing the pathogen spike solution was changed from low density plastic to glass. In addition, the spike solution injection line composition was changed from a low density polyethylene plastic to a higher density polypropylene plastic since the use of glass here was impossible (Figure 3.14). These two modifications addressed the tendency of the (oo)cysts adhere to or adsorb into the walls of lower density plastics. These modifications improved the delivery of the entire spike solution to both filtration systems with minimal loss. The sampling equipment utilized in the field was also improved by mounting all of the interrelated pathogen sampling components onto a single board. This improved our ability to monitor the status of the pathogen sampling and filtration. Also tested and implemented were the use of diaphragm pumps to inject the pathogen seed material into each respective filtration system. This modification allowed us to inject each system with spike material with maximum precision which allowed for identical pathogen loading on each system for the duration of each spike experiment. Finally, 1 micron absolute rated filters were adopted for effluent filtration due to their superior particle retention qualities and their improved filter to housing interface which minimized filter leakage.

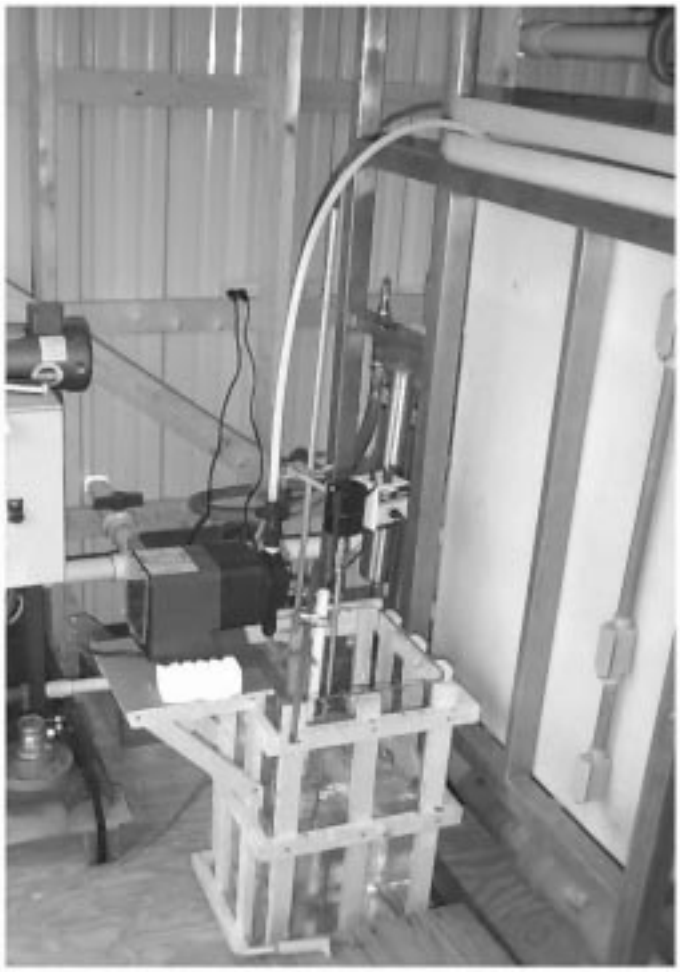


Figure 3.14 Equipment used to inject (oo)cysts into the influent of each system.

3.5.2 Spiking methodology

Spike material to be used:

The spiking material for these tests consisted of Formalin fixed (oo)cysts (Figure 3.15). Although Formalin fixed (oo)cysts may have different surface properties than live (oo)cysts, there was general agreement that live (oo)cysts pose too much of a health risk to project staff and the public to warrant its use. In addition, Formalin fixed (oo)cysts have been identified as the material most often used in pathogen spike tests based on reports in the literature. The formalin fixed (oo)cysts are obtained from research laboratories set up to provide these biological materials.

Dose:

The concentration of spike material and the duration of its application should be sufficient to exceed the detection limits for the samples to be collected at the effluent of each system. Based on previous DEP studies and the availability of spike material the spiked dose was maximum number of (oo)cysts that could be purchased commercially - 1×10^8.

Figure 3.15 Spiking material.

3.5.3 Monitoring configuration

Figure 3.16 provides the locations of the sampling points for the facilities tested in this study. Samples were collected at the influent, effluent and backwash of each system. More than one filter may be required for a location if the filter clogs. Two replicates of all effluent samples and one replicate of the influent samples will be collected for quality assurance purposes.

3.5.4 Sample filtration

Filter Type

Based on previous DEP studies, 10% of the (oo)cysts are able to break through the standard ICR polypropylene spiral wound filters which has a pore size which is nominally rated. Accordingly, all samples collected at the effluents of both the MF and CBUD sand filtration systems were filtered through absolute rated 10 inch filters (Nuclepore #641505) depicted in Figure 3.17. The membrane of this type of filter is bombarded by sub-atomic particles filters for a specific period of time to create specific pore sizes. This precise pore size minimizes the possibility of (oo)cysts penetrating and escaping through the filter matrix. In addition, the interface between the filter and the filter housing is augmented by the use of double O-rings. This positive seal minimizes the risk of particles escaping through the filter- filter housing interface.

Filter Volumes

Every effort was made to keep the effluent volumes sampled (filtered) from the output of each filtration system constant. Since it had been agreed that both systems would be monitored during "steady state" spiking conditions, the window for sampling time was set at 80 minutes. In

addition, to minimize the possibility of (oo)cysts escaping the effluent filter matrix, a maximum flowrate of 1.6 gallons per minute was established for effluent sample filtration. In practice, sample volumes varied from only 109 to 116 gallons filtered per effluent sample (based upon the established filtration interval and flowrate). This variability was minimized by constantly adjusting the filtration flowrates to specifications.

