Monitoring for Protozoan Pathogens in Reclaimed Water: Florida's Requirements and Experience

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The 1999 revisions to Chapter 62-610, Florida Administrative Code (F.A.C.), added requirements for pathogen monitoring to Florida's reuse rules (1). This paper provides background information on the protozoan pathogens *Giardia lamblia* and *Cryptosporidium parvum*. The new monitoring requirements, sampling and analysis methods, and laboratory certification requirements are discussed. Results from this ongoing monitoring program are presented. In addition, this paper includes brief summaries of two *Giardia* infectivity studies.

Protozoan Pathogens

The protozoan pathogens (notably *Cryptosporidium* and *Giardia*) have received much attention over the last decade. The massive, 1993 outbreak of Cryptosporidiosis in Milwaukee (2) captured the attention of water resource managers worldwide. About 400,000 individuals became ill as a result of *Cryptosporidium* in the public drinking water system in Milwaukee, Wisconsin. Both *Giardia* and *Cryptosporidium* are recognized as the causes of significant diarrheal disease in the United States, as well as elsewhere in the world.

Although one species of *Cryptosporidium* was first identified in 1907 in mice, *Cryptosporidium* was not known to be a human pathogen until 1976 (3). This gastrointestinal infection results in a watery diarrhea, which may be accompanied by abdominal pain, nausea, anorexia, dehydration, and weight loss. The organism is spread by a fecal/oral route. Infection by *Cryptosporidium* is regarded as being self-limiting in immunocompetant individuals. Most individuals become asymptomatic within two weeks. It is a more serious infection in immunocompromised individuals. Patients with AIDS typically are unable to clear the infection and the infection is frequently fatal.

Giardia lamblia is a protozoan pathogen that is found worldwide (4). It infects the intestinal tract and can result in a variety of symptoms, such as chronic diarrhea, bloating, abdominal cramps, frequent greasy malodorous stools, fatigue, and weight loss. *Giardia* is transmitted by a fecal/oral route. Infections by *Giardia* are regarded as being self-limiting and frequently are asymptomatic. When symptoms occur, they typically last two to six weeks.

In discussing pathogenic organisms, it is important to distinguish between infection and disease. Infection means the invasion of the human body by pathogenic organisms in sufficient quantities that the organisms are able to carry out their normal life cycles within the human body. Infection essentially relates to "colonization." Once a pathogen has invaded and colonized (infected) an individual, a number of factors will determine whether or not the individual will experience symptomatic disease. These factors include the type of organism and it's characteristics, the numbers of organisms present, and the health and immune status of the infected individual. Not all infections will result in symptomatic disease.

Both *Cryptosporidium* and *Giardia* have low infective doses. Some public health officials have stated that ingestion of as few as one oocyst of *Cryptosporidium* may result in infection in some individuals. While a possibility, this probably has been somewhat overstated. Applying the dose-response model for *Cryptosporidium* to ingestion of one viable oocyst yields an estimated probability of infection of about 0.5 percent. A 1995 study (5) established the median infectious dose (ID_{50}) for humans at 132 oocysts. The median infectious dose for *Giardia* is between 50 and 100 cysts (6).

There are significant animal reservoirs of these protozoan pathogens, particularly for *Cryptosporidium*. Calves, dogs, cats, and rodents are among the more than 40 mammals that serve as hosts (calves are a particularly significant source). While man represents the main reservoir of *Giardia*, other animals, particularly beavers, may serve as hosts.

Exhibits A and B present information on the prevalence of *Cryptosporidium* and *Giardia*, respectively, in the environment and in treated drinking water (7,8). Available data on Florida waters are included in both exhibits. These exhibits focus on relatively high quality waters. Polluted waters tend to exhibit higher concentrations of both pathogens. For comparison purposes, both exhibits include a characterization of reclaimed water in St. Petersburg, Florida. Exhibit A also includes limited data on irrigation canals in Arizona, which are located within ranch lands.

