IMSL

INDUSTRIAL MICROBIOLOGICAL SERVICES LTD

STUDY REPORT:	**Determination of the Ability of the Shower-Safe S-S886 Filter Unit at Preventing Transfer of *Legionella pneumophila* to Shower Outlets.**
CLIENT:	**Shower-Safe** **Units 1-2 Gilbert Way** **Burma Road Industrial Estate** **Blidworth** **Mansfield** **Nottinghamshire** **NG21 0RT** **United Kingdom**
REPORT NO:	**IMSL2008/09/009.2A**
DATED:	**27th October 2008**

Study: **Determination of the Ability of the Shower-Safe S-S886 Filter Unit at Preventing Transfer of *Legionella pneumophila* to Shower Outlets.**

Number: **IMSL 2008/09/009.2A**

Client: **Helibright**

The above study was conducted in the laboratories of Industrial Microbiological Services Ltd at Pale Lane Hartley Wintney, Hants, RG27 8DH, UK. This report represents a true and accurate account of the results obtained.

Start Date 14th October 2008

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1 Introduction

Legionella spp can colonise many manmade environments and proliferate in tanks (*eg.*calorifiers) and water distribution systems usually as a component of a biofilm. Both fragments of such biofilms as well as planktonic daughter cells are distributed by intermittent flow through systems (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. Once colonised, the dispersion of *Legionella spp* must be prevented and systems must be cleaned and disinfected and be demonstrated to be no longer contaminated before being returned to service.

The intended purpose of the Helibright Shower-Safe S-S886 shower head filters is to act as a barrier to prevent aerosols from such colonisation and growth being released prior to system disinfection and reinstatement. This report documents a study to examine the barrier properties of the filters to *Legionella pneumophila* both when first used and after simulation of use for up to 4 weeks.

2 Test Materials / Pre-Conditioning of Filters

Four shower heads and 12 filter units were supplied by Helibright Ltd. All were held at 20°C in the dark prior to use.

Shower heads fitted with filters (Shower-Safe S-S886) were aged to simulated 0, 1 week, 2 weeks and 4 weeks use (see Table 1). The volume of water employed to simulate this usage was calculated based on an average 5 minute shower using 26 litres of water with 10 showers taken per day. Individual shower heads fitted with a Shower-Safe filter unit were coupled to a domestic UK mains water supply. Water was allowed to flow through groups of units at a rate of 607 l $hour^{-1}$ for either 3, 6 or 12 hours. These pre-conditioned filters along with a group of 3 fresh filters were then used to determine the effect of water flow on their barrier properties to *Legionella pneumophila* as described in Section 3 below.

Table 1: Pre-Conditioning of Filters

Simulated Use (Weeks)‡	Flow Rate (l $hour^{-1}$)	Total Volume (l)	Time (hours)
0	0	0	0
1	607	1820	3
2	607	3640	6
4	607	7280	12

‡ 10 showers per day of 5 minutes duration using 26 litres of water each.

3 Method

The microorganism used is listed in Table 1. It was held as a primary stock culture at 4°C prior to use. Four - ten days prior to testing, a secondary sub-culture was prepared on Buffered Charcoal Yeast Extract Selective Agar with Glycine, Vancomycin, Polymyxin B, and Cycloheximide (GVPC Agar) and incubated at 37°C. Immediately prior to use, a cell suspension of the test species was prepared by suspending a number of colonies in a tryptone based diluent as described in EN1040 (Ref 4) to achieve a cell density of 1.5 - 5.0 x 10^8 cells ml^{-1} (extreme

care was taken to ensure no media was transferred with the colonies to the diluent). All cell densities were measured using a counting chamber (Thoma $^{1}/_{400}$ mm^2 x 0.02 mm depth) under 400 X magnification and phase contrast illumination. Where too low a cell count was achieved, further cells were added and the suspension was re-counted. Where too high a cell count was achieved, the cells suspension was diluted with tryptone based diluent as appropriate to achieve the cell densities specified above. The resulting, corrected cell suspension was then diluted 1:999 in WHO standard hard water (300 ppm hardness - see Appendix A) to give a suspension containing *ca* 2 x 10^5 cells ml^{-1}.

A sub-sample (100 ml) of a suspension of cells of *Legionella pneumophila* Serotype 1 (ca 10^5 CFU ml$^{-1)}$ in WHO Standard Hard Water (300 ppm hardness) was passed through each of the filter / head assemblies described in Section 2 above and the filtrate collected. The number of colony forming units of *Legionella pneumophila* in the filtrate was then analysed by spiral dilution and spread plate (0.1 ml) onto plates of GVPC medium. A sub-sample (50 ml) of the filtrate was also passed through a membrane filter (0.22 µm pore diameter) to concentrate the sample. The filter was transferred immediately to the surface of plates of GVPC medium. All plates were then incubated at 37°C for 4 days under conditions of high humidity and colonies consistent with the morphology of *L pneumophila* were counted.

Table 1: Test Microorganism

Species	Strain Reference
Legionella pneumophila	NCTC 11192

4 Results / Discussion

The results are shown in Table 2 below.

Table 2: Results as Colony Forming Units (CFU) *Legionella pneumophila*

Simulated Usage	*Legionella pneumophila* in Filtrate‡		
	Replicate 1	Replicate 2	Replicate 3
None	< 20 l^{-1}	< 20 l^{-1}	< 20 l^{-1}
1 week	< 20 l^{-1}	< 20 l^{-1}	< 20 l^{-1}
2 weeks	< 20 l^{-1}	< 20 l^{-1}	< 20 l^{-1}
4 weeks	< 20 l^{-1}	< 20 l^{-1}	< 20 l^{-1}
No filter fitted	4.0 x 10^8 CFU l^{-1}		

‡ A 50 ml sub-sample of the filtrate was analysed giving a limit of detection of 20 CFU l^{-1}.

It can be seen from the results above that the size of the population on the supply-side of filter units was 4.0 x 10^5 CFU ml^{-1}. No colonies of *Legionella pneumophila* were detected on any of the plates / membrane filters used to analyse the filtrate that had passed through the filter units. The Shower-Safe S-S886 filter units were effective as a barrier to the transmission of *Legionella pneumpohila* from the supply-side to the shower heads.

5 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

4 Anon, EN1040: 2005; Chemical disinfectants and antiseptics - Quantitative Suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics.

6. Raw Data

The raw data for this study will be held in files IMSL2008/09/009 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 . . . . . 700 ml
0.05 M Borax . . . . . 300 ml
Solution A . . . . . 1.5 ml
Solution B . . . . . 1.5 ml

Adjust the pH to 8.0 +/- 0.2. Filter sterilize with 0.22 µm filter as required. Use within 8 hours.

0.2 M Boric acid
Boric acid (61.83) . . . . . 12.4 g / 1000 ml Distilled Water

0.05 M Borax
Borax (201.2) . . . . . 10.06 g / 1000 ml Distilled Water

Solution A

Magnesium Chloride (Anhydrous) . . . . . 4.9 g

Make up to 50 ml with Distilled Water

Solution B

Calcium Chloride (Anhydrous) . . . . . 10.8 g

Make up to 50 ml with Sterile Distilled Water