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Automated Dead-end Ultrafiltration for Enhanced Surveillance of *Legionella pneumophila* and *Legionella* spp. in Cooling Tower Waters

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ABSTRACT

Detection of *Legionella pneumophila* in cooling towers and domestic hot water systems involves concentration by centrifugation or membrane filtration prior to inoculation onto growth media or analysis using techniques such as PCR or immunoassays. The Portable Multi-use Automated Concentration System (PMACS) was designed for concentrating microorganisms from large volumes of water in the field and was assessed for enhancing surveillance of *L. pneumophila* at the Savannah River Site, SC. PMACS samples (100 L; n = 28) were collected from six towers between August 2010 and April 2011 with grab samples (500 ml; n = 56) being collected before and after each PMACS sample. All samples were analyzed for the presence of *L. pneumophila* by direct fluorescence immunoassay (DFA) using FITC-labeled monoclonal antibodies targeting serogroups 1, 2, 4 and 6. QPCR was utilized for detection of *Legionella* spp. in the same samples. Counts of *L. pneumophila* from DFA and of *Legionella* spp. from qPCR were normalized to cells/L tower water. Concentrations were similar between grab and PMACS samples collected throughout the study by DFA analysis ($P = 0.4461$; repeated measures ANOVA). The same trend was observed with qPCR. However, PMACS concentration proved advantageous over membrane filtration by providing larger volume, more representative samples of the cooling tower environment, which led to reduced variability among sampling events and increasing the probability of detection of low level targets. These data highlight the utility of the PMACS for enhanced surveillance of *L. pneumophila* by providing improved sampling of the cooling tower environment.

48 **INTRODUCTION**

49 *Legionella pneumophila* causes Legionnaires' disease and has been found in
50 cooling towers and domestic hot water systems (1). Several outbreaks of legionellosis in
51 the past decade have been linked to cooling towers (23) with transmission possibly
52 occurring via exposure to aerosols containing the bacteria (1, 23). However, current
53 procedures for the detection of *L. pneumophila* in cooling towers lack standardization,
54 with variable approaches in culturing, diverse interpretations of infectious dose, and
55 widely varying protocols for disinfection (2, 20). For example, the CDC does not
56 recommend routine testing of cooling tower waters while the Association of Water
57 Technologies (AWT) supports tower surveillance for proactive outbreak prevention (1).
58 Furthermore, the exact source of *L. pneumophila* in outbreaks attributed to cooling towers
59 remains unclear. Towers were first implicated by CDC investigators as a reservoir in a
60 hospital outbreak in Memphis, Tennessee in 1978 with aerosolization being the presumed
61 mode of transmission (7). A recent outbreak of Legionnaires' disease in New Zealand
62 implicated a cooling tower as the source following environmental testing (19).

63 If such testing is performed, recovery of *L. pneumophila* generally involves
64 concentration by membrane filtration or centrifugation of small volumes prior to
65 inoculation onto growth media. Analysis may also include techniques such as PCR or
66 immunoassays (5). Regardless of the exact methodology chosen, detection in cooling
67 tower waters remains problematic with limitations likely related to sampling error created
68 by the small volumes of sample normally processed for analysis. For example, a third of
69 tower samples in a recent study of Italian hotel cooling towers were positive for *L.*
70 *pneumophila* (4), but since only 1 L samples were analyzed, additional towers could

71 have contained the microorganisms. Also, Lee et al. noted that 10% of cooling tower
72 samples in China were positive for *L. pneumophila* (14). Again the amount of water
73 collected for testing was 1 L. Collection of microorganisms from larger volumes
74 increases the likelihood of detection of low-level contaminants and provides more
75 representative sampling of a water body, such as a cooling tower.

