



ZANAQUA BIOLOGICAL TESTING



ZANAQUA VAPOR COMPRESSION DISTILLATION SANITIZATION EFFICIENCY
WHEN TREATING HIGHLY CONTAMINATED, STEADY-STATE SOURCES

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I. PURPOSE OF THE TEST

The industry standard for producing laboratory-grade water is a combination treatment of municipal tap water by reverse osmosis (RO) to filter and ultra-violet (UV) radiation to kill bacteria. Such combinations of RO and UV claim a biological rejection rate of 99.99% (4 Log). While this performance is considered adequate for many uses, it would be valuable to find a technology that performed to a higher standard for uses where the elimination of bacteria is of particular concern. Traditional distillation can remove all bacteria and endotoxins, but the use of distillation has been largely abandoned due to the high energy cost. The ability of ZanaAqua's low-energy, vapor compression distillation (VCD) technology to dramatically reduce the viability of live bacteria from the intake water supply, at low specific power density, has been observed to be substantial. The purpose of this biological reduction test is to establish a predictable minimum measure of effectiveness of the ZanaAqua technology to eliminate bacteria from contaminated source water.

II. MATERIALS AND METHODS

Chemicals and Reagents. Distilled water was obtained from WalMart in one-gallon jugs. It was consistently negative in tests for endotoxin (LAL), coliforms (Colilert-18) and total bacteria (SimPlate for HPC) and served as the general water source for preparative and analytic procedures. For larger volumes, Hudson Town Water (HTW, Hudson, NH) was used without further treatment. Sodium hypochlorite was obtained as a 5.2% (v/v) solution in water in a standard commercial preparation (Chlorox). All other reagents and media are described in the SOPs listed in this section.

Bacteria. A widely used wild-type *E. coli* strain (ATCC#25922) was cloned from an Idexx Quanti-Cult kit (Idexx Cat. No. WKIT-1001, prepared by Remel, Inc., Lake Charles, LA) by Zeptomatrix (Buffalo, NY), grown and then aliquoted for frozen storage. 30 vials were shipped to ZanAqua and reserves are held at Zeptomatrix for future need. The *E. coli* aliquots are each 1.0 mL and contain 2×10^{10} cfu/mL. This concentration of cfu was confirmed in experiments #DL031209-10A and B. These aliquoted cells are intended to be a standardized source for future experiments.

Preparation of *E. coli* for steady-state injection. Because the objective of these experiments was to assess killing and removal of *E. coli* from a heavily contaminated water source, *E. coli* cells were mixed directly into the Influent tanks which hold a total volume of 20-25 gallons. Added cells were first added to 100 mL of WalMart water and then mixed into the much larger volume of HTW by aggressive stirring with a plastic pipet. Samples of the Intake levels of *E. coli* were collected by dipping sterile 100-120 mL collection vessels into the tank directly. *E. coli* cells entered the Distiller directly through the Influent tank port. The highly contaminated influent was the sole source of intake water for the 30 minutes during which samples were collected.

Sample Collection. Samples of 100-120 mL were collected directly from the Concentrate and Distillate drain lines into pre-labeled, sterile, sealable, EPA-approved containers using a “clean catch” technique, i.e., not touching the collection vessel to the drain hose itself. The timing of sample collection was intended to identify early and steady-state changes in the Distillate (every five minutes) and the relatively slow changes in the Concentrate (every 10 minutes) and to collect enough samples to demonstrate that a steady-state had been attained.

Colilert-18. An EPA-approved method for enumerating coliforms in general and *E. coli* in particular is Idexx’s Colilert-18. The method is described in detail in the ZanAqua SOP titled COLILERT-18 ASSAY FOR ENUMERATION OF COLIFORM BACTERIA AND *ESCHERICIA COLI*(4). Briefly, 100 mL of a water sample is mixed with a lactose-rich bacteriological medium containing colorless dyes that are turned into detectable products by β -gal, ortho-nitrophenyl- β -D-galactopyranoside, (ONPG) which turns yellow, and β -gluc, 4-methylumbelliferyl- β -D-glucuronide (MUG) which turns fluorescent. The sample is then distributed into 51 small cultures in a Quanti-Tray, sealed and incubated overnight at 35°C. After 18 hours,

wells are inspected for cultures that have turned yellow and/or fluorescent. The number of cultures that are both yellow and fluorescent indicates the number of *E. coli* in the original sample. After correction for the probability of multiple *E. coli* cells in a single culture by using a Most Probable Number table, *E. coli* cfus per 100 mL are reported.