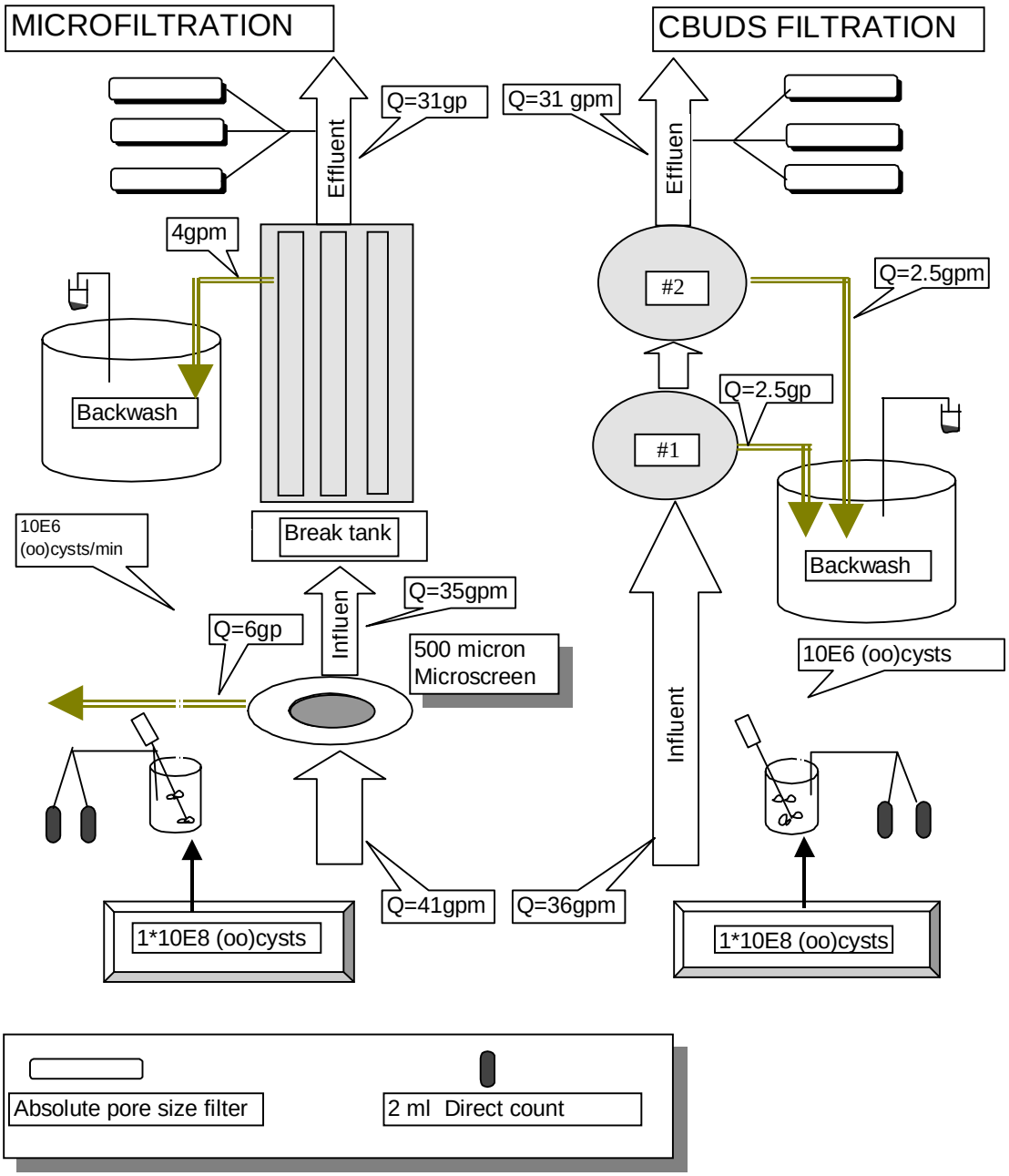


Figure 3.16 Schematic of sampling scheme to test equivalency of CBUD sand filtration with microfiltration.

Figure 3.17 Absolute pore filter used to collect effluent samples.

3.6 Laboratory Analysis

(Summary - for detailed procedure see Appendix A.)

3.6.1 ICR Method for *Giardia spp*. and *Cryptosporidium spp.*

(Used for effluent samples.)

Assay Procedure

The decision to use the ICR method for analyzing effluent samples was based on EPA, NYSDOH, and DEP's agreement that the ICR method is the most widely recognized method for detecting (oo)cysts in water samples.

The ICR method includes elusion from the collection filter, which is accomplished by using a stomacher. The combined eluate and residual water is then concentrated into a single pellet by centrifugation. The sample is further prepared by flotation purification. If necessary, sample volume is adjusted based upon microscopic examination of particulate distribution. Final sample preparation includes membrane filtration of one mL of suspension using a 25 mm diameter cellulose acetate filter, 0.2 mm pore size, and the Indirect Fluorescent Antibody (IFA) staining procedure. IFA control procedures are used to assure that the assay reagents are functioning, that the assay procedures have been properly performed, and that the microscope has been adjusted and aligned properly. Samples are examined using epifluorescence for detection of (oo)cysts. The DIC portion of the ICR Procedure was not implimented in this study since the source of (oo)cysts were known. The calculation method is based upon these observations and the percent of floated sample examined, and is detailed in Appendix A.

3.6.2 Direct Count Method for *Giardia spp*. and *Cryptosporidium spp.*

(Used for influent and backwash samples.)

The decision to use the direct count method for analysis of influent and backwash samples was based on preliminary studies which showed that for samples with high (oo)cyst concentrations the direct count method was superior in (oo)cyst recovery and variability. This can be attributed to the elimination of the variable losses associated with the filtering, concentration and flotation purification procedures.

The direct count method is similar to the ICR method above, but because the sample is not collected by a field filter, it does not include the stomacher process, or the flotation purification steps. Instead, the sample is collected directly, and an aliquot of approximately 2 mL is received by the laboratory. A 0.75 mL sample of this aliquot is then prepared and examined in an identical manner to the purified pellet in the ICR method above, including membrane filtration, IFA staining and control procedures, and epifluorescent microscopy.

4. Results

Tables 4.1 through 4.3. summarizes the data which resulted from the twelve (oo)cyst spike challenge tests described in this protocol. The table presents the results using several statistical methods to summarize the data. These methods have been used often in reports found in the literature. Some observations of the data include:

1. Both systems provided consistent (oo)cyst removal of greater than four logs.
2. The results of effluent sampling indicate counts and concentrations of similar magnitude, with the CBUD sand filtration system providing less detection and lower average concentration than microfiltration. The statistical significance of these findings are provided in a separate report prepared for EPA by Research Triangle Institute.
3. Results for backwash sampling indicate that most of the time large numbers of (oo)cysts are found in the backwash. The CBUD sand filtration system tended to have lower numbers. One speculative explanation for this could be that the shearing forces within the CBUD sand filtration system breaks apart the (oo)cysts.

Table 4.1. Detection of (oo)cysts from testing the effluent of two systems during 10E6/min spike challenge. (N=35)[1]

	GIARDIA		CRYPTO	
	CBUD	MICRO	CBUD	MICRO
No. of Detects	3	6	3	5
% Detection	8.6	17.1	8.6	14.3
Values of effluent samples ((oo)cysts/100L) where there was detection				
	0.244	6.058	0.231	0.229
	0.233	0.244	0.44	0.896
	0.233	0.224	0.223	1.178
		0.235		0.705
		0.235		0.46
		0.46		

1. The study generated 35 effluent samples for each system during the 12 spike challenge tests. The first test included 2 samples per system with the remaining 11 tests generating 3 samples per test per system.

