

**—FINAL REPORT—**

**ANALYSIS**

**OF**

**DEL AGW-0500 MOBILE OZONE SURFACE SANITATION SYSTEM  
AL SSS 500 MOBILE OZONE SURFACE SANITATION SYSTEM  
DEL AGW-1500G MOBILE RECIRCULATING OZONE SANITATION SYSTEM  
AL SSS 1500 MOBILE RECIRCULATING OZONE SANITATION SYSTEM  
DISINFECTION EFFECTIVENESS ACCORDING TO  
AOAC OFFICIAL METHOD 961.02**

Prepared for DEL Agricultural and  
Air Liquide America Corporation



**April 25, 2002**

**Submitted by:**

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A Division of NSF International  
Ann Arbor, Michigan

**SIGNATURE PAGES**

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## Executive Summary

The Toxicology Group, LLC, a Wholly Owned Company of NSF International, was contracted by DEL Agricultural, Inc. and Air Liquide to perform testing on the DEL AGW-0500/AL SSS 500 Mobile Ozone Surface Sanitation System against the test and performance requirements formulated by the US Environmental Protection Agency (EPA)'s Office of Pesticide Programs in the Disinfectant Technical Science Section Efficacy Document DIS/TSS-1 (Efficacy Data Requirements/Disinfectants for Use on Hard Surfaces, January 22, 1982). This document stipulates the use of AOAC Official Method 961.02, Germicidal Spray Products as Disinfectants, for both broad-spectrum and hospital/medical environment efficacy claims. By this method, the AGW-0500/AL SSS 500 unit was able to effectively disinfect glass surfaces coated with greater than  $1 \times 10^6$  colony forming units (cfu) *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella choleraesuis* at exposure times of ten (10) minutes, five (5) minutes, and three (3) minutes, respectively. For each organism type, greater than 59 out of 60 inoculated glass slides were disinfected over the aforementioned exposure periods. Based on these findings, the AGW-0500/AL SSS 500 system meets the performance requirements for both broad-spectrum and hospital/medical environment efficacy claims as specified in the US EPA DIS/TSS-1.

The unit was also evaluated against the procedures outlined in the US EPA DIS/TSS-1 with *Listeria monocytogenes*, *Aspergillus flavus*, *Campylobacter jejuni*, *Brettanomyces bruxellensis* and *Trichophyton mentagrophytes* as the test organisms. The AGW-0500/AL SSS 500 system was able to successfully disinfect 20 out of 20 glass slides inoculated with  $1 \times 10^4$  cfu of *Listeria monocytogenes*, *Campylobacter jejuni*, and *Brettanomyces bruxellensis* at an exposure period of three (3) minutes, 20 out of 20 glass slides inoculated with  $1 \times 10^4$  cfu of *Aspergillus flavus* at an exposure period of five (5) minutes, and 60 out of 60 glass slides inoculated with  $1 \times 10^6$  cfu of *Trichophyton mentagrophytes* at an exposure period of thirty (30) seconds based on AOAC 961.02 method requirements.

Additionally, the validity of the aforementioned efficacy claims extends to the AGW-1500G/AL SSS 1500 Mobile Recirculating Ozone Sanitation System, a unit which delivers an applied ozone dose identical to the one delivered by the AGW-0500/AL SSS 500 unit (i.e. 3-3.5 ppm in water).



**Analysis of**  
**DEL AGW-0500 Mobile Ozone Surface Sanitation System**  
**AL SSS 500 Mobile Ozone Surface Sanitation System**  
**DEL AGW-1500G Mobile Recirculating Ozone Sanitation System**  
**AL SSS 1500 Mobile Recirculating Ozone Sanitation System**  
**Disinfection Effectiveness According to**  
**AOAC Official Method 961.02**

**Prepared for DEL Agricultural, Inc. and Air Liquide**  
**By The Toxicology Group, LLC, A Wholly Owned Company of NSF International**  
**April 25, 2002**