The number of total coliforms is determined in the identical manner except that the total number of yellow cultures is used.

A point of clarification: Samples of Intake water where the cfu count is expected to be very large are diluted to the indicated level and then 1.0 mL is added to 100 mL of sterile water for enumeration in the Colilert-18 test so the numbers are reported as cfu/mL. Samples of the Distillate and Concentrate where the numbers are expected to be near zero are 100 mL samples and the reported values are in the industry standard cfu/100 mL.

Endotoxin and SimPlate for HPC. Because the test that has been consistently failed was the Colilert-18 test, it was the only test used in these experiments. Endotoxin and SimPlate for HPC will be added in future experiments.

ZanAqua Distillers. For the purpose of demonstrating that our observations are representative of ZanAqua distillers, two randomly selected ZanAqua purification units were used: #111-01 and #111-08. The objective was the measurement of ZanAqua purification units performance running at steady-state. Each unit had been recently disassembled, cleaned and reassembled. Both units were run at standard conditions at least several hours prior to each experiment. Intake flow rates (Fint) were 27-31 gph, Distillate flow rates (Fdis) were 24-27 gph, and Concentrate flow rates (Fcon) were 3-4 gph. The system pressures were 5-6 inwc in 111-01, and 11-13 inwc in 111-08. The observed ? P values were between 25 and 30 inwc in both units.

III. RESULTS AND DISCUSSION

ZanAqua Distiller #	Experiment #	Intake <i>E. coli</i> cfu /100 mL	Distillate <i>E. coli</i> cfu/100 mL	Distillate/Intake	Log Kill
111-01	DL042109-12A	4.5 x 10 ⁷	0	0	=7.6
111-01	DL042209-09A	3 x 10 ⁷	0	0	=7.5
111-08	DL042109-12B	4.5 x 10 ⁷	0	0	=7.6
111-08	DL042209-09B	3 x 10 ⁷	0	0	=7.5

Table 1. Elimination of live bacteria from the Distillate by ZanAqua water purification units: Compiled performance of two experiments in each of two distillers. In each experiment, the Intake water was contaminated with 3-5 x 10⁷ live *E. coli*/100 mL and samples of the Distillate were collected at 5 minute intervals over 30 minutes.

No live *E. coli* were detected in the Distillate in either Distiller. Presented in Table 1 (the experiments beginning with DL04) and in the Appendices are the key data on the performance of ZanAqua units 111-01 and 111-08. Both of the test units were challenged with 3- 5 x 10⁷ *E. coli* cfu/mL for 30 minutes, for a total inoculum of about 2 x 10¹⁰ live *E. coli*. No live cell was detected in the Distillate of 111-08 in two separate experiments. The same results were obtained in 111-01. The ZanAqua Distillers may operate at a greater efficiency than a 7 Log bacteria rejection rate, but the test would need to challenge the systems with still higher concentrations of *E. coli*.

REFERENCES CITED

- Dussault D (2009) A Steady State Influent Conductivity Study. *ZanAqua Technical Reports.*
 Daiss J (2009) Colilert-18 Assay for the Enumeration of Coliform Bacteria and *Eschericia coli*.
ZanAqua Standard Operating Procedures.