Table 4.2. Mean concentration of (oo)cysts from testing the effluent of two systems during 10E6/min spike challenge.[1]

	GIARDIA		CRYPTO	
	CBUD	MICRO	CBUD	MICRO
Arith. Mean (ND=0)[2]	0.020	0.213	0.026	0.099
Arith. Mean (ND=DL)[3]	0.232	0.406	0.239	0.299
Geo. Mean (POS)[4]	0.237	0.451	0.283	0.601

1. Average concentration was calculated using the various methods that have been reported in the literature.
2. Not detected values were treated as equal to zero. This is the least biased method to calculate an average value for this data (according to Parkhurst and Stern, 1998 [in publication]).
3. Not detected were treated as equal to the detection limit.
4. Only detected values were included in the calculation.

Table 4.3. Log removal of (oo)cysts from testing the effluent of two systems during 10E6/min spike challenge.

	GIARDIA		CRYPTO	
	CBUD	MICRO	CBUD	MICRO
80 Min. Influent (oo)cysts/100L	5.77e+07	5.91e+07	6.62e+07	6.78e+07
Ave. Effluent (oo)cysts/100L	0.020	0.213	0.026	0.099
Avg. Log Removal	9.46	8.44	9.41	8.84
Max Observed Log Removal[1]	8.39	8.42	8.47	8.47
Min Observed Log Removal[2]	8.37	6.99	8.18	7.76

1. .Log removal was based on the minimum concentration (above zero) detected in the effluent during study.
2. Log removal was based on the maximum concentration seen in the effluent during study.

Appendices

A. DETAILED PROTOCOLS

A.1 FIELD COLLECTION

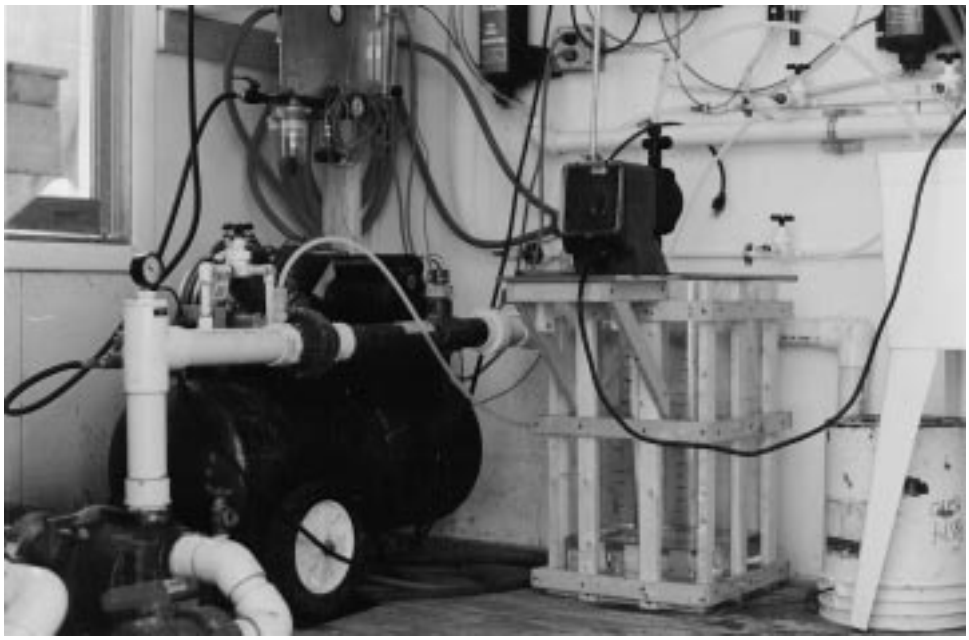
A.1.1 Introduction

The purpose of this series of experiments is to evaluate the suitability of two types of recognized filtration methodologies for removing pathogens from a treated wastewater stream. High concentrations of *Giardia lamblia* (or *Giardia muris*) and *Cryptosporidium parvum* will be injected into the feed line supplying each filtration system. This feed is the effluent line discharging from the sewage treatment plant's activated sludge aeration tank. As the highly concentrated pathogen seed material is slowly injected into the feed line over 100 minutes, pathogen samples are collected at strategic points in both filtration systems. Log removal , overall removal efficiency, mass balance analysis, pathogen concentration at various filtration train locations, potential pathogen breakthrough and pathogen concentrations in reject water in both systems will be analyzed over a series of twelve individual experiments. The results of these spike challenges will provide USEPA, NYSDOH and DEP management with information regarding the most efficient and reliable way to remove pathogens from a treated wastewater stream .

