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Clinical Evaluation

Study Report Determination of the Activity of ShowerSafe against *Legionella pneumophila* in a Simulated Shower Head Application

Client Ackw Ltd. T/A SAS (Safe & Secure)
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Report No. IMSL 2007/01/007.01A

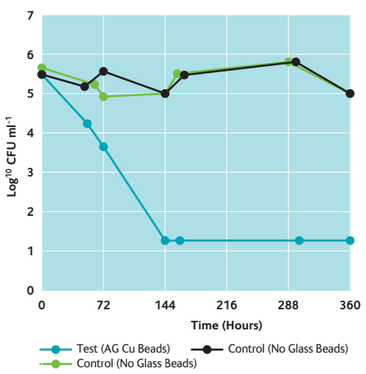
Dated 30th May 2007

At–a–glance

On the 30th May 2007, Industrial Microbiological Services Ltd (IMSL) published their findings from their research of ShowerSafe beads, a product designed to prevent the occurrence of legionella bacteria in showers.

Treatment

Figure 2: Effect of ShowerSafe Beads on Bacterial Population in Standard Hard Water (Results as Log10 CFU ml-1)



Legionella pneumophila

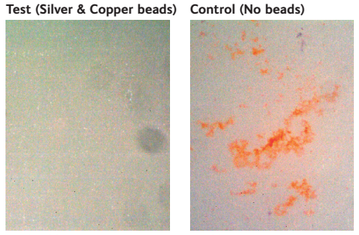
Effect of ShowerSafe beads on *legionella pneumophila* and *legionella aeruginosa*, showing a big reduction of legionella bacteria in standard hard water after as little as 36 hours. After 6 days the level of legionella bacteria was "below the limit of detection after incubation for 6 days."

Prevention

More significantly IMSL found that ShowerSafe prevented the formation of the biofilm in showers on which legionella lives.

Biofilm formation following incubation at 20° for 48 hours

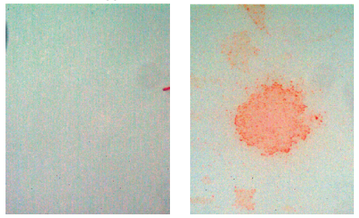
Test (Silver & Copper beads) **Control (No beads)**



No biofilm **Biofilm present**

Biofilm formation following incubation at 20° for 408 hours (17 days)

Test (Silver & Copper beads) **Control (No beads)**



No biofilm* **Biofilm present**

* A few stained particles of organic debris were detected but no bacterial colonisation was observed.

Contents

Study	Determination of the Activity of ShowerSafe against Legionella pneumophila in a Simulated Head Application
Number	IMSL 2007/01/007.01A
Client	Ackw Ltd. T/A SAS (Safe & Secure) Birchwoodmoor House Roston Ashbourne DE6 2EH The above study was conducted in the laboratories of Industrial Microbiological Services Ltd at Pale Lane, Hartley Wintey, Hants, RG27 8DH, UK. This report represents a true and accurate account of the results obtained.
Start Date	23rd January 2007
Report Issued	30th May 2007
Responsible Scientist	Gillian W Iredale Senior Microbiologist G W Iredale
Study Director	Peter D Askew Managing Director

Reprint of the Study Report to Determine the Activity of ShowerSafe against Legionella pneumophila

1 Introduction

Legionella spp rarely grow alone in systems and are often associated with relatively complex biofilms (Ref 1). In the case of water distribution systems, they colonise and proliferate in tanks (eg calorifiers) and in biofilms on pipework and are distributed by intermittent flow through the system (Ref 1 and 2). They have been shown to be partially aerosolised from shower heads (Ref 3) and it is reasonable to assume that they have the potential, along with other microbial species, to colonise the hose fittings and heads of showers that are used infrequently via small reservoirs of contaminated water.

The intended purpose of the SAS ShowerSafe beads is to prevent such colonisation and growth. This report described a study performed to examine this ability by both determining whether the presence of the beads could remove a standing planktonic population of *Legionella pneumophila* and prevent the formation of a biofilm of it in the presence of a commensal species (*Pseudomonas aeruginosa* was used as the model).