**1.0 Introduction**

DEL Agricultural, Inc. and Air Liquide contracted the Toxicology Group, LLC, a Wholly Owned Company of NSF International, to perform testing according to the US Environmental Protection Agency (EPA)'s Office of Pesticide Programs Disinfectant Technical Science Section Efficacy Document DIS/TSS-1 (Efficacy Data Requirements/Disinfectants for Use on Hard Surfaces, January 22, 1982). This document stipulates the use of AOAC Official Method 961.02, Germicidal Spray Products as Disinfectants, for both broad-spectrum and hospital/medical environment efficacy claims. AOAC Official Method 961.02 determines the effectiveness of sprays and pressurized spray products as spot disinfectants for contaminated surfaces. The testing was performed at NSF International by a representative from the Toxicology Group, LLC, during the period of May 2001 through February 2002. This report serves to detail the methodology and findings associated with assessing the performance of DEL Agricultural's AGW-0500/AL SSS 500 Mobile Ozone Surface Sanitation System against AOAC Method 961.02.



## 2.0 Materials and Methods

The procedures described in AOAC Official Method 961.02 were followed with the following modifications.

### A. Challenge Organisms

To satisfy the requirements of broad-spectrum disinfectant claims, *Salmonella choleraesuis* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538) were utilized as the challenge organisms. For hospital/medical environment efficacy claims, *Pseudomonas aeruginosa* (ATCC 15442) was employed. DEL Agricultural, Inc. requested that in addition to the organisms specified in DIS/TSS-1, *Trichophyton mentagrophytes* (ATCC 9533), *Aspergillus flavus* (ATCC 9296), *Campylobacter jejuni* (ATCC 35250), *Brettanomyces bruxellensis* (ATCC 10560), and *Listeria monocytogenes* (ATCC 7644) also be tested.

The culturing methodology provided in 961.02 was followed for all organisms, except for *L. monocytogenes*, *C. jejuni*, *B. bruxellensis* and *A. flavus*. As these organisms were not specified in the AOAC method, *L. monocytogenes*, *C. jejuni*, *B. bruxellensis* and *A. flavus* were cultivated in the following manners.

The vial of *L. monocytogenes* received from ATCC was resuspended in 10 mL Listeria Enrichment Media (LEM). The suspension was incubated for 48 hours at 30°C. 1 mL of the resulting culture was transferred to a fresh 10 mL of LEM. The tube was incubated for 48 hours at 30°C. The procedure was repeated three more times for a total of five passages. After the final incubation, 0.1 mL of the culture suspension was spread onto each of six PALCAM Listeria selective agar plates (EM Science, Gibbstown, NJ; [www.emscience.com](http://www.emscience.com)). The plates were incubated for 48 hours at 30°C. Each plate was washed with 5 mL of sterile buffered deionized water and the cells were harvested into a sterile test tube. Using epifluorescent microscopy, it was determined that approximately  $5 \times 10^9$  cfu/mL were obtained.



The vial of *C. jejuni* received from ATCC was resuspended in 10 mL Brucella Broth. The suspension was incubated for 48 hours at 35°C. 1 mL of the resulting culture was transferred to a fresh 10 mL of Brucella Broth. The tube was incubated for 48 hours at 35°C. The procedure was repeated three more times for a total of five passages. After the final incubation, 0.1 mL of the culture suspension was spread onto each of six Campylobacter Selective Agar plates (REMEL). The plates were incubated for 48 hours at 35°C under microaerophilic conditions using a Mitsubishi Gas Pack container and 5% CO<sub>2</sub> sachets (REMEL). Each plate was washed with 5 mL of sterile buffered deionized water and the cells were harvested into a sterile test tube. Using epifluorescent microscopy, it was determined that approximately  $1 \times 10^9$  CFU/mL were obtained.

