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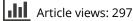
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## Microbiological Benefits of Ozone in Laundering Systems

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A major benefit of ozone in commercial laundry systems is the control, disinfection, and/or total eradication of microorganisms normally found in/on soiled laundry. In hospitals, health care, retirement facilities, as well as in locker rooms of academic and professional athletes, in particular, certain microorganism strains exist and proliferate that are particularly resistant to modern medications. Numerous infections from the two currently prevalent "superbugs" - Methicillin-Resistant Staphylococcus aureus (MRSA) and Clostridium difficile (C. difficile, or C. diff) have created panics in recent years. Detailed studies conducted in the United Kingdom on both the "routine" microorganisms found in hospital and health care facilities (E. coli, Pseudomonas aeruginosa, etc.) as well as four types of viruses, and in particular, the two superbugs (MRSA and C. difficile) are rapidly eradicated by ozone cold water laundering within a few minutes. Not only are microorganisms eradicated, but the laundering with ozone saves about half of the cost of current conventional laundering systems not incorporating ozone. Details of many studies conducted in the UK on the microbiological benefits of ozone in disinfecting and ensuring the absence of microorganisms are presented in this paper. These have shown that C. difficile spores are not consistently eliminated from microfibre mops and wiping cloths by conventional laundering processes. This means that this superbug can be spread around the facility when reused after conventional laundering, thus increasing the potential for possible re-infection of patients, staff, and visitors. Fortunately, ozone laundering totally eradicates C. difficile spores and eliminates this danger. Studies of the effects of repeated ozone laundering of microfibre mops and wiping cloths showed that ozone laundering has much lesser effects on the longevity of microfibre mops and cloths than do the conventional laundering systems of today.

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Address correspondence to Dr. Rip G. Rice, RICE International Consulting Enterprises, 1710 Hickory Knoll Road, Sandy Spring, MD 20860, USA. E-mail: RGRice4ozone@aol.com Keywords Ozone, Ozone Laundry Systems, Laundry Applications, Microbiological Benefits, Methicillin-Resistant *Staphylococcus aureus*, *C. difficile*, Microfibre Mops and Wiping Cloths

# MICROBIOLOGICAL BENEFITS OF OZONE LAUNDERING

#### Background

In hospitals, nursing homes, and health care centers, bedding, linens, and garments worn by patients and caregiving staff all are exposed constantly to numerous microorganisms. The ubiquitous "usual suspects" include, e.g., Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Escherichia coli, Streptococcus faecaelis, Aspergillus niger, Clostridium perfringens, Campylobacter jejuni, Aeromonas mixed species, Acinetobacter sps, and Lactobacilli sps. In addition, the two current "superbugs" are recognized as living rampantly in many of these types of facilities as well. These are MRSA (Methicillin-Resistant Staphylococcus aureus) and Clostridium difficile (C. difficile). Both super-bugs are harder to destroy than are the more well-known microorganisms listed just above. In addition, several strains of viruses also are encountered in health-care establishments, including Saccharomyces virus ScV-L-BC.

These microorganisms have the potential to proliferate in athletic locker rooms as well as in hotels and motels, although the risks are not as great as in health-care facilities, where incontinent patients confined to beds continually are contaminating bed linens and hospital clothing.

At any time, approximately 1 in 10 patients in acute hospitals have a hospital-acquired infection (HAI). At the same time, an unquantified number of patients discharged into the community from hospitals have an infection related to their hospital stay. HAI imposes both a financial and non-financial burden upon society (Hook, 2007a).

A clean hospital environment is vital to provide a background to acceptable hygiene standards, as well as maintaining the confidence and morale of patients, health care staff and visitors. Hospital floors and surfaces become contaminated by settlement of airborne bacteria, by contact with items such as shoes, trolley wheels and other solid objects, and occasionally by the spilling of urine, pus, sputum and other body fluids. Some of the bacteria lie loosely in dust while others become ingrained into the surface and between cracks. Pathogens commonly present include *Staphylococcus aureus* dispersed by patients and staff, and in much smaller numbers Gram-negative rods, such as *Pseudomonas aeruginosa. Clostridium difficile* is transmitted between patients, health care workers and the environment.

Environmental contamination with *C. difficile* spores, often widely dispersed, has been demonstrated in 34-58% of sites in hospital wards. Commodes, bed frames, sluice rooms and toilet floors are the most frequently contaminated sites and the floor areas showed heaviest contamination. Although it can be difficult to distinguish between cause and effect, in most studies correlation can be shown between environmental contamination and infection rates (Hook, 2007a).

OTEX, an ozone laundering system developed by JLA Limited, the UK's largest supplier of laundry equipment, has gained success within the hotel and care industry. It has received approval from the Director of Nursing, Director of Prevention Control, Infection East and North Hertfordshire NHS. CSCI (Commission for Social Care Inspection) has officially confirmed that OTEX is fully compliant with the National Minimum Standards (2002). Ozone gas is injected directly into the water employed in the wash process via JLA's patented interfusor system. This provides a continual replenishment flow of ozonated water throughout the wash and rinse cycles. The advantage of this system is that the bioburden is continually treated with ozone, thus providing constant disinfection.