2 Test Materials

ShowerSafe silver and copper beads were supplied by Ackw Ltd. T/A SAS (Safe & Secure). Samples were held in the dark at 20°C prior to testing. Each ShowerSafe application contains 20 silver beads and 20 copper beads. This is designed to treat 80 ml of static water within a shower head / hose assembly. In the tests, 5 beads of each material were used in combination with 20 ml of WHO Standard Hard Water (300 ppm hardness - see Appendix 1). Coupons of untreated polypropylene were supplied by IMSL to act a model surfaces for studies on biofilm formation.

3 Methods

Two methods were employed to assess the performance of the ShowerSafe beads. In the first, the ability of the presence of the ShowerSafe beads to disinfect water containing a planktonic population of *Legionella pneumophila* was investigated. A population of *L pneumophila* in standard hard water was exposed to the beads for up to 24 hours and the water was then analysed for the presence of viable bacteria at intervals. In the second method, the effect of the presence of the ShowerSafe beads on the colonisation of a plastic surface by a biofilm comprised of *L pneumophila* in combination with *Pseudomonas aeruginosa* was determined over a 17 day period. Planktonic populations were also monitored.

3.1 Disinfection Test Method

Replicate (3) sterile containers (100 ml capacity) containing either standard hard water (19.8 ml) plus 5 silver and 5 copper beads or standard hard water (19.8 ml) were inoculated with an aliquot (200 μl) of a suspension of cells of *Legionella pneumophila* (3.5 x 107 cells ml^{-1}) prepared in standard hard water. After 6 and 24 hours incubation at 20°C (to simulate temperature in a shower room) the suspensions were analysed for the number colony forming units of *L pneumophila* present by dilution plate count onto BCYE agar. The plates were then incubated at 37°C for 4 days and then the colonies were counted.

3.2 Biofilm Determination Method

Replicate (7 per treatment) sterile containers (100 ml capacity) containing either standard hard water (19.8 ml) plus 5 silver and 5 copper beads plus a coupon (20 x 20 mm) of polypropylene, standard hard water (19.8 ml) plus 10 glass beads of similar dimension to the silver and copper beads used in ShowerSafe or standard hard water (19.8 ml) alone were inoculated with an aliquot (200 μl) of a suspension of cells of *Legionella pneumophila* (3.5 x 107 cells ml^{-1}) prepared in standard hard water. After 6, 24, 48 and 72 hours, 6 days, 13 days and 17 days incubation at 20°C (to simulate temperature in a shower room) under constant, gentle agitation (minimal rotational speed on an orbital shaker) the suspensions were analysed for the number colony forming units of both *L pneumophila* and *Ps aeruginosa* present by dilution plate count onto BCYE and Trypcase Soya agar. The plates were then incubated at 37°C for either 4 days (*Legionella*) or 24 hours (*Pseudomonas*) and then the colonies were counted. In addition, at each sampling interval from 2 days onwards a coupon was removed, gently washed and then stained with a solution of safranin, rinsed and then examined under the microscope for the presence of biofilm. Typical fields of view were recorded photographically. Sampling was destructive and neither individual samples nor coupons were re-used / re-analysed.

Table 1: Test Microorganisms

Species	Strain Reference
Legionella pneumophila *Pseudomonas aeruginosa*	NCIMB 10008

4 Results / Discussion

4.1 Disinfection Test

The results are shown in Table 2 and Figure 1 below.