The culturing methods for *A. flavus* and *B. bruxellensis* were similar to the cultivation method for *T. mentagrophytes*, with the following exceptions. The vials of *A. flavus* and *B. bruxellensis* received from ATCC were resuspended in 10 mL Potato Dextrose Broth (PDB). The suspensions were incubated for 5 days at 30°C. 1 mL of each resulting culture was transferred to a fresh 10 mL of PDB. The tubes were incubated for 5 days at 30°C. The procedure was repeated three more times for a total of five passages. After the final incubation, 0.1 mL of each culture suspension was spread onto each of six Potato Dextrose Agar plates. The plates were incubated for 10 days at 30°C. The directions outlined in AOAC 955.17 for preparation of a spore suspension was followed. Using a hemocytometer, it was determined that approximately,  $2 \times 10^7$  spores/mL and  $2 \times 10^8$  spores/mL were obtained for *A. flavus* and *B. bruxellensis*, respectively.

## B. Test Procedure

Culture preparation and slide inoculation / drying was carried out as prescribed in AOAC 961.02. As *L. monocytogenes*, *C. jejuni*, *B. bruxellensis* and *A. flavus* are not specified in the AOAC method, it was necessary to ensure that greater than  $1 \times 10^4$



organisms per slide survived the drying step. A loopful (approximately 10  $\mu$ l) of a 1:10 dilution of the microorganism suspension was aseptically placed on a sterile glass slide in duplicate. The slides were dried at 30°C for 45 minutes. Each slide was transferred to 10 mL of sterile growth media (LEM for *L. monocytogenes*, Brucella Broth for *C. jejuni*, and PDB for *A. flavus* and *B. bruxellensis*). The suspension was placed on a platform shaker for 10 minutes at a medium setting to dislodge the inoculated organisms.  $10^{-1}$  and  $10^{-2}$  dilutions of the suspension were spread plated onto selective growth media plates (PALCAM for *L. monocytogenes*, CSA for *C. jejuni*, and PDA for *A. flavus* and *B. bruxellensis*). The plates were incubated at the following temperatures and times: 30°C for 48 hours for *L. monocytogenes*, 35°C for 48 hours under microaerophilic conditions for *C. jejuni*, and 30°C for 5 days, for *A. flavus* and *B. bruxellensis*. The resulting colonies were enumerated and the average surviving concentrations were calculated. The individual slide concentrations for the duplicates and average surviving concentrations are provided in Appendix A.

For each test organism, the dried challenge slides were aseptically transferred to a sterile spray platform. Before challenge testing was initiated, the AGW-0500/AL SSS 500 System was plumbed-in to a temperature controlled water line (tempered to approximately 20°C). The water line passed through a carbon filter (model F400 bulk; US Filter, Warrendale, PA; [www.usfilter.com](http://www.usfilter.com)) to remove residual chlorine prior to entrance in the AGW-0500/AL SSS 500 System. The unit was turned on and allowed to warm up for 15 minutes. Prior to testing, the dissolved ozone concentration emitted from the spray unit was measured using both the AccuVac Ozone High Range Test Kit (Cat. no. 2518025; Hach Company, Loveland, CO; [www.hach.com](http://www.hach.com)) and a dissolved ozone monitor (Model no. A15/64; ATI, Oaks, PA; [www.analyticaltechnology.com](http://www.analyticaltechnology.com)). Dissolved ozone concentrations consistently measured in the range of 1.85 to 2.25 ppm. Challenge slides were sprayed at a distance of 18 inches. Each challenge organism was run in sixty replicates with the