76 The Savannah River Site (SRS) has been monitoring cooling towers for the
77 presence of *Legionella* since the early 1980s. *Legionella pneumophila* was first observed
78 in well-maintained cooling towers and air conditioning systems at SRS by Fliermans et
79 al. (10). The concentrations detected were deemed acceptable because the authors
80 believed it was unreasonable to assume that *L. pneumophila* could be completely
81 eradicated from a given system since the cells are members of the "natural aquatic
82 community." Disease risk levels for SRS towers were established following an extensive
83 review of cooling tower-associated Legionnaires' disease outbreaks by SRS Biosafety
84 and Industrial Hygiene Committees and SRS medical personnel (21). Expanding the
85 surveillance program to testing of larger volumes of tower water would provide SRS
86 managers with valuable information for protecting the health of Site workers.

87 The Portable Multi-use Automated Concentration System (PMACS, (IntelliSense
88 Design, Inc., Tampa, FL; Fig. 1) collects microorganisms from large volumes of water
89 using dead-end ultrafiltration and recovers them in a small amount of buffer to produce a
90 concentrated sample or retentate. The unit was developed at the University of South
91 Florida and has been modified for field collection (13, 16). The PMACS is a software-
92 driven system with a small footprint to provide portability for on-site collection. The
93 collection module weighs less than 11.5 kg and has dimensions of 0.25 m x 0.28 m x 0.91

94 m (L x W x H). The system also contains transducers for digital monitoring of pressure
95 within the system and a flow meter to monitor flow rate and total filtered volume. The
96 objective of this study was to establish if surveillance for *L. pneumophila* in SRS cooling
97 towers waters could be enhanced by collection of larger volume samples with the
98 PMACS. The current surveillance program at SRS involves detection in grab samples
99 using direct immunofluorescence assay (DFA). Quantitative PCR (qPCR) was included
100 in this study as confirmation of PMACS concentration of *Legionella* spp. for samples
101 collected from January through April 2011.
102

103 **MATERIALS AND METHODS**

104 **Antibodies.** FITC-labeled monoclonal antibodies for *L. pneumophila* serogroups 1, 2, 4
105 and 6 (Monoclonal Technologies, Inc., Alpharetta, GA) were used in the direct
106 immunofluorescence assay (DFA). These serogroups were chosen for analysis since they
107 are most commonly linked with disease, especially serogroup 1 (9).
108 **Bacteria.** Inactivated *L. pneumophila* serogroups 1, 2, 4 and 6 were acquired from
109 Monoclonal Technologies, Inc. and served as positive controls for the DFA. *Serratia*
110 *marcescens* (ATCC#13880) served as the DFA negative control. Genomic DNA for *L.*
111 *pneumophila* serogroup 1 was acquired from American Tissue and Culture Collection
112 (ATCC 33152D-5) and served as positive control for qPCR. *Serratia marcescens*
113 (ATCC#13880) genomic DNA served as the qPCR negative control.
114 **Sampling location and dates.** The SRS, a 499 km^2 U.S. Department of Energy facility,
115 is located in the Southeastern U.S. near Aiken, SC and is bordered to the west by the
116 Savannah River and Georgia. Several cooling towers were selected for evaluation of

117 PMACS enhancement of the SRS *L. pneumophila* surveillance program. A total of
118 twenty-eight 100 L samples and fifty-six 500 ml grab samples were collected between
119 August 2010 and April 2011. Both sample types were collected from the same area and
120 depth of the cooling tower basins, which had water flow rates of approximately 9,500
121 L/min. Grab samples were taken prior to and immediately after PMACS samples. Water
122 from two tower basins was sampled in August to assess the most appropriate DFA
123 protocol for the PMACS retentates. Due to the variety of tower types at SRS, those tower
124 basins were sampled again in September together with an additional three towers to
125 evaluate the general applicability of the PMACS to a *Legionella* surveillance program.
126 Finally, one tower basin was sampled exclusively in 2011 to provide statistical validation
127 of the PMACS' performance for *Legionella* detection over an extended timeframe. All
128 samples were transported immediately to the laboratory or were placed on ice until the
129 time of processing, which was performed within three hours of collection.
130 **Physical and chemical water quality parameters.** Water quality parameters of tower
131 basin waters were collected to evaluate potential influences on the concentration of *L.*
132 *pneumophila* within tower waters in grab or retentate samples. Temperature, dissolved
133 oxygen, conductivity and pH of cooling tower water on each sampling date were taken
134 with a YSI 556MPS Sonde (YSI, Yellow Springs, OH). Free chlorine and total bromine
135 within tower waters were analyzed with a Palintest 1000 Bromometer Duo Kit (Palintest
136 USA, Erlanger, KY). Turbidity of tower water was determined with a Hach 2100P
137 Turbidimeter (Hach, Loveland, CO).
138 **Dead-end ultrafiltration and sample recovery with the PMACS.** The PMACS was
139 transported to the test cooling towers at SRS for each sampling event. A new Fresenius