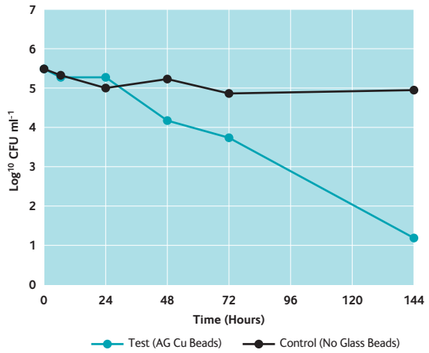
Table 2: Results as Colony Forming Units (CFU) ml^{-1} *(Legionella pneumophila)*

Formulation Variant	Contact Time (Hours)					
	0	6	24	48	72	144
ShowerSafe Beads	3.5x10^5	2.9x10^5	2.0x10^5	1.8x10^4	4.3x10^3	< 20
Control	3.5x10^5	3.0x10^5	9.5x10^5	1.6x10^5	8.6x10^4	9.2x10^4

Key: Tested in combination with *Pseudomonas aeruginosa*

It can be seen from the results above that ShowerSafe beads did not demonstrate any rapid disinfection activity however, prolonged exposure of a population containing both *L pneumophila* and *Ps aeruginosa* to standard hard water containing the beads resulted in a reduction in the population (to below the limit of detection after incubation for 6 days) in comparison with standard hard water alone. The slow activity observed may be a result of the time taken for ions of silver and copper to be liberated from the beads.

Figure 1: Effect of ShowerSafe Beads on *L pneumophila* in Standard Hard Water (Results as Log$_{10}$ CFU ml^{-1})



4.2 Biofilm Determination Method

The results are shown in Tables 3 and 4 and in Plates 1 and 2 below.

Table 3: Results as Colony Forming Units (CFU) ml^{-1} *(Legionella pneumophila)*

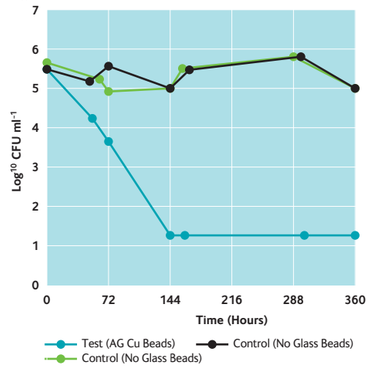
System	Contact Time (Hours)						
	0	48	72	144	168	312	408
ShowerSafe Beads	3.5x10^5	1.8x10^4	4.3x10^3	< 20	< 20	< 20	< 20
Glass Beads	3.5x10^5	1.5x10^5	3.1x10^5	8.6x10^4	2.9x10^5	6.0x10^5	8.6x10^4
Control	3.5x10^5	1.6x10^5	8.6x10^4	9.2x10^4	3.7x10^4	4.3x10^4	6.1x10^3

Table 4: Results as Colony Forming Units (CFU) ml^{-1} *(Pseudomonas aeruginosa)*

System	Contact Time (Hours)						
	0	48	72	144	168	312	408
ShowerSafe Beads	5.0x10^6	< 20	< 20	< 20	< 20	< 20	< 20
Glass Beads	5.0x10^6	4.6x10^6	3.5x10^6	3.6x10^6	4.2x10^6	8.6x10^5	1.7x10^6
Control	5.0x10^6	2.4x10^6	2.7x10^6	2.4x10^6	2.5x10^6	1.6x10^6	1.6x10^6

Figure 2: Effect of Shower Safe Beads on Bacterial Population in Standard Hard Water (Results as Log10 CFU ml^{-1})

Legionella pneumophila



Pseudomonas aeruginosa

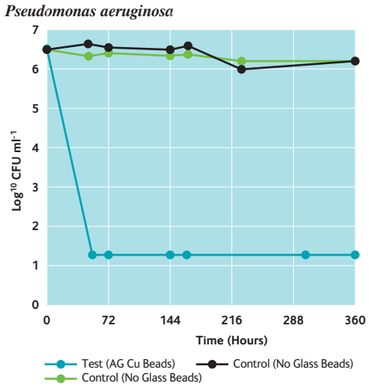


Plate 1: Biofilm Formation Following Incubation at 20°C for 48 Hours.