Inspection of Exhibits A and B reveals that these organisms are rather widespread. *Cryptosporidium* has come to be regarded as being ubiquitous in the environment. In reviewing these exhibits and in evaluating data for protozoan pathogens, it must be noted that all cysts and oocysts are not viable. Microscopic examination of cysts and oocysts may enable investigators to make estimates of the percentage of cysts and oocysts that have complete internal structure and that may be presumed to be infective. This is normally accomplished through DAPI staining techniques, as outlined in EPA Method 1623. However, many existing data sets (including those in Exhibits A and B) do not include this level of sophistication. More definitive studies of infectivity of cysts and oocysts involve animal feeding studies and involve significant effort and costs.

It also should be noted that the percentages of samples testing positive for these organisms reflect the detection limits of the individual sample collections. It is likely that these percentages would increase if larger volumes of water had been processed in all sampling activities.

Filtration and passage through soils are effective in removing cysts and oocysts from water (3,9,10,11,12). Filters in a St. Petersburg water reclamation facility removed about two logs (99 percent) of cysts and oocysts (13,14). Removals of cysts and oocysts by soil systems should be comparable or greater. One study found loamy sand exhibited better removals than either silty loam or clay loam. A study of riverbank filtration in the Netherlands (15) found that passage through 25 to 30 meters of sandy soils removed greater than 2.6 logs of enterovirus and greater than 4.7 logs of Reoviruses. Given the larger sizes of the protozoan pathogens, removals are expected to be greater than that observed for viruses.

Several protozoan (*Giardia*, *Cryptosporidium*, and *Cyclospora*) and other pathogens (*Escherichia coli* 0157:H7, *Legionella*, and *Salmonella*) were evaluated in a study of a large agricultural reuse project in Monterey County, California (16). This treatment facility provides a level of treatment and disinfection similar to what is required in Florida for Part III reuse projects. Neither *E. coli* 0157:H7 nor *Legionella* were detected in the untreated wastewater. *Legionella*, *E. coli* 0157:H7, *Salmonella*, *Cryptosporidium*, and *Cyclospora* were never detected in the reclaimed water. *Giardia* was detected in 80 percent of the reclaimed water samples at concentrations ranging from 3 to 9 cysts per 100 L. However, all *Giardia* cysts were devoid of internal structure and were considered to be non-viable.

Florida's Pathogen Monitoring Requirements

This section describes Florida's rule requirements governing pathogen monitoring in reclaimed water.

Purpose: In 1999, requirements for pathogen monitoring were added to Chapter 62-610, F.A.C., in an effort to learn more about the presence of these organisms in reclaimed water. Depending on what this monitoring shows, the DEP may, or may not, move toward additional rulemaking that could involve placing controls or limits on these organisms.

Effective Date: The requirements for pathogen monitoring became effective on August 8, 1999. This requirement is being added to all new, renewed, and revised permits issued by the DEP after the effective date. Existing facilities will not be subject to this monitoring requirement until the standard permit condition that requires this monitoring has been added to the permit.

Sample Frequency: The following table lists the types of facilities that must monitor for the protozoan pathogens and the required frequencies for this monitoring:

Part	Type of System	F.A.C. Rule	Frequency
III	Public access, residential, edible crop, and other Part III projects	62-610.463(4)	Once every 2 yr. (a) Once every 5 yr. (b)
III	Surface water used as a supplemental water supply	62-610.472(3)(d)	Once every 2 yr. (a) Once every 5 yr. (b)
IV	"Other" rapid-rate systems subject to Rule 62-610.525, F.A.C.	62-610.525(13)	Once every 2 yr.
V	Discharge to Class I surface waters & waters tributary or contiguous (travel time < 4 hr.)	62-610.568(11)	Quarterly
V	Injection for ground water recharge or salinity barriers	62-610.568(11)	Quarterly
V	Discharge to surface waters that are directly connected to ground water	62-610.568(11)	Quarterly
V	Discharges upstream from Class I surface waters (travel time 4-24 hr.)	62-610.568(12)	Once every 2 yr.
VII	Use of reclaimed water in open cooling towers	62-610.652(6)(c)	Once every 2 yr.