140 Optiflux F200NR Polysulfone High-flux Capillary Dialyzer (Fresenius Medical Care
141 North America, Lexington, MA) was installed into the PMACS for each tower sample.
142 Each 200NR filter has a total surface area of 2.0 m^2 and a molecular weight cut-off of
143 approximately 30 kDa. Cooling tower water was fed into each filter by a Masterflex I/P
144 Precision Brushless peristaltic pump drive with a Masterflex I/P High Performance pump
145 head (Cole Parmer Instrument Co., Vernon Hills, IL). The pump was powered in the
146 field by a portable Xantrex XPower Powerpack 600 HD (Xantrex Technology Inc.,
147 Burnaby, BC, Canada). Fifteen feet of Masterflex I/P BioPharm platinum-cured silicone
148 I/P 70 tubing (Cole Parmer), with a nylon mesh screen attached to the uptake end,
149 transported the water from the cooling tower basins to the PMACS. PMACS
150 performance was monitored throughout each collection cycle using system parameters,
151 including flow rate and pre- and post-filter pressure. Recovery of the concentrated
152 samples was performed by backflushing with 0.1% sodium phosphate buffer augmented
153 with 0.01% sodium polyphosphate (PB+NaPP) to produce retentates of 350-400 ml. All
154 PMACS components and sample tubing were cleaned between sampling events with 180
155 ppm acidified bleach (c. pH 6.5) and rinsed with distilled water (DI) water. Residual
156 chlorine was removed from the PMACS by rinsing with 2.5% sodium thiosulfate
157 followed by two flushes with DI water.
158 **Direct immunofluorescence assay (DFA) enumeration of *L. pneumophila*.** The entire
159 500 ml volume of each grab sample was concentrated by membrane filtration through
160 one to three 47 mm diameter, 0.4 µm pore size filters (Whatman Nucleopore, GE
161 Healthcare Life Sciences, Piscataway, NJ) prior to DFA processing. Each filter or
162 membrane set, if more than one filter was required for a sample, was aseptically cut and

163 placed into a 15 ml conical tube (Thermofisher Scientific), one milliliter 0.2 µm-
164 sterilized nanopure water was added to each, and tubes were vortexed for 4 min at high
165 speed. Secondary concentration by filtration of aliquots (1 ml and 10 ml) of PMACS
166 retentates was tested in August 2010 to determine the best method for DFA processing of
167 retentates. The remaining retentates throughout the study were processed by direct
168 application of retentate to DFA slides.

