IMSL

INDUSTRIAL MICROBIOLOGICAL SERVICES LTD

STUDY REPORT: **Determination of the Antibacterial Activity of Polyurethane Screed Formulations against *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes* using ISO 22196 (Formally JIS Z 2801)**

Flowfresh HF (6 - 9 mm) 20 Washes

CLIENT: **Flowcrete UK Limited**
Flowcrete Buisness Park
Booth Lane
Moston
Sandbach
Cheshire
CW11 3QF

REPORT NO: **IMSL2015/02/014.1A**

DATED: **06th March 2015**

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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.

Report Issued 06th March 2015

Reason Consolidation and updating of normative references.

Supervisor Kyle Allison
Senior Microbiologist

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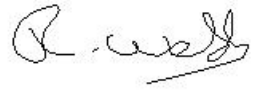


Contents

1 Introduction

This report summarises studies (Ref 1 and 2) performed to assess the antibacterial performance of a range of polyurethane screed formulations against *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* using the method described in the standard ISO 22196 (formerly JIS Z 2801).

2 Test Materials

Plaques which had been prepared using polyurethane resin floor screed formulated either with or without an antibacterial agent were supplied by Flowcrete UK Limited. Samples were processed at 50°C in a dishwasher without detergent for 20 cycles. All samples were held in the dark at 20°C prior to testing.

3 Methods

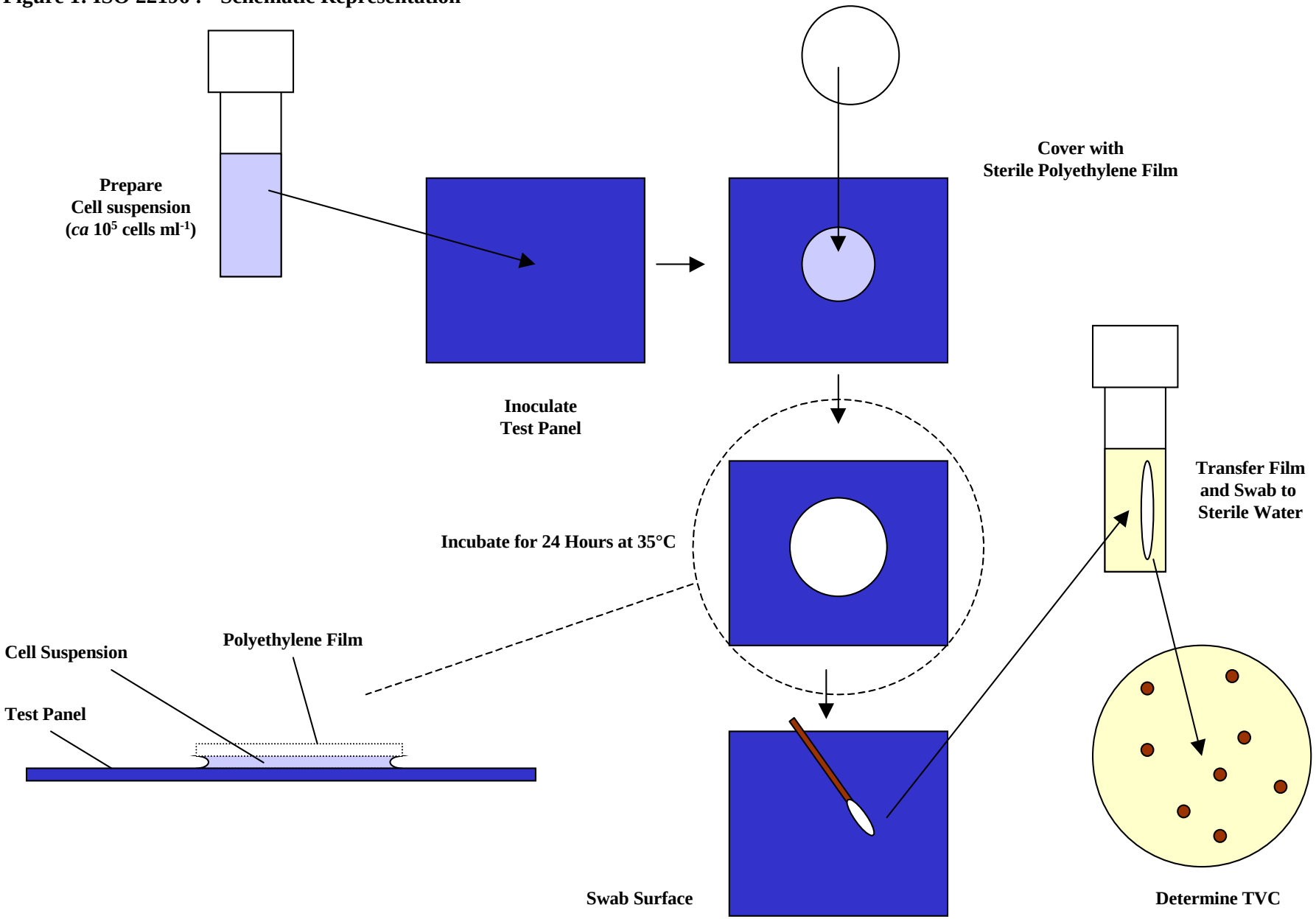
Antibacterial activity was determined using the method described in ISO 22196 / JIS Z 2801 (Ref 3 and 4).

