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INDUSTRIAL MICROBIOLOGICAL SERVICES LTD

STUDY REPORT: Determination of the Antibacterial Activity of Samples against *Escherichia coli* and *Staphylococcus aureus* using ISO 22196 : 2007

Counter sample no.10313

Test sample no.10313

CLIENT: Merino Industries Limited (India)

REPORT NO: IMSL 2010/08/009.1A

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1 Introduction

This report summarises a study performed to assess the antibacterial performance of samples against *Escherichia coli* and *Staphylococcus aureus* using the method described in ISO 22196:2007.

2 Test Materials

Samples either unfortified or fortified with antimicrobial were supplied by Merino Industries Limited (India). A sample of unfortified polystyrene 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

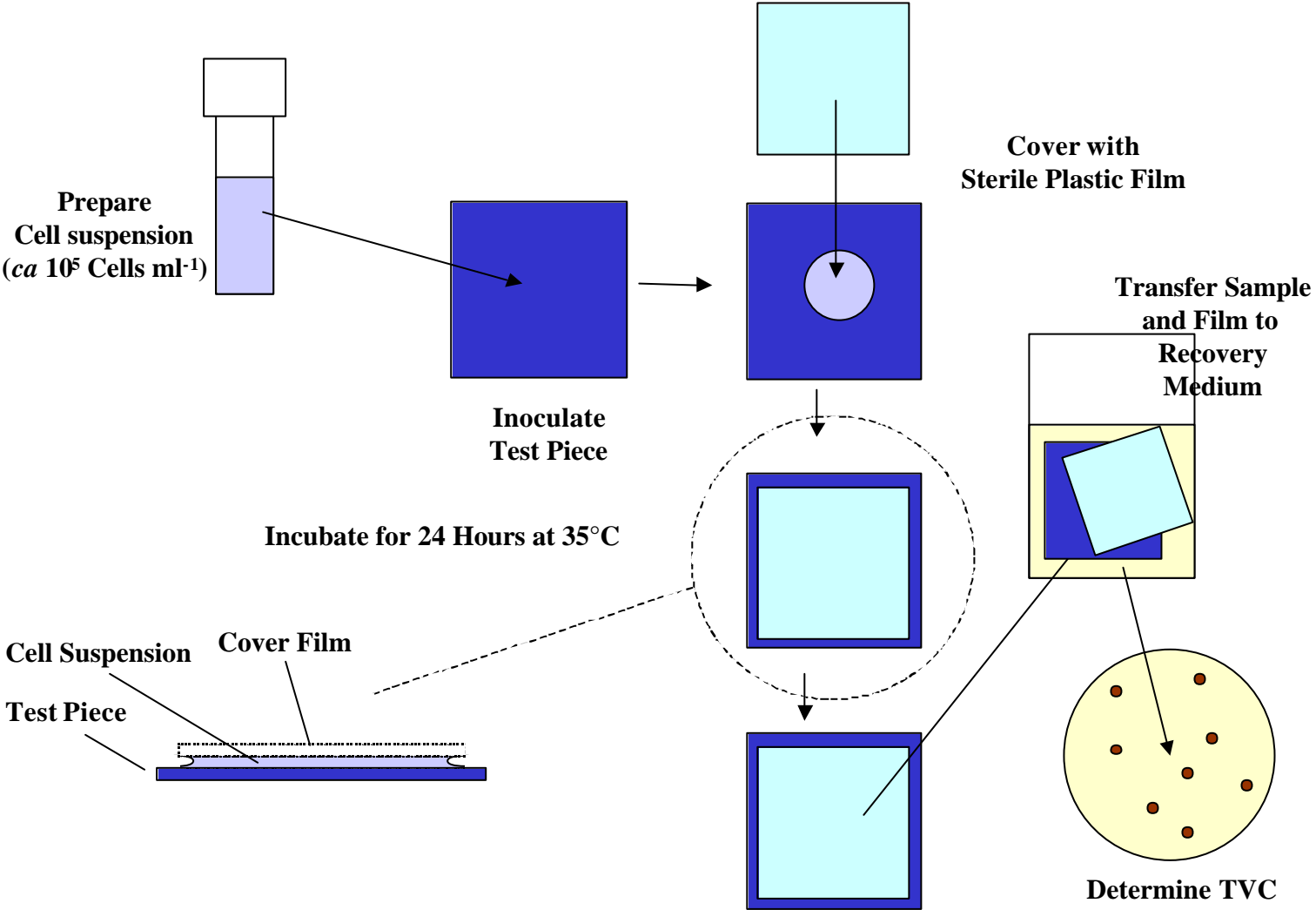
Antibacterial activity was determined using the method described in ISO 22196 : 2007 (Ref 1).

