IMSL

INDUSTRIAL MICROBIOLOGICAL SERVICES LTD

STUDY REPORT:	**Determination of the Antibacterial Activity of Blank Plates against *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes,* Methicillin resistant *Staphylococcus aureus, Salmonella typhimurium and Klebsiella pneumoniae* using ISO 22196:2011**
CLIENT:	**Hager Ltd** **Hortonwood 50** **Telford** **Shropshire** **TF1 7FT**
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Study: Determination of the Antibacterial Activity of Blank Plates against *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes,* Methicillin resistant *Staphylococcus aureus, Salmonella typhimurium and Klebsiella pneumoniae* using ISO 22196:2011

Number: IMSL 2021/05/039.1A

Client: Hager Ltd
Hortonwood 50
Telford
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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 09th June 2021

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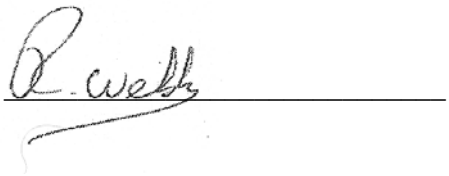

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1 Introduction

This report summarises a study performed to assess the antibacterial performance of Blank Plates against *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes,* Methicillin resistant *Staphylococcus aureus, Salmonella typhimurium and Klebsiella pneumoniae* using ISO 22196:2011

2 Test Materials

Samples of Blank Plates were supplied by Hager Group. A sample of unfortified polypropylene was supplied by IMSL to act as a reference material. 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: 2011 (Ref 1).

3.1 Determination of Antibacterial Activity

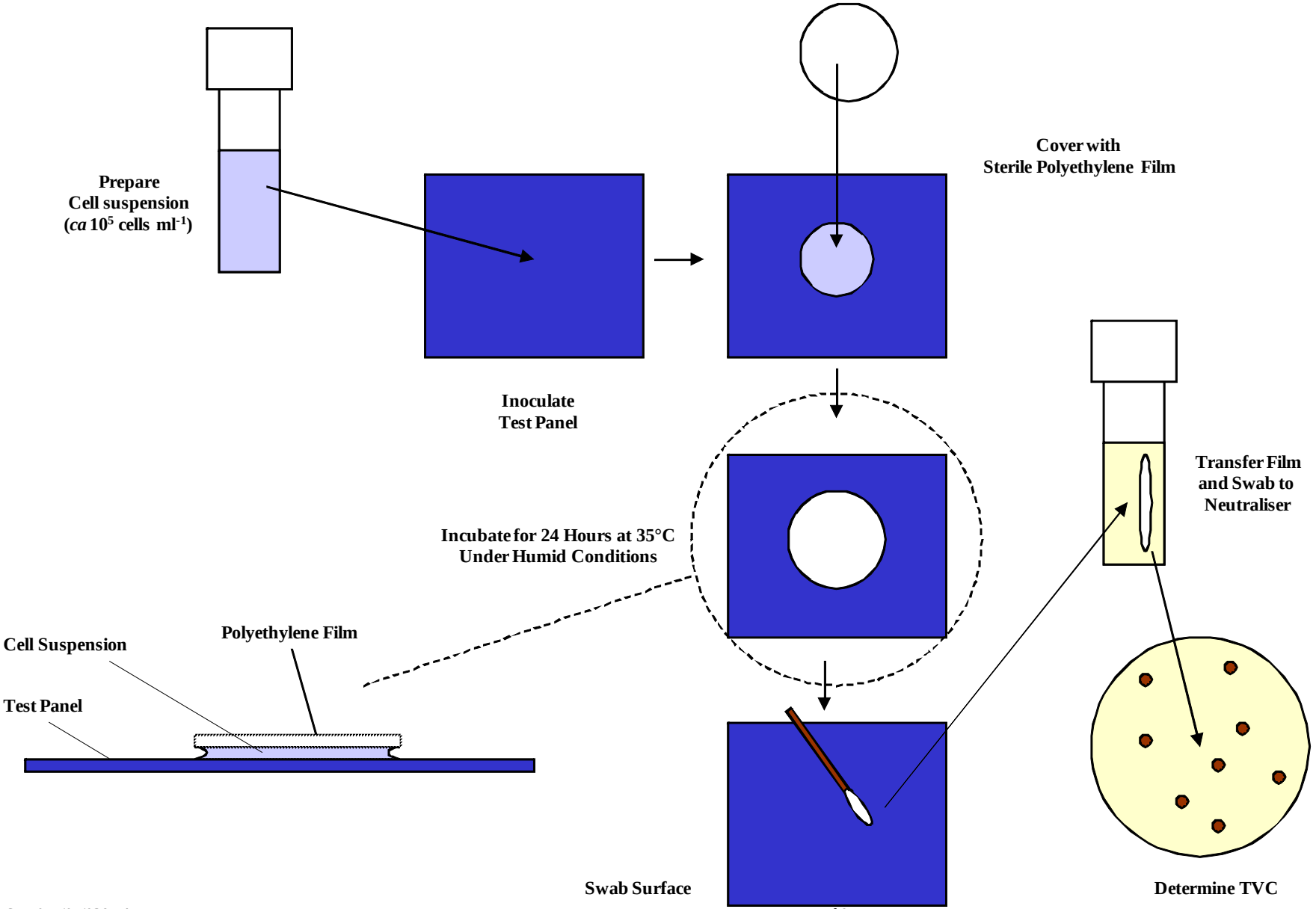
An aliquot (225µl) of a log phase cell suspension of either *Escherichia coli* (7.0×10^5 cells mL^{-1};ATCC 8739), *Staphylococcus aureus* (4.5×10^5 cells mL^{-1}; ATCC 6538p), *Listeria monocytogenes* (7.2×10^5 cells mL^{-1}, NCTC 12023), Methicillin resistant *Staphylococcus aureus* (7.2×10^5 cells mL^{-1}, ATCC BAA-2313), *Salmonella typhimurium* (7.6×10^5 cells mL^{-1}, NCIMB 13284), or *Klebsiella pneumonia* (6.9×10^5 cells mL^{-1}, NCIMB 10104) prepared using the method described in ISO 22196 : 2011 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 : 2011. 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 then incubated at 35°C for 24 hours and then the colonies present are 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 (Log_{10}) to provide a data set 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 - 6 and Figures 2 and 3.