Microfibres, used to manufacture mops and wiping cloths, are a blend of polyester and polyamide fibers, which have undergone a process of splitting the yarn into thousands of tiny fibers each less than a 100th the size of a human hair. This creates the potential for a huge surface area with unique absorbency properties enabling them to be used effectively in both wet and dry conditions. When used dry, static electricity attracts soil to the fibers, while in damp applications soil is drawn into the fibers by capillary action. Their effectiveness at cleaning surfaces and removing bacteria, yeast and molds is well documented (Association of Domestic Management, UK, 2003, 2005; Moore and Griffith, 2006; Wren et al., 2008; United Kingdom Dept. of Health, undated).

Ozone is very effective against bacteria, viruses and other microorganisms. In aqueous environments, the key to attaining a desired level of disinfection lies in achieving a "Ct" value (contact time in minutes multiplied by ozone concentration in mg/L) of 1 mg/L-min or more. That is to say, develop a residual level of ozone in the water and maintain that residual ozone level for a sufficient number of minutes so that the product of ozone concentration in water times the number of minutes required to maintain that residual concentration equals 1.0 mg/L-min or higher. This Ct value is readily attained in properly designed ozone laundry systems.

## Microbiological Tests and Studies of Ozone Laundering Systems Conducted in the United Kingdom

Cardis et al. (2006) described a series of evaluations designed to determine the extent of inactivation during ozone laundering of the microorganisms associated with soiled linens and microfiber mops and wiping cloths encountered in hospitals, nursing homes, care centers, and the like. Briefly, these investigators obtained the following major results:

1. Efficacy of Hot Water (75°C) vs Ambient Water With Ozone on *C. difficile* Spores

The survivability of *C. difficile* spores in a commercial laundering machine in hot water only (linens absent) at temperatures required by UK health authorities (75 °C = 167 °F) for the UKrequired 15 minutes was found to be insignificant. A second test compared the exposure of *C. difficile* spores in cold water containing ozone provided by the JLA Ltd. OTEX equipment over a similar time period. In this case (ozone treatment), after only 2.5 minutes of exposure to aqueous ozone, no viable trace of *C. difficile* spores could be found.

2. Efficacy of Four OTEX Laundering Cycles on MRSA and *C. difficile* Survival

Various garments were challenged with MRSA and *C. difficile* microorganisms and then subjected to four ozone laundering cycles (heavy soil, light soils, delicate items, and a rewash cycle designed for oil/ grease-stained articles). Ozone levels were constant, but each laundering cycle requires differing amounts of detergent, because of the differing degrees of soil. All ambient temperature ozone launderings resulted in > 5 logs reduction (> 99.999%) in MRSA and *C. difficile* levels, whereas washing without ozone at the UK-specified thermal laundering temperature of 75 °C (167 °F) resulted in < 5-log reductions.

3. Nurses' Uniforms Contaminated with MRSA Care labels of nurses' uniforms commonly carry the recommendation that they be laundered at 40 °C (104 °F). Therefore a comparison of thermal washing (40 °C) with ambient temperature ozone washing was conducted on soiled nurse uniforms into which were implanted membranes impregnated with MRSA. After 40 °C laundering, MRSA was clearly present on the membrane samples, but totally absent after ozone laundering. Data obtained indicated a > 8-log reduction (> 99.999999%) in MRSA on garments washed with ozone, but only a 3.3-log reduction (99.93%) after thermal washing at 40 °C (104 °F).

4. Efficacy of OTEX Laundering Against E. coli Validation trials of OTEX equipment operating at 60% of the maximum ozone output were conducted (20 minutes laundering for each test) to determine the efficacy of ozone kill in water (absent garments or linens) of E. coli at an initial contamination level of log-7 cells/mL. Each trial was preceded by a hot sanitizing wash and rinse cycle.

In the control experiment, with no additives or ozone treatment, an *E. coli* log-reduction of ca 1-log cycle was found. In the ozone trials, *E. coli* could not be found after the initial ozone dosing period. In fact, by the 10-minute mark, the data indicated that a 7-log reduction of *E. coli* was obtained in 7 minutes of ozone dosing at only 60% of the maximum ozone output of the OTEX machine.

5. OTEX Bacteriological and Viral Efficacy Study

Another test program compared the OTEX system (using ambient temperature water) against thermal washing at 75 °C (167 °F), which is 4 °C higher than the UK-recommended thermal disinfection temperature. No linens or detergent were employed in this investigation. Three wash solutions were employed: (a) a control ambient temperature water, no ozone; (b) hot water (75 °C) containing no ozone; (c) ambient temperature water containing 0.2 mg/L (at the start) to 0.6 mg/L (after 15 minutes) of dissolved ozone. Samples were withdrawn for analysis from the wash drum after 3, 7, 11 and 15 minutes of washing.

Challenge organisms employed are listed in Table 1. These include 12 bacterial strains and 4 types of virus particles. Without ozone and at ambient temperature, only small amounts of bacterial kills were noted, as expected. With thermal washing, 3 strains of bacteria remained in the water even after 15 minutes (*Pseudomonas aeruginosa*, *C. difficile*, and *Clostridium perfringens*). However, with ozone washing, no bacteria were present after only 3 minutes of washing.