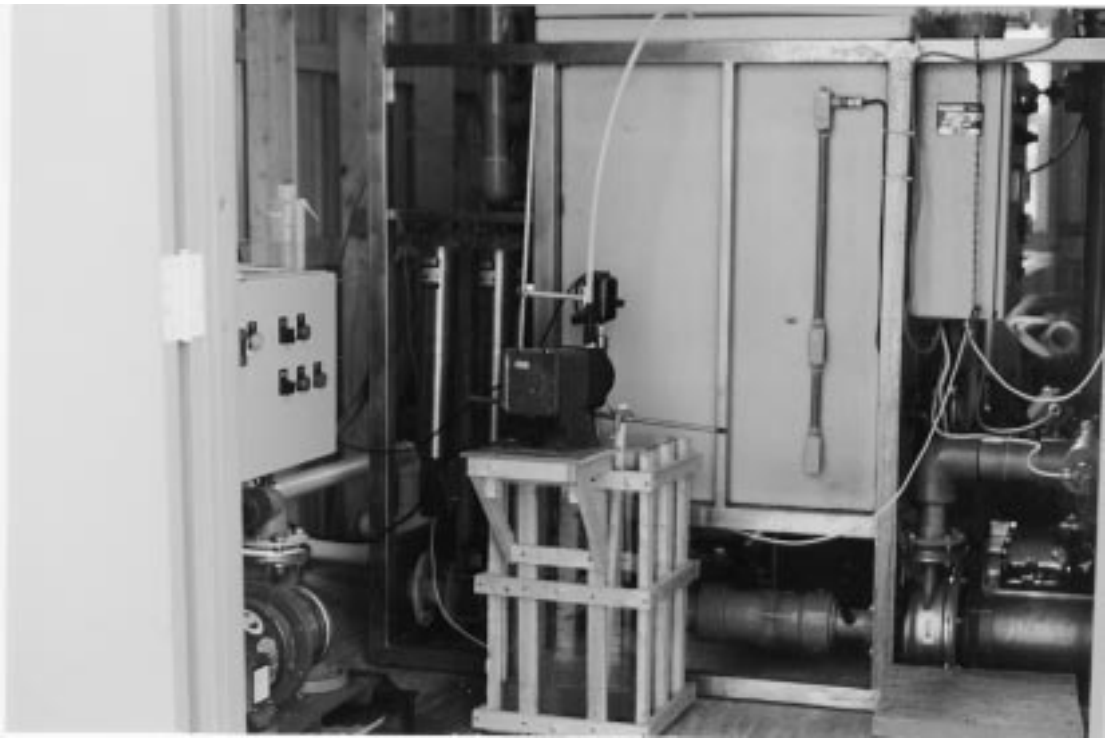
A.1.2 Setup

Two twelve gallon glass vessels will be set up prior to the actual injection. These tanks are completely cleaned, sanitized and rinsed prior to and after use as are all other materials which come into contact with the spike material. Field personnel are required to practice extreme caution and wear proper safety gear when coming into contact with spike material. For spiking, one billion formalinized *Giardia muris* cysts and one billion formalinized *Cryptosporidium parvum* oocysts previously obtained will be placed into each vessel with 10 gallons of untreated surface water obtained from Taylor Reservoir or the West Branch of the Delaware River. Using dilution water with dissolved ionic material limits the tendency of (oo)cysts to adhere to the side walls of the vessel containing them. The use of the surface water as a dilution medium is based on a NYSDOH recommendation due to the mixed ionic nature of surface water. In addition, one gallon of treated wastewater effluent from the Stamford STP was also added to the spike solution to further enhance its ionic mixture. Pathogen concentrations will be verified prior to spiking by siphoning off 150 microliters of the concentrated spike material. This analysis is performed at the DEP Pathogen Laboratory utilizing a hemacytometer. The seed material is vortexed for three minutes prior to deposition in the spike solution tank and mixed for 10 minutes prior to injection. Each filtration system will have an individual feed line which is fed by the effluent of the aeration basin of the activated sludge treatment plant. To create a spike material inlet port, each feed line is tapped with a half inch pipe nipple and is fitted with a valve to prevent air from entering the feed line through the spike inlet port. This is to be the seed material input point. The location of the inlet port is to be down stream of the feed pumps for both the Memcor and CBUD system to eliminate the possibility of (oo)cyst destruction in the pumps. The spike solution feed line is placed into the spike solution vessel. The elevation of each feed line in each tank is kept at a constant 2

inches above the bottom of each spike solution tank. A small electric lab mixer is placed into each glass vessel for stirring the concentrated spike solution. Two 1.5 inch propellers are situated equidistant on a 24 inch shaft to facilitate complete mixing. Diaphragm pumps are used to inject the spike solution into each feed line over 100 minutes. Actual effluent sampling will occur for 80 minutes at pre-specified times to allow for travel time through the CBUD system. Also, since it is our goal to sample both systems while at a state of equilibrium while being dosed with 1×10^{6} (oo)cysts per minute, effluent sampling will only be performed while the systems are receiving the maximum dose and not during the initial and final stages of (oo)cyst injection.



Appendix Figure 1.1 (oo) cyst spike injection system for CBUD sand filtration unit.



Appendix Figure 1.2 (oo) cyst spike injection system for microfiltration unit.

Appendix Figure 1.3 Collection of aliquot

Appendix Figure 1.4 Collection of Backwash.

A.1.3 Sample locations

Parkson Filtration System:
(Refer to Figure 3.16 for schematic of sample locations)

- Influent tank aliquot at 33 and 66 minutes.
- Backwash composite aliquot.
- Final Effluent - 3 absolute pore filters

Memcor Microfiltration System:

- Influent tank aliquot at 33 and 66 minutes.
- Backwash composite aliquot.
- Final Effluent - 3 absolute pore filters

A.1.4 Sampling apparatus

Each pathogen sampling apparatus will consist of a portable low flow pump supplying approximately 1.5 gallons per minute of sample stream to a filter housing contains a 10 inch, 1 micron, absolute rated, polycarbonate track etched filter membrane (Nuclepore # 641505). Effluent samples from each system are collected in triplicate for quality assurance purposes. Following the filters is a digital flowmeter/totalizer which measures the current flowrate and total volume

filtered for each sample. These flowmeters are calibrated prior to each use. Samplers are connected to each filtration (sampling) point using hose connectors. Samplers are to be sanitized and rinsed prior to and after each use. At each filtration system effluent point, the flow will be split to the three pathogen samplers and equalized. The sampling protocol, filtration rate, and sample volume filtered will be identical for each split sample collected. Effluent sampler start up and shut down times will be predetermined for each spike based upon spike injection times for both the Memcor and CBUD system.

Backwash sampling will be performed at six of the twelve spike experiments, specifically at every other spike. For the Memcor system, backwash sampling will consist of collecting a series of discrete 1 liter samples at every backwash cycle, which occurs once every 18 minutes. The entire backwash flow is directed to a single 500 gallon tank. Following the completion of each backwash cycle, a 1 liter discrete sample is collected from this tank. These samples are then deposited into a single 5 gallon vessel. Following the entire backwash sampling sequence, this composite sample is homogenized. A 2 milliliter aliquot of this composite sample is then collected representing the average backwash of the Memcor unit for the entire spike challenge. The backwash sampling of the CBUD unit is performed differently due to the continuous nature of its backwashing as opposed to the cyclic nature of the Memcor backwashing. At each 18 minute interval when Memcor is backwashing, one 500 milliliter sample is collected from the backwash of each CBUD filtration unit. These samples are composited into a single five gallon vessel for the duration of the spike experiment. Following collection, the composite sample in the vessel is homogenized. A 2 milliliter sample of this composite sample is collected representing the average backwash of the CBUD unit for the entire spike challenge. These backwash samples are analyzed using the same direct count procedure applied to the 2 milliliter influent samples.

Appendix Figure 1.5 Sampling effluent with absolute pore filters.

