IMSL

INDUSTRIAL MICROBIOLOGICAL SERVICES LTD

RAPPORT D'ESSAI **CERTIFICAT N°** **1020811.001C-1**

CLIENT **REF. CLIENT**

PPG Coatings Deutschland GmbH
An der Halde 1
44805 Bochum
Deutschland

REF. ECHANTILLON **ECHANTILLON RECU EN DATE** 11-Feb-13

Sigmaresist Immun Mat

METHODE. Determination de l'Activité Antibacterienne selon ISO 22196:2011

DATE DU TEST 13-Feb-13 **DATE CERTIFICAT** 27-May-13

RESULTATS (CFU CM-2)

ECHANTILLON		TEMP DE CONTACTE		REDUCTION BACTERIENNE	
		TEMP 0 Heures	TEMP 24 Heures	Log	%
Polypropylene Control (IMSL)	*E coli*	1.8E+04	1.7E+05	-	-
Sigmaresist Immun Mat	*E coli*	1.8E+04	≤ 1.0	≥ 4.2	≥ 99.99
Polypropylene Control (IMSL)	*S aureus*	1.6E+04	8.6E+03	0.3	47.25
Sigmaresist Immun Mat	*S aureus*	1.6E+04	≤ 1.0	≥ 4.2	≥ 99.99
Polypropylene Control (IMSL)	MRSA	1.6E+04	4.3E+03	0.6	72.61
Sigmaresist Immun Mat	MRSA	1.6E+04	≤ 1.0	≥ 4.2	≥ 99.99
Polypropylene Control (IMSL)	*Ps aeruginosa*	1.7E+04	2.6E+05	-	-
Sigmaresist Immun Mat	*Ps aeruginosa*	1.7E+04	≤ 1.0	≥ 4.2	≥ 99.99

Le test montre que après un contact de 24 heures à 35° C sur la surface de l'échantillon une réduction supérieure à 95% de la population initiale des bactéries à lieu

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MANAGING DIRECTOR

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RAPPORT D'ESSAI **CERTIFICAT N°** **1020811.001D-1**

CLIENT **REF. CLIENT**

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An der Halde 1
44805 Bochum
Deutschland

REF. ECHANTILLON **ECHANTILLON RECU EN DATE** 11-Feb-13

Sigmaresist Immun Mat

METHODE. Determination de l'Activité Antibacterienne selon ISO 22196:2011

DATE DU TEST 13-Feb-13 **DATE CERTIFICAT** 27-May-13

RESULTATS (CFU CM-2)

ECHANTILLON		TEMP DE CONTACTE		REDUCTION BACTERIENNE	
		TEMP 0 Heures	TEMP 24 Heures	Log	%
Polypropylene Control (IMSL)	*E hirae*	1.9E+04	1.9E+04	-	-
Sigmaresist Immun Mat	*E hirae*	1.9E+04	≤ 1.0	≥ 4.3	≥ 99.99
Polypropylene Control (IMSL)	*A baumannii*	2.5E+04	9.7E+05	-	-
Sigmaresist Immun Mat	*A baumannii*	2.5E+04	≤ 1.0	≥ 4.4	≥ 99.99
Polypropylene Control (IMSL)	S pneumoniae	2.2E+04	1.1E+01	3.2	99.95
Sigmaresist Immun Mat	S pneumoniae	2.2E+04	≤ 1.0	≥ 4.3	≥ 99.99
Polypropylene Control (IMSL)	*K pneumoniae (CPE)*	1.8E+04	2.8E+05	-	-
Sigmaresist Immun Mat	*K pneumoniae (CPE)*	1.8E+04	≤ 1.0	≥ 4.3	≥ 99.99

Le test montre que après un contact de 24 heures à 35° C sur la surface de l'échantillon une réduction supérieure à 95% de la population initiale des bactéries à lieu

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STUDY REPORT: **Determination of the Antibacterial Activity of Paint Samples against *Escherichia coli, Staphylococcus aureus,* MRSA, *Pseudomonas aeruginosa, Enterococcus hirae, Acinetobacter baumannii, Streptococcus pneumoniae* and Carbapenemase Producing *Klebsiella pneumoniae* using Modified ISO 22196: 2011.**

CLIENT: **PPG Coatings Deutschland GmbH**
An der Halde 1
44805 Bochum
Deutschland

REPORT NO: **IMSL 2012/11/012.1A-2**

DATED: **27th May 2013**

Study: Determination of the Antibacterial Activity of Paint Samples against *Escherichia coli, Staphylococcus aureus,* MRSA, *Pseudomonas aeruginosa, Enterococcus hirae, Acinetobacter baumannii, Streptococcus pneumoniae* and Carbapenemase Producing *Klebsiella pneumoniae* using Modified ISO 22196: 2011.