Similar results were found for the four virus and phage strains tested. With thermal or ozone washing, viral inactivation was obtained after five minutes of washing. This test shows that ozone washing is as effective as thermal washing (at 75 °C). However, since ozone washing is conducted at ambient temperature, energy costs are significantly higher for thermal washing.

6. Six-Month QE-II Hospital Bacterial Testing of OTEX Laundering System

The Queen Elizabeth II Hospital (Welwyn Garden City, Hertfordshire, UK) first conducted testing

**TABLE 1.** Solution Challenge Test Organisms

Microorganism	cfu/mL
Staphylococcus aureus	1.3E+08
Pseudomonas aeruginosa	3.1E+09
Candida albicans	3.1E+08
Escherichia coli	5.2E+08
Streptococcus faecalis	5.0E+08
Aspergillus niger	3.1E+08
Clostridium difficile	4.2E+08
Clostridium perfringens	9.2E+08
Campylobacter jejuni	6.0E+08
Aeromonas mixed species	8.2E+08
Actinobacter sps	4.3E+08
Lactobacilli sps	3.9E+08
Virus particle	Particles/mL
Lambda phage	3.8E+24
FCoV <sup>A</sup>	2.6E+24
Saccharomyces virus ScV-L-BC	3.1E+23
Vibrio phage fs1	2.6E+28

of microfibre mops and wiping cloths contaminated with various microorganisms found in hospitals by conventional laundering (thermal disinfection at 71 °C = 160 °F over 60 minutes) and with detergent. Microbiological analyses showed the mops and cloths to be still contaminated. *C. difficile* counts were over 150,000 TVC (total viable counts). This means that even after the recommended thermal laundering, microfibre mops and wiping cloths were simply distributing *C. difficile* throughout the hospital.

An OTEX ozone laundering system was installed (by JLA Ltd.) in the QE II Hospital, and a 6-month trial of this system began on May 17, 2005. Table 2 shows the microbiological status of microfibre mops and cloths sampled at the QE-II hospital during April 2005 (before OTEX laundering testing began) to establish a "control base line". Many problematic bacterial species were present.

The OTEX system at the QE-II hospital utilized ozone throughout an entire 47 minute laundering programme, to wit:

Ambient
47 minutes (22% less than Thermal
Disinfection Cycle of 60 min)
40 mL (50% less than conventional
Thermal Disinfection Cycle)
Continuously throughout
the wash cycle

TABLE 2. Pretreatment Microbiological Status of Microfibre Mops and Cloths (Microsearch Laboratories, Ltd., 2005)

Test - cfu/cm <sup>2</sup>	Soiled mop	Soiled blue cloth*	Soiled red cloth*
TVC	9.30E +14	5.20E + 13	4.60E +12
Enterobacteriaceae	6.20E +13	9.10E +10	8.40E +09
E. coli	5.10E +07	1.00E + 07	3.20E +07
Pseudomonas	6.80E +13	8.30E +12	9.20E +12
S. aureus	9.70E +06	4.50E +09	6.10E +11
Total Streptococci	4.00E +11	700E +12	1.30E +13
Listeria	8.20E +04	700	6.20E +04
Clostridia species	7.10E +12	6.20E +09	9.30E +08
Salmonella	4.10E +03	2.00E + 02	1.30E +03

\*Red Cloths are used for bathrooms, washrooms, showers, toilets, basins and bathroom floors. Blue Cloths are used for general areas, including wards, departments, offices and basins in public areas. These are requirements of the NPSA National Color Coding Scheme that became effective throughout the UK by March 2008.

Samples with no contamination. The samples of used microfibre utilities examined in this trial were not challenged. The levels of recovered targets reflected the actual levels of contamination due to usage. However, on some occasions, tests indicated an absence of target organisms from one or more categories.

## **Test Protocol**

Diluent for plate counts:	Difco Universal Quenching
	Agent (DUQA)
Target Organisms:	Methicillin-Resistant
	Staphylococcus aureus
	(MRSA)
	Clostridium difficile
	(C. difficile)
	Yeasts
	Molds (Aspergillus niger
	initially requested)

Acceptable results - no microorganisms isolated on samples.

Details of the wash program installed are given in Table 3, together with the Mediclean thermal disinfection program details for comparison.

*Loading criteria.* The following loading matrix was employed:

Item	Maximum per net bag	Maximum per washload	
Microfibre Mops	20	4	
Microfibre Cloths	40	6	

Microfibre mops and wiping cloths taken randomly from the existing "live laundry" bins were processed every week of the 6-month trial period in the OTEX (ozone-ambient temperature) washing system in the numbers shown just above. One sample of mop or cloth from

#### TABLE 3. OTEX and Mediclean Laundry Wash Program Details

	OTEX	Thermal disinfection
Program		Temperature
Pre Wash	Cold	Warm 40 °C (104 °F)
Main Wash	Cold	Hot 75 °C (167 °F)
Rinse 1	Cold	Cold
Rinse 2	Cold	Cold
Rinse 3	Cold	
Cycle Time	47 min	1 hour
Detergent Volumes	40 mL	80 mL

a bag washed was analyzed before and after ozone laundering. One of the mops laundered is called a "Spanky". This is a flexible mop, made for hard-to-reach dusting in areas such as curtain rails, doors, window frames, etc. The Spanky is solid, akin to a foamed plastic-covered paint brush designed to cover corners and joints between walls and floors or ceilings. The Spanky also comes with a washable microfibre cover.