3.1 Determination of Antibacterial Activity

An aliquot (225µl) of a log phase cell suspension of either *Escherichia coli* (5.6×10^5 cells ml^{-1};ATCC 8739), *Staphylococcus aureus* (6.4×10^5 cells ml^{-1}; ATCC 6538p) or *Listeria monocyogenes* (6.9×10^5 cells ml^{-1}; NCTC 11994) prepared using the method described in ISO 22196 were held in intimate contact with each of 3 replicates of the test surfaces supplied using a 30 x 30 mm polyethylene film (cut from a sterile Stomacher bag) for 24 hours at 35°C. The size of the surviving population was determined using the method described in ISO 22196. The viable cells in the suspension were enumerated by spiral dilution on to Trypcase Soya Agar and by the pour plate method described in ISO 22196. These plates were incubated at 35°C for 24 hours and then counted. An additional 3 replicate unfortified surfaces were also inoculated in the manner described above but were then analysed immediately for the size of microbial population present to provide 0-time control data. The method is described schematically in Figure 1 below.

All data were converted to colony forming units (CFU) cm^{-2} and then transformed (Log10) to provide a data set that conformed to a Gaussian distribution.

Figure 1: ISO 22196 : - Schematic Representation



4 Results / Discussion

The results are shown in Tables 1 - 3 and Figure 2 below.

Table 1: Activity of Coatings Against *Escherichia coli* after 20 Wash Cycles (Geometric Mean of 3 Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time		Reduction From Initial	
	0 hours	24 hours ‡	Log_{10}	%
IMSL Reference Material	1.4×10^4	6.8×10^5	-	-
Polyurethane Resin Control	1.4×10^4	5.9×10^1	2.4	99.58
Flowfresh HF (6 - 9 mm)	1.4×10^4	6.7×10^0	3.3	99.95

‡ The theoretical limit of detection is 1 CFU cm^{-2}

It can be seen from the results above that the population of *Escherichia coli* held in contact with the IMSL reference material increased in size by 1.7 orders of magnitude during the 24 hour contact interval. This is considered a normal response for this organism on an inert surface under the conditions imposed by ISO 22196. The population of *Escherichia coli* exposed to the surfaces of the Polyurethane Resin Control after 20 wash cycles declined by 2.4 orders of magnitude compared to the initial population after 24 hours. The population of *Escherichia coli* held in contact with the Sample of Flowfresh HF (6 - 9 mm) after 20 wash cycles declined by a further 0.9 orders of magnitude after 24 hours compared to the initial population.

Table 2: Activity of Coatings Against *Staphylococcus aureus* after 20 Wash Cycles (Geometric Mean of 3 Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time		Reduction From Initial	
	0 hours	24 hours ‡	Log_{10}	%
IMSL Reference Material	1.6×10^4	1.8×10^4	-	-
Polyurethane Resin Control	1.6×10^4	4.5×10^0	3.6	99.97
Flowfresh HF (6 - 9 mm)	1.6×10^4	≤ 1.0	≥ 4.2	≥ 99.99

‡ The theoretical limit of detection is 1 CFU cm^{-2}

The results in Table 2 show that the population of *Staphylococcus aureus* held in contact with the unfortified IMSL reference material remained relatively constant over the 24 hour contact interval. This is again considered a normal response for most Gram positive organisms under the conditions imposed by ISO 22196. The population of *Staphylococcus aureus* exposed to the Polyurethane Resin Control after 20 wash cycles declined by 3.6 orders of magnitude. The population of *Staphylococcus aureus* exposed to the surface of Flowfresh HF (6 - 9 mm) after 20 wash cycles declined by a further 0.6 orders of magnitude to below the limit of detection over the 24 hour contact period.