169 Eight-well glass slides (Carlson Scientific, Peotone, IL) were pretreated at 90°C
170 for a minimum of 4 h and then washed with 70% ethanol prior to sample deposition.
171 Cleaned slides were heated to 80- 90°C on a heat block and 10 µl of each sample was
172 added to a slide well. Separate slides were prepared for assay controls for each sampling
173 event. Samples were added to 4 separate wells of a slide and heat fixed at 80-90°C for
174 10-15 min. Twenty microliters of 2% hydrolyzed gelatin (Difco, Thermofisher
175 Scientific, Waltham, MA) was added to each well while the slide remained on the heat
176 block and slides were allowed to dry completely. Slides were placed into a 25°C
177 humidified chamber for 20 min. The four slide aliquots of each sample were then stained
178 with 20 µl of antibody to one serogroup for 20 min at 25°C in the dark. Slides were
179 rinsed with DI water and stored in FA buffer (Difco, Thermofisher Scientific) at 25°C
180 overnight. Slides were dipped into fresh 5% sodium pyrophosphate (Difco, Thermofisher
181 Scientific) buffer and allowed to dry completely in the dark at 25°C. Slides were stored
182 at 4°C until examined microscopically.

183 **Quantitative PCR (qPCR) enumeration of *Legionella* spp.** DNA was extracted from
184 grab samples and PMACS retentates with the Mo Bio PowerSoil DNA Isolation Kit (Mo
185 Bio Laboratories, Inc., Carlsbad, CA). The presence of *Legionella* spp. in the 2011

186 samples was confirmed by qPCR using the primers LEG-225 and LEG-858, which
187 amplify approximately 654 bp of the 16S rRNA gene (17). Thermal cycling conditions
188 were as follow: 95°C for 3 min., 95°C for 10 s., 48°C for 30 s., and 72°C for 30 s. with
189 plate read for 40 cycles. Primers were obtained from Integrated DNA Technologies
190 (Coralville, IA) and mixed with IQ SYBR Green Supermix (Bio-Rad Laboratories,
191 Hercules, CA). Reactions for qPCR were performed on a CFX96 Real-Time PCR
192 Detection System (Bio-Rad Laboratories, Hercules, CA). C_T values were calculated
193 automatically by software package (Bio-Rad CFX Manager v. 1.6.541.1028). A positive
194 control (*L. pneumophila* serogroup 1), negative control (*S. marcescens*) and co non-
195 template controls (PCR grade water and mastermix) were included in all assays. Each
196 sample was tested in triplicate and the mean value was used for statistical analysis.
197 Melting curve analysis was performed to evaluate positive and negative results.
198 **Statistical analysis.** Concentrations of *L. pneumophila* as determined by DFA were
199 normalized per liter of tower water to allow direct comparisons between samples and
200 across sampling dates. Differences in $\log_{10}$-transformed grab sample cell concentrations
201 from DFA taken before and after PMACS ultrafiltration samples were compared to those
202 in PMACS retentates using parametric or nonparametric repeated measures ANOVA
203 when appropriate based on the number of samples collected for a given test set
204 (GraphPad InStat 3.0, GraphPad Software Inc., San Diego, CA). Linear regression was
205 used to compare *Legionella* concentrations in grab and retentate samples to all chemical
206 and physical water quality data. Statistical significance for all data was accepted at the
207 95% confidence level ($\alpha \leq 0.05$).
208

209 **RESULTS**

210 **DFA procedure for PMACS retentates.** Ten samples were tested in August 2010 to
211 determine the best DFA procedure for PMACS retentates. Waters within the tower
212 basins were approximately 30°C with free chlorine levels of 1 ppm and turbidities of 2
213 NTU (nephelometric turbidity unit) during the sampling period. Samples collected
214 included grab samples (four) to provide a comparison with the standard SRS procedure.
215 The PMACS processed an average of 100 L with an average flow rate of 2.86 $\pm$ 0.20
216 L/min. The retentate samples processed by direct deposition onto DFA slides had similar
217 concentrations of *L. pneumophila* to the grab samples after all concentrations were
218 normalized to the volume of water in the towers (P = 0.1750, Friedman Test; Table 1).
219 Grab sample levels were approximately 6.0 $\log_{10}$ cells/L while directly deposited
220 PMACS samples contained 6.17 $\log_{10}$ cells/L. Processing retentate aliquots using the
221 SRS standard membrane filtration procedure resulted in either decreased levels of *L.*
222 *pneumophila* (1 ml) or levels that were not substantially increased over the directly
223 deposited retentate samples (10 ml). Secondary concentration of the retentates using the
224 SRS procedure required additional processing time without substantially increasing
225 detectable normalized levels; therefore, retentate samples were processed for DFA by
226 direct deposition onto slides for the remainder of the study.