#### Recovery

- a) Multiple 20 gram samples of microfibre utility were stomached for 1 minute in 180 mL of DUQA.
- b) Decimal serial dilutions down to  $10^8$  were prepared.
- c) Aliquots of all dilutions were plated out and incubated as per Table 4.
- d) 100 mL of DUQA was subjected to membrane filtration and then was examined using the incubation conditions detailed in Table 4.
- e) Positive and negative controls were employed for all determinations. NCC or ATCC. strains were used at  $10^1$  and  $10^4$  levels of inoculation for positive controls.
- f) Confirmation and identification strategies are summarized in Table 4.

Note: All protocols are based on UKAS approved methodology conducted under a BS17025 quality system.

#### RESULTS

Table 5 summarizes the microbiological data obtained for the microfibre mops, Spankies, and wiping cloths before laundering. Throughout the 6-month OTEX trial, no residual target organisms, as set by the East and North Hertfordshire NHS Trust Infection Control, were detected, including MRSA and *Clostridium difficile*, after laundering with the OTEX system. In addition the OTEX system provided a simple laundering process with one cycle, which can also accommodate traditional cotton mops while using less detergent and being energy efficient.

The data listed in Table 5 comprise a total of 53 individual samples of microfibre mops and wiping cloths taken weekly from in-use laundry bins of the QE II Hospital. Most samples (but not all) were contaminated with MRSA, *C. difficile*, *A. niger*, yeasts and molds. After ozone laundering under the conditions stated in Table 3, every single one of these 53 samples showed zero cfu in each of the microorganism categories analyzed. Based on this 6-month evaluation, the Queen Elizabeth II hospital adopted the OTEX ozone laundering system as their method of laundry decontamination on Dec. 12, 2005.

#### Degradation Analysis of Microfibre Cloths Within the Healthcare Environment (Hook, 2007b, 2007c)

With the adoption of ozone laundering at the QE-II Hospital in late 2005, the question arose as to the stability of physical properties and colors of microfibre mops and wiping cloths during repeated ozone launderings. A detailed investigation was undertaken to resolve this uncertainty (Hook, 2007b).

#### Work Performed

New microfibre cloths together with samples of cloths from a variety of "live" laundry sources (i.e., from hospital and nursing homes utilizing either traditional thermal or OTEX washing processes) were tested. In order to maintain confidentiality, the identity of the sites was withheld. The cloths were subjected to a number of physical tests. Further physical testing to assess performance concentrating on absorbency and loss in surface area, i.e., shrinkage, was considered necessary to provide corroborative data. In addition, electron microscope imaging was performed on the materials by an independent laboratory to provide both photographic evidence and expert opinion on the condition of the materials.

#### **Chemical Resistance**

The environment and the methodology in which these cloths are used (i.e., no additional chemicals used for

Target organism	Culture media	Incubation	I.D. Confirmation
MRSA	Baird Parker	48 hours, 37°C aerobic	Morphology Probe
MRSA	Biomerieux Chromogenic Agar	24 and 48 hours, 37°C, aerobic	Chromogenic Reaction Probe (DNA)
C. difficile	Cycloserine Agar	24 and 48 hours, 35°C, anaerobic	Microscopy Biochemical Profile
Yeasts, Molds, A. niger	R.B.C.A. Rosebengal Chloramphenicol Agar	5 days - 25°C	Microscopy

TABLE 4. Microorganism Recoveries - Culture, Incubation, Confirmation

TABLE 5. Microbiological Counts on Microfibre Mops, Spanky Mops, and Wiping Cloths to be Laundered (cfu/cm<sup>2</sup>)

Items tested (no. samples)	MRSA	C. difficile	Yeasts	Molds	A. niger
Microfibre Mops (15 samples	)				
Mean count pre-OTEX	489	1,023	61,770,000	76,000	647
Range	0-3,000	0-13,000	0-920,000,000	0-730,000	0-2,800
Counts post- OTEX	0	0	0	0	0
Microfibre Spanky Mops (8 s	amples)				
Mean count pre-OTEX	607	33	5,626,000	83,450	866
Range	0-4,500	0-230	0-43,000,000	2,100-400,000	0-4100
Counts post- OTEX	0	0	0	0	0
Microfibre Cloths (30 samples	s)				
Mean count pre-OTEX	853	1073	296,200	329,000	238,000
Range	0-19,000	0-26,000	0-6,200,000	0-8,300,000	0-7,100,000
Counts post- OTEX	0	0	0	0	0

surface cleaning) emphasizes the need to maintain condition and integrity of the microfibre throughout its service life. In addition, the use of color-coding of cloths and mops is widely used to help prevent cross-contamination by the transfer of bacteria when cleaning different surfaces and areas. It is therefore equally important to maintain a degree of color during the life of these items. However, color loss will occur over a period of time, irrespective of the wash process adopted. Exposure to chemicals such as hypochlorite or sodium dichloroisocyanurate, either through use or misuse, also will affect the extent of color loss.

