



FINAL REPORT

Efficacy Testing of Laundry Systems

PROTOCOL

Efficacy of an Ozone Laundry System

ORDER Number

371011678

PREPARED FOR:

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ArtiClean Ozone Laundry Systems

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Certificate of Analysis

Client: ArtiClean Ozone Laundry Systems

Contact: Danny Kirk

Project: Efficacy Testing of an Ozone Laundry System

Product : Ozone Laundry System EconOzone Model EC-10

EMSL NO: 371011678

Sample received: 9/29/10

Start date: 9/29/10

Report date: 10/7/2010

Challenge Bacteria: Methicillin resistant *Staphylococcus aureus* (MRSA)
Clostridium difficile (vegetative cells and spores)

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 (EconOzone Model EC-10) methicillin resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile* (both vegetative cells and spores) were used to establish a relative log reduction of such organisms when compared to a normal hot water laundry system. MRSA was 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 (~10⁶ CFU/mL). *C. difficile* was prepared similarly by growing it on TSA + 5% blood agar (TSAB) at 35°C in anaerobic conditions using a BD gaspak for 48 h, before being inoculated into 100 mL of phosphate buffer. In order to obtain *C. difficile* spores the organism was grown on brain heart infusion agar + yeast extract and cysteine, which has been shown to promote spore formation, at 35°C in anaerobic conditions using a BD gaspak for 48 h. Cells were then inoculated into 200 mL of phosphate buffer to obtain a 1.0 McFarland. Immediately following, the solution was placed in a water bath at 58°C for 12 minutes, before placing on ice for 5 minutes in order to kill any vegetative cells. Afterwards, the spore solution was ready for inoculation onto the washing material.



After preparation of cells each organism was individually inoculated onto a 10 x 10 cm² piece of towel by placing 10 mL of a ~ 10⁶ CFU/mL solution. Each cloth was allowed to air dry for 1 hr before being placed into the washing machine for testing. Simultaneously, a 100 µL solution of cells was inoculated onto a 2 x 2 cm² piece of firefighter jacket material, and allowed to dry for 1 hr.

Once the inoculated test materials were sufficiently dried they were placed into the washing machine. Three separate cycles were run for each organism: 1) hot water (155°F) plus detergent and bleach, 2) cold water (75°F) plus ozone with detergent and bleach, and 3) cold water (75°F) plus ozone. MRSA and *C. difficile* vegetative cells inoculated test material were tested simultaneously, whereas *C. difficile* spores were tested separately. Immediately following inoculated cloth material was placed into stomacher bags with 100 mL of phosphate buffer and placed into the stomacher for 30 seconds. MRSA inoculated towels were then plated onto TSAB and 10 mL were placed into 90 mL of Tryptic soy broth and incubated at 35°C for 48 h. *C. difficile* vegetative cells and spores similarly plated onto TSAB and 10 mL were placed into Reinforced clostridial medium and incubated at 35°C for 72 h.

Experimental Results:

Table 1

Material	Wash Type	MRSA		<i>C. difficile</i> vegetative cells		<i>C. difficile</i> spores	
		Log10	Initial CFU	Log10	Initial CFU	Log10	Initial CFU
Firefighter Cloth	Control	5.55	3.57x10 ⁵	4.67	5.00x10 ⁴	4.97	9.67x10 ⁴
		LR	%Reduction	LR	%Reduction	LR	%Reduction
	Heat w/	5.55	>99.999%	4.67	>99.99%	4.97	>99.99%
	Ozone w/	5.55	>99.999%	4.67	>99.99%	4.97	>99.99%
	Ozone w/o	5.55	>99.999%	4.67	>99.99%	4.97	>99.99%
Towel	Control	7.31	2.10 x10 ⁷	4.66	5.33 x10 ⁴	4.71	5.67 x10 ⁴
		LR	%Reduction	LR	%Reduction	LR	%Reduction
	Heat w/	6.31	>99.9999%	4.24	>99.99%	3.86	>99.986%
	Ozone w/	7.31	>99.99999%	3.74	>99.98%	3.79	>99.983%
	Ozone w/o	6.89	>99.9999%	3.81	>99.98%	2.49	99.68%

