IMSL

INDUSTRIAL MICROBIOLOGICAL SERVICES LTD

STUDY REPORT: **Determination of the Antibacterial Activity of Plastic Samples Treated with Antimicrobial Agents against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Legionella pneumophila and Campylobacter jejuni* using ISO 22196 : 2011.**

CLIENT:
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REPORT NO: **IMSL LRN 1025835.1A**

DATED: **14th November 2014**

Study: Determination of the Antibacterial Activity of Plastic Samples Treated with Antimicrobial Agents against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Legionella pneumophila and Campylobacter jejuni* using ISO 22196 : 2011.

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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.

Start Date: 21st October 2014

Report Issued: 14th November 2014

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Senior Scientist

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Contents

1 Introduction

This report summarises a study performed to assess the antibacterial performance of film samples against *Escherichia coli Staphylococcus aureus, Pseudomonas aeruginosa, Legionella pneumophila and Campylobacter jejuni* using the method described in ISO 22196 : 2011

2 Test Materials

Samples of the plastic fortified with an antibacterial agent were supplied by Addmaster Ltd. All samples were held in the dark at 20°C prior to analysis. A sample of unfortified polypropylene was supplied by IMSL to act as a reference material.

3 Methods

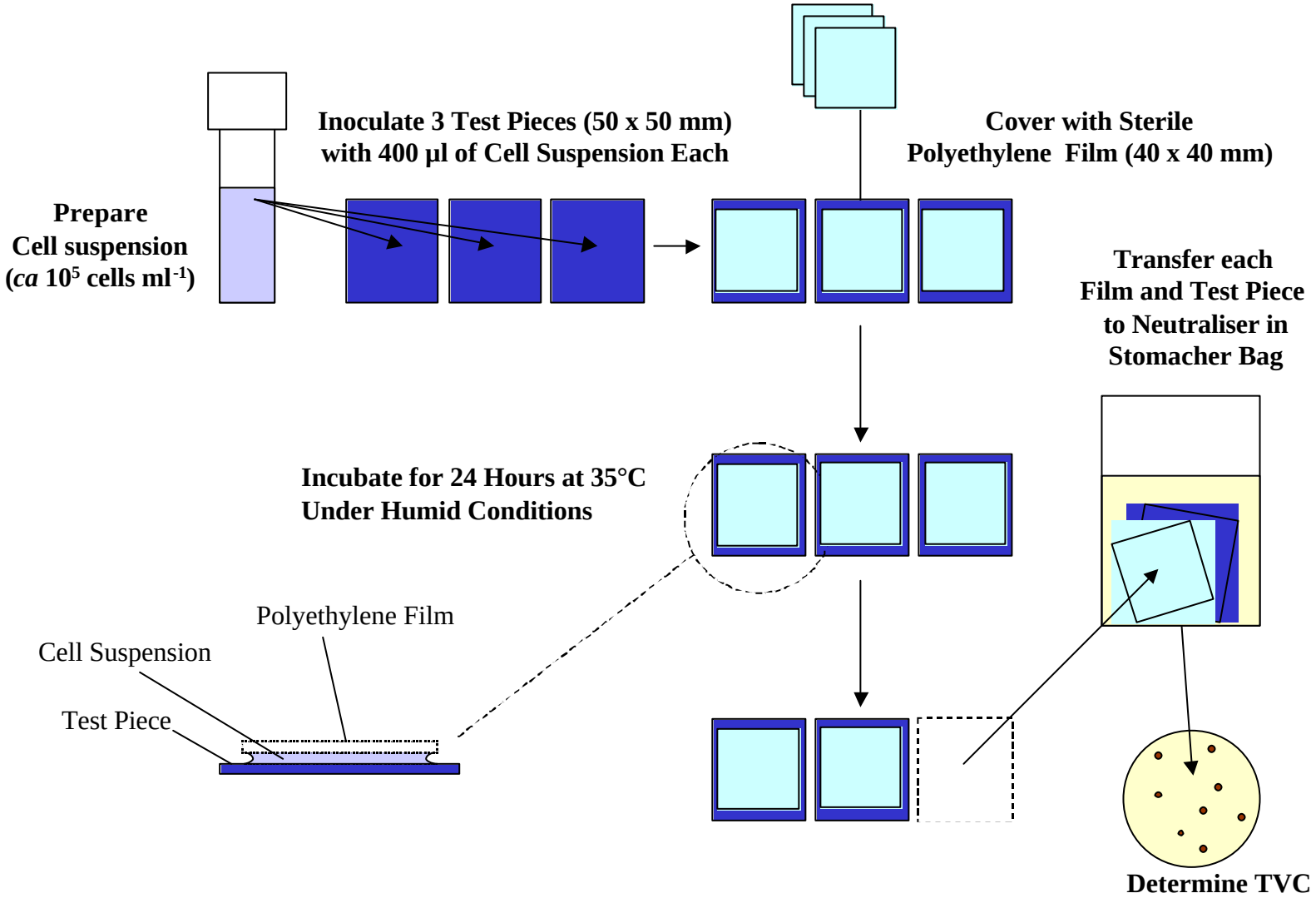
Antibacterial activity was determined using the method described in ISO 22196 : 2011 (Ref 1).