Notes: (a) For treatment facilities having capacities of 1.0 mgd or larger.
(b) For treatment facilities having capacities less than 1.0 mgd.

The rules referenced in the above table place limitations on the amount of time allowed between sampling events. However, these rules do not place limits on the time within which the initial sampling must be done after the initial permit containing the sampling requirement is issued. That is, a permittee required to sample once every two years must collect the first sample sometime within two years of issuance of the first permit that contains this sampling requirement.

Form: DEP Form 62-610.300(4)(a)4 is to be used to report the results of the pathogen monitoring. This form is available in Word and pdf formats from the DEP's reuse webpage at www.dep.state.fl.us/water/reuse (follow the clickable link to "Legal Documents"). The instructions included in this form are important, since they specify how data is to be reported. The instructions also include detection limit requirements, which are binding as rule.

Submittal of Monitoring Data: Results of this monitoring are to be submitted to both the DEP district office and to the DEP's Reuse Coordinator in Tallahassee. The mailing addresses are included in the DEP permit and on DEP Form 62-610.300(4)(a)4.

Pathogen Monitoring: The Basics

Methods: The best available method for sampling and analysis for *Giardia* and *Cryptosporidium* is EPA Method 1623, which can be found at www.epa.gov/nerlcwww/1623ap01.pdf. This method will require modification to achieve the detection limits required by DEP's form. EPA Method 1623 calls for filtering 10 L of water in the laboratory. With this volume, the lowest detection limit that could possibly be obtained is 10 per 100 L. In most cases, compliance with the detection limit recommendations in DEP's form will necessitate filtration of larger volumes of water (generally 100 L or more) in the field as samples are collected.

Dechlorination of samples is recommended as a means of avoiding possible interference with the analytical methods.

EPA Method 1623 includes relatively extensive discussion of quality assurance (QA) provisions for the sampling and analysis of Giardia and Cryptosporidium. Compliance with these QA measures is an integral component of meeting the requirements of EPA Method 1623.

The analytical methods used in the analysis and related QA activities must be documented when the permittee submits pathogen data on DEP Form 62-610.300(4)(a)4. In addition, deviations from or modifications to EPA Method 1623 should be documented.

Rule 62-160.330, F.A.C., includes requirements for DEP approval of analytical methods (17).

Detection Limits: DEP Form 62-610.300(4)(a)4 requires that the detection limit not be greater than 10 per 100 L. The form recommends use of detection limits on the order of 1 per 100 L. At this time, about the best that can be achieved is a detection limit on the order of 0.1 per 100 L. Filtering increased volumes of reclaimed water lowers the detection limit. In order to achieve detection limits less than 1 cyst per 100 L, at least 100 L would have to be filtered for analysis. To achieve a detection limit of 0.1 cyst per 100 L, at least 1000 L would have to be filtered for analysis. Note that, in some cases, the use of multiple filters may be needed to achieve the appropriate detection limit.

Viability: Not all cysts or oocysts in a disinfected reclaimed water will be viable (capable of causing infection). DEP Form 62-610.300(4)(a)4 requires that the permittee sample, analyze, and report total numbers of cysts and oocysts. This form allows the permittee to also report concentrations of viable cysts and viable oocysts (this is in addition to the reporting of total cysts and oocysts). The enumeration of viable cysts or oocysts typically involves microscopic examination of cysts or oocysts that are present with an evaluation of the sufficiency of the internal structure. This is commonly done using DAPI staining methods, which are included within EPA Method 1623. Cysts or oocysts that are stained DAPI positive are regarded as being potentially viable. Other, more definitive viability tests involve animal infectivity studies and are significantly more elaborate and expensive.