Control = Inoculated test material not exposed to a washing cycle

LR = Log Reduction; %Reduction = compares initial CFU and final CFU



Fig. 1.1 – Log Reduction of MRSA Inoculated onto Towels

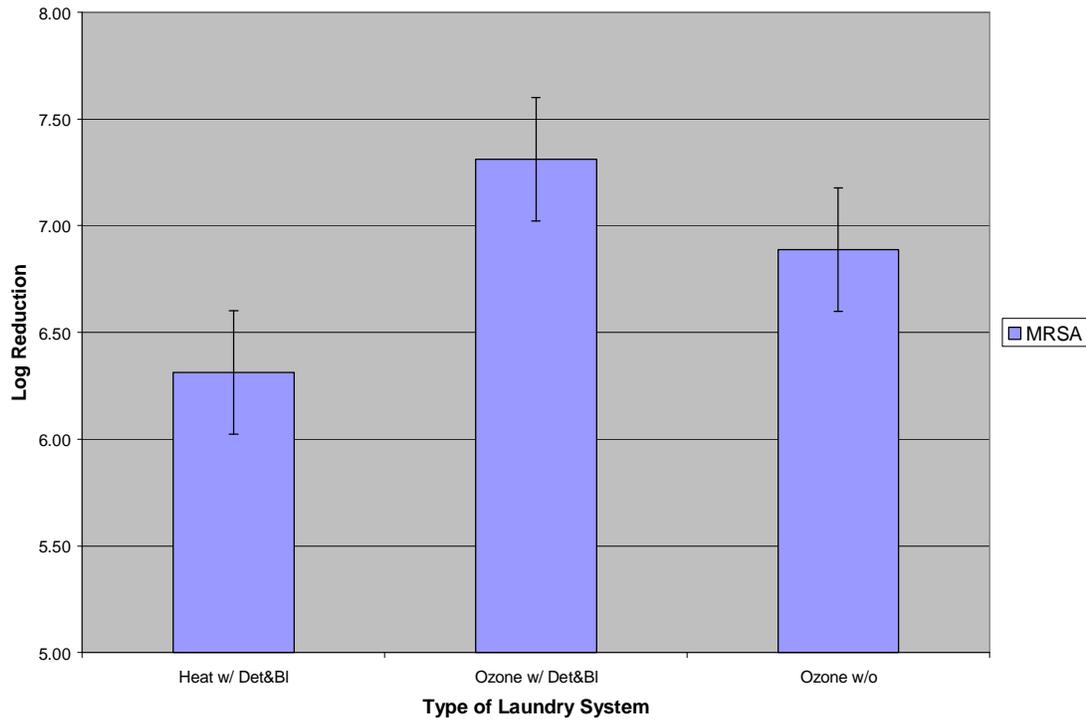
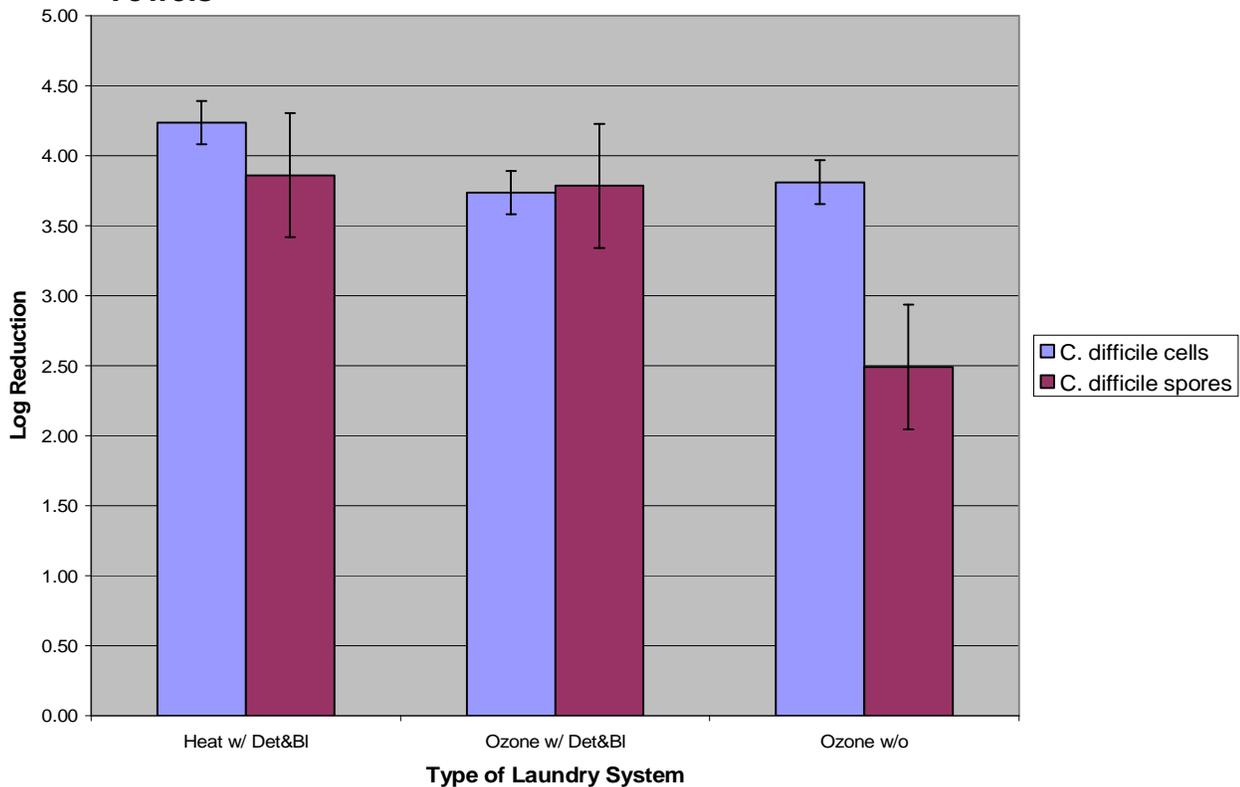