A.1.5 Procedure

1) Ensure that all sampling apparatus is properly cleaned, calibrated and installed at all predetermined sampling locations with filters loaded and ready to sample (filter). Set up tanks (as needed) to collect samples from backwash lines and tanks.
2) Vortex spike solution for three minutes and mix pathogen spike material (1 billion *Giardia* sp. cysts and 1 billion *Cryptosporidium* sp. oocysts) into each 12 gallon glass vessel containing 10 gallons of Taylor Reservoir or West Branch Delaware River water and 1 gallon of treated Stamford STP effluent. Measure temperatures of all solutions. Homogenize with stirrer for at least 10 minutes prior to injection. Attach spike material inlet lines to each respective pipe tap in each filtration system (Parkson and Memcor) feed line.
3) Verify flow rate through each filtration system. Correct and document all sampler start up and shut down times. Double check all equipment and connections.
4) Begin spiking by opening valves at each spike solution inlet port, starting injection pumps and adjust to 0.11 gallons per minute. This will deliver the entire spike solution to each system over 100 minutes.
5) Collect a 2 milliliter influent sample from each spike tank at 33 and 66 minutes into the spike injection. Package samples in a refrigerated cooler after collection.
6) Start sampling effluent of CBUD and Memcor systems synchronously at prespecified times. Record sampler start up and spike start up times. Record sampler and system flowrates. Verify 0.11 gpm spike solution injection flowrate periodically and adjust as necessary. Constantly monitor pathogen filtration apparatus and adjust as necessary.
7) Collect backwash samples as required as per established protocol.
8) Shut effluent sampling apparatus down after 80 minutes have passed. Record all pertinent sample data and package samples (filters) in ziploc bags and store in a refrigerated cooler. After 100 minutes have passed and the entire spike solution has been injected into each filtration system feed line, shut down spike solution injection pumps. Record exact time and any other pertinent spike information.
9) Continue to sample each filtration systems backwash for 15 minutes after spike termination. Homogenize and collect backwash samples. Package backwash samples in a refrigerated cooler after collection.
10) Remove and sanitize all spiking and sampling apparatus. Samples are to be delivered blind to the laboratory with no information other than the date and a code number or letter for our own sample identification purposes. Deliver all samples to NYCDEP Pathogen laboratory immediately to expedite analysis.

A.2 LABORATORY ANALYSIS

A.2.1 ICR Method for *Giardia spp*. and *Cryptosporidium spp*.

(Used for <u>effluent</u> samples)

Assay Procedure

(See Appendix Figures A2.1 and A2.2 for images showing several of the following steps.)

Filter Elution And Concentration

The initiation of sample collection and elusion from the collection filter must be performed within 96 hours.

Stomacher Washing:

Step 1. Receive sample and give lab #.

Step 2. Place in pan and cut down center with a sharp blade.

Step 3. Cut around ends and remove plastic casing.

Step 4. Cut around each end of the pleated filter as close as possible to the end caps.

Step 5. Remove pleated material and open.

Step 6. Place material in a stomacher bag

Step 7. Use a stomacher with a bag capacity of 3500 mL. Remove the filter from the inner bag and place it in a glass or stainless steel tray. Pour the residual solution in either the inner or outer bags into a pooling beaker, rinse the bags with eluting solution, add the rinse solution to the beaker and discard the bags. Using a razor knife or other appropriate disposable cutting instrument, cut the filter fibers lengthwise down to the core. Discard the blade, after the fibers have been cut.

Step 8. After loosening the fibers, place all the filter fibers in a stomacher bag. To insure against bag breakage and sample loss, place the filter fibers in the first stomacher bag into a second stomacher bag.

Step 9. Add 1.75 L of eluting solution to the fibers. Homogenize for 2-five minute intervals. Between each homogenization period, hand knead the filter material to redistribute the fibers in the bag.

Step 10. Pour the eluted particulate suspension into a 4 L pooling beaker. Wring the fibers to express as much of the liquid as possible into the pooling beaker.

Step 11. Put the fibers back into the stomacher bag, add 1.0 L more eluting solution, and homogenize, as in Step 3 above, for 2-five minute intervals. Between each homogenization period, hand knead the filter material to redistribute the fibers in the bag.

Step 12. Add the eluted particulate suspension to the 4 L pooling beaker. Wring the fibers to express as much of the liquid as possible into the pooling beaker. Discard the fibers. Rinse the stomacher bag with eluting solution and place this rinse water into the pooling beaker.

Eluate Concentration:

Concentrate the combined eluate and residual water into a single pellet by centrifugation at 1,050 xg for 10 min using a swinging bucket rotor and plastic conical centrifuge bottles. Carefully aspirate and discard the supernatant fluid and resuspend the pellet in sufficient elution solution by vortexing. After pooling the particulates in one conical bottle, centrifuge once more at

1,050 xg for 10 min and record the packed pellet volume. Carefully aspirate and discard the supernatant fluid and resuspend the pellet by vortexing in an equal volume of 10% neutral buffered formalin solution. If the packed pellet volume is less than 0.5 mL, bring the pellet and solution volume to 0.5 mL with eluting solution before adding enough 10% buffered formalin solution to bring the resuspended pellet volume to 1.0 mL.

At this point, a break may be inserted if the procedure is not going to progress immediately to the FLOTATION PURIFICATION procedure below. If a break is inserted at this point, be sure to store the formalin treated sample at 4˚C for not more than 72 hours.

Flotation Purification

Step 1. In a clear plastic 50 mL conical centrifuge tube(s), vortex a volume of resuspended pellet equivalent to not more than 0.5 mL of packed pellet volume with a sufficient volume of eluting solution to make a final volume of 20 mL.

Step 2. Using a 50 mL syringe and 14 gauge cannula, underlay the 20 mL vortexed suspension of particulates with 30 mL Percoll-sucrose flotation solution (sp. gr. 1.10).

Step 3. Without disturbing the pellet suspension/Percoll-sucrose interface, centrifuge the preparation at 1,050 xg for 10 min using a swinging bucket rotor. Slowly accelerate the centrifuge over a 30-sec interval up to the speed where the tubes are horizontal to avoid disrupting the interface. Similarly, at the end of centrifugation, decelerate slowly. DO NOT USE THE BRAKE.

Step 4. Using a polystyrene 25 mL pipet rinsed with eluting solution, draw off the top 20 mL particulate suspension layer, the interface, and 5 mL of the Percoll-sucrose below the interface. Place all these volumes in a plastic 50 mL conical centrifuge tube.

Step 5. Add additional eluting solution to the plastic conical centrifuge tube (Step 4) to a final volume of 50 mL. Centrifuge at 1,050 xg for 10 min.

Step 6. Aspirate and discard the supernatant fluid down to 5 mL (plus pellet). Resuspend the pellet by vortexing and save this suspension for further processing with fluorescent antibody reagents.