3.1 Determination of Antibacterial Activity

An aliquot (225µl) of a log phase cell suspension of either *Escherichia coli* (6.3×10^5 cells ml⁻¹; ATCC 8739) or *Staphylococcus aureus* (7.4×10^5 cells ml⁻¹; ATCC 6538p) 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 was converted to colony forming units (CFU) cm⁻² and then transformed (Log10) to provide a data set that conformed to a gaussian distribution. Potential outliers were tested using Dixon's *Q*-test (*P* = 0.05). Statistical significance of any effects in the residual data set was detected by analysis of variance (ANOVA, *P* = 0.05) and the confidence intervals of the means was calculated and displayed as Box and Whisker plots.

Figure 1: ISO 22196 - Schematic Representation



4 Results / Discussion

The results are shown in Tables 1 - 2 and Figure 2 below. The statistical analysis of the data is shown in Tables 3 - 4 and Figure 3 below.

Table 1: Activity of Coatings Against *E coli*
(Geometric Mean of 3 Replicates as Colony Forming Units cm⁻²)

Sample	Contact Time		Reduction from Control Foam		Reduction from initial	
	0 hours	24 hours	Log ₁₀	%	Log ₁₀	%
Polystyrene	1.6 x 10 ⁴	3.6 x 10 ⁵	-	-	-	-
Counter sample no. 10313	1.6 x 10 ⁴	7.1 x 10 ⁰	-	-	3.3	99.96
Test sample no. 10313	1.6 x 10 ⁴	2.8 x 10 ⁰	0.4	60.96	3.8	99.98

‡ The theoretical limit of detection is 1 CFU cm⁻²

It can be seen from the results above that the population of *E coli* held in contact with unfortified polystyrene increased in size by *ca* 1 orders of magnitude during the 24 hour contact interval. In contrast, the populations held in contact with both the Counter sample no.10313 and the Test sample no.10313 were reduced by 3.3 and 3.8 orders of magnitude respectively. Due to this large reduction in the populations held in contact with the control sample (Counter sample no.10313) no statistically significant differences were observed between these populations and the ones recovered from the Test sample no.10313.

**Table 2: Activity of Coatings Against *Staphylococcus aureus*
(Geometric Mean of 3 Replicates as Colony Forming Units cm⁻²)**

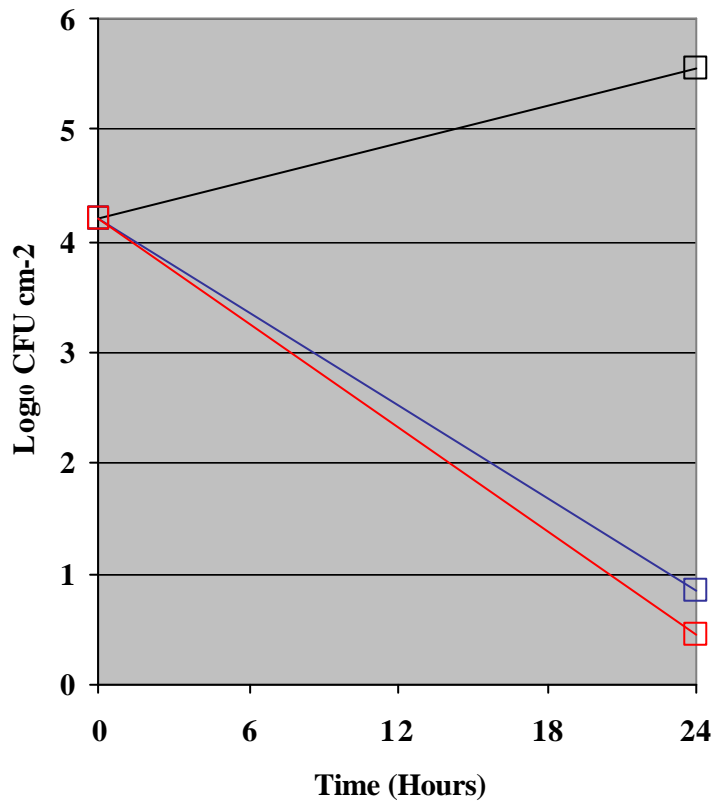
Sample	Contact Time		Reduction from Control Foam		Reduction from initial	
	0 hours	24 hours	Log ₁₀	%	Log ₁₀	%
Polystyrene	1.9 x 10 ⁴	9.0 x 10 ³	-	-	0.3	51.61
Counter sample no. 10313	1.9 x 10 ⁴	> 1.00	-	-	> 4.3	> 99.99
Test sample no. 10313	1.9 x 10 ⁴	> 1.00	-	-	> 4.3	> 99.99