Laboratories: Rule 62-160.300, F.A.C. (17), requires that all laboratories generating environmental data for submission to the DEP shall hold certification from the Department of Health's (DOH) Environmental Laboratory Certification Program (ELCP). The DOH ELCP is in a position to grant certification to laboratories offering analytical services for *Giardia* and Cryptosporidium. Laboratory certification for these parameters will reflect modifications made to EPA Method 1623 to enable attainment of recommended detection limits. Laboratories interested in learning more about the DOH ELCP program should contact Mr. Steve Arms at 904/791-1502.

Significance of Pathogen Data

As reclaimed water utilities sample for *Giardia* and *Cryptosporidium*, it is likely that some utilities occasionally will find relatively low concentrations of these organisms in their reclaimed water. Florida does not have numeric standards for *Giardia*, *Cryptosporidium*, or other pathogens. As a result, questions may arise about the significance of the data.

The only state that once had numeric pathogen standards is Arizona (18). Arizona once required that reclaimed water used to irrigate food crops that are consumed raw or for use for full body contact recreation (swimming) have *Giardia* concentrations less than detection. The detection limit was not specified. No *Giardia* limits were applied to the use of reclaimed water for landscape irrigation, irrigation of pastures, or for livestock watering. It should be noted that the Arizona standards were not based on risk assessment and that the state recently restructured their reuse regulations, which eliminated their pathogen standards.

York and Walker-Coleman (19,20) used risk assessment methodologies to develop guidelines for pathogens in reclaimed water used to irrigate public access areas (focus was on residential irrigation). They suggested basing "average" pathogen limits on consumption of 1.0 mL of reclaimed water on each of the 365 days in a year. They suggested basing "maximum" pathogen limits on a single, worst case ingestion of 100 mL of reclaimed water on one occasion during a year. An annual risk of infection of $1x10^{-4}$ was used in the analysis. This analysis was based on the assumption that all cysts or oocysts contained complete internal structure and were fully viable and capable of causing infection. This analysis suggested the following pathogen guidelines:

Organism	Units	Suggested Guidelines	
		Average	**Maximum**
Giardia	Viable cysts/100 L	1.4	5.0
Cryptosporidium	Viable oocysts/100 L	5.8	22
Enterovirus (a)	PFU/100 L	0.044	0.165

Note: (a) Assumes all viruses are highly-infective Rotavirus.

While not standards, these guidelines may prove useful to permittees as a frame of reference.

A 1992 study provided a good data set for the pathogen content of reclaimed water at a treatment facility in St. Petersburg (13,14). This data, which follows, also may prove useful as a frame of reference:

Organism	St. Petersburg's Reclaimed Water Quality		
	% Positive	Average	Maximum
Giardia (a)	25	0.49	3.3
Cryptosporidium (b)	17	0.75	5.35
Enterovirus	8	0.01	0.133
Helminthes (c)	0	--	--

Notes: (a) In terms of total cysts. No attempt was made to identify viable cysts.
(b) In terms of total oocysts. No attempt was made to identify viable oocysts.
(c) No helminthes were detected at any time at any location after the secondary clarifier.

The data in Exhibits A and B also provides a frame of reference on the occurrence of these pathogens in other high quality water sources.

Finally, Haas and Rose (21) suggested an "action level" for *Cryptosporidium* of between 10 and 30 oocysts/100 L in treated drinking water. They suggest that at concentrations above these levels outbreak conditions may result. They suggested that utilities consider modifications of plant operations and public notification above the action level.