Indirect Fluorescent Antibody (IFA) Procedure

Determining Sample Volume per Filter (optional):

Step 1. Determine the volume of sample concentrate from the Flotation Purification procedure above that may be applied to each 25-mm diameter membrane filter used in the IFA assay.

Step 2. Vortex the sample concentrate and apply 40 µL to one 5-mm diameter well of a 12-well red heavy Teflon-coated slide.[1]

Step 3. Allow the sample to sit approximately two min at room temperature.

1.Cel-line Associates, Inc., 33 Gorgo Lane, Newfield, NJ 08344, C

Step 4. Examine the flooded well at 200X total magnification. If the particulates are distributed evenly over the well surface area and are not crowded or touching, then apply 1 mL of the undiluted sample to a 25 mm diameter membrane filter in Step 6 of Sample Application below.

Step 5. Adjust the volume of the sample accordingly if the particulates are too dense or are widely spread. Retest on another well. Always adjust the sample concentrate volume so that the density of the particulates is just a little sparse. If the layer of sample particulates on the membrane filters is too dense, any cysts or oocysts present in the sample may be obscured during microscopic examination. Make sure the dilution factor, if any, from this Step is recorded.

Preparing the Filtration Manifold:

Step 1. See diagram of the filtration manifold assembly (Figure A2.1)

Step 2. Connect the filtration manifold to the vacuum supply using a vacuum tube containing a "T"-shaped tubing connector. Attach a Hoffman screw clamp to 4-6 cm of latex tubing and then attach the latex tubing to the stem of the "T"- connector. The screw clamp is used as a bleeder valve to regulate the vacuum to 2-4 inches (5-10 cm) of Hg.

Step 3. Close all the manifold valves and open the vacuum all the way. Using the bleeder valve on the vacuum tubing, adjust the applied vacuum to 2-4 inches (5-10 cm) of Hg. Once adjusted, do not readjust the bleeder valve during filtration. If necessary, turn the vacuum on and off during filtration at the vacuum source.

Membrane Filter Preparation:

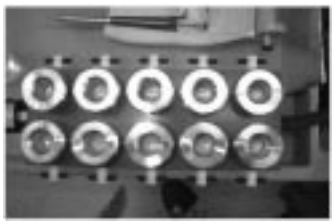
Step 1. One Sartorius 25 mm diameter cellulose acetate filter, 0.2 µm pore size and one 25 mm diameter ethanol compatible membrane support filter, any porosity, are required for each 1 mL of adjusted suspension obtained in the Determining Sample Volume per Filter section. Soak the required number of each type of filter separately in Petri dishes filled with 1X PBS. Drop the filters, handling them with blunt-end filter forceps, one by one flat on the surface of the buffer. Once the filters are wetted, push the filters under the fluid surface with the forceps. Allow filters to soak for a minimum of one minute before use.

Step 2. Turn the filtration manifold vacuum source on. Leaving all the manifold well support valves closed, p1ace one support filter on each manifold support screen. This filter ensures even distribution of sample.

Received samples

Washing with stomacher

Centrifugation



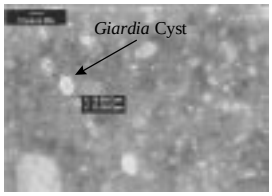
Final manifold filtration

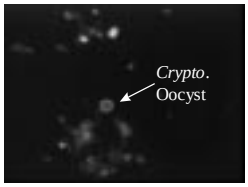
Filters mounted on slides

Appendix Figure 2.1 Concentration and separation.

MICROSCOPE EVALUATION

Epiflourescence





Appendix Figure 2.2 Microscope evaluation.

Step 3. Place one Sartorius 25 mm diameter cellulose acetate filter on top of each support filter. Use a rubber policeman to adjust the cellulose acetate filter, if necessary. Open the manifold well support valves to flatten the filter membranes. Make sure that no bubbles are trapped and that there are no creases or wrinkles on any of the filter membranes.

Step 4. For the positive controls, add 500-1000 *Giardia lamblia* cysts and 500-1000 *Cryptosporidium parvum* oocysts or use the Ensys positive control antigen as specified in the kit to a well.

Step 5. For a negative control, add 1.0 mL 1X PBS to one well.

Step 6. Add 1.0 mL of the vortexed, adjusted water sample (Determining Sample Volume per Filter section) to a well. If the optional step to determine sample volume was not performed, add the volume determined by the principal analyst to be appropriate to a well.

Step 7. Open the manifold valve under each membrane filter to drain the wells. Rinse each stainless steel well with 2 mL 1% BSA. Do not touch the pipet to the membrane filter or to the well. Close the manifold valve under each membrane filter.

Indirect Fluorescent Antibody Staining:

Step 1. Dilute the primary antibody mixture and labeling reagent according to the manufacturer's instructions using 1X PBS.

Step 2. Pipet 1.0 mL of the diluted primary antibody onto each membrane and allow to remain in contact with the filter for 25 minutes at room temperature.

Step 3. At the end of the contact period, open the manifold valve to drain the antisera.

Step 4. Rinse each well and filter 5 times with 2 mL 1X PBS. Do not touch the tip of the pipet to the membrane filter or to the stainless steel wells. Close all manifold valves after the last wash is completed.

Step 5. Pipet 1.0 mL labeling reagent onto each membrane and allow to remain in contact with the filter for 25 minutes at room temperature. Cover all wells with aluminum foil to shield the reagents from light and to prevent dehydration and crystallization of the fluorescein isothoicyanate dye during the contact period.

Step 6. At this point, start the **Filter Mounting** procedure below.

Step 7. At the end of the contact period, open the manifold valves to drain the labeling reagent.

Step 8. Rinse each well and filter 5 times with 2 mL 1X PBS. Do not touch the tip of the pipet to the membrane filter or to the stainless steel wells. Close all manifold valves after the last wash is completed.

Step 9. Dehydrate the membrane filters in each well by sequentially applying 1.0 of 10, 20, 40, 80 and 95% ethanol solutions containing 5% glycerol. Allow each solution to drain thoroughly before applying the next in the series.

Filter Mounting:

Step 1. Label glass slides for each filter and place them on a slide warmer or in an incubator calibrated to 37°C.

Step 2. Add 75 µL 2% DABCO-glycerol mounting medium to each slide on the slide warmer or in the incubator and allow to warm for 20-30 minutes.