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

Sample	Contact Time		Reduction from Initial	
	0 hours	24 hours ‡	Log_{10}	%
IMSL Polypropylene	1.8×10^4	9.3×10^5	-	-
Hager Blank Plate	1.8×10^4	≤ 1.0	≥ 4.3	≥ 99.99

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

It can be seen from the results above that the population of *Escherichia coli* exposed to the IMSL polypropylene increased by 1.7 orders of magnitude over the 24 hour contact interval compared to the initial population. This is considered a normal response for this organism on an inert surface under the conditions imposed by ISO 22196.

In contrast, the populations of *Escherichia coli* in contact with the samples of the Hager Blank Plate declined by ≥ 4.3 orders of magnitude to below the limit of detection over the 24 hour contact interval compared to the initial population.

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

Sample	Contact Time		Reduction from Initial	
	0 hours	24 hours ‡	Log_{10}	%
IMSL Polypropylene	1.1×10^4	8.4×10^3	0.13	25.84
Hager Blank Plate	1.1×10^4	≤ 1.0	≥ 4.1	≥ 99.99

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

It can be seen from the data above that the population of *Staphylococcus aureus* exposed to the unfortified IMSL polypropylene remained at a relatively constant magnitude during the 24 hour contact interval. This is again considered a normal response for this organism 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 Hager Blank Plate declined by ≥ 4.1 orders of magnitude to below the limit of detection over the 24 hour contact interval compared to the initial population.

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

Sample	Contact Time		Reduction from Initial	
	0 hours	24 hours ‡	Log_{10}	%
IMSL Polypropylene	1.8×10^4	5.0×10^3	0.56	72.21
Hager Blank Plate	1.8×10^4	≤ 1.0	≥ 4.3	≥ 99.99

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

It can be seen from the data above that the population of *Listeria monocytogenes* exposed to the unfortified IMSL polypropylene declined by 0.6 orders of magnitude over the 24 hour contact interval compared to the initial population. This is considered a normal response for this organism on an inert surface under the conditions imposed by ISO 22196.