Number: IMSL 2012/11/012.1A-2

Client: PPG Coatings Deutschland GmbH

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.

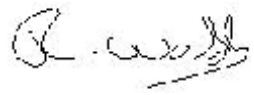
Start Date 13th February 2013

Report Issued 08th May 2013

Report Reissued 27th May 2013 (Name Change)

Supervisor Kyle Allison
Senior Microbiologist

Operator Richard Webb
Microbiologist



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1 Introduction

This report summarises a study performed to assess the antibacterial performance of Paint Formulations against *Escherichia coli, Staphylococcus aureus,* MRSA, *Pseudomonas aeruginosa, Enterococcus hirae, Acinetobacter baumannii, Streptococcus pneumoniae* and Carbapenemase Producing *Klebsiella pneumoniae* using the method described in ISO 22196: 2011.

2 Test Materials

Leneta scrub resistance test panels coated with paint formulations prepared with an antimicrobial agent were supplied by PPG Coatings Deutschland GmbH. All samples were held in the dark at 20°C prior to testing. An inert sample of Polypropylene was supplied by IMSL to act as a reference material.

3 Methods

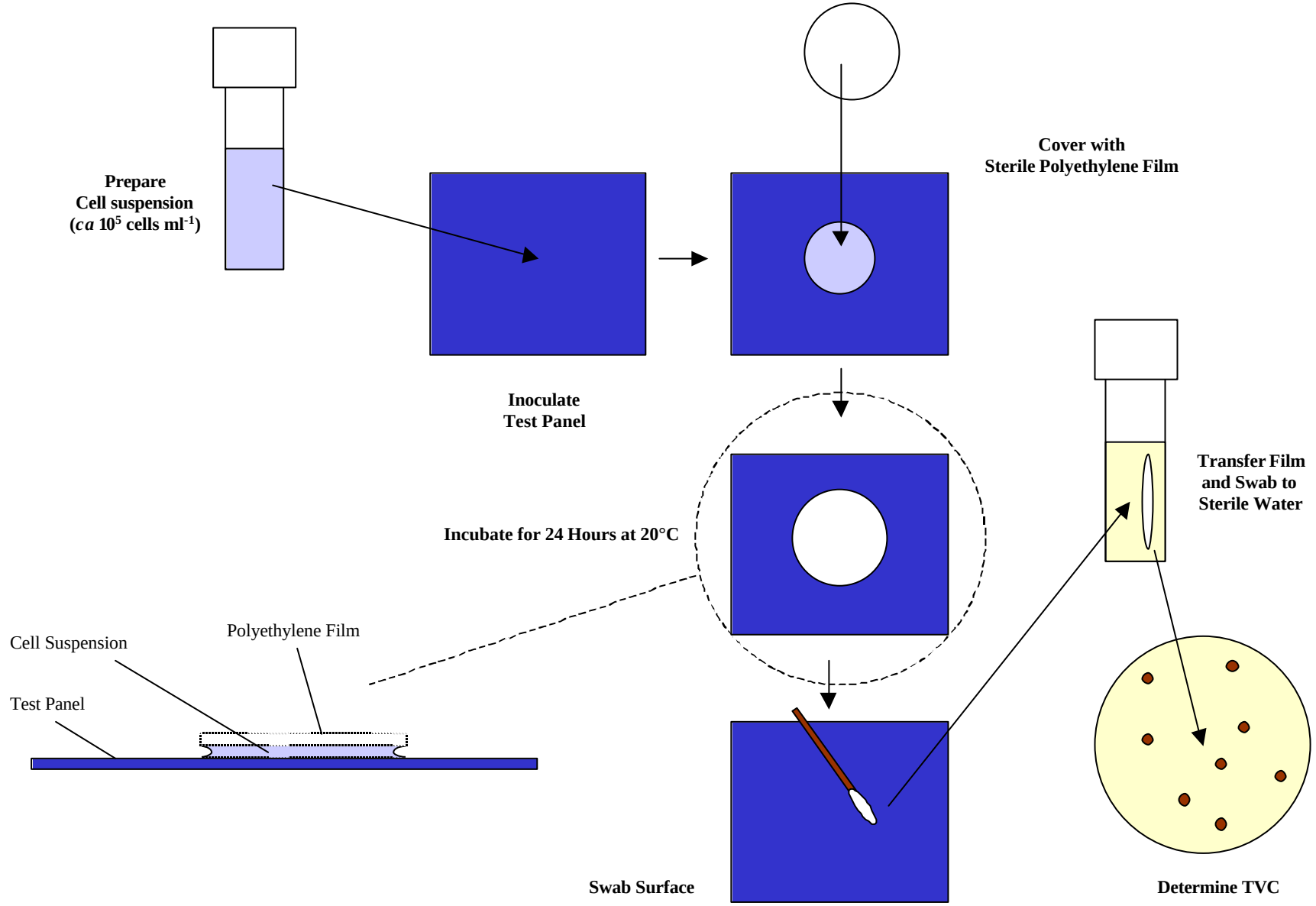
Antibacterial activity was determined using the method described in ISO 22196: 2011 (Ref 1).