Step 3. Remove the top cellulose acetate filter with fine-tip forceps and layer it over the correspondingly labeled DABCO-glycerol mounting medium prepared slide. Make sure the sample application side is up. If the entire filter is not wetted by the DABCO-glycerol mounting medium, pick up the membrane filter with the same forceps and add a little more DABCO-glycerol mounting medium to the slide under the filter. Place the mounted filter either on the slide warmer or in the incubator for a clearing period of 20 minutes.

Step 4. Use a clean pair of forceps to handle each membrane filter. Soak used forceps in a beaker of diluted detergent cleaning solution.

Step 5. After the 20 minute clearing period, the filter should become transparent and appear drier. After clearing, if the membrane starts to turn white, apply a small amount of DABCO-glycerol mounting medium under the filter.

Step 6. After the 20 minute clearing period, apply 20 µL DABCO-glycerol mounting medium to the center of each membrane filter and cover with a 25 mm x 25 mm cover glass. Tap out air bubbles with the handle end of a pair of forceps. Wipe off excess DABCO-glycerol mounting medium from the edge of each cover glass with a slightly moistened Kimwipe.

Step 7. Seal the edge of each cover glass to the slide with clear fingernail polish.

Step 8. Store the slides in a “dry box”. A dry box can be constructed from a covered Tupperware-type container to which a thick layer of anhydrous calcium sulfate has been added. Cover the desiccant with paper towels and lay the slides flat on the top of the paper towels. Place the lid on the dry box and store at 4°C.

Step 9. Examine the slides microscopically as soon as possible but within 5 days of preparation, because they may become opaque if stored longer.

Microscopic Examination:

1. General: Microscopic work by a single analyst should not exceed four hours per day nor more than five consecutive days per week. Intermittent rest periods during the four hours per day are encouraged.

Step 1. Remove the dry box from 4°C storage and allow it to warm to room temperature before opening.

Step 2. Adjust the microscope to assure that the epifluorescence optics are in optimal working order. Make sure that the fluorescein isothiocyanate cube is in place in the epifluorescent portion of the microscope.

2. IFA Controls: The purpose of these IFA controls is to assure that the assay reagents are functioning, that the assay procedures have been properly performed, and that the microscope has been adjusted and aligned properly.

a. Negative IFA Control for *Giardia/Cryptosporidium*

Step 1. Using epifluorescence, scan the negative control membrane at no less than 200X total magnification for apple-green fluorescence of *Giardia* cyst and *Cryptosporidium* oocyst shapes.

Step 2. If no apple-green fluorescing cyst or oocyst shapes are found, and if background fluorescence of the membrane is very dim or non-existent, continue with examination of the water sample slides.

If apple-green fluorescing cyst or oocyst shapes are found, discontinue examination since possible contamination of the other slides is indicated. Clean the equipment, recheck the reagents and procedure and repeat the assay using additional aliquots of the sample.

b. Positive IFA Control for *Giardia/Cryptosporidium*

Step 1. Using epifluorescence, scan the positive control slide at no less than 200X total magnification for apple-green fluorescence of *Giardia* cyst and *Cryptosporidium* oocyst shapes. Background fluorescence of the membrane should be either very dim or non-existent. *Cryptosporidium* oocysts may or may not show evidence of oocyst wall folding, which is characterized under epiflluorescence by greater concentrations of FITC along surface fold lines, depending upon the manner in which the oocysts have been treated and the amount of turgidity they have been able to maintain.[1]

Step 2. If no apple-green fluorescing *Giardia* cyst or *Cryptosporidium* oocyst shapes are observed, then the fluorescent staining did not work or the positive control cyst preparation was faulty. Do not examine the water sample slides for *Giardia* cysts and *Cryptosporidium* oocysts. Recheck reagents and procedures to determine the problem.

3. Sample Examination

Scanning Technique - Scan each slide in a systematic fashion beginning with one edge of the mount and covering the entire coverslip. An up-and-down or a side-to-side scanning pattern may be used.

a. When appropriate responses have been obtained for the positive and negative controls, use epifluorescence to scan the entire coverslip from each sample at not less than 200X total magnification for apple-green fluorescence of cyst and oocyst shapes.

b. When brilliant apple-green fluorescing round to oval objects (8 to 18 µm long by 5 to 15 µm wide) are observed with brightly highlighted edges, count and record as total IFA *Giardia* cyst count

1. Roberson, L.J., et all. 1993. Induction of folds or sutures on the walls of *Cryptosporidium* parvum oocysts and their importance as a diagnostic feature. Appl. Environ. Microbiol. 59(8):2638-2641.

Calculation:

Step 1. Percentage of Floated Sample Examined - Record the percentage of floated sediment examined microscopically. [Calculate this value from the total volume of floated pellet obtained, the number of 25-mm membrane filters preparted together with the volume of floated pellet represented by these membrane filters, and the number of membrane filters examined.]

The following values are used in calculations:

V = volume (liters) of original water sample

P = eluate packed pellet volume

F = fraction of eluate packed pellet volume (P) subjected to flotation, determined as

$$F = \frac{\text{mL P subjected to flotation}}{P}$$

R = Percentage (expressed as a decimal) of floated sediment examined

TG = Total *Giardia* IFA cyst count

TC = Total *Cryptosporidium* IFA oocyst count

Step 2. For positive samples, calculate the number of cysts or oocysts per 100 L of sample as follows:

$$\frac{X}{100L} = \frac{(TG \text{ or } TC)(100)}{FVR}$$

For samples in which no cysts or oocysts are detected, (TG or TC) = <1. Calculate the detection limit as follows:

$$\frac{<X}{100L} = \frac{(<1)(100)}{FVR}$$

A.2.2. Direct Count Method for *Giardia spp*. and *Cryptosporidium spp*.

(Used for influent and backwash samples)

Sample Preparation and Slide Staining Procedure

**** The following instructions are to be used with the Ensys Inc. Hydrofluor Combo test kit for staining samples to detect Giardia and Cryptosporidium.

1. A small vial is received by the Pathogen Laboratory containing approximately 2 ml of sample. This vial is given the next available number from the Pathogen Log Book.

2. Prepare the Hoefer manifold as described in the proceeding section or the U.S. EPA ICR Microbial Laboratory Manual.
3. Vortex each vial for at least one minute immediately prior to dispensing the liquid sample onto the filter membrane. For each vial received, perform the Hoefer manifold method using 750 µL of vortexed sample.
4. Follow the ICR Staining Procedure and Microscopy procedure for sample completion
5. Routine quality control samples should be analyzed accordingly, including positive and negative controls as well as duplicates (See laboratory SOP).
6. Filters must be read five days from processing.

