



FINAL REPORT

Efficacy Testing of Laundry Systems

PROTOCOL

Efficacy of an Ozone Laundry System

ORDER Number

371117064

PREPARED FOR:

Danny Kirk

ArtiClean Ozone Laundry Systems

129 Fieldview Dr., P.O. Box 455

Versailles, KY 40383

Jason Dobranic, Ph.D.

EMSL Analytical, Inc.

200 Route 130 North, Cinnaminson, NJ 08077

Phone: (856) 858-4800 Fax: (856) 786-0262 Web: <http://www.emsl.com>





Certificate of Analysis

Client: ArtiClean Ozone Laundry Systems

Contact: Danny Kirk

Project: Efficacy Testing of an Ozone Laundry System

Product : Ozone Laundry System

EMSL NO: 371117064

Sample received: 11/9/11

Start date: 11/9/11

Report date: 12/1/11

Challenge Bacteria: Methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 33591
Vancomycin resistant *Enterococcus faecalis* (VRE) ATCC

Experimental Summary: The testing procedure was designed after discussions between EMSL Analytical, the testing company, and the client, ArtiClean Ozone Laundry Systems. The testing was conducted on an ozone laundry system as well as a normal hot water laundry system. The testing was conducted in our Cinnaminson Microbiology Laboratory.

Procedure:

In order to determine the efficacy of an ozone laundry system methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus faecalis* (VRE) were used to establish a relative log reduction of such organisms when compared to a normal hot water laundry system. Both MRSA and VRE were prepared by culturing it on Tryptic soy agar (TSA) at 35°C for 24 h. It was then aseptically inoculated into 100 mL of phosphate buffer to reach a McFarland 0.5 standard (~10⁸ CFU/mL). After preparation of cells each organism was individually inoculated onto a 5 x 5 cm² piece of towel by placing 0.2 mL of a ~10⁸ CFU/mL solution. Each cloth was allowed to air dry for 1 hr before being placed into the washing machine for testing. Simultaneously, a 200 µL solution of MRSA was also inoculated onto a 5 x 5 cm² piece of firefighter jacket material, and allowed to dry for 1 hr at 42°C. VRE was not inoculated onto the firefighter jacket material.

Once the inoculated test materials were sufficiently dried they were placed into the washing machine. Four separate cycles were performed with MRSA: 1) Warm (105°F) with Turnout Gear Cleaner 1851, 2) Cold (63°F) with Ozone and



Citrisolv, 3) Warm with Citrisolv, and 4) Cold with Ozone and Turnout Gear Cleaner 1851. The fourth cycle was only performed with the firefighter material. Two separate cycles were performed with the VRE: 1) Hot (155°F) with Alkaline detergent and Bleach, 2) Cold (63°F) with Ozone, Alkaline detergent and Bleach.

Experimental Results:

Table 1.1 Wash cycles efficacy against MRSA

Sample	Wash Cycle	CFU	LR	%Reduction
Firefighter Material	None	2.43x10 ⁷		
	Warm (105°F) w/ Turnout Gear Cleaner 1851	7.16x10 ⁴	2.54	99.71%
	Cold (63°F) w/ Ozone and Citrisolv	1.64x10 ³	4.35	99.995%
	Warm(105°F) w/ Citrisolv	3.5x10 ⁴	2.92	99.88%
	Cold (63°F) w/ Ozone and Turnout Gear Cleaner 1851	1.41x10 ⁴	3.40	99.96%
Towel	None	7.63x10 ⁶		
	Warm(105°F) w/ Turnout Gear Cleaner 1851	5.07x10 ³	3.19	99.94%
	Cold (63°F) w/ Ozone and Citirsolv	6.67x10 ²	4.29	99.994%

Control = Inoculated test material not exposed to a washing cycle
 LR = Log Reduction; %reduction = compares initial CFU and final CFU

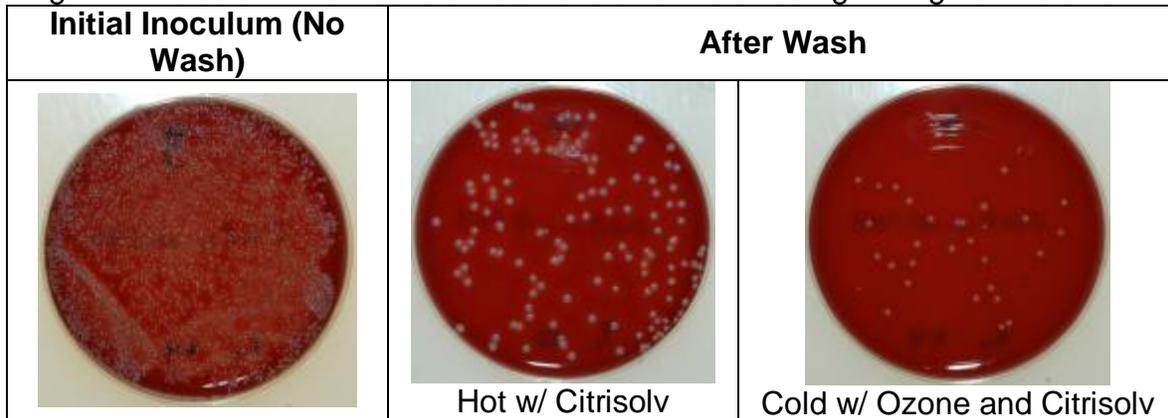
Table 1.2 Wash cycles efficacy against VRE

Sample	Wash Cycle	CFU	LR	%Reduction
Towel	None	5.17x10 ⁶		
	Cold (63°F) w/ Ozone, Alk and Bleach	3	6.37	99.99996%
	Hot (155°F) w/ Alk and Bleach	6	6.27	99.99995%

Control = Inoculated test material not exposed to a washing cycle
 LR = Log Reduction; %reduction = compares initial CFU and final CFU

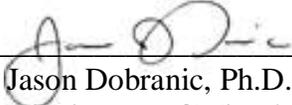


Fig 1.1 Photos of MRSA before wash and after wash using Firefighter material



Conclusions/Observations:

The purpose of this study was to compare the efficacy of a normal hot water (155°F) laundry wash cycle, normal warm water (105°F) wash cycle, and a cold water (63°F) ozone wash cycle. Two different material types were tested, a 10 x 10 cm² towel and a firefighter's jacket material, against MRSA and solely the towel against VRE. Against MRSA both the firefighter's jacket and towel material were disinfected best with the cold wash plus ozone and citrisolv producing a 4.35 log reduction (jacket) and 4.29 log reduction (towel) as shown in Table 1.1. Similarly, VRE was observed to have a greater log reduction in the cold wash plus ozone, bleach and an alkaline detergent producing a 6.37 log reduction while the hot wash plus bleach and alkaline detergent produced a 6.27 log reduction (Table 1.2). Additionally, photos were taken of the firefighter's jacket material comparing the hot wash with cirtisolv and the cold wash with ozone and citrisolv. The photos clearly demonstrate the greater reduction with the use of a cold wash and ozone as compared to a hot wash and ozone. In conclusion, the cold (63°F) wash plus ozone and citrisolv produced the greatest percent reduction of MRSA on both the firefighter's jacket material (99.995%) and the towel material (99.994%) as compared to the warm (105°F) wash with cirtisolv. Furthermore, it was observed that the VRE was almost completely disinfected (killed) by the both the hot (155°F) and cold (63°F) wash, however the cold wash plus ozone, bleach and an alkaline detergent produced a slightly higher percent reduction (99.99996%).



Jason Dobranic, Ph.D.
National Director of Microbiology