3.1 Determination of Antibacterial Activity

An aliquot (225 µl) of a cell suspension of either *Escherichia coli* (7.6×10^5 cells ml^{-1}; ATCC 8739), *Staphylococcus aureus* (8.5×10^5 cells ml^{-1} ; ATCC 6358p) or *Pseudomonas aeruginosa* (7.0×10^5 cells ml^{-1}; ATCC 15442) prepared using the method described in ISO 22196 or *Legionella pneumophila* (6.1×10^5 cells ml^{-1}; NCIMB 50008) prepared in simulated hard water containing a level of 1:250 nutrient broth or *Campylobacter jejuni* (5.6×10^5 cells ml^{-1}; ATCC 33921) prepared in 1:100 nutrient broth were held in intimate contact with each of 3 replicates of the plastic sample 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 : 2011. The viable cells in the suspension were enumerated by spiral dilution on to Trypcase Soya Agar except in the case of *Legionella pneumophila* where spiral dilution onto GVPC agar was employed and *Campylobacter jejuni* where spiral dilution onto Columbia Blood Agar was employed.. These plates were then incubated at 35°C for 24 hours or 4 days in the case of *Legionella pneumophila* 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 to provide a dataset that conformed to a Gaussian distribution. Potential outliers were tested using Dixon's *Q*-test (P = 0.05).

Figure 1: ISO 22196 : 2011 - Schematic Representation



4 Results / Discussion

The results are shown in Tables 1-5 and Figures 2 - 4 below.

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

Sample	Contact Time		Reduction from initial	
	0 hours	24 hours	Log_{10}	%
Polypropylene	1.9×10^4	3.7×10^5	-	-
Medi Shower (Biomaster 612 @ 2%)	1.9×10^4	$\leq$ 11.1	$\geq$ 3.2	$\geq$ 99.94

‡ The theoretical limit of detection is 11.1 CFU cm^{-2}

It can be seen from the results in Table 1 that the population of *Escherichia coli* held in contact with the surface of unfortified polypropylene increased by 1.3 orders of magnitude during the 24 hour exposure interval. This is considered a normal response of this species on an inert surface under the conditions imposed by ISO 22196. In contrast, the populations of *Escherichia coli* held in contact with the sample of Medi Shower treated with Biomaster 612 @ 2% declined by $\geq$ 3.2 orders of magnitude to below the limit of detection during the 24 hour contact period compared to the initial population.

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

Sample	Contact Time		Reduction from initial	
	0 hours	24 hours	Log_{10}	%
Polypropylene	2.1×10^4	1.3×10^4	0.2	39.34
Medi Shower (Biomaster 612 @ 2%)	2.1×10^4	$\leq$ 11.1	$\geq$ 3.3	$\geq$ 99.95

‡ The theoretical limit of detection is 11.1 CFU cm^{-2}

It can be seen from the data in Table 2 above that the population of *Staphylococcus aureus* held in contact with unfortified polypropylene declined by *ca* 39% over the 24 hour contact interval. This is again considered a normal response for this species on an inert surface under the conditions imposed by ISO 22196. In contrast, the populations of *Staphylococcus aureus* held in contact with the samples of Medi Shower treated with Biomaster 612 @ 2% declined by $\geq$ 99.95% to below the limit of detection during the 24 hour contact period compared to the initial population.