B. Operating History

The (oo)cyst spike challenge tests were conducted from May 27 through October 31, 1997. The CBUD sand filtration facility became operational on May 1; the MF facility went on line May 16. Both systems were optimized before the first spike challenge on May 27.

The entire pilot project equipment was installed by the operations and engineering staff of Delaware Engineering, P.C. The two systems were powered by a dedicated 90-amp service off of the existing Stamford Wastewater Treatment Plant main circuit panel, with 10 amps dedicated to the main control building (i.e. feed pumps for both systems, all monitoring equipment, air compressor and chemical feed equipment for the CBUD sand filtration system, and data loggers for the data acquisition system computer), and 80 amps dedicated to the separate MF building and equipment (i.e. 500 micron strainer, the 6M10C CMF, the air compressor, and chemical feed equipment for the MF system).

The pilot was staffed by operations staff on a seven-day basis, with Memcor service technicians visiting the site weekly. The DualSand representative visited the site periodically. Representatives of Memcor were on site for each of the twelve (12) spike challenges, and various representatives of the USEPA, NYCDEP, NYSDEC, and NYSDOH visited the pilot site.

Cbud Sand Filtration System

The CBUD sand filtration facility was moved to the wastewater treatment plant site from the recently completed DualSand potable water pilot at the Village of Stamford's upland reservoir. The CBUD sand filtration system was treating water on May 1, and continued to treat water uninterrupted, except the night of July 16 for an approximate four-hour power interruption due to a lightning strike. Actually, the lightening strike knocked out power temporarily, as recorded by the computer's recording software, and the CBUD sand filtration system began operating again once power was restored. However, the MF unit does not restart automatically after a power interruption, so the MF supply pump was pumping at 50 gpm against a closed valve for several hours until the weakest pipe connection failed and flooded the neighboring compressor, causing the operator to manually shut down the CBUD sand filtration system. With the compressor head dried out and back on line, the CBUD sand filtration was back up and running. As the net water production section indicates, the CBUD sand filtration system consistently produced filtrate at approximately 49,000 gpd [(36 gpm x 1440 min./day) - reject of (1.8 gpm x 1440 min./day) = 49,248 gpd]. The reject flow rate was measured by depth of flow past the overflow weir, verified by timed flow volume in the reject line.

The CBUD sand filtration system was monitored by an influent flow meter, an influent turbidimeter and particle counter, and an effluent particle counter. Sodium hypochlorite and the coagulant PASS (poly-aluminum-sulicate-sulfate) were injected into the influent line and manually adjusted to provide optimal effluent quality (i.e. typically three (3) gallons per day of PASS and a total chlorine residual of 0.5 ppm). The air flow rates, influent flow rates, chlorine residual,

effluent particle counts, and effluent turbidity were monitored manually several times daily; the particle count data and turbidity data were archived through the ChemTrac particle counter software.

Microfiltration System

The Memcor 6M10C skid-mounted unit was an older pilot unit, which after reconditioning at the Memcor Timonium, Md., facility, arrived on site May 15 and was installed in the dedicated structure. The Memcor structure also housed the self-cleaning 500-micron strainer, the air compressor, and related piping and controls; the energy dissipation tank (i.e. backwash tank) was housed externally to the building. The MF system was serviced by an 80-amp sub panel, as well as an influent feed pump in the instrumentation building. Sampling lines were connected from the filtrate line to the bubble trap and finally the monitoring equipment. The sampling line flow was manually adjusted to provide adequate pressure for filtrate to reach the bubble trap, approximately twelve (12) feet above grade, and to minimize the impact of post backwash filtrate dissolved air entrainment.

During the majority of the pilot, the MF facility was processing influent at approximately 36 gpm, on a 90% operational frequency (i.e. the unit backwashed for approximately two minutes every 20 minutes, or produced filtrate 1296 minutes daily). Therefore, on a daily basis, with no scheduled cleaning downtime or unscheduled maintenance time, the unit produced approximately 46,600 gallons per day (36 gpm x 1296 min./day = 46,656 gpd). Chemical cleaning was scheduled on a bi-weekly basis. The addition of coagulant for phosphorus removal resulted in a weekly chemical cleaning cycle, or a reduction in flow rate to 30 gpm. The net water production section includes the data log for the MF facility, indicating a downtime (e.g. chemical cleaning, malfunction, and repair time) of 378 hours or almost 16 days for the 159-day pilot.

Pursuant to the timing of the 18-minute cycle for MF unit gas backwash, a non-net water production time of 2 minutes for every 20-minute period translates to a 90% production rate, or a total downtime of approximately 16 days for the 159-day pilot. Extrapolated to an annual basis, the individual MF unit would be non productive 36.5 days.

Relative to the history of repairs on the Memcor unit, there was a situation early on in the pilot, wherein one of the six modules was replaced on July 7. The replacement was mandated due to a failure by the technicians in getting the questionable module apart in order to address a perceived problem with one of the O-rings. The replacement was completed without prior approval by the NYCDEP. No other module replacement/rehabilitation was done during the remainder of the pilot

On June 25, the Memcor service technicians installed the equipment to conduct the DAF (diffusive air flow) test on the unit (Appendix Figure 1.6). The DAF test is more definitive in assessing the membrane integrity than in the standard membrane test. The standard membrane test was conducted by Delaware operating staff on a daily basis; the DAF test was performed only by Memcor personnel. Based upon these two tests, the Memcor Process Engineer concluded that one of the module's O- rings had been "rolled" during a routine service inspection. (Note: each module has a total of 16 O- rings.) The O-ring situation was discussed among the NYCDEP, USEPA, Memcor, and Delaware personnel, and the decision was made to leave the subject O-ring as it was since the unit was still meeting the membrane integrity test provided through the pressure hold test. Since this was the standard operating procedure for testing membrane integrity at other MF facilities it was decided that actions should not be taken based on advanced testing methods which are not routinely available at MF facilities.

On September 9, the Memcor service technicians identified a potential problem with one of the polypropylene strands. However, the pressure hold test continued to indicate membrane integrity. Accordingly, the strand was left in its existing condition.

When the project team from the NYCDEP and USEPA toured the site on October 3, the Memcor Process Engineer identified the potential problem module by listening to the "whooshing" sound of the air rushing past the rolled O-ring during the standard membrane test. All membrane tests indicated an acceptable pressure decay, accounting for a theoretical log removal in excess of 5 log (99.999%) which was confirmed with the results from the (oo)cyst spike challenge tests.

Appendix Figure 1.6 Equipment installed for DAF test to measure membrane integrity.

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