APPENDIX: DETAIL OF PURIFICATION UNIT PERFORMANCE

DL042209-09A										
Inoculum	2 x 1.0 mL vials of ATCC #25922 prepared by Zeptomatrix (BacT-050, Lot #304996); 2 x 10 ¹⁰ cfu per mL; mixed into 100 mL WalMart water									
Injection Method	Contents of two vials added to ~20 g HTW in large white open vessel (influent tank) to yield a cell suspension estimated at ~ 5 x 10 ⁵ cfu/ mL(I0).									
Instrument Parameters	ZanAqua Distiller 111-01. Fint = 28 gph; Fdis = 24 gph; Fcon = 4.0 gph; P _{sep} = 30 inwc; P _{sys} = 5 inwc. Instrument had been run for Dussault/Lucchesi aerosol experiments, cleaned and reassembled; 113 regulator added; C _{intake} = 130 μS; C _{distillate} = 0.5 μS									
Sample Collection	100-120 mL of each of the samples were collected into sterile vessels at the indicated time points.									
Sample#	Sample description	Colilert-18					Simplate	Endotoxin		Comments
Intake		ONPG+ (yellow)	MUG+ (Fluor.)	ONPG+ MPN/ mL	MUG+ MPN/ mL	Corrected for Volume and Dilution	Positive Wells	EU/mL	Total EU	For abbreviations see Summary Page
WalMart	Distilled Water from Walmart	0	0	0	0		ND	ND		
Ipre	Influent tank water before inoculation	2	2	2	2	2 cfu/mL	ND	ND		
I0/10 ⁴	I0 diluted by 10,000	22	22	29	29	2.9 x 10 ⁵ cfu/mL	ND	ND		Good agreement with Zepto estimates
I0/10 ⁵	I0 diluted by 100,000	8	8	9	9	9 x 10 ⁵ cfu/mL	ND	ND		Total inoculum = 20 Gal x 3.78 Lpg x 1000 x cfu/mL = 2.2 x 10 ¹⁰ cfu
Concentrate				MPN per 100 mL	MPN per 100 ML	Cfus Corrected for Volume				Cfus corrected for volume = MPN x Fcon(gph) x t(hr) x 3.78 (L/g) x 10 (L/100mL)
C0	Pre-inoculum (Background)	0	0	0	0	0	ND	ND	ND	
C10	t = 10 min	0	0	0	0	0	ND	ND	ND	
C20	t = 20 min	0	0	0	0	0	ND	ND	ND	
C30	t = 30 min	0	0	0	0	0	ND	ND	ND	
					Total	0				No cfus in Concentrate
Distillate										Cfus corrected for volume = MPN x Fdis(gph) x t(hr) x 3.78 (L/g) x 10 (L/100mL)
D0	Pre- inoculum (Background)	0	0	0	0	0	ND	ND	ND	
D5	t = 5 min	0	0	0	0	0	ND	ND	ND	
D10	t = 10 min	0	0	0	0	0	ND	ND	ND	
D15	t = 15 min	0	0	0	0	0	ND	ND	ND	
D20	t = 20 min	0	0	0	0	0	ND	ND	ND	
D25	t = 25 min	0	0	0	0	0	ND	ND	ND	
D30	t = 30 min	0	0	0	0	0	ND	ND	ND	
					Total	0				No cfus in Distillate
Endotoxin Standard								ND	ND	Estimated concentration is 1.0 EU/mL; sensitivity of LAL kit is 0.03 EU/mL.