‡ The theoretical limit of detection is 1 CFU cm⁻²

It can be seen from the results in Table 2 that the population of *Staph aureus* held in contact with the unfortified polystyrene declined by 0.3 orders of magnitude during the 24 hour contact period. In contrast, the populations held in contact with both the Counter sample no.10313 and the Test sample no.10313 were reduced by > 4.3 orders of magnitude to below the limit of detection. Due to this large reduction in the populations held in contact with the control sample (Counter sample no.10313) no statistically significant differences were observed between these populations and the ones recovered from the Test sample no.10313.

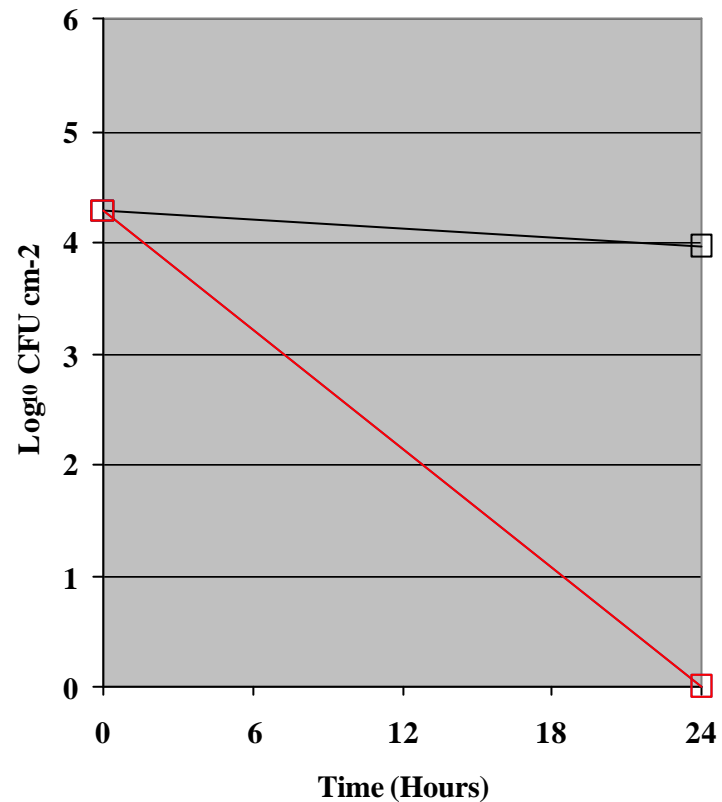
Figure 2: Results as Log_{10} CFU cm^{-2}

Escherichia coli



- Polystyrene
- Counter sample no.10313
- Test sample no.10313

Staphylococcus aureus



- Polystyrene
- Counter sample no.10313
- Test sample no.10313

Table 3: Statistical Analysis of Data (ANOVA P = 0.05) - *Escherichia coli*

Log ₁₀ CFU cm ² by Treatment	n	Mean	SE	Pooled SE	SD
Counter sample no.10313	3	0.907	0.2953	0.2331	0.511
Polystyrene	3	5.550	0.0223	0.2331	0.039
Test sample no.10313	3	0.576	0.2745	0.2331	0.475

Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	46.406	2	23.203	142.33	< 0.0001
Residual	0.978	6	0.163		
Total	47.385	8			

Contrast	Difference	95% CI
Test sample no.10313 v Counter sample no.10313	-0.332	-1.138 to 0.475