Fig. 1.2 – Log Reduction of *C. difficile* Cells and Spores Inoculated onto Towels





Conclusions/Observations:

The purpose of this study was to compare the efficacy of a normal hot water (155°F) laundry wash cycle with added detergent and bleach to a cold water (75°F) ozone laundry wash cycle without detergent and separately with added detergent and bleach. Two different material types were tested, a 10 x 10 cm² towel and a firefighter's jacket material, against all wash types and bacterial species.

MRSA

Complete reduction of MRSA was observed in all wash cycles for the tested firefighter's jacket material. The normal hot water wash cycle with added detergent and bleach was observed to reduce MRSA by a greater than 6 log reduction (>99.9999%), the cold water ozone wash cycle with added detergent and bleach was observed to reduce MRSA by a greater than 7 log reduction (>99.99999%), and the ozone wash without added detergent and bleach was observed to reduce MRSA by a greater than 6 log reduction (>99.9999%), as shown in Table 1. Furthermore, the ozone wash cycle with added detergent and bleach was observed to reduce the amount of MRSA by 1 log more than the normal hot water wash cycle with equivalent amounts of added detergent and bleach (Fig. 1.1).

In conclusion, all three wash cycles were able to eliminate (kill) MRSA from the firefighter material whereas, the cold water ozone wash cycle with added detergent and bleach was observed to produce the greatest log reduction of MRSA on the towel when compared to the other wash cycles (the normal hot water wash cycle with added detergent and bleach and the cold water ozone wash cycle without added detergent and bleach).

C. difficile

All three wash cycles produced similar results for the tested firefighter's jacket material, demonstrating complete reduction of *C. difficile* cells and spores; however differences in the reduction of *C. difficile* cells and spores inoculated onto the towels were observed. The normal hot water wash cycle with added detergent and bleach was observed to reduce *C. difficile* by a greater than 4 log reduction (99.99%) and *C. difficile* spores by a greater than 3 log reduction (99.986%), the cold water ozone wash cycle with added detergent and bleach was observed to reduce *C. difficile* by a greater than 3 log reduction (99.98%) and *C. difficile* spores by a greater than 3 log reduction (99.983%), and the cold water ozone wash without added detergent and bleach was observed to reduce *C. difficile* by a greater than 3 log reduction (99.98%) and *C. difficile* spores by a greater than 2 log reduction (99.68%), as shown in table 1. Comparing the two ozone washes, produced similar results in the log reduction of *C. difficile* vegetative cells but the cold water ozone wash without detergent and bleach demonstrated a drastic difference of 1.5 logs in the reduction of *C. difficile* spores



when compared to the cold water ozone wash with detergent and bleach (Fig. 1.2).

In conclusion, all three wash cycles were able to eliminate (kill) *C. difficile* cells and spores from the firefighter material, whereas on the towel the normal hot water wash with added detergent and bleach was observed to produce a larger log reduction (0.5 log) of *C. difficile* cells than the other two washes tested (Fig. 1.2). However, both the normal hot water wash with added detergent and bleach and the cold water ozone wash with added detergent and bleach were observed to produce comparable log reductions of the *C. difficile* spores.

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