DL042209-09B

Inoculum	2 x 1.0 mL vials of ATCC #25922 prepared by Zeptomatrix (BacT-050, Lot #304996); 2 x 10 ¹⁰ cfu per mL; added to 100 mL WalMart water									
Injection Method	Contents of two vials added to ~20 g HTW in large white open vessel (influent tank) to yield a cell suspension estimated at ~ 5 x 10 ⁵ cfu/ mL(I0).									
Instrument Parameters	ZanAqua Distiller 111-08. Fint = 27 gph; Fdis = 23 gph; Fcon = 4.0 gph; P _{scp} = 38 inwc; P _{sys} = 11 inwc. Instrument had been run for Dussault/Lucchesi aerosol experiments, cleaned and reassembled; new 113 regulator and new scoops; C _{intake} = 294 μS; C _{distillate} = 0.7 μS									
Sample Collection	100-120 mL of each of the samples were collected into sterile vessels at the indicated time points.									
Sample#	Sample description	Colilert-18					Simplate	Endotoxin		Comments
Intake		ONPG+ (yellow)	MUG+ (Fluor.)	ONPG+ MPN/ mL	MUG+ MPN/ mL	Corrected for Volume and Dilution	Positive Wells	EU/mL	Total EU	For abbreviations see Summary Page
WalMart	Distilled Water from Walmart	0	0	0	0		ND	ND	ND	
Ipre	Influent tank water before inoculation	5	5	5	5	5 cfu/mL	ND	ND	ND	
I0/10 ⁴	I0 diluted by 10,000	22	22	29	29	2.9 x 10 ⁵ /mL	ND	ND	ND	Total inoculum = 20 Gal x 3.78 L/g x 1000 x cfu/mL
I0/10 ⁵	I0 diluted by 100,000	4	4	4	4	4.0 x 10 ⁵ /mL	ND	ND	ND	Total inoculum = 2.2 x 10 ¹⁰ cfus; good agreement with Zepto estimate
Concentrate				MPN per 100 mL	MPN per 100 ML	Cfus Corrected for Volume				Cfus corrected for volume = MPN x Fcon(gph) x ? t(hr) x 3.78 (L/g) x 10 (L/100mL)
C0	Pre-inoculum (Background)	0	0				ND	ND		
C10	t = 10 min	0	0				ND	ND		
C20	t = 20 min	0	0				ND	ND		
C30	t = 30 min	0	0				ND	ND		
					Total	0				No cfus in Concentrate
Distillate										Cfus corrected for volume = MPN x Fdis(gph) x ? t(hr) x 3.78 (L/g) x 10 (L/100mL)
D0	Pre- inoculum (Background)	0	0				ND	ND		
D5	t = 5 min	0	0				ND	ND		
D10	t = 10 min	0	0				ND	ND		
D15	t = 15 min	0	0				ND	ND		
D20	t = 20 min	0	0				ND	ND		
D25	t = 25 min	0	0				ND	ND		
D30	t = 30 min	0	0				ND	ND		
					Total	0				No cfus in Distillate!
Endotoxin Standard										Estimated concentration is 1.0 EU/mL; sensitivity of LAL kit is 0.03 EU/mL.

DL042109-12A										
Inoculum	2 vials of ATCC #25922 prepared by Zeptomatrix (BacT-050, Lot #304996); 2 x 10 ¹⁰ cfu per mL; added to 100 mL WalMart water									
Injection Method	Contents of two vials added to ~20 g HTW in large white open vessel (influent tank) to yield a cell suspension estimated at ~ 5 x 10 ⁵ cfu/mL(10).									
Instrument Parameters	ZanAqua Distiller 111-01. Fint = 31 gph; Fdis = 28 gph; Fcon = 3.0 gph; P _{sep} = 36 inwc; P _{sys} = 6 inwc. Instrument had been run for Dussault/Lucchesi aerosol experiments, cleaned and reassembled; 113 regulator added; C _{intake} = 783 µS; C _{distillate} = 0.3 µS									
Sample Collection	100-120 mL of each of the samples were collected into sterile vessels at the indicated time points.									
Sample#	Sample description	Colilert-18					Simplate	Endotoxin		Comments
Intake		ONPG+ (yellow)	MUG+ (Fluor.)	ONPG+ MPN/mL	MUG+ MPN/mL	Corrected for Volume and Dilution	Positive Wells	EU/mL	Total EU	For abbreviations see Summary Page
WalMart	Distilled Water from Walmart	0	0	0	0		ND	ND		
Ipre	Influent tank water before inoculation	0	0	0	0		ND	ND		
I0/10 ⁴	I0 diluted by 10,000	22	22	29	29	2.9 x 10 ⁵ cfu/mL	ND	ND		Good agreement with Zepto estimates
I0/10 ⁵	I0 diluted by 100,000	0	0	0	0		ND	ND		Total inoculum = 20 Gal x 3.78 Lpg x 1000 x cfu/mL = 2.2 x 10 ¹⁰ cfu
Concentrate				MPN per 100 mL	MPN per 100 ML	Cfus Corrected for Volume				Cfus corrected for volume = MPN x Fcon(gph) x t(hr) x 3.78 (L/g) x 10 (L/100mL)
C0	Pre-inoculum (Background)	0	0	0	0	0	ND	ND	ND	
C10	t = 10 min	0	0	0	0	0	ND	ND	ND	
C20	t = 20 min	0	0	0	0	0	ND	ND	ND	
C30	t = 30 min	0	0	0	0	0	ND	ND	ND	
					Total	0				No cfus in Concentrate
Distillate										Cfus corrected for volume = MPN x Fdis(gph) x t(hr) x 3.78 (L/g) x 10 (L/100mL)
D0	Pre- inoculum (Background)	0	0	0	0	0	ND	ND	ND	
D5	t = 5 min	0	0	0	0	0	ND	ND	ND	
D10	t = 10 min	0	0	0	0	0	ND	ND	ND	
D15	t = 15 min	0	0	0	0	0	ND	ND	ND	
D20	t = 20 min	0	0	0	0	0	ND	ND	ND	
D25	t = 25 min	0	0	0	0	0	ND	ND	ND	
D30	t = 30 min	0	0	0	0	0	ND	ND	ND	
					Total	0				No cfus in Distillate
Endotoxin Standard								ND	ND	Estimated concentration is 1.0 EU/mL; sensitivity of LAL kit is 0.03 EU/mL.