3.1 Determination of Antibacterial Activity

An aliquot (225µl) of a log phase cell suspension of either *Escherichia coli, Staphylococcus aureus,* MRSA, *Pseudomonas aeruginosa, Enterococcus hirae, Acinetobacter baumannii, Streptococcus pneumoniae* or Carbapenemase Producing *Klebsiella pneumoniae* prepared using the method described in ISO 22196 : 2011 were held in intimate contact with multiple replicates of the test surfaces supplied using a 30 x 30 mm polyethylene film (cut from a sterile Stomacher bag) for 12 and 24 hours at 35°C, except in the case of *Klebsiella pneumoniae* which was assessed after 6, 12, 18 and 24 hours. 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 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- 8 and Figures 2 - 4.

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

Sample	Contact Time		
	0 hours	12 hours	24 hours
Polypropylene Control (IMSL)	1.8×10^4	1.4×10^4	1.7×10^5
Sigmaresist Immun Mat	1.8×10^4	≤ 1.0	≤ 1.0

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

It can be seen from the data above that the population of *Escherichia coli* exposed to the unfortified IMSL polypropylene control remained relatively constant during the initial 12 hour contact interval and then increased by 1 order of magnitude after 24 hours. This is considered a normal response for this organism on an inert surface under the conditions imposed by ISO 22196. A reduction of ≥ 4.2 orders of magnitude was observed in the populations held in contact with the samples of Sigmaresist Immun Mat after 12 hours.

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

Sample	Contact Time		
	0 hours	12 hours	24 hours
Polypropylene Control (IMSL)	1.6×10^4	1.4×10^4	8.6×10^3
Sigmaresist Immun Mat	1.6×10^4	≤ 1.0	≤ 1.0

It can be seen from the data above that the population of *Staphylococcus aureus* exposed to the unfortified IMSL polypropylene control remained relatively constant during the initial 12 hour contact interval and then decreased by 0.3 orders of magnitude during the second 12 hour interval. This is again considered a normal response for this organism on an inert surface under the conditions imposed by ISO 22196. A reduction of ≥ 4.2 orders of magnitude was observed in the populations of *Staphylococcus aureus* held in contact with the samples of Sigmaresist Immun Mat after 12 hours.

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

Sample	Contact Time		
	0 hours	12 hours	24 hours
Polypropylene Control (IMSL)	1.6×10^4	1.0×10^4	4.3×10^3
Sigmaresist Immun Mat	1.6×10^4	≤ 1.0	≤ 1.0

The data in Table 3 above shows that the population of Methicillin Resistant *Staphylococcus aureus* exposed to the unfortified IMSL polypropylene control declined by 0.2 orders of magnitude during the initial 12 hour contact interval and by a further 0.4 orders of magnitude over the remaining 12 hours. This is again considered a normal response for this organism on an inert surface under the conditions imposed by ISO 22196. A reduction of ≥ 4.2 orders of magnitude was observed in the populations of MRSA held in contact with the samples of Sigmaresist Immun Mat after 12 hours.