227 **PMACS concentration across towers.** Testing the utility of the PMACS for enhancing
228 the SRS *Legionella* surveillance program was expanded in September 2010 to include
229 five towers (A-E). The purpose of these tests was to assess the robustness of the PMACS
230 and to characterize any problems that might occur during sampling of the large number of
231 tower types (towers in different areas have different maintenance protocols (e.g., biocide

232 treatment, anti-corrosion additives, water turnover), source water (well vs. treated water)
233 and levels of total particulates (construction activity and pollen) on the Site. Physical and
234 chemical water quality parameters were similar across the towers for most of the
235 measured values. Temperatures ranged from 22.2-26.0^{o}C and free chlorine levels were
236 below 2 ppm. Turbidity was the most variable parameter across towers and ranged from
237 0.5-4.3 NTU. The PMACS processed an average of 100.3 $\pm$ 0.67 L with an average flow
238 rate of 2.08 $\pm$ 0.09 L/min. Some differences were noted in the concentrations of *L.*
239 *pneumophila* across towers regardless of the collection method (Table 2). Levels were
240 highest in Tower A (7.41-7.57 $\log_{10}$ cells/L) and lowest in Tower C (4.66-4.96 $\log_{10}$
241 cells/L). Generally, concentrations were slightly higher for PMACS retentates, but the
242 difference in grab samples and retentate concentrations was not significant when the data
243 were combined by collection method ($P = 0.28$; repeated measures ANOVA). No
244 relationships were observed between the measured water quality parameters and the
245 concentration of *L. pneumophila* detected in either grab or retentate samples (data not
246 shown).
247 **Dedicated PMACS sampling of a single SRS cooling tower.** The PMACS was tested
248 in a series of experiments conducted from January to April 2011 to better characterize
249 PMACS-enhanced detection of *Legionella*. Twenty-one retentate samples were
250 generated during this time, together with concurrent grab samples taken both before and
251 after each PMACS sample (grab sample n = 42). Most of the water quality parameters
252 varied during this portion of the study that ranged from winter to spring of 2011 (Table
253 3). Temperatures increased in the tower basin water from an average of 12.89^{o}C to
254 20.17^{o}C during this time ($P = 0.004$; one-way ANOVA). Free chlorine and total bromine

255 levels within the tower basin water were highest in January (both $P = 0.0001$; one-way
256 ANOVA). Turbidity was the only parameter that remained unchanged over the course of
257 the study ($P = 0.7581$; one-way ANOVA). The PMACS processed an average of 98.6 $\pm$
258 4.3 L with an average flow rate of 2.72 $\pm$ 0.38 L/min. Concentrations of *L. pneumophila*
259 generally increased during the study period for both collection methods. The highest
260 levels were recorded in April, which had an average level of 7.5-7.7 $\log_{10}$ cells/L (DFA)
261 and 6.2-8.4 $\log_{10}$ cells/L (qPCR), while the lowest values (5.0-5.5 $\log_{10}$ cells/L by DFA
262 and 3.4 $\log_{10}$ cells/L by qPCR) were recorded in February for both collection methods.
263 No differences were found in the $\log_{10}$ normalized *L. pneumophila* concentrations for the
264 two collection methods across the four month sampling period (Fig. 2; $P = 0.2319$,
265 Friedman test). The median value of each sample type was very similar. However, the
266 distribution across PMACS samples collected over the course of the study was less than
267 that observed for grab samples. Furthermore, *L. pneumophila* was detected in the
268 PMACS-enhanced samples during the February 2011 sampling when levels were below
269 the limit of detection for the DFA in the corresponding grab samples (data not shown).
270 No relationships were observed between the measured water quality parameters and the
271 concentration of *L. pneumophila* detected in either grab or retentate samples, which
272 agreed with results from the 2010 samplings (data not shown).
273

