



685 Stone Road, Unit 6 • Benicia, CA 94510 • (707) 747-5906 • 1-800-GIARDIA • FAX (707) 747-1751 • WEB: www.biovir.com

DATE: August 25, 2010

TO: Mike Kirkland  
Del Industries, Inc.  
3580 Sueldo Street  
San Luis Obispo, CA 93401  
(805) 541-1601

FROM: Richard Danielson, Ph.D.  
Laboratory Director/Vice President

SUBJECT: The Evaluation of the Del Industries, Inc. Del Shield, Ozone Generator, BioVir #101613.

SUMMARY. Del Industries, Inc., requested that BioVir Laboratories, Inc. (BioVir) test their portable ozone generator, Del Shield, Model LT268-XB01 to demonstrate efficacy against the bacterium *E. coli*. The effectiveness of the water disinfectant system was measured by culture of the organism on selective and differential media. The goal of the challenge study was to describe the log reduction of *E. coli* relative to multiple exposure periods and different volumes of water. Ozone exposures in one liter of water of 2 min or more resulted in > 4-log reduction (> 99.99%) of *E. coli*.

Discussion. BioVir was requested to see if the Del Industries, Inc. Del Shield, Model LT268-XB01 was capable of killing *E. coli*. One unit was delivered to BioVir on June 22, 2010. It came equipt with a power supply. An air flow meter (Dwyer Instruments, Michigan City, IN) was supplied earlier by Del Industries and used for this study.

In this test format, one water type were used, a modified City of Benicia tap water.

Please refer to the attached protocol for the conditions and execution of the study. There were two studies conducted, the test conditions are summarized below.

Challenge	Exposure Time	Volume
Test 1	2 min	1 L
	10 min	1 L
	30 min	1 L
Test 2	0.5 min	1 L
	1 min	1 L
	2 min	2 L
	2 min	4 L

All challenges were conducted in a biological safety cabinet (Figure 1).

Description of Challenge Organism and Assay. There were two strain-types of the bacteria *Escherichia coli* used in this study, *E. coli* (ATCC# 11229) and *E. coli* O157:H7 (ATCC# 700728). From

-80°C freezer stocks, each was propagated in Trypticase Soy Broth with at least two 24-hr transfers at 35°C (see attached protocol). The assay was by Standard Methods 9222, membrane filtration, with inoculum volumes of 100, 10 and 1 mL per sample. Additional dilutions were prepared in Standard Methods buffer as necessary. Inoculated membrane filters were placed onto mFC agar (BBL/Difco) and samples were incubated for 24 hrs ± 2 hrs at 44.5°C. Colonies with blue centers were counted as positive *E. coli* and recorded as CFU (colony-forming unit) per 100 mL.

In order for the client to determine the dose of ozone generated, the air flow rate, temperature and relative humidity were recorded.

Results.

Water Quality of the Test Matrix:

Parameter	Test 1	Test 2
Temperature (°C)	20.3	21.5
pH	8.13	8.2
Chlorine (mg/L)	None Detected	None Detected
Turbidity (NTU)	0.30	0.16
TDS (mg/L)	138	145

Environmental Air Quality:

Parameter	Test 1			Test 2*			
	2 min	10 min	30 min	1L/0.5	1L/1	2L/2	4L/2
Temperature (°C)	24	24	24.5	23	23.7	23.9	24.2
Air Flow Rate (SCFH)	4.5	4.5	4.5	4.5	4.7	4.5	4.5
Relative Humidity (%)	45	45	45	45	43	42	42

\* = designation is volume (L) per minutes of exposure

The results are presented in Table 1. There were no detectable *E. coli* observed after 10 min exposure to the ozone generated in 1 L of water (> 7-log reduction). At 2 min exposure in 1 L there was a 4-log reduction (99.99%). There was little log reduction (about 0.5-log) observed under the other conditions tested.

Please contact me if you have any questions regarding this information.

**TABLE 1**  
**SUMMARY OF RESULTS**  
*E. coli* Reduction  
 with Del Industries, Inc. Model LT268-XB01

Sample No	Test Condition	E. coli* (CFU/100 mL)	Log Reduction
Test 1	1 Untreated	1.40E+07	
	2 2 min / 1L	1.16E+03	4.10
	3 10 min / 1 L	<1	> 7.14
	4 30 min / 1 L	<1	> 7.14
Test 2	5 Untreated	8.70E+07	
	6 0.5 min / 1L	2.30E+07	0.58
	7 1 min / 1 L	2.50E+07	0.54
	8 2 min / 2 L	2.10E+07	0.62
	9 2 min / 4 L	2.10E+07	0.62

\* Test 1 conducted with *E. coli* ATCC 11229. Test 2 conducted with *E. coli* O157:H7 ATCC# 700728

**Figure 1**  
 Del Industries Model LT268-XB01