The populations of *Listeria monocytogenes* held in contact with the samples of Hager Blank Plate declined by ≥ 4.3 orders of magnitude to below the limit of detection over the 24 hour contact interval compared to the initial population.

Table 4: Activity Against Methicillin Resistant *Staphylococcus aureus*
(Geometric Mean of Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time		Reduction from Initial	
	0 hours	24 hours ‡	Log_{10}	%
IMSL Polypropylene	1.8×10^4	1.8×10^4	0.01	1.15
Hager Blank Plate	1.8×10^4	≤ 1.0	≥ 4.3	≥ 99.99

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

It can be seen from the data above that the population of Methicillin Resistant *Staphylococcus aureus* exposed to the unfortified IMSL polypropylene remained at a constant 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.

In contrast, the populations of Methicillin Resistant *Staphylococcus aureus* held in contact with the samples of the Hager Blank Plate declined by ≥ 4.3 orders of magnitude to below the limit of detection over the 24 hour contact interval compared to the initial population.

Table 5: Activity Against *Salmonella typhimurium*
(Geometric Mean of Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time		Reduction from Initial	
	0 hours	24 hours ‡	Log_{10}	%
IMSL Polypropylene	1.9×10^4	2.8×10^5	-	-
Hager Blank Plate	1.9×10^4	≤ 1.0	≥ 4.3	≥ 99.99

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

It can be seen from the data above that the population of *Salmonella typhimurium* exposed to the unfortified IMSL polypropylene increased by 1.2 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.

In contrast, the populations of *Salmonella typhimurium* in contact with the samples of Hager Blank Plate declined by ≥ 4.3 orders of magnitude to below the limit of detection over the 24 hour contact interval compared to the initial population.

Table 6: Activity Against *Klebsiella pneumoniae*
(Geometric Mean of Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time		Reduction from Initial	
	0 hours	24 hours ‡	Log_{10}	%
IMSL Polypropylene	1.7×10^4	3.6×10^4	-	-
Hager Blank Plate	1.7×10^4	≤ 1.0	≥ 4.2	≥ 99.99

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

It can be seen from the results above that the population of *Klebsiella pneumoniae* exposed to the IMSL polypropylene increased by 0.3 orders of magnitude over the 24 hour contact interval compared to the initial population. This is considered a normal response for this organism on an inert surface under the conditions imposed by ISO 22196.

In contrast, the populations of *Klebsiella pneumoniae* held in contact with the samples of Hager Blank Plate declined by ≥ 4.2 orders of magnitude to below the limit of detection over the 24 hour contact interval compared to the initial population.