Table 3: Activity of Systems Against *Pseudomonas aeruginosa* (Geometric Mean of 3 Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time		Reduction from initial	
	0 hours	24 hours	Log_{10}	%
Polypropylene	1.7×10^4	1.6×10^5	-	-
Medi Shower (Biomaster 612 @ 2%)	1.7×10^4	≤ 11.1	≥ 3.1	≥ 99.93

‡ The theoretical limit of detection is 11.1 CFU cm^{-2}

It can be seen from the results in Table 3 that the population of *Pseudomonas aeruginosa* held in contact with the surface of unfortified polypropylene increased by 1 order of magnitude during the 24 hour exposure interval. This is considered a normal response of this species on an inert surface under the conditions imposed by ISO 22196. In contrast, the populations of *Pseudomonas aerugionsa* held in contact with the sample of Medi Shower treated with Biomaster 612 @ 2% declined by ≥ 3.1 orders of magnitude after 24 hours compared to the initial population.

Table 4: Activity of Systems Against *Legionella pneumophila* (Geometric Mean of 3 Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time		Reduction from initial	
	0 hours	24 hours	Log_{10}	%
Polypropylene	1.5×10^4	1.0×10^5	-	-
Medi Shower (Biomaster 612 @ 2%)	1.5×10^4	≤ 11.1	≥ 3.1	≥ 99.93

‡ The theoretical limit of detection is 11.1 CFU cm^{-2}

The data in Table 4 shows that the population of *Legionella pneumophila* held in contact with unfortified polypropylene increased by *ca* 85% over the 24 hour contact interval. In contrast, the populations of *Legionella pneumophila* held in contact with the sample of Medi Shower treated with Biomaster 612 @ 2% declined by $\geq 99.93\%$ to below the limit of detection during the 24 hour contact period compared to the initial population.

Table 5: Activity of Systems Against *Campylobacter jejuni* (Geometric Mean of 3 Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time		Reduction from initial	
	0 hours	24 hours	Log_{10}	%
Polypropylene	1.4×10^4	4.5×10^4	-	-
Medi Shower (Biomaster 612 @ 2%)	1.4×10^4	3.1×10^1	2.7	99.78

‡ The theoretical limit of detection is 11.1 CFU cm^{-2}

It can be seen from the data in Table 5 above that the population of *Campylobacter jejuni* held in contact with unfortified polypropylene increased by *ca* 69% over the 24 hour contact interval. In contrast, the populations of *Campylobacter jejuni* held in contact with the Medi Shower treated with Biomaster 612 @ 2% declined by 99.78% during the 24 hour contact period compared to the initial population.