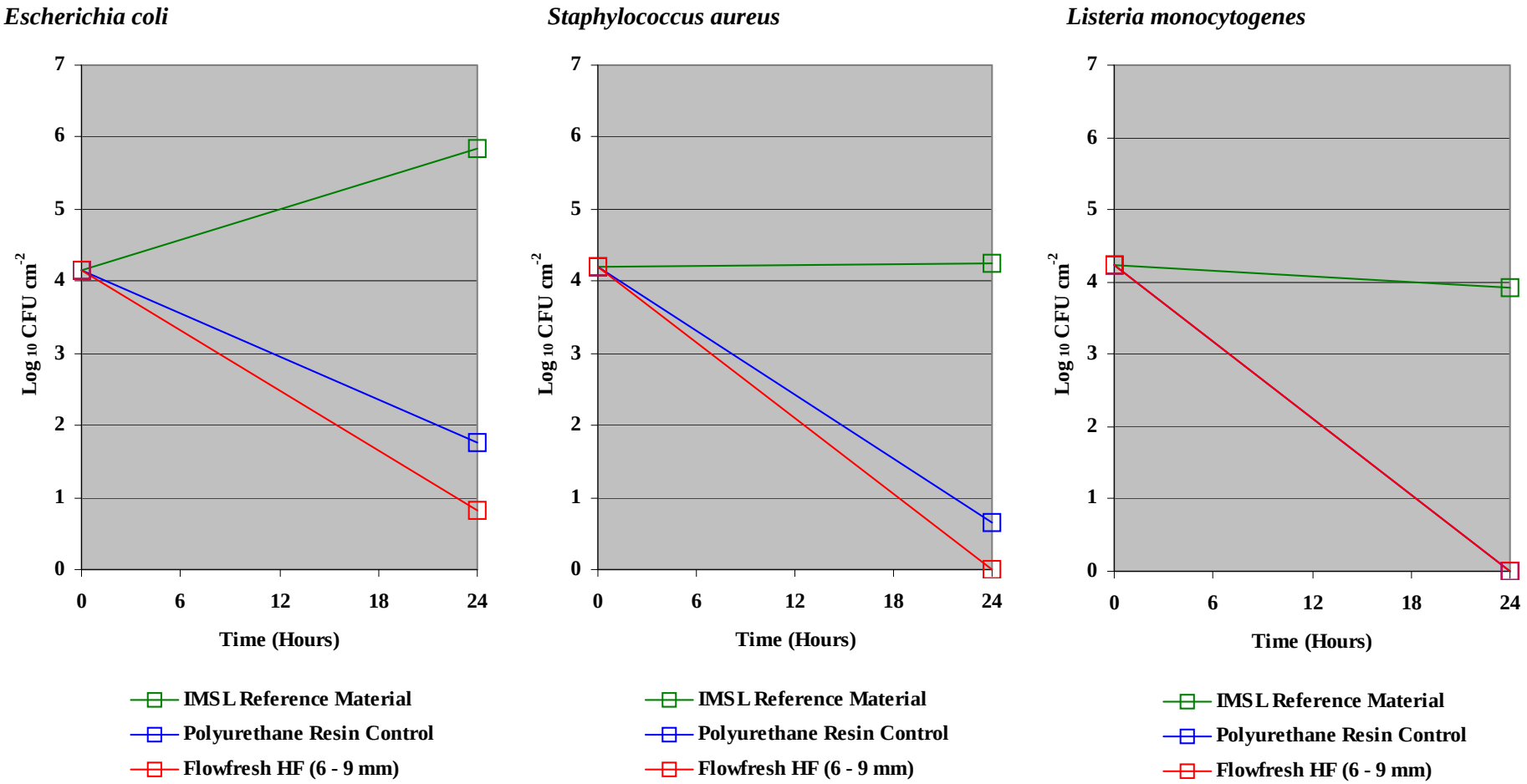
Table 3: Activity of Coatings Against *Listeria monocytogenes* after 20 Wash Cycles (Geometric Mean of 3 Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time		Reduction From Initial	
	0 hours	24 hours ‡	Log_{10}	%
IMSL Reference Material	1.7×10^4	8.2×10^3	0.2	51.78
Polyurethane Resin Control	1.7×10^4	≤ 1.0	≥ 4.2	≥ 99.99
Flowfresh HF (6 - 9 mm)	1.7×10^4	≤ 1.0	≥ 4.2	≥ 99.99

‡ The theoretical limit of detection is 1 CFU cm^{-2}

The results in Table 3 above show that the population of *Listeria monocytogenes* held in contact with the unfortified IMSL Reference Material remained relatively constant over the 24 hour contact interval. This is considered a normal response for this species under the conditions imposed by ISO 22196. The population of *Listeria monocytogenes* exposed to the Polyurethane Resin Control after 20 wash cycles and Flowfresh HF (6 - 9 mm) after 20 wash cycles both declined by ≥ 4.2 orders of magnitude to below the limit of detection over the 24 hour contact interval.

Figure 2: Results as Log_{10} CFU cm^{-2} after 20 Dishwasher Cycles



5 Raw Data

The raw data for this study will be held in file IMSL 2015/02/014 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.

6 References

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1 Technical Letter: IMSL 2006/11/013.1 - Antibacterial Activity of Cast Flooring Systems

2 Report: IMSL 2008/11/013.1B - Determination of the Antibacterial Activity of Polyurethane Screed Formulations against *Listeria monocytogenes* using Japanese Industrial Standard JIS Z 2801: 2000.

3 Anon, ISO 22196 : 2007, Measurement of antibacterial activity on plastic surfaces.

4 Japanese Industrial Standard JIS Z 2801: 2000 (E)

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