274 **DISCUSSION**

275 This study is the first to couple dead-end ultrafiltration to detection of *L.*
276 *pneumophila* and *Legionella* spp. in cooling tower waters. Detection of targets in the
277 PMACS concentrates was possible with both DFA and qPCR. PMACS collection of

278 SRS tower water resulted in a 200-fold increase in sampled volume compared to the
279 standard membrane filtration collection method. Additionally, PMACS concentration
280 proved advantageous over membrane filtration by providing larger volume, more
281 representative samples of the cooling tower environment, which led to reduced variability
282 among sampling events. The absence of *L. pneumophila* in February 2011 grab samples
283 highlights another advantage of PMACS concentration, increasing the probability of
284 detection of low level targets. Furthermore, use of the PMACS did not require additional
285 sample processing time since retentate could be directly deposited onto DFA slides for
286 analysis and many of the tower waters that were difficult to filter using the standard
287 membrane filtration (frequently requiring more than one filter due to pollen and other
288 suspended solids) were more easily processed using the PMACS filtration method.
289 Generally speaking, sampling for *Legionella* spp. and *L. pneumophila* in cooling
290 tower waters remains reactionary and the probability of detection is limited by sampling
291 error resulting from collection of small volumes. The CDC protocol for detection in
292 environmental water samples calls for collection of 1 L, if possible (5), and was
293 employed in two recent survey studies of cooling towers (4, 14). *Legionella*
294 *pneumophila* was detected in both studies, but in fewer than a third of towers tested.
295 Collection of microorganisms from larger volumes increases the likelihood of detection
296 of low-level contaminants and provides more representative samples of a water body,
297 such as a cooling tower. Enhanced detection of low level targets has been demonstrated
298 with manual versions of the concentration system (15) and it is possible that a survey
299 using PMACS concentration would increase the frequency with which *L. pneumophila* is
300 detected in cooling tower water.

301 The ability to concentrate particulates, including microorganisms, from large
302 volumes of water is also dependent on the ability to recover the collected microbes from
303 the filter. Furthermore, sampling SRS cooling towers operating year round offers many
304 challenges due to shifting environmental (temperature changes, pollen levels) and
305 operational conditions (addition of corrosion inhibitors and biocides to tower water).
306 Since there were no differences in the normalized concentrations of *L. pneumophila*
307 between the standard grab samples and retentates throughout the study, this demonstrates
308 effective recovery of the target bacteria from the PMACS. Recovery of target
309 microorganisms from the filter is also an issue for the standard method and should be
310 taken into consideration when implementing monitoring programs. Differences in
311 recovery from PMACS retentates filtered using the standard SRS membrane filtration
312 method (Table 1) highlight the difficulty of recovering *L. pneumophila*, which may
313 contribute to increased sample variability. Filtering 1 ml of retentate using the standard
314 SRS method yielded much lower levels of *L. pneumophila* by DFA than direct
315 application of PMACS retentate. Filtering 10 ml of retentate yielded levels that were
316 only slightly higher than direct application. Inconsistent recovery from standard method
317 filters could partially explain the higher variability observed in grab samples during the
318 single tower extended sampling stage of this study. By comparison, PMACS samples
319 showed reduced distribution across the study period, which supports the case for
320 improved surveillance of *L. pneumophila* when the PMACS is used for concentration of
321 cooling tower waters. The ability to automatically collect larger volume samples
322 followed by software-driven recovery of retentates made possible with the PMACS is the
323 likely cause of the reduced variability observed.