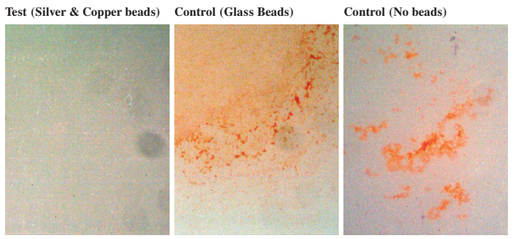
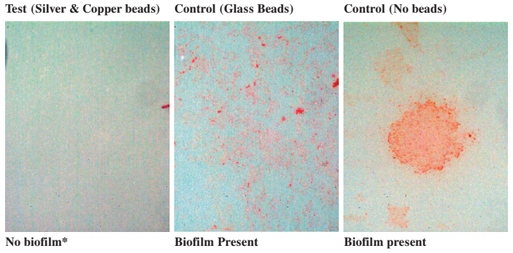


Plate 2: Biofilm Formation Following Incubation at 20°C for 17 days (408 Hours).



* A few stained particles of organic debris were detected but no bacterial colonisation was observed.

It can be seen from the results above that the population of *L pneumophila* in combination with *Ps aeruginosa* remained viable in the standard hard water suspensions prepared either with or without the presence of glass beads for up to 17 days. In contrast, the population of L neumophila in standard hard water containing ShowerSafe beads was, as discussed above, reduced to below the limit of detection after incubation for 6 days. Similarly, the population of *Ps aeruginosa* present in the consortium was reduced to below the limit of detection after exposure for 2 days. It can be seen from Plates 1 and 2 above that a biofilm was formed on the polypropylene coupons in the systems prepared either with or without glass beads. Biofilm formation was slightly heavier in the systems that contained glass beads, possibly due to the increase in turbulent flow that resulted from their presence. In contrast, no biofilm was detected on any of the coupons exposed to water containing ShowerSafe beads at any of the sampling intervals over the 17 day exposure interval.

5. Conclusions

It would appear from the results of this study that the presence of ShowerSafe beads in water does not result in rapid disinfection of moderate to large populations of either *Legionella pneumophila* or *Pseudomonas aeruginosa* probably due to the dynamics of silver and copper ion release. Their presence does result in a biologically significant reduction following extended contact. More importantly, the presence of ShowerSafe beads did inhibit the formation of a biofilm containing *Pseudomonas aeruginosa* in combination with *Legionella pneumophila* over a 17 day interval. This could be significant in preventing the colonisation of shower hoses and heads by these and similar species.

6. References

1 Murga1 R, Forster TS, Brown E, Pruckler JM, Fields BS Donlan RM (2001) Role of Biofilms in the Survival of *Legionella pneumophila* in A Model Potable-Water System, Microbiology, 147, 3121 - 3126.

2 Wadowsky RM, Yee RB, Mezmar L, Wing EJ and Dowling JN (1982), Hot Water Systems As Sources of *Legionella pneumophila* in Hospital and Nonhospital Plumbing Fixtures. Appl Environ Microbiol. 43(5) 1104 - 1110

3 Bollin GE, Plouffe JF, Para MF and Hackman B (1985), Aerosols Containing *Legionella pneumophila* Generated by Shower Heads and Hot-water Faucets Appl Environ Microbiol; 50(5), 1128 - 1131

7. Raw Data

The raw data for this study will be held in files IMSL2006/01/007 in the Archive of IMSL at Pale Lane, Hartley Wintney, Hants, RG27 8DH, UK for 6 years from the date of this report unless other specific instructions are given.

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Appendix A

WHO Standard Hard Water (No Fe)

0.2 M Boric acid	70ml
0.05 M Borax	30ml
Solution A	6ml
Solution B	8ml

The solutions above were added to 600 ml of distilled water (DW) and adjusted to 1 litre and the pH was then adjusted to 8 +/- 0.2. The solution was sterilised by membrane filtration (0.45μm pore) and used within 8 hours.

0.2 M Boric acid	
Boric acid	1.24g / 100ml DW
0.05 M Borax	
Borax	1.0g / 100ml DW

Solution A

Magnesium chloride	0.99g
Calcium chloride	2.3g

Made up to 50 ml with DW

Solution B

Sodium bicarbonate	1.75g

Made up to 50 ml with DW