Figure 2: Results as Log_{10} CFU cm^{-2}

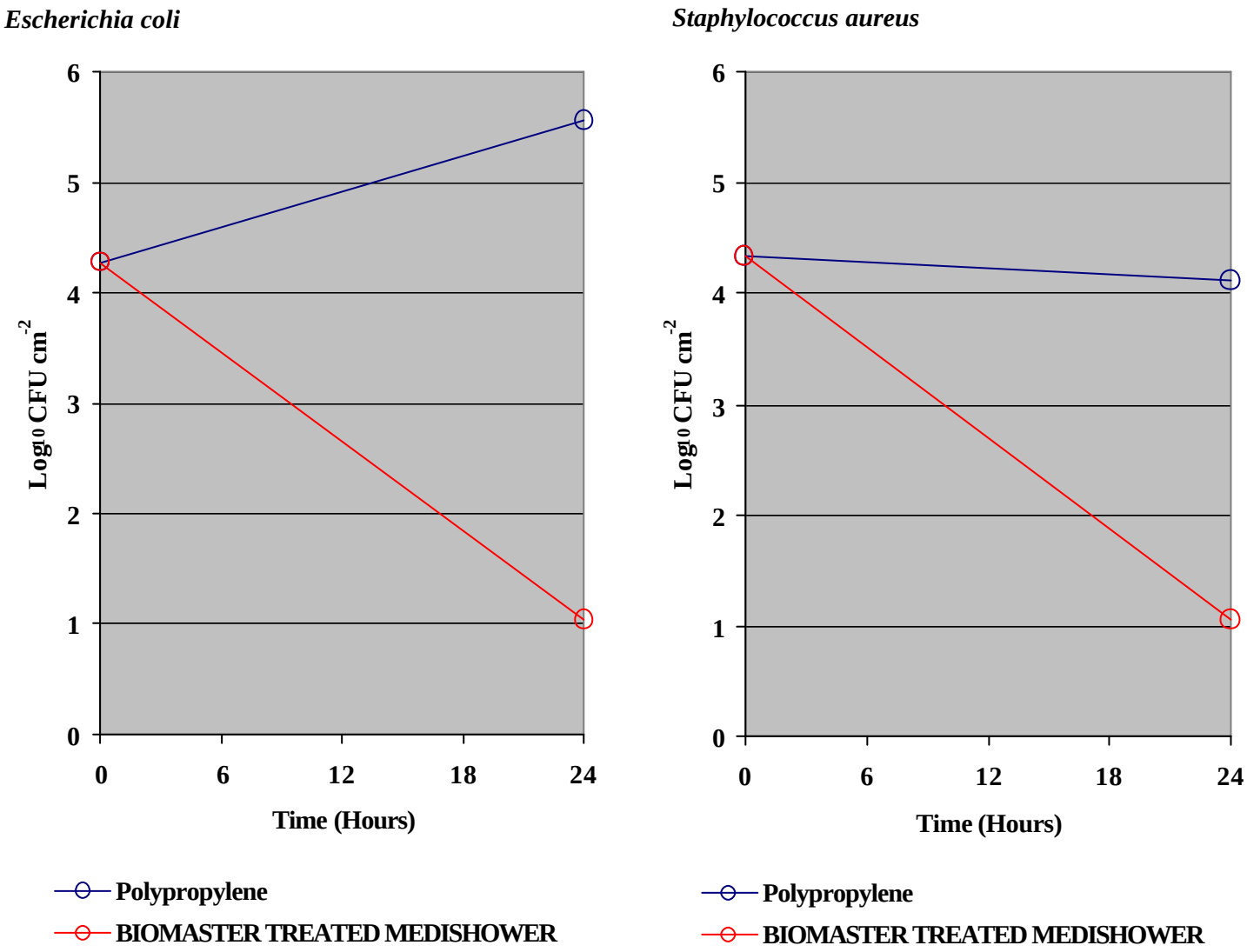


Figure 3: Results as Log_{10} CFU cm^{-2}

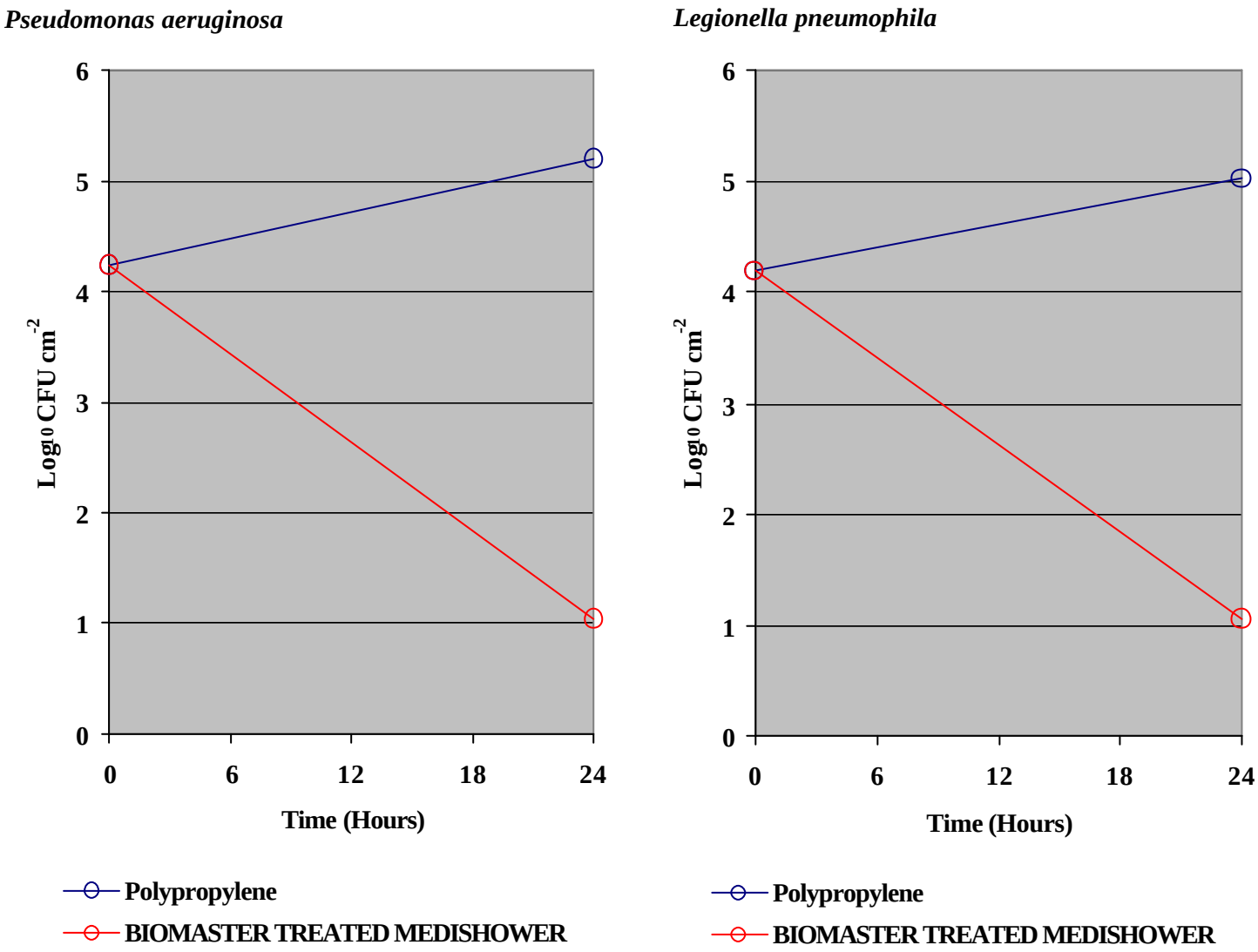
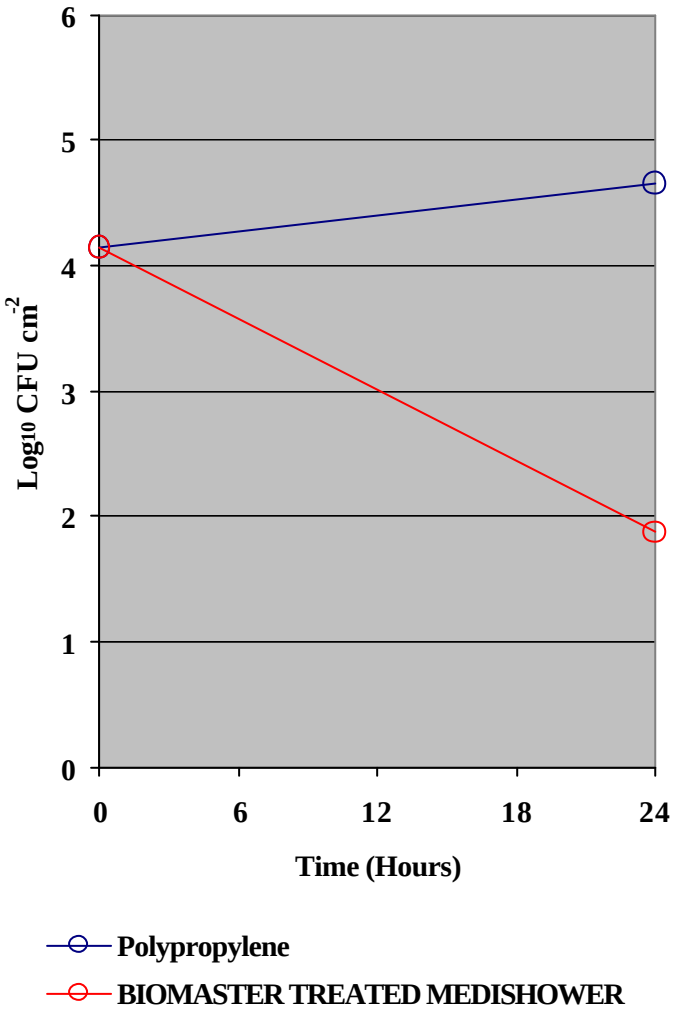


Figure 4: Results as Log_{10} CFU cm^{-2}

Campylobacter jejuni



5 Raw Data

The raw data for this study will be held in file IMSL 1025835 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

1 Anon, ISO 22196 : 2011, Measurement of antibacterial activity on plastics and other non-porous surfaces.

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