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

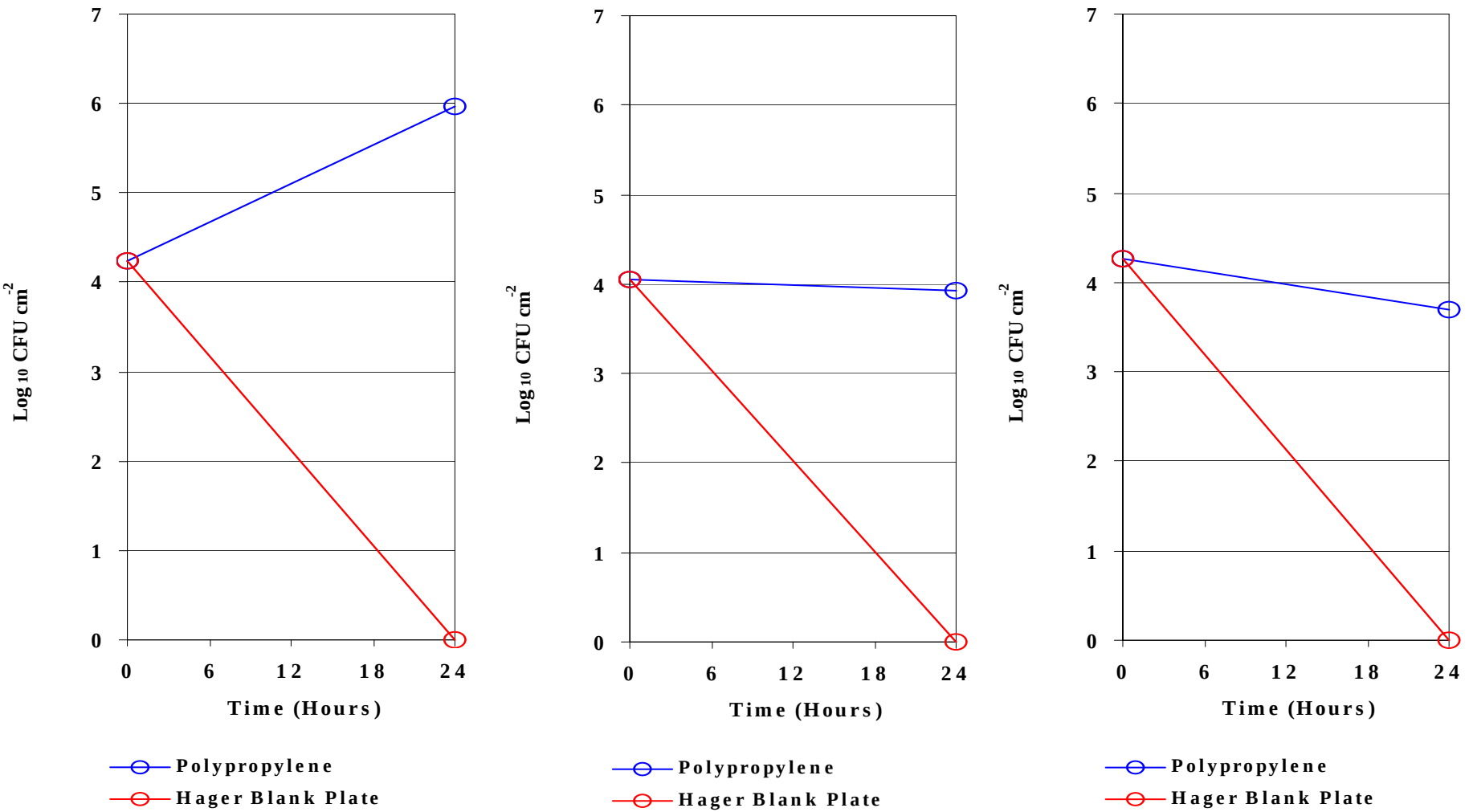
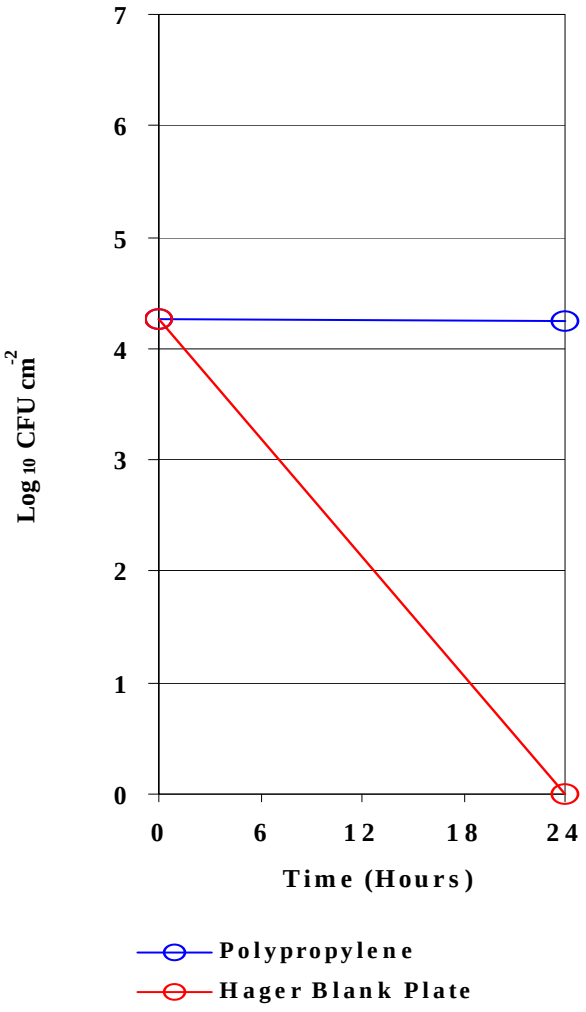
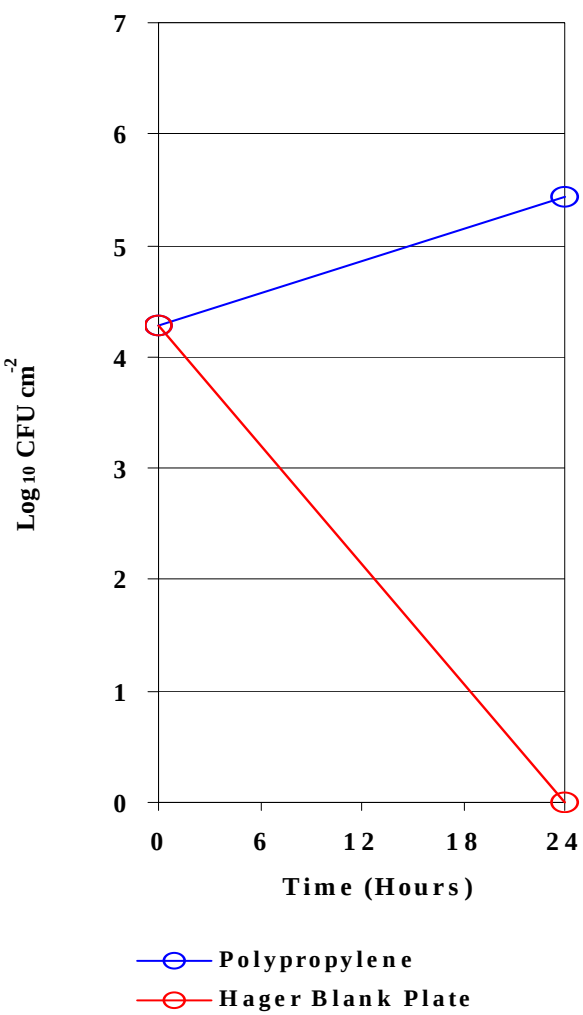