The microfibre cloths generally are produced from a blend of 80% polyester and 20% polyamide fibers, as a narrow fabric with over-locking to both ends and "fabric" dyed. The susceptibility of the dye to fade will depend upon the particular dye process used. Certain dyes are more susceptible to fading from exposure to chemicals and differing pH conditions. Many dyes, especially blues, are prone to ozone fading. Some disperse dyes used with nylon exhibit the tendency of ozone fading (Burkinshaw, 1995). One cloth manufacturer advises that color may run if colored cloths/mops are washed together with uncolored ones, but states that discoloration will **not** affect the cleaning characteristic of the material.

Both polyester and polyamides have good chemical resistance. In particular, polyester can withstand a range of chemicals including oxidizing agents and is normally only affected by strong alkalis. In strong alkaline solutions, such as sodium hydroxide, polyester fibers can be broken. Its outer layer is peeled away. The rate of peeling increases with increasing alkalinity and increasing water temperature over 40 °C (104 °F). If a cationic disinfectant, such as a quaternary ammonium compound (QAC), is added to the alkaline solution, a catalytic breakdown of the polyester material will take place, causing it to quickly lose its strength (Burkinshaw, 1995). Polyamides tend to be more susceptible to acids but also can be weakened by strong alkalis.

Manufacturers of the microfibres recommend that chemicals not be used with microfibres for surface cleaning. During the low temperature ozone wash process, a neutral pH is maintained throughout the entire cycle. No hot water is used within the wash cycle.

## Laboratory Testing

This study was conducted to compare the condition of microfibre cloths following differing disinfection wash cycles within the healthcare environment. The objective was to establish whether thermal or ozone disinfection wash processes have any detrimental effects on the integrity of the microfibre and subsequent effectiveness.

Laboratory tests were conducted on new microfibre cloth samples to assess the effect from exposure to ozone gas and ozonated water. In addition, numerous samples from a broad range of hospital and nursing home sites also were examined to determine the extent of color loss and potential fiber damage. Independent examination of the samples was conducted by Scientifics Ltd., Forensic Section at Derby.

## **Controlled Exposure Tests**

Samples of microfibres were exposed both to ozone gas and ozone dissolved in water. This resulted in excessive fading, particularly on the sample exposed to ozone gas. Samples then were forwarded together with a new cloth for comparative purposes to Scientifics Ltd. for independent examination to confirm whether there was any relationship between color loss and microfibre condition. Microscopic examination of ozone-treated microfibre cloths showed microfibres still in good condition following exposure to both ozone gas and immersion in ozone–containing water.

#### **Controlled Wash Tests**

Cumulative wash programs were run under extreme conditions, i.e., high concentrations of ozone, with/

without tumble-drying, and no detergent, to provide information on the degree of color loss under extreme conditions. The samples then were studied by electron microscope imaging to ascertain whether any chemical damage had occurred. The imaging laboratory concluded, "Visual examination of these samples showed no evidence of systematic chemical damage to the structure of the cloths or to the individual fibers. The damage that was noted is entirely more consistent with physical 'wear and tear', that is to say the twisting of individual fibers and general stretching of the loops of arranged fibers."

#### Surface Area and Absorbency

Surface area and absorbency of the cloths are pivotal to the performance and effectiveness of these items. A reduction in either of these parameters is likely to reduce the ability of the cloths to remove soil from the surface, with obvious implications. Shrinkage and fiber damage also may affect the effectiveness of the laundering process, with organic matter becoming embedded in the fiber structure. Laboratory tests were conducted on both site samples and laboratory controlled test samples measuring water absorbency and surface area in comparison to new cloths.

After 100 laundering cycles, the surface area losses were:

OTEX-washed only (not dried)	4%
OTEX washed and tumble dried	10%
at 180 °F (82 °C)	
Thermal disinfection at 75 °C	17%
(167 °F) and tumble-dried at	
180 °F (82 °C)	

After 100 laundering cycles, the absorbency losses were:

OTEX-washed only (not dried)	5%
OTEX washed and tumble dried	18%
at 180 °F (82 °C)	
Thermal disinfection at 75 °C (167 °F)	26%
and tumble-dried at 180 °F (82 °C)	

#### CONCLUSIONS

The results obtained in this study indicate the following:

- Color loss is experienced irrespective of either washing under current HSG (Health Safety Guidelines) guidelines utilizing thermal disinfection, or OTEX, ozone disinfection wash cycles.
- No association between color loss and fibre damage resulting in a reduced performance was found. This finding is supported by information on color loss supplied by one of the microfibre manufacturers.

- No chemical damage/erosion was found in any of the samples submitted to Scientifics Ltd.
- There is evidence supported by Scientifics Ltd. that physical damage occurred during laundering. However, the Scientifics Ltd. report shows that the cloths processed with OTEX exhibit less damage than found on samples processed by thermal laundering. The damage is localized on the tips of the fibers and is indicative of exposure to high temperatures during the drying process.
- The effect of physical damage can be seen in the loss of the original surface area together with a corresponding reduction in the original absorbency. The physical damage is likely to be as a result of drying at high temperatures for prolonged periods, since the cloths are polymers or "plastic" and are therefore susceptible to heat. Processing mops and cloths together also will have a detrimental effect on the cloths by increasing the physical action or abrasion of the materials.

The results obtained show clearly that the use of OTEX laundering does not result in any detrimental effect to the microfibre effectiveness or integrity and is a viable alternative to thermal disinfection. In contrast, there is evidence that the use of ozone maintains the microfibre integrity, with the added benefit of an improved disinfection process and additional utility savings.