324 While techniques have been developed for detection of *L. pneumophila*, DFA is
325 the preferred technique for environmental samples compared to PCR or culture, which
326 supports utilization of DFA in the SRNL surveillance program (6, 8, 12). Culturing *L.*
327 *pneumophila* is difficult and requires up to seven days for results (11). DFA does not
328 require extraction as is necessary for PCR, which can present problems with interfering
329 environmental chemicals (6), and it is less subject to failure from environmental
330 inhibitors as has been documented to occur with PCR and can lead to false negative
331 results (3, 18, 22). The results from the qPCR in this study further support the
332 preferential use of DFA in environmental monitoring. The concentrations of *Legionella*
333 spp. from qPCR were similar to those of *L. pneumophila* by DFA for all of the samples
334 collected in 2011 although the qPCR analysis was performed on a genus level and DFA
335 on a species and serogroup level.
336 The ability of PMACS concentration to enhance detection of low levels of targets
337 in cooling tower water can increase the sensitivity of monitoring methods for these
338 environmental waters. A more thorough assessment of potential contaminants in these
339 waters is possible with the combined methodology presented here. The portable format
340 of the PMACS facilitates field monitoring programs such as those at SRS and could
341 potentially contribute to the implementation of routine monitoring programs for cooling
342 towers as supported by the AWT. Public health would benefit from the enhanced ability
343 to detect the presence of *Legionella* spp. within cooling towers and domestic hot water
344 systems prior to an outbreak of legionellosis. The capabilities demonstrated in the
345 described research may also be applicable to the monitoring of other water types and
346 other problematic microorganisms in water.

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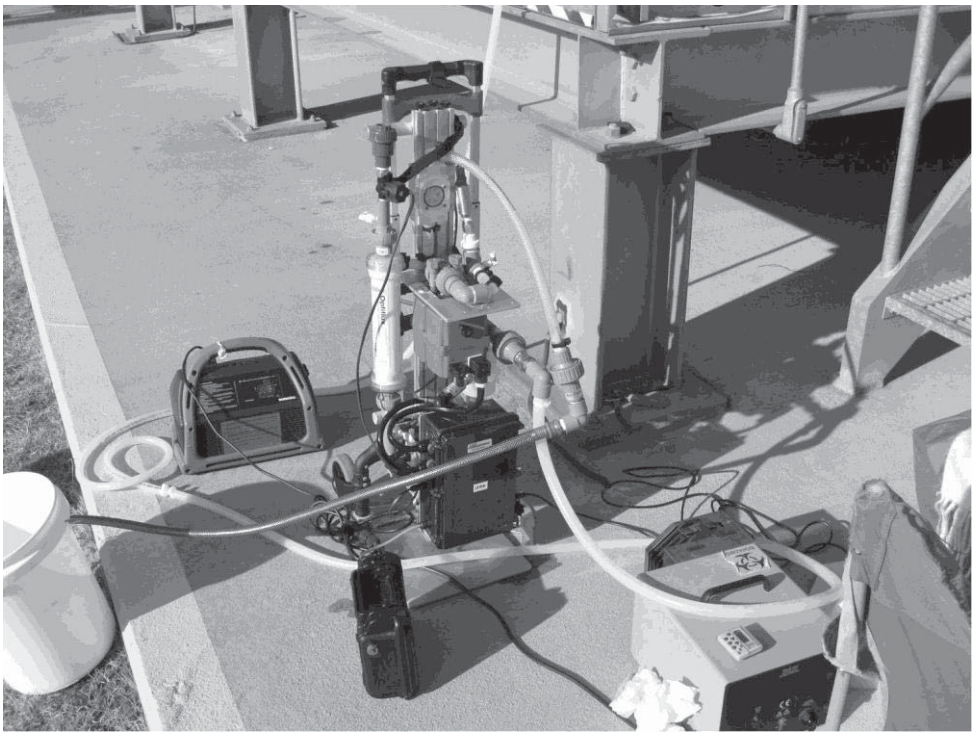

FIG. 1. Photograph of PMACS at SRS for collection of cooling tower waters.