exception of *L. monocytogenes*, *C. jejuni*, *B. bruxellensis* and *A. flavus* (twenty replicates each), with ten slides treated simultaneously. The exposure times for each organism were as follows: 30 seconds for *T. mentagrophytes*, 3 minutes for *L. monocytogenes*, 3 minutes for *S. choleraesuis*, 3 minutes for *B. bruxellensis*, 3 minutes for *C. jejuni*, 5 minutes for *P. aeruginosa*, 5 minutes for *A. flavus*, and 10 minutes for *S. aureus*. After each exposure period, the slides were allowed to sit in a horizontal position for 2 minutes to allow for residual disinfection by any remaining dissolved ozone. The slides were then aseptically transferred to 20 mL of sterile growth media (Nutrient broth for all organisms except *L. monocytogenes*, *C. jejuni* and *A. flavus* and *B. bruxellensis* which utilized LEM, Brucella Broth and PDB, respectively). For all organisms except *L. monocytogenes*, *B. bruxellensis* and *A. flavus*, the tubes were incubated at 35°C for 48 hours. The tubes containing *L. monocytogenes* were incubated at 30°C for 48 hours. The tubes containing *A. flavus* and *B. bruxellensis* were incubated at 30°C for 5 days. Growth present in the positive controls and any samples was confirmed as the challenge organism through the use of selective growth media, microscopic characteristics, and biochemical characterization.

### 3.0 Results and Discussion

Table 1 contains the test results, and includes exposure times, the number of slides tested per organism, and percentage of challenge slides successfully disinfected. The AGW-0500/AL SSS 500 unit was able to effectively disinfect glass surfaces coated with greater than  $1 \times 10^6$  cfu per slide of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis* and *Trichophyton mentagrophytes* at exposure times of ten (10) minutes, five (5) minutes, three (3) minutes, and thirty (30) seconds, respectively. For each organism type, greater than 59 out of 60 inoculated glass slides were disinfected over the aforementioned exposure periods. The AGW-0500/AL SSS 500 system was also able to successfully disinfect 20 out of 20 glass slides inoculated with a minimum of  $1 \times 10^4$  cfu per slide of *Listeria monocytogenes*, *Campylobacter jejuni* and *Brettanomyces bruxellensis* at an exposure period of three



(3) minutes, and 20 out of 20 glass slides inoculated with a minimum of  $1 \times 10^4$  cfu per slide of *Aspergillus flavus* at an exposure period of five (5) minutes. Based on these findings, the AGW-0500/AL SSS 500 system meets the requirements for both broad-spectrum and hospital/medical environment efficacy claims as specified in the US EPA DIS/TSS-1.

Additionally, the validity of the aforementioned efficacy claims extends to DEL AGW-1500G/AL SSS 1500 Mobile Recirculating Ozone Sanitation System, a unit which delivers an applied ozone dose identical to the one delivered by the AGW-0500/AL SSS 500 unit (i.e. 3-3.5 ppm in water).



TABLE 1: AGW-0500/AL SSS 500 OZONE DISINFECTION RESULTS			
Challenge Organism	Exposure Time	# Of positive slides / total # of slides	Percent Disinfected
<i>Trichophyton mentagrophytes</i>	30 seconds	60 / 60	> 99.9999%
<i>Salmonella choleraesuis</i>	3 minutes	60 / 60	> 99.9999%
<i>Pseudomonas aeruginosa</i>	5 minutes	59 / 60	> 99.9999%
<i>Staphylococcus aureus</i>	10 minutes	60 / 60	> 99.9999%
<i>Listeria monocytogenes</i>	3 minutes	20 / 20	> 99.99%
<i>Aspergillus flavus</i>	5 minutes	20 / 20	> 99.99%
<i>Campylobacter jejuni</i>	3 minutes	20 / 20	> 99.99%
<i>Brettanomyces bruxellensis</i>	3 minutes	20 / 20	> 99.99%

**Table 1.** Disinfection results for the DEL AGW-0500/AL SSS 500 Mobile Ozone Surface Sanitation System.



**APPENDIX A**

<b>Challenge Organism</b>	<b>Individual Concentrations for Duplicate Slide Analysis (CFU per slide)</b>	<b>Average Concentration (CFU per slide)</b>
<i>Listeria monocytogenes</i> Slide 1 Slide 2	29,000 47,000	38,000
<i>Aspergillus flavus</i> Slide 1 Slide 2	50,000 66,000	58,000
<i>Campylobacter jejuni</i> Slide 1 Slide 2	70,000 110,000	90,000
<i>Brettanomyces bruxellensis</i> Slide 1 Slide 2	110,000 60,000	80,000