Measurement of Inactivation of *Cryptosporidium parvum* and *Bacillus subtilis* Spores using the Clear Comfort System

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Introduction:

Cryptosporidiosis is a highly contagious gastrointestinal illness caused by the protozoa known as *Cryptosporidium*. Both the microbe and the disease are commonly known as "Crypto." *Cryptosporidium* is the cause of one of the most common recreational waterborne diseases in the United States, and the protozoa is characterized by an outer shell resistant to chlorine and many other disinfection chemicals.
Increased occurrences of this chlorine tolerant microorganism have directly resulted in higher downtimes with longer duration in public pools. A single *Cryptosporidium* oocyst may be sufficient to cause symptoms in immunocompromised individuals and infants. The net result is lower revenues at higher operational costs while instilling fear into the patrons that rely on clean and safe pool environments. Next generation technologies and actionable solutions for pools are in high demand to control such outbreak occurrences.

Aim:

Test the dose response of the Clear Comfort with *Cryptosporidium* oocysts and Bacillus spores over a 400-minute time period at a 2-Liter per minute air flow rate in a dechlorinated tap water matrix.

Method Summary:

Cryptosporidium parvum Iowa isolate (Harley Moon) was obtained from Waterborne Inc. passed through mice. 10^8 oocysts of viable *C. parvum* were harvested and stored in 10 mM phosphate buffer and the oocysts were used within 30 days from shedding to experiments.
Exposed *Cryptosporidium* oocysts to a range of doses (0 min, 15 min, 60 min, 150 min, 300 min and 420 min) of Clear Comfort airflow in a 4-liter reactor, with sterile chlorine-free tap water. Chlorine free tap water was chosen to ensure disinfection efficacy from the Clear Comfort system was not compromised by water quality issues, so as to gain insights into the fundamental mechanisms of the disinfection process. A warm up period of 30 minutes where the disinfection system was run in the water was used before spiking the microbes into the disinfection chamber. pH, conductivity, calcium hardness, total alkalinity, and water temperature (20°C) were held constant for the duration of the experiment. A mixing bar was used to agitate the sample volume and ensuring complete mixing. Samples were removed at 0 min, 15 min, 60 min, 150 min, 300 min and 420min. Sterile bottles were used for sampling during the investigation and sample volumes and frequencies were chosen to not significantly affect experimental working volumes. An additional test was performed using *Bacillus subtilis* spores at similar doses (0min, 15min, 30 min, 60 min, 150 min, and 300 min) under identical conditions. Duplicate samples were taken at 3 time points for each microbe.
All biological samples were transported overnight to the University of Washington School of Public Health for microbiological assays. *Cryptosporidium* was assayed using cell culture infectivity with cell line HCT-8 to evaluate infectivity and inactivation. Infectious foci, identified with an immunofluorescent stain, were counted using an inverted epifluorescent microscope. Bacillus spores were assayed using a spread plate agar method and counting colony forming units.

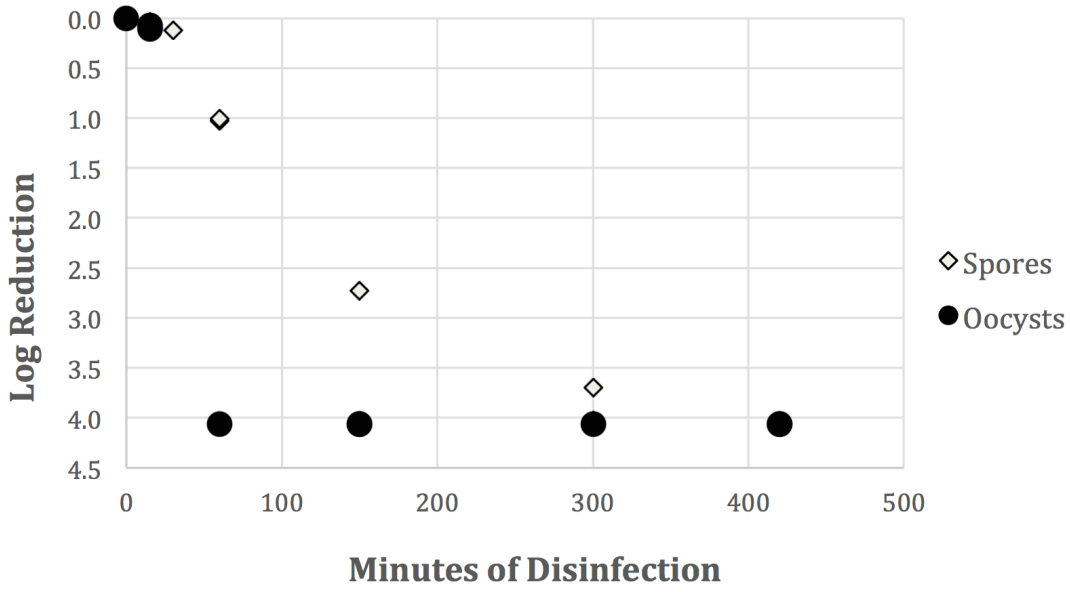

Figure 1. Log reduction in *Cryptosporidium* oocysts over time of exposure to the Clear Comfort system. Note that results at 4 log indicate complete inactivation

Results:

Figure 1 presents the results from the disinfection testing. All control tests came out as expected.

Conclusions

The Clear Comfort disinfection system was able to achieve greater than 4 log inactivation (>99.99%) of *Cryptosporidium* in under 60 minutes of disinfection exposure time in a clean water matrix of dechlorinated tap water. Bacillus spores were disinfected to greater than 4 log reduction in approximately 300 minutes in an identical water matrix. The Clear Comfort system is capable of disinfecting these microorganisms in a matter of hours. Because the Bacillus spores exhibited more resistance to the disinfection process compared to *Cryptosporidium*, the spores can be used as a conservative surrogate to represent *Cryptosporidium*
disinfection in this water matrix. These findings should be verified with a test in a water matrix as would be used in practice.

Significance and Impact of this Study:

This study is the first report on the investigation of *Cryptosporidium parvum* inactivation by oxidant production from the Clear Comfort system. Results demonstrate disinfectant produced from the Clear Comfort system achieve complete inactivation.

Appendix

Table A1. Log inactivation data for *Cryptosporidium* oocysts and Bacillus spores

Exposure (min)	Log Inactivation (log(Co/C)	
	Spores	Oocysts
0	0.00	0.00
15	0.04	0.07
15	0.09	0.11
30	0.12	-
60	1.03	>4.06
60	1.01	-
150	2.73	>4.06
150	-	>4.06
300	3.73	>4.06
300	>4.03	-
420	-	>4.06
420	-	>4.06