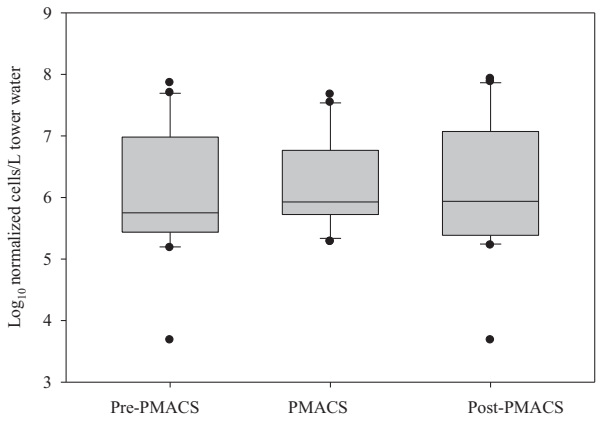

FIG. 2. Distribution of $\log_{10}$-transformed concentrations of normalized *L. pneumophila* cells per liter of the selected Savannah River Site cooling tower water in grab samples collected before and after concentration and in retentate samples. The tower was sampled from January to April (n = 21 for each sample type).

Table 1. Differences in concentrations of *L. pneumophila* cells per liter of Savannah River Site (SRS) cooling tower water by sample processing type as determined by DFA[a].

Sample type (n = 2 each)	Log_{10} normalized cells/L[b]
Pre-PMACS grab[c] (500 ml MF[d])	6.05 ± 0.13
PMACS (10 µl direct)	6.17 ± 0.14
PMACS (1 ml MF, 10 µl direct)	5.98 ± 0.77
PMACS (10 ml MF, 10 µl direct)	6.29 ± 0.08
Post-PMACS grab	5.93 ± 0.09

[a] DFA- direct fluorescence immunoassay

[b] Concentrations of grab and concentrated samples were normalized to liters of water per cooling tower.

[c] Grab samples were collected before (Pre-PMACS) and after (Post-PMACS) PMACS concentrated samples (100L).

[d] MF- membrane filtration (standard SRS protocol).

Table 2. Differences in concentrations of *L. pneumophila* cells per liter of Savannah River Site (SRS) cooling tower water by sample type as determined by DFA[a].

Tower	Log_{10} normalized cells/L[b]		
	Pre-PMACS[c]	PMACS	Post-PMACS
A	7.43	7.57	7.41
B	7.33	7.48	7.40
C	4.96	4.96	4.66
D	6.18	6.16	5.60
E	6.03	6.06	6.24

[a] DFA- direct fluorescence immunoassay

[b] Concentrations of grab (500 ml) and concentrated (100 L) samples were normalized to liters of water per cooling tower.

[c] Grab samples were collected before and after PMACS concentrated samples.

Table 3. Physical and chemical water quality parameters of SRS cooling tower basin water during dedicated single tower sampling, January to April 2011.

Date	Temp (^{o}C)[a]	pH	Conductivity(µS)	DO (mg/L)	Total Br_2 (ppm)	Free Cl_2 (ppm)	Turbidity (NTU)
Jan.	12.89 ± 3.85	8.69 ± 0.05	0.56 ± 0.04	9.08 ± 1.42	22.45 ± 5.90	9.83 ± 2.63	3.94 ± 0.80
Feb.	15.21 ± 2.40	8.83 ± 0.13	0.93 ± 0.27	11.02 ± 5.01	9.58 ± 2.84	4.22 ± 1.20	4.80 ± 1.24
March	17.86 ± 3.43	8.11 ± 0.82	0.67 ± 0.09	7.44 ± 2.47	9.04 ± 8.45	7.47 ± 2.92	4.02 ± 2.00
April	19.49 ± 2.91	8.58 ± 0.11	0.65 ± 0.06	7.90 ± 0.44	6.15 ± 1.78	3.33 ± 1.70	3.89 ± 3.70

[a-] Parameters were recorded prior to sample collection.