Table 4: Activity Against *Ps aeruginosa*
(Geometric Mean of Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time		
	0 hours	12 hours	24 hours
Polypropylene Control (IMSL)	1.7×10^4	5.6×10^3	2.6×10^5
Sigmaresist Immun Mat	1.7×10^4	≤ 1.0	≤ 1.0

Table 4 shows that the population of *Pseudomonas aeruginosa* exposed to the unfortified IMSL polypropylene control declined by 0.5 orders of magnitude during the initial 12 hour contact interval and then increased by 1.7 orders of magnitude over the remaining 12 hours. This could be attributed to the surviving population receiving a nutrient source from the dead bacterial population allowing it to recover and grow. In contrast, the populations of *Pseudomonas aeruginosa* exposed to the surfaces of Sigmaresist Immun Mat declined by ≥ 4.2 orders to below the limit of detection after 12 hours.

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

Sample	Contact Time		
	0 hours	12 hours	24 hours
Polypropylene Control (IMSL)	1.9×10^4	1.3×10^4	1.9×10^4
Sigmaresist Immun Mat	1.9×10^4	3.7×10^0	≤ 1.0

The data in Table 5 above shows that the population of *Enterococcus hirae* exposed to the unfortified IMSL polypropylene control declined by 0.2 orders of magnitude during the initial 12 hour contact interval and recovered back to its original level over the remaining 12 hours. In contrast, the population of the population of *Enterococcus hirae* exposed to Sigmaresist Immun Mat declined by 3.7 orders of magnitude after 12 hours and to below the limit of detection after 24 hours.

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

Sample	Contact Time		
	0 hours	12 hours	24 hours
Polypropylene Control (IMSL)	2.5×10^4	1.8×10^5	9.7×10^5
Sigmaresist Immun Mat	2.5×10^4	≤ 1.0	≤ 1.0

The data in Table 6 shows that the population of *Acinetobacter baumannii* exposed to the unfortified IMSL polypropylene control increased after 12 and 24 hours by a total of 1.6 orders of magnitude. In contrast, the populations of *Acinetobacter baumannii* exposed to the surfaces of Sigmaresist Immun Mat declined by 4.4 orders to below the limit of detection after 12 hours.

Table 7: Activity Against *S pneumoniae*
(Geometric Mean of Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time		
	0 hours	12 hours	24 hours
Polypropylene Control (IMSL)	2.2×10^4	1.1×10^3	1.1×10^1
Sigmaresist Immun Mat	2.2×10^4	≤ 1.0	≤ 1.0

It can be seen from the data above that the population of *Streptococcus pneumoniae* exposed to the polypropylene reference material declined by 1.8 orders of magnitude during the first 12 hours and then by a further 1.9 orders of magnitude during the second 12 hour interval. After 12 hours, the population of *Streptococcus pneumoniae* exposed to the samples of Sigmaresist Immun Mat declined by 4.3 orders of magnitude to below the limit of detection.

Table 8: Activity Against Carbapenemase Producing *K pneumoniae*
(Geometric Mean of Replicates as Colony Forming Units cm^{-2})

Sample	Contact Time				
	0 hours	6 hours	12 hours	18 hours	24 hours
Polypropylene Control (IMSL)	1.8×10^4	1.3×10^4	7.5×10^4	5.7×10^4	2.8×10^5
Sigmaresist Immun Mat	1.8×10^4	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0

The data in Table 8 shows that the population of Carbapenemase Producing *Klebsiella pneumoniae* exposed to the unfortified IMSL polypropylene control gradually increased by up to 1.1 orders of magnitude after 24 hours. In contrast, a reduction of 4.4 orders of magnitude to below the limit of detection of *Klebsiella pneumoniae* was observed on the samples of Sigmaresist Immun Mat after 6 hours.