Florida Monitoring Results

Table 1 presents a summary of pathogen monitoring data obtained from Florida's reuse facilities from the inception of Florida's pathogen monitoring requirement through May 1, 2002 (22). Earlier papers by York, et al. (23,24) presented earlier results of Florida's monitoring. It is interesting to note that the frequencies of detectable observations are greater than previously reported in the St. Petersburg (13,14) and Monterey County (16) studies. This is particularly true for *Giardia*, where 58 percent of the observations reported as part of Florida's ongoing monitoring had detectable concentrations. Reported concentrations for *Giardia* also were significantly higher than in the previous studies. About 48 percent of all observations for *Giardia* were greater than 5 cysts per 100 L, 25 percent of all observations were greater than 76 per 100 L, and 10 percent of observations exceeded 333 per 100 L.

From the data in Table 1, it appears that *Cryptosporidium* poses less concern than does *Giardia* (22). While the maximum value observed (282 oocysts/100 L) is larger than data reported in previous studies, the frequency of detecting *Cryptosporidium* (22% of observations) was relatively low. Only six observations were greater than 5 oocysts per 100 L (actual values were 11, 14, 39.1, 60.7, 70, and 282 oocysts/100 L).

In general, the facilities that have reported pathogen data have been well operated (22). This is shown by the total suspended solids (TSS), turbidity, and total chlorine residual data contained in Table 2. The median values for these parameters were 1.0 mg/L, 0.99 NTU, and 4.1 mg/L, respectively. Only two values (3.2% of all observations) were reported that constitute possible violations of state standards. Both were TSS observations of 6.0 mg/L (Florida's single sample maximum standard for TSS is 5.0 mg/L).

Table 1. Summary of Florida Pathogen Monitoring Data

Statistic	*Giardia*	*Cryptosporidium*
Number of observations	69	68
% having detectable concentrations	58%	22%
25 percentile (#/100 L)	ND	ND
50 percentile (#/100 L)	4	ND
75 percentile (#/100 L)	76	ND
90 percentile (#/100 L)	333	2.3
Maximum (#/100 L)	3,096	282
% greater than 5/100 L	48%	9%

Notes: (a) ND indicates a value less than detection.
(b) All numeric data are total numbers of cysts or oocysts per 100 L.
(c) Source: Walker-Coleman, et al. (22)

Table 2. Operational Data for Florida Facilities

Statistic	TSS (mg/L)	Turbidity (NTU)	Chlorine Residual (mg/L)
Minimum	0.19	0.31	1.01
10 percentile	0.40	0.45	1.9
25 percentile	0.80	0.65	2.32
50 percentile	1.0	0.99	4.1
75 percentile	1.76	1.36	5.0
90 percentile	2.1	1.8	7.1
Maximum	6.0	4.5	10.67

Source: Walker-Coleman, et al. (22)

It is interesting to note that the facilities reporting the highest concentrations of pathogens appeared to be providing effective filtration and disinfection (22). For example, the facility reporting the highest concentration of *Giardia* (3,096 cysts/100 L) reported TSS of 1 mg/L, turbidity of 1.5 NTU, and a chlorine residual of 3.5 mg/L. This facility, which uses contact stabilization and deep bed filters, also reported a Cryptosporidium concentration of 11 oocysts/100 L.

The facility reporting the second highest *Giardia* concentration (2,575 cysyts/100 L) reported turbidity of 0.87 NTU (22). TSS and chlorine residual were not reported. This facility, which uses a biological nutrient removal process and upflow filters, did not detect Cryptosporidium in their reclaimed water (detection limit was 3.4 oocysts/100 L).

Results from one utility in Southeast Florida are of interest. Initially, this facility reported concentrations of *Giardia* and *Cryptosporidium* of 120 cysts/100 L and 70 oocysts/100 L, respectively (22). At that time, TSS was 0.7 mg/L, turbidity was 0.6 NTU, and the chlorine residual was 3 mg/L. After refining their filtration process (adjusting the backwash rates on the upflow filters and the addition of a polymer) this utility was successful in reducing concentrations of the protozoan pathogens to less than detection.