#### RECOMMENDATIONS

Loss in color was experienced with both laundering processes. Given the importance of retaining color throughout the life of the cloth, it is recommended that an investigation into alternative dyes be carried out. The use of dyes resistant to oxidizing agents (i.e., vat dyes) should be explored.

Clear guidance needs to be given by the supplier and manufacturer as to the recommended drying temperature. Care labels attached to the cloths give inconsistent guidelines on washing and drying temperatures; these should be standardized throughout the industry. Specific drying programs have now been developed by JLA Ltd., and are being employed at a number of sites laundering micro-fibre. These use a lower drying temperature of 130 °F (55 °C) in comparison to the common practice of drying at 180 °F (82 °C). No detrimental effects on the micro-fibres have been experienced at any of these sites.

It is strongly recommended that staff become fully conversant with the laundering needs of microfibres. Full training and advice should be introduced at all sites. All dryers on-site should have clear instruction for their programs, including drying temperatures, laundry sorting and times. Where applicable, microfibre-specific drying programs should be adopted.

#### Microbial Analysis of Microfibre Cloths Employed Within a Hospital Environment (Hook, 2007a)

In order to compare the effectiveness of the ozone laundering process to the standard thermal disinfection process, analysis of microfibre cloths from two UK hospitals (having been used for cleaning and contaminated with various microorganisms) was conducted before and after washing by the two procedures. Individual microfibre cloths were selected randomly and cut in half. One portion was retained as the "pre-sample", and the remaining half was processed with OTEX (ozone laundering). Three separate microbiological laboratories were employed to provide independent data.

The standard OTEX microfibre laundering program was employed at each site. This is a cold water program with minimal detergent dosed within the main wash step. Details of the OTEX wash program installed are given in Table 6 together with details of a typical thermal disinfection program for comparison. Data reported by the three microbiological laboratories are presented in Table 7. At one of the laboratories, difficulties were encountered with detecting *C. difficile* spores.

Microfibre laundering via the OTEX process has been carried out at several sites including nursing homes and hospitals with no adverse reports on their performances. Indeed, one installation that currently has 12 OTEX trial **TABLE 6.** Details of OTEX and Thermal Disinfection Washing of Microfibre Cloths (Hook, 2007a)

	OTEX	Thermal disinfection
Program		Temperature
Pre-Wash	cold	Warm – 40 °C (104 °F)
Main Wash	cold	Hot – 75 °C (167 °F)
Rinse 1	cold	cold
Rinse 2	cold	cold
Rinse 3	cold	cold
Cycle Time	47 min	60 min

sites has been laundering microfibre items for over three years with ozone, with no apparent detrimental effects (Hook, 2007c).

# COMPARATIVE TESTING OF OZONE VS STANDARD LAUNDERING

Based on an August 2006 evaluation of the JLA Ltd. OTEX ozone laundering system, Reid et al., 2007 conducted a phase 1, single blind, randomized, controlled series group study of standard laundry disinfection techniques using the current standard VIKING machine versus the OTEX validated ozone disinfection system, set up at the laundry at Woodend Hospital, Aberdeen, Scotland.

TABLE 7. Microbiological Test Results on Laundered Microfibre Cloths (Hook, 2007a)

				TVC		C. dį	fficile
Test lab	Site	Sample*	Date	Before	After	Before	After
1 1 1 1 1 2 2 2 2 2	Scottish Hospital Scottish Hospital	red cloth red cloth blue cloth blue cloth red cloth red cloth red cloth blue cloth	13 Apr 06 13 Apr 06	1.90E+07 3.20E+07 3.50E+07 8.32+06 4.12E+06 2.90E+06 4.00E+07 9.10E+06 8.30E+06	8.50E+03 2.26E+04 1.00E+04 2.17E+03 3.50E+02 190 300 180 59	Analytical p No data c 57 60 13 9	
2 3 3 3 3 2 2 2 2 2	North West Hospital North West Hospital	blue cloth red cloth blue cloth red cloth blue cloth red cloth blue cloth red cloth	2 May 07 2 May 07	$\begin{array}{l} 3.50 \pm +00\\ 2.9 \pm +09\\ 8.8 \pm +08\\ < 10\\ < 10\\ 1.3 \pm +08\\ 2.1 \pm +07\\ 1.90 \pm +07\\ 2.10 \pm +08\end{array}$	1.1E+02 < 10 < 10 < 10 80 420 < 1 < 1	1.7E+02 6.5E+03 no growth no growth 18 94 < 1 27	no growth no growth no growth no growth < 1 < 1 < 1 < 1 < 1 < 1

<sup>\*</sup>Red Cloths are used for bathrooms, washrooms, showers, toilet, basins and bathroom floors. Blue Cloths are used for general areas, including wards, departments, offices and basins in public areas. These are requirements of the NPSA National Color Coding Scheme that became effective throughout the UK by March 2008.

The OTEX laundry process system utilizing ozone complies with the Commission for Social Care Inspection (CSCI) national minimum standard regarding disinfection of laundry. This system does not rely on current standard thermal disinfection temperatures of 65 °C for 10 minutes or 71 °C for 3 minutes to clean or disinfect laundry. Nonetheless, the OTEX system does produce satisfactory cleaning and antibacterial results through appropriate application of ozone.