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

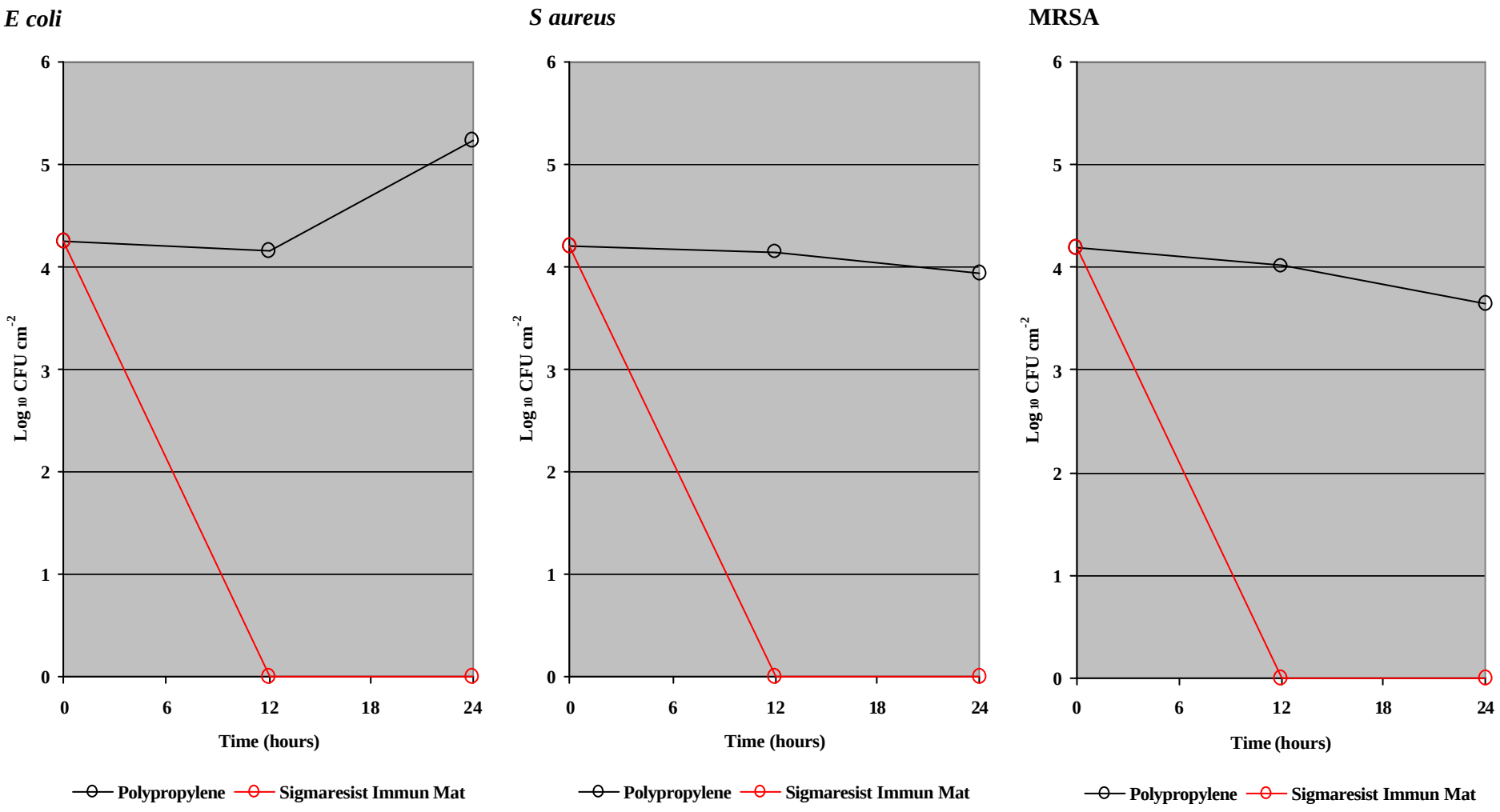


Figure 3: Results as Log_{10} CFU cm^{-2}

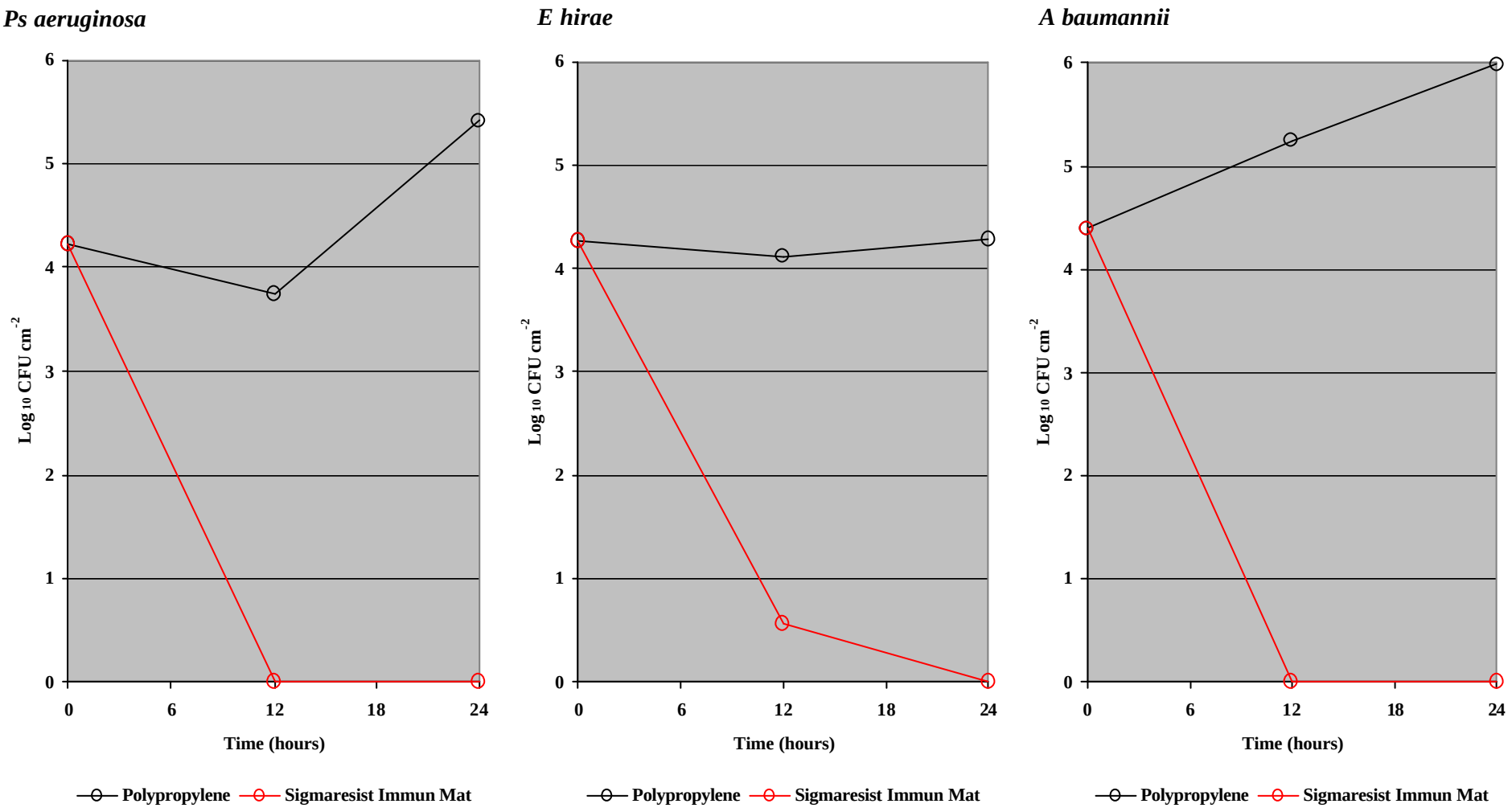
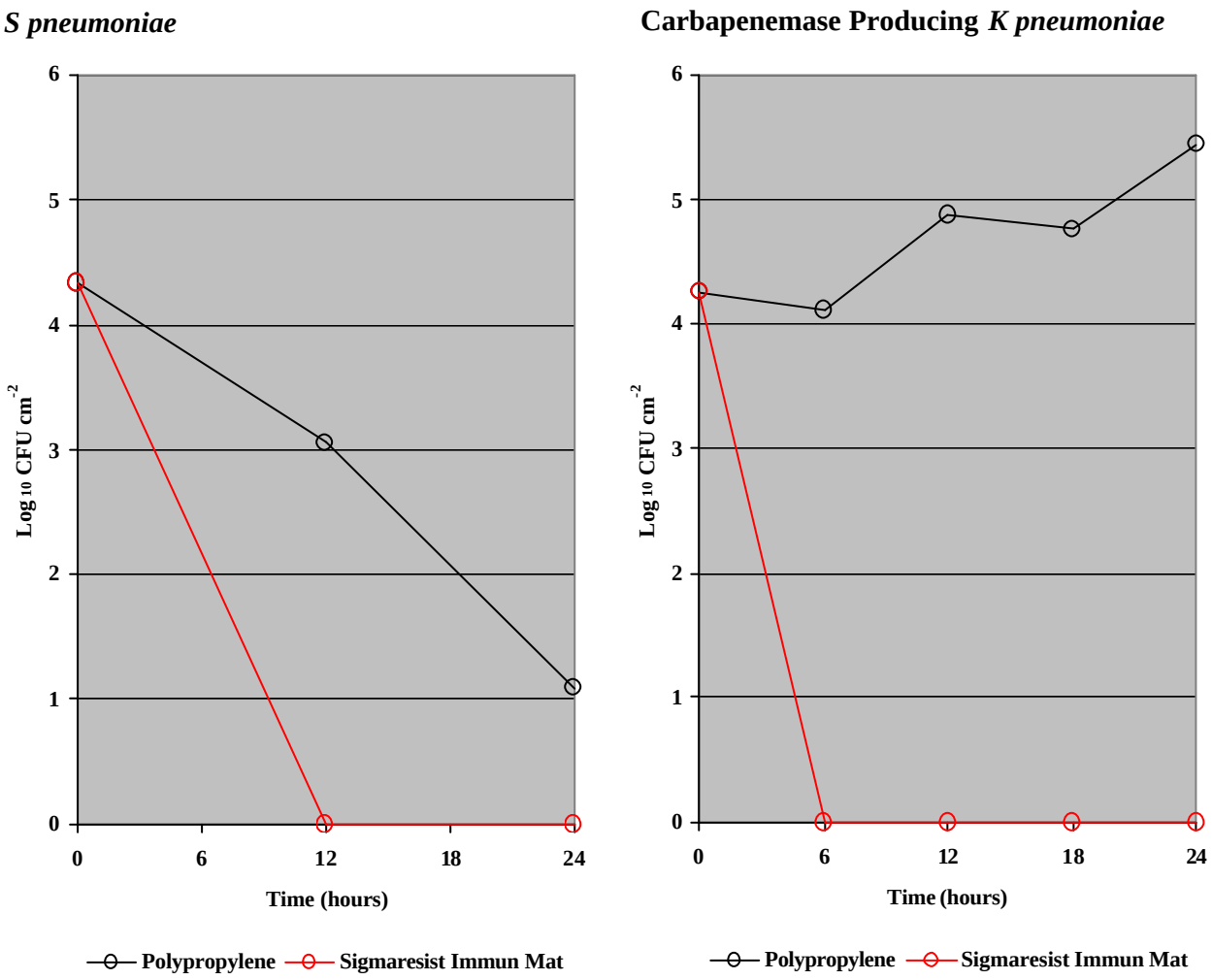


Figure 4: Results as Log_{10} CFU cm^{-2}



5 Raw Data

The raw data for this study will be held in file IMSL 2012/11/012 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

7 Exclusion of Liability

The contents of this report are subject to the standard terms and conditions of IMSL as displayed on the reverse of the invoice. Specific attention is drawn to Section 10 restated below.

(a) IMSL warrants that the results as stated in this Report are accurate in so far as they relate to the Samples as received in the laboratory of IMSL. Except in respect of death or personal injury caused by IMSL's negligence IMSL accepts no other liability or responsibility to any party whatsoever (whether caused by the negligence of IMSL, its employees, or agents or otherwise) arising out of or in connection with the provision of this Report. In particular, but without prejudice in the generality of the foregoing IMSL shall have no liability or responsibility whatsoever in respect of or in any way by reference to:-

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(ii) the interpretation of the Report and / or the application of the results as stated and / or the accuracy of any advices based thereon

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(iv) incorrect information or data supplied by the Customer relating to the Samples

(v) loss of or damage to the Samples when in the possession of IMSL

(vi) delay in provision of the Service or mis-delivery or non-delivery of any Report or Sample.

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