Two facilities reported TSS of 6 mg/L, which is above the state standard of 5.0 mg/L (22). However, these facilities each reported turbidities of about 1.2 NTU. *Giardia* concentrations at these two facilities were 58 and 99.3 cysts/100 L, while *Cryptosporidium* was less than detection. Chlorine residuals were about 2.3 and 4.5 mg/L.

While Florida does not require assessment of whether or not cysts or oocysts are viable, utilities may report numbers of potentially viable cysts or oocysts in addition to the total numbers (22). Thirteen facilities that reported detectable levels of *Giardia* also reported concentrations of potentially viable *Giardia* cysts and three facilities reported total and potentially viable concentrations of *Cryptosporidium* (based on microscopic examination and vital staining). The percentage of *Giardia* cysts reported as potentially viable ranged from 10 to 90 percent with an average of 61 percent. For *Cryptosporidium*, the viable fraction ranged from 70 to 90 percent with an average of 77 percent.

Table 3 presents statistics for *Giardia* concentrations related to treatment, filtration, and disinfection technologies employed. It is interesting to note that facilities that nitrify appeared to offer increased potential for removing *Giardia* than do the facilities that do not nitrify.

Florida's Ongoing Activities

The DEP has implemented follow-up procedures for contacting facilities that report relatively high concentrations of the protozoan pathogens. Although pathogen standards have not been established in Florida, the DEP writes to facilities that report concentrations of *Giardia* greater than 5 cysts per 100 L or of *Cryptosporidium* greater than 5 oocysts per 100 L. The 5 per 100 L threshold used by DEP generally reflects the work of York and Walker-Coleman (19,20). Contacts are made to alert the facilities to the significance of these pathogens and to encourage refinement of their filtration processes.

Staff in DEP's district offices evaluate facilities reporting concentrations of 5 per 100 L or greater and conduct follow-up inspections of the facilities. These inspections provide opportunities to discuss the significance of these pathogens and ascertain whether the facilities have implemented corrective actions to reduce pathogen concentrations. Facilities reporting relatively high concentrations are encouraged, but not required, to resample upon implementation of operational refinements. The DEP also sends follow-up letters to facilities reporting relatively high pathogen concentrations to augment the district offices' efforts.

Table 3. *Giardia* Data for Treatment Technologies from Florida Monitoring Data

Technology	No. of Observ.	% less than Detect.	*Giardia* (#/100L) Median	Max.	% >5
All Facilities	60	40	6.0	3096	50%
Facilities that Nitrify	46	48%	3.5	2575	41%
Facilities that do not Nitrify	20	20%	11.74	3096	65%
Conventional Activ. Sludge	28	22%	6.78	287.1	53%
Extended Aeration	19	63%	ND	710	37%
Biological Nutrient Removal	12	42%	6	2575	50%
Contact Stabilization	4	0%	223	3096	100%
Pure Oxygen	2	100%	ND	ND	0%
SBR	1	100%	ND	ND	0%
Chlorine Gas	52	40%	6.0	3096	50%
Chlorine, Other	8	37%	6.0	120	50%
Deep Bed Filters	15	67%	ND	3096	27%
Shallow Bed ABW Filters	25	20%	6.0	411	52%
Upflow Filters	16	50%	17	2575	50%
Other Filter Types	6	17%	61	137	83%

Source: York, et al. (24)

Other Monitoring Data

A reclaimed water utility in Southwest Florida that is pursuing a reclaimed water aquifer storage and recovery (ASR) system sampled for the protozoan pathogens on six occasions in 1998-2000 (22,24). All observations of Giardia were less than detection. Of the six Cryptosporidium samples, only one was positive (2 oocysts/100 L).

Data are also available from another utility in Southwest Florida that is pursuing a reclaimed water ASR system (22,24). Two treatment facilities were sampled 16 times each in the 1998-2000 period. Of the 32 total samples, 13 were positive for Giardia (41%) and 3 were positive for Cryptosporidium (9%). The maximum values observed were 10.3 oocysts/100 L for Cryptosporidium and 264.3 cysts/100 L for Giardia.