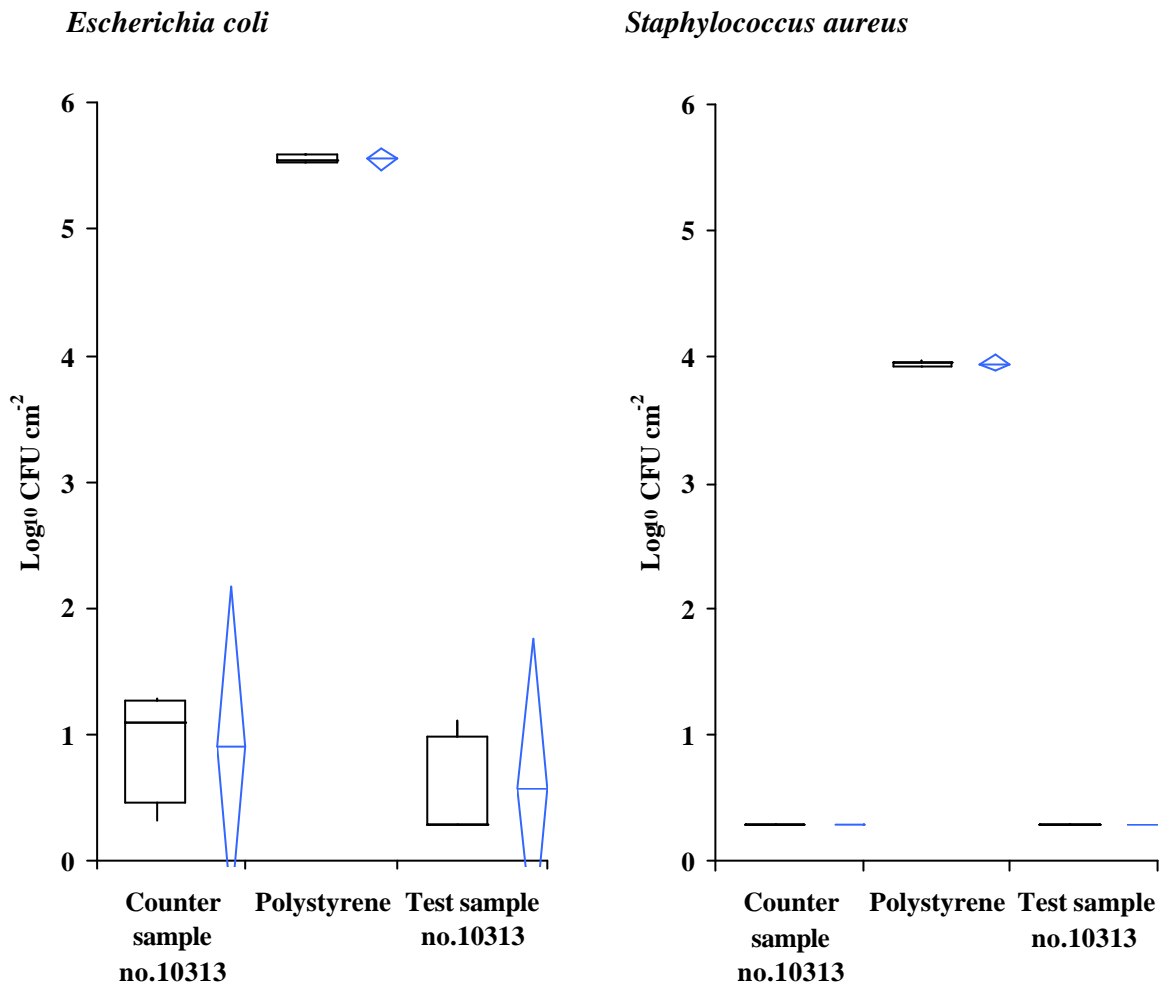
Table 4: Statistical Analysis of Data (ANOVA P = 0.05) - *Staphylococcus aureus*

Log ₁₀ CFU cm ² by Treatment	n	Mean	SE	Pooled SE	SD
Counter sample no.10313	3	0.301	0.0000	0.0075	0.000
Polystyrene	3	3.952	0.0130	0.0075	0.022
Test sample no.10313	3	0.301	0.0000	0.0075	0.000

Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	26.666	2	13.333	79329.21	< 0.0001
Residual	0.001	6	0.000		
Total	26.667	8			

Contrast	Difference	95% CI
Test sample no.10313 v Counter sample no.10313	0.000	-0.026 to 0.026

Figure 3: Confidence Intervals (95%) of Means -



5 Raw Data

The raw data for this study will be held in file IMSL2010/08/009 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.

6 References

- 1 ISO 22196: 2007, Plastics - Measurement of antibacterial activity on plastics surfaces.

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