Figure 3: Results as Log_{10} CFU cm^{-2}

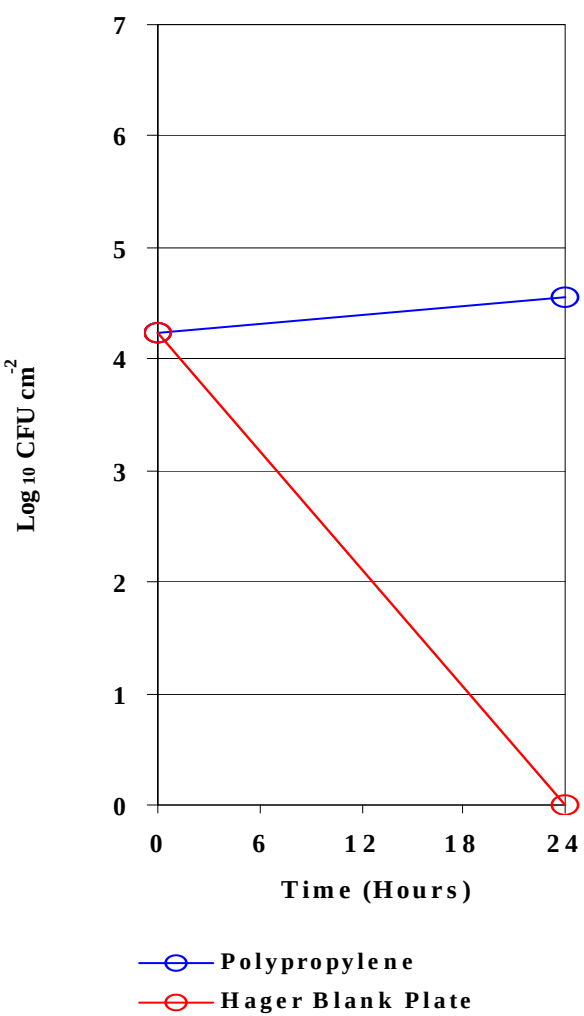
Methicillin resistant *Staphylococcus aureus*



Salmonella typhimurium



Klebsiella pneumoniae



5 Raw Data

The raw data for this study will be held in file IMSL 2021/05/039.1 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 ISO 22196:2011 Measurement of antibacterial activity on plastics and other non-porous surfaces.

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