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The Efficacy of an Ozone Laundry Wash System in Reducing the Levels of Microorganisms on Textile Garments.

Prepared for Ozone Technologies Pty. Ltd.
Unit 15 9 Kilto Crescent
Glendenning NSW 2761

Prepared by Silliker Microtech Pty. Ltd.

Tass Karalis *BSc (Hons) PhD MAIFST MASM*
Laboratory Director

1 BACKGROUND

Ozone Technologies Pty. Ltd. has developed a system for the delivery of ozone to laundry machines. The general premise of ozone treatment is that the reactivity of the ozone acts to reduce or destroy organic and inorganic matter. This application could be suitable for the laundry of various textile garments used domestically and commercially.

To determine the antimicrobial capability of the ozone laundry system, Ozone Technologies Pty. Ltd has requested an evaluation of the survival of various microorganisms introduced onto textile garments. This study will evaluate the efficacy of an ozone wash compared to a traditional hot/thermal wash in reducing or destroying *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E.coli* inoculated onto washable garments.

The Australian Standard for Laundry Practice, AS/NZ 4146:2000, does not specify parameters of disinfection for chemical based laundry treatments. Section 3.5.3 of the standard states that any selected chemical disinfection process must be validated and illustrated to be at least equivalent to the thermal process described in section 3.5.2 of the same Standard.

As such, the aim of this study is to illustrate this equivalence via a validation protocol, which can withstand reasonable scientific scrutiny.

The study aims to evaluate the reduction of medium-high levels of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E.coli* inoculated onto polyester overalls and subjected to the wash systems described above.

2 METHODOLOGY

Garments

The polyester overalls used in the study were supplied by Ozone Technologies Pty. Ltd. The overalls were stored in the laboratory at Silliker Microtech until required. The overalls used in the study for the validation of inocula and for both the ozone and thermal wash treatments were identical. This removed any scientific bias based on garment variation.

Cultures and culture preparation

Staphylococcus aureus, E.coli and *Pseudomonas aeruginosa* were obtained from the Silliker Microtech culture collection. All cultures were maintained and used within 5 generations of ATCC mother strains.

All test microorganisms were subcultured into Tryptone soy broth (TSB) and incubated at 30℃ for 48h. After incubation the brot hs was subcultured onto Tryptone soy agar (TSA) and incubated at 30℃ for 3 days. These we re checked for purity and subsequently used in the study.

Preparation of inocula

Prior to use in the challenge study the isolates were re-cultured in TSB, incubated at 30℃ for 48h and serial dilutions made in saline to pr epare the challenge concentrations of 100-500 cfu/item.

Validation of the inoculation of the test garments

To validate the inoculation of the garments and to determine the concentration of mciroorganisms required to meet the challenge dose, cultures were prepared as described in the section above.

A garment was then selected and 3 areas, each approximately 15cm x 15cm were marked on the chest area. Each marked square was inoculated with a target microorganism. That is, 1 area was inoculated with *S. aureus* , the next with *E.coli* and so forth. The organisms were introduced by spreading 1.0mL of the appropriated broth culture dilution. The garment was allowed to dry overnight and the organisms recovered to demonstrate the viability and number of each microorganism.

Microbial challenge

Having demonstrated that each challenge organisms could be successfully inoculated onto the garment, remain viable and be recovered, the testing was performed in duplicate for each of the 3 challenge organisms and a control.

Five (5) garments were inoculated with the challenge microorganisms as described above. One garment remained in the laboratory as a positive control. The other garments were individually packed and delivered to Ozone Technologies who inturn supervised the washing at a 3rd party premises.

Two of the inoculated garments were treated with traditional thermal washes and 2 by the OTEK laundry system developed by Ozone Technologies Pty. Ltd. Each garment was washed in a separate wash trial and as such, each system was assessed in duplicate.

Washing

The wash treatments were conducted at 3rd party premises using 22kg load machines with 120L water. The parameters of the thermal and ozone wash cycles are outline in the document “Wash Formulas used to determine the comparison of thermal disinfection to ozone disinfection” (Ex-Ozone Technologies Pty. Ltd.) and is provided in the appendix of this report.

Analysis of the garments post treatment

On completion of the washes, the garments were returned Silliker Microtech and analysed on the same day. Recovery for each of the challenge organisms was performed using contact plates over the marked and inoculated areas. An adequate number of plates were used to cover the entire marked areas. The contact plates used in the study were gamma sterilised Tryptone Soy agar.

Once tested, the contact plates were incubated at 30°C for 48h in accordance with Silliker Method M17 and recovery counts made for each of the test organisms and for each of the wash variants.

The counts for each wash treatment (ozone and thermal) were compared determine equivalence and comparison was also made with the untreated control to determine the degree of reduction of each microorganism for wash treatment.

3 RESULTS

Table 3.1: Effect of ozone and thermal washes on the survival of *P.aeruginosa* on polyester overalls

Organism	**No. cells inoculated[1] cfu/garment**	**Cell counts after each treatment cfu/garment**			
		Ozone treatment 1	**Ozone treatment 2**	**Thermal treatment 1**	**Thermal treatment 2**
P.aeruginosa	260	0	0	0	0
% Destruction		100	100	100	100

1. Inoculum determined by performing counts on the control sample

Table 3.2: Effect of ozone and thermal washes on the survival of *S.aureus* on polyester overalls

Organism	**No. cells inoculated[1] cfu/garment**	**Cell counts after each treatment cfu/garment**			
		Ozone treatment 1	**Ozone treatment 2**	**Thermal treatment 1**	**Thermal treatment 2**
S.aureus	280	2	2	1	
% Destruction		99.3	99.3	99.7	100

1. Inoculum determined by performing counts on the control sample

Table 3.3: Effect of ozone and thermal washes on the survival of *E.coli* on polyester overalls

Organism	**No. cells inoculated[1] cfu/garment**	**Cell counts after each treatment cfu/garment**			
		Ozone treatment 1	**Ozone treatment 2**	**Thermal treatment 1**	**Thermal treatment 2**
E.coli	260	0	0	0	2
% Destruction		100	100	100	99.2

1. Inoculum determined by performing counts on the control sample

4 DISCUSSION

The experimental findings of this study suggest that the ozone and thermal heat treatments were at a minimum, comparable in their disinfection capabilities. A reduction and/or destruction of each of the challenge microorganisms, was observed on the garments after each treatment.

Bacterial counts from the inoculated, untreated control sample indicated that the inocula used were able to survive on the polyester overall and that the reductions observed in the test samples were indicative of the effect of the process used.

A 100% reduction/disinfection of *E.coli* was observed on the test samples using both the ozone and thermal washes. Similar findings were observed for *S.aureus* and *P.aeruginosa* using both wash treatments with the % reduction of the challenge organisms on the garments ranging from 99.2-100%.

The results of the study clearly meet the requirements of AS/NZS 4146:2000 and show that the level of disinfection of the ozone system is at least equivalent to that of the thermal wash. As such, this study validates the OTEK ozone wash system as compliant with the current standard and as effective as thermal washing in terms of disinfection properties.

5 APPENDIX

1. AS/NZS 4146:2000 “Laundry Practice” Section 3.5.2 and 3.5.3
2. Product Trial “On-Premise Laundry”. OTEK Laundry Systems. Ozone Technologies Pty. Ltd.
3. Wash Formulas used to determine the comparison of thermal disinfection to ozone disinfection. Ex-Ozone Technologies Pty. Ltd.