A utility along Florida’s East Coast operates two water reclamation facilities – one uses chlorination, the other ultraviolet (UV) disinfection (22,24). During the 1999 to 2001 period, 11 samples were taken at each facility for the protozoan pathogens. The UV facility recorded two positive observations of Giardia (0.1 and 20 cysts/100 L), while the chlorination facility recorded seven positive observations (range: 0.4 to 210/100 L). For Cryptosporidium, the UV facility recorded two positive observations (0.1 and 10 cysts/100 L), while the chlorination facility recorded four positives (0.1 to 270/100 L). Ten observations of enteric virus also were made at each facility and all twenty observations were less than detection.

Giardia Infectivity Studies

As demonstrated by the Los Angeles County Sanitation District in California (22,25,26,27), cysts found in reclaimed water probably are not capable of causing infection. In the first phase of this study, it was determined that the Giardia infective dose (ID_{50}) for gerbils was less than 10 cysts. Gerbils were then fed doses of Giardia taken from primary effluent and from reclaimed water. Of the six gerbils fed a dose of 300 cysts (of which 78 were potentially viable, as determined by vital staining) from the primary effluent, four became infected, indicating that cysts found in undisinfected primary effluent probably are capable of causing infection. In the reclaimed water studies, eight gerbils received a dose of 1,000 cysts (240 potentially viable) and six gerbils received 200 cysts (48 potentially viable). None of these 14 animals became infected. It is interesting to note that even at doses many times larger than the infective dose, cysts found in the final reclaimed water were not capable of causing infection.

The City of Calgary, Alberta, Canada conducted a separate infectivity study at a biological nutrient removal treatment plant (28,29). The Calgary treatment facility, which does not include filters, uses UV for disinfection. The design UV dose was 30 mW-sec/cm^2, and the design UV transmittance is 55 percent. Gerbils were inoculated with water containing Giardia and were tested for infection one week after inoculation. Gerbils inoculated with primary effluent became infected with Giardiasis, as did gerbils inoculated with pre-disinfected effluent. The mean concentration of Giardia in the primary effluent was 4,625 cysts/100 L, of which 63 percent were potentially viable. Potential viability was determined through DAPI positive staining and microscopic examination. The mean concentration of Giardia in the pre-disinfected effluent was 474 cysts/100 L, of which 33 were potentially viable. Gerbils also were inoculated with Giardia that had passed through the treatment plants UV disinfection system. The mean concentration of Giardia in the post UV-disinfected reclaimed water was 443 cysts/100 L, of which 15 percent were potentially viable. However, none of the gerbils inoculated with post UV-disinfected reclaimed water became infected with Giardiasis.

Reuse and the Absence of Disease

It must be noted that there is no evidence or documentation of any disease associated with water reuse systems in the United States or in other countries that have reasonable standards for reuse. This is true for protozoan, viral, helminthic, and bacterial pathogens.

Summary

In 1999, Florida established requirements for monitoring of the protozoan pathogens in reclaimed water. This paper reviewed Florida's rule requirements and presented information related to the sampling, analysis, and reporting of the pathogen monitoring. In addition, background information on *Giardia* and *Cryptosporidium* was presented. This included notes on the significance of pathogen data that may be useful to permittees as they conduct this monitoring. Results from ongoing monitoring activities have been presented along with the results of two interesting infectivity studies.