The objectives of this study were to assess the safety, tolerability and efficacy of ozone applied in the OTEX *versus* standard laundry cleaning procedures (VIKING machine). In addition, it was deemed important to assess the reproducibility of the OTEX ozone disinfection system on a standardized series of heavily fouled laundry loads contaminated with hospital-acquired bacteria, fungi and/or viruses in comparison to a matched series of heavily fouled laundry loads using the Standard VIKING laundry machine.

## STUDY DESIGN

The processes used for washing highly contaminated hospital linen can be summarized as follows:

- a) The execution of one washing cycle with conventional chemical products (detergent, alkalis and 150 ppm of chlorine).
- b) One washing cycle with ozone (up to 4 g per hour).

Water samples were collected using standard sterile 1-liter collection bottles (each containing a measured volume of sodium bisulfate solution) from access ports on each washing machine at the relevant phases of each laundry wash on each machine. Pre-wash samples were taken after three minutes of agitation without any additives. Post-wash samples (sterile 1-liter collection bottles) were collected similarly from each machine following the final cycle of the laundry load.

All collection bottles were stored (no more than 4 hours) in a receiving refrigerator at 4 °C to 8 °C before collection. The bottles containing contaminated fluid (1-liter) were transported directly to the Public Health Laboratory within the Department of Microbiology, Aberdeen Royal Infirmary. The 1-liter samples of laundry liquor were plated in the usual way using routine standardized laboratory procedures. The plates then were read in the usual way for the standard contaminants of hospital laundry using Total Viable Count (TVC) as an index of contamination of each laundry wash.

Forty (40) loads were tested for each of the two washing methods with 20 loads over 3 days consisting of personal laundry. The same laundry operator was in charge over the entire trial. This design ensured that each method was treated equitably in all other respects. In addition, the laboratory personnel who undertook the

analysis both of TVC and each of the four organisms (*E. coli*, coliforms, *C. difficile* and *Staphylococcus aureus*, including MRSA) also were constant throughout. All of the methods adopted in the Public Health Laboratory as the responsible laboratory for analysis remained the same. Standard laboratory procedures were adopted throughout in accordance with the Certificate of Compliance for all Public Health Laboratories.

All loads of personal laundry showed contamination with respect to TVC, *E. coli* and coliforms. *C. difficile* was not present in two OTEX loads and three VIKING loads. MRSA was not present in four OTEX loads and six VIKING loads. Pre-wash levels were not significantly different between OTEX and VIKING loads for all categories of contamination, giving the desired similarity between methods at the start of the washing process.

In all, 20 loads of personal laundry were divided on an alternating basis between the OTEX machine and the VIKING machine. It was recognized at the outset that 40 loads of laundry was a very small sample. This can therefore be described as a pilot study. The setting in which the study was placed accounted for 35,000–40,000 items of personal laundry serviced per week by the standard VIKING machine. Nonetheless, it was felt useful that in this instance, a formal comparison between standard operating standard-based laundry procedures would be useful when compared with the ozone generating OTEX machine in exactly the same circumstances.

All loads were delivered during the course of any one morning and each machine (of similar size) was filled to approximately 15–17 kilos of laundry on each occasion. For the personal laundry, the VIKING machine was programmed to run on a chemical disinfection programme. The OTEX machine used the same programme throughout at 40  $^{\circ}$ C (low temperature) for the laundry washes. A standard SPSS output was used throughout and recognized for its good presentation of results and similarly for detecting statistical differences and presenting these graphically.

#### RESULTS

The mean reductions in log levels achieved by the two different methods were compared by t-tests, since the reductions were normally distributed. No statistically significant differences in reduction levels by OTEX and VIKING conventional processes were evidenced in any of the five categories compared. Mean reductions fluctuated for the two methods over the five categories, but not in a significant manner.

#### Significance Tests – Post-Wash Levels

The mean post-wash levels on the log scale achieved by the two different methods are compared by *t*-tests (Table 8). OTEX laundering gave significantly lower mean levels for *C. difficile*, eliminating this microorganism

TABLE 8. t-Tests (Personal Laundry: Post-Wash Levels) - Group Statistics (Reid et al., 2007)

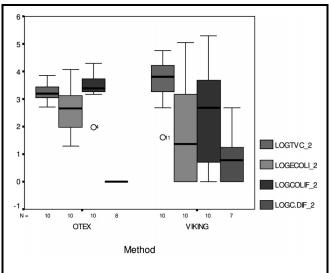
Method	Ν	Mean	Std. deviation	Std. error – mean
Log TVC_2	10	3.2446	0.32062	0.10139
OTEX	10	3.6135	0.90162	0.28512
VIKING				
Log E. coli_2	10	2.6732	0.90525	0.28626
OTEX	10	1.6912	1.68678	0.53341
VIKING				
Log Colif_2	10	3.4446	0.65652	0.20761
OTEX	10	2.4330	1.79228	0.56677
VIKING				
$Log C. diff_2$	8	0.0000	0.00000	0.00000
OTEX	7	0.8496	1.02195	0.38626
VIKING				
Log MRSA_2	6	0.0000	$0.00000^{\rm a}$	0.00000
OTEX	4	0,0000	$0.00000^{\rm a}$	0.00000
VIKING				

 $^{a}t$  cannot be computed because the standard deviations of both groups are 0.

completely. OTEX also delivered significantly less variable results for three of the categories. The table of means shows OTEX having a zero mean for *C. difficile* and much lower standard deviations than by the VIKING conventional process. MRSA has a zero mean for both methods. The *C. difficile* difference and the differences in variations are visually clear in the box plot (Figure 1).