DL042109-12B										
Inoculum	2 vials of ATCC #25922 prepared by Zeptomatrix (Bact-050, Lot #304996); 2 x 10 ¹⁰ cfu per mL; added to 100 mL WalMart water									
Injection Method	Contents of two vials added to ~20 g HTW in large white open vessel (influent tank) to yield a cell suspension estimated at ~ 5 x 10 ⁵ cfu/ mL(10).									
Instrument Parameters	ZanAqua Distiller 111-08. Fint = 27 gph; Fdis = 23 gph; Fcon = 4.0 gph; P _{scp} = 38 inwc; P _{sys} = 13 inwc. Instrument had been run for Dussault/Lucchesi aerosol experiments, cleaned and reassembled; new 113 regulator and new scoops; C _{intake} = 783 µS; C _{distillate} = 0.3 µS									
Sample Collection	100-120 mL of each of the samples were collected into sterile vessels at the indicated time points.									
Sample#	Sample description	Colilert-18					Simplate	Endotoxin		Comments
Intake		ONPG+ (yellow)	MUG+ (Fluor.)	ONPG+ MPN/ mL	MUG+ MPN/ mL	Corrected for Volume and Dilution	Positive Wells	EU/mL	Total EU	For abbreviations see Summary Page
WalMart	Distilled Water from Walmart	0	0	0	0		ND	ND	ND	
Ipre	Influent tank water before inoculation	0	0	0	0		ND	ND	ND	
I0/10 ⁴	I0 diluted by 10,000	30	30	45	45	4.5 x 10 ⁵ /mL	ND	ND	ND	Total inoculum = 20 Gal x 3.78 Lpg x 1000 x cfu/mL
I0/10 ⁵	I0 diluted by 100,000	2	2	2	2	2.0 x 10 ⁵ /mL	ND	ND	ND	Total inoculum = 3.4 x 10 ¹⁰ cfus; good agreement with Zepto estimate
Concentrate				MPN per 100 mL	MPN per 100 ML	Cfus Corrected for Volume				Cfus corrected for volume = MPN x Fcon(gph) x ? t(hr) x 3.78 (L/g) x 10 (L/100mL)
C0	Pre-inoculum (Background)	0	0				ND	ND		
C10	t = 10 min	0	0				ND	ND		
C20	t = 20 min	0	0				ND	ND		
C30	t = 30 min	0	0				ND	ND		
					Total	0				No cfus in Concentrate
Distillate										Cfus corrected for volume = MPN x Fdis(gph) x ? t(hr) x 3.78 (L/g) x 10 (L/100mL)
D0	Pre- inoculum (Background)	0	0				ND	ND		
D5	t = 5 min	0	0				ND	ND		
D10	t = 10 min	0	0				ND	ND		
D15	t = 15 min	0	0				ND	ND		
D20	t = 20 min	0	0				ND	ND		
D25	t = 25 min	0	0				ND	ND		
D30	t = 30 min	0	0				ND	ND		
					Total	0				No cfus in Distillate!
Endotoxin Standard										Estimated concentration is 1.0 EU/mL; sensitivity of LAL kit is 0.03 EU/mL.