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Exhibit A. *Cryptosporidium* in the Environment

Water Type	% Positive	Average (oocysts/100 L)	Range (oocysts/100 L)	Ref.	Notes
Reclaimed water (St. Petersburg)	17	0.75	ND-5.35	13	12 samples
Irrigation canals (in AZ)	100	555,000	530,000-580,000	30	2 samples
Surface waters (all categories)	51	43	ND-29,000	10	181 samples in 17 states
Rivers (pristine)	32	29	ND-24,000	10	59 samples
Lakes (pristine)	53	9.3	ND-307	10	34 samples
Springs	---	4	---	10	7 samples
Ground water	5.5	0.3	ND-4	10	12 samples
Ground waters	17	41	---	31	74 samples
Source waters of surface water treatment plants	87	270	ND-48,400	32	66 water treatment plants in 14 states and 1 Canadian province - 85 samples
Surface water supplies for drinking water plants	51.5	240	ND-6510	33	1991-1993, 262 samples at 72 water plants
Rivers in a protected watershed (Western USA)	83	2	ND-13	34	6 samples
Catskill Watershed Delaware Malcolm Brook	46 37 52	1.4 0.8 1.0	ND-17.3 ND-15 ND-48	35	3 protected watersheds that serve as sources of drinking water for New York City.
Filtered drinking water	26.8	1.52	ND-48	36	66 water treatment plants in 14 states & 1 Canadian province - 85 samples
Treated drinking water	13.4	3.3	ND-57	33	1991-1993, 262 samples at 72 water plants
Filtered drinking water (Western USA)	20	0.1	---	34	10 samples
Non-filtered drinking water (Western USA)	50	0.6	---	34	4 samples
Treated drinking water	17	0.1	---	10	36 samples
Phillippi Creek (FL)	13	16	ND-158	37	An urban stream in Sarasota, 16 samples
5 streams (FL)	4	6.6	ND-157	37	In the vicinity of Sarasota, 24 samples
Sarasota Bay (FL)	0	ND	ND	37	4 samples at 1 point in a high-quality estuary.
Tampa Bypass Canal (FL)	43	3.1	ND-11	9	7 samples

Notes: ND = Less than detection. Information adapted from (7,8).

Exhibit B. *Giardia* in the Environment

Water Type	% Positive	Average (cysts/100 L)	Range (cysts/100 L)	Ref.	Notes
Reclaimed water (St. Petersburg)	25	0.49	ND-3.3	13	12 samples
Surface waters (all categories)	15	3	ND-625	10	181 samples in 17 states
Rivers (pristine)	6.8	0.35	ND-12	10	59 samples
Lakes (pristine)	12	0.5	ND-7	10	34 samples
Springs	0	<0.25	---	10	7 samples
Ground water	0	<0.25	---	10	12 samples
Ground waters	9.5	16	---	31	74 samples
Source waters of surface water treatment plants	81.2	277	ND-6600	32	66 water plants in 14 states & 1 Canadian province - 85 samples
Surface water supplies for drinking water plants	45	200	ND-4380	33	262 samples at 72 water plants, 1991-1993
Rivers in protected watershed (Western USA)	17	0.6	---	34	6 samples
Catskill Watershed Delaware Malcolm Brook	36 29 46	1.2 0.7 1.3	ND-9.3 ND-8.2 ND-23.4	35	3 protected watersheds that serve as sources of drinking water for New York City
3 pristine river systems (near Seattle, WA)	42	6.3	ND-520	38	222 samples at 17 sites over 9 months
Portland, OR water supply reservoir	19	0.34-2.77	---	39	A protected reservoir. Several data sets
Filtered drinking water	17.1	4.45	ND-64	36	66 water plants in 14 states & 1 Canadian province - 82 samples
Treated drinking water	4.6	2.6	ND-9	33	262 samples at 72 water plants, 1991-1993
Treated drinking water	0	<0.25	---	10	36 samples
Phillippi Creek (FL)	6	9.8	ND-157	37	an urban stream in Sarasota, 16 samples
5 streams (FL)	0	ND	ND	37	24 samples from streams in the vicinity of Sarasota
Sarasota Bay (FL)	0	ND	ND	37	4 samples at 1 point in a high-quality estuary.
Tampa Bypass Canal (FL)	14	0.42	ND-2.9	9	

Notes: ND = Less than detection. Information adapted from (7,8).