#### DISCUSSION

This is the first in-house hospital comparative study in which the validated ozone disinfection system



**FIGURE 1.** Box plot of post-wash levels by method of wash (personal laundry) (Reid et al., 2007).

produced by JLA Ltd has been compared directly using identical loads against a standard laundry cleaning procedure, in this instance using a VIKING machine. Both machines were modified slightly in that sample procedures using pre-wash and post-wash aspirations of identical materials and identical volumes into identical bottles were carried out for all loads throughout the entire clinical study.

The methods for OTEX and VIKING were compared with respect to the reduction of contamination in soiled laundry and also for pre-wash and post-wash levels. For each wash load the contamination level was measured pre-wash and post-wash for each of the five categories agreed – TVC, *E. coli*, coliforms, *C. difficile* and MRSA.

In the personal laundry washes where the data collected is well balanced, it is clear that OTEX showed a significantly better end wash for *C. difficile* with complete elimination. Also, OTEX residual contaminants exhibit significantly lower variation. Both of these factors are attributes of OTEX only and both methods, i.e., OTEX and VIKING successfully eliminated MRSA.

At the present time, the use of the VIKING machine involves approximately 20 different programmes and 6 different chemicals, whereas the OTEX system utilizes a biolochemical detergent only (detergent plus enzymes) to break down stains. On day-to-day work quality issues. This approach has substantial benefits for all the operatives involved in the study. In particular, the benefits both at present and in the future, as defined by the laundry operators, have indicated that OTEX does disinfect articles that are difficult to disinfect in a VIKING machine. The staff training is straightforward and there are substantially fewer damaged items because of human error in the programming of the OTEX equipment as opposed to the standard and similar hospital laundry equipment.

Further, all of the personnel indicated that the quality of the finished personal articles, particularly jumpers, was both softer and had a pleasant, fresh odor as opposed to a number of items of personal clothing delivered by conventional cycles in the VIKING laundry processing machine.

There are a number of points of advantage in favor of OTEX as a laundry process, both in terms of cleanliness, disinfection and end product production. The use of the OTEX laundry system is not only more straightforward with a better outcome in the view of the laundry staff, but is simpler and more straightforward to use for all staff involved.

## CONCLUSIONS

This study has shown the current valid differences between the OTEX Validated Ozone Disinfection System and the standard laundry processing programmes in the VIKING machine.

Overall, the OTEX system produced a significantly better end wash in heavily fouled personal laundry for *C. difficile* with complete elimination. In addition, the residual contaminants following OTEX laundry processing were significantly more consistent than from the VIKING conventional laundry system.

OTEX, like the VIKING laundry processing system, also completely eliminated MRSA from the personal laundry washes. In all other respects the two laundry systems appear to be similar for personal laundry. This is certainly worthy of further evaluation in a larger number of hospital laundry loads of similar type, i.e., heavily fouled loads.

Overall, this study provided good evidence both from the point of view of comparative laundry processes and the views of the personnel involved that the OTEX Validated Ozone Disinfection System was the preferred and safer based laundry processing system to those systems, and in particular VIKING, currently in use.

#### SUMMARY AND CONCLUSIONS

1. Extensive microbiological testing conducted in the United Kingdom of cold water ozone laundering systems compared to current conventional laundering (thermal, with chemicals) has shown that ozone systems eradicate twelve of the usual microorganisms (plus four virus strains) found in hospital and health care facilities.

- 2. Ozone laundry systems also are capable of eradicating Methicillin-Resistant *Staphylococcus aureus* and *Clostridium difficile* in laundry wash water and on contaminated garments, within 3 minutes (> 5-logs reduction). By comparison, standard thermal laundering procedures (75 °C = 167 °F) are not able to provide a 5-logs reduction in either of these superbugs.
- 3. The Queen Elizabeth-II Hospital conducted a 6-month study of the efficiency of an ozone-laundry system to wash and disinfect microfibre mops and wiping cloths contaminated with MRSA and *C. difficile*. Conventional laundering left significant counts of *C. difficile* on these materials. However, none of the many samples of microfibre mops and wiping cloths laundered using cold water ozone showed any viable microorganism counts. The Hospital installed ozone laundering systems in Dec. 2005.
- 4. Following installation of ozone laundering systems in the QE-II Hospital, the question of possible ozone degradation of microfibre mops and wiping cloths upon repeated ozone laundering was studied in detail. Shrinkage, absorbency, color stability of the red and blue cleaning products, and effects on microfibre stability were less after 100 cycles of ozone laundering than during 100 cycles of conventional, higher temperature laundering.
- 5. A similar study comparing microbiological efficiency of ozone vs conventional laundering, plus effects of both laundering processes on physical properties of microfibre mops and cloths conducted at three UK hospitals employing ozone laundering showed similar results.

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