

IMSL

INDUSTRIAL MICROBIOLOGICAL SERVICES LTD

STUDY REPORT: **Determination of the Antibacterial Activity of Coated Polypropylene against *Escherichia coli* and *Staphylococcus aureus* after 1 and 24 Hours using ISO 17094 : Test Method for Antibacterial Activity of Semiconducting Photocatalytic Materials Under Indoor Lighting Environment.**

CLIENT: **Singlotech Ltd
28 Trentlea Way
Sandbach,
Cheshire,
CW11 3AZ
UK**

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Study: Determination of the Antibacterial Activity of Coated Polypropylene against *Escherichia coli* and *Staphylococcus aureus* after 1 and 24 Hours using ISO 17094 : Test Method for Antibacterial Activity of Semiconducting Photocatalytic Materials Under Indoor Lighting Environment.

Number: IMSL 2019/01/011.1A

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Sandbach,
Cheshire,
CW11 3AZ
UK

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.

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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 coated polypropylene against *Escherichia coli* and *Staphylococcus aureus* after 1 and 24 hours using the method described in ISO 17094 : Test Method for Antibacterial Activity of Semiconducting Photocatalytic Materials Under Indoor Lighting Environment.

2 Test Materials

Samples of an inert polypropylene was supplied by IMSL to Singlotech Ltd which was coated with SingloTex™ Photocatalytic Dye. A sample of uncoated inert polypropylene was included as a control. 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 17094: 2014 (Ref 1).

3.1 Determination of Antibacterial Activity

An aliquot (150µl) of a log phase cell suspension of either *Escherichia coli* (1.3×10^6 cells ml⁻¹; ATCC 8739) or *Staphylococcus aureus* (1.4×10^6 cells ml⁻¹; ATCC 6538p) prepared in 1/500 Nutrient Broth (NB) using the method described in ISO 17094: 2014 were held in intimate contact with each of 12 replicates of the test surfaces supplied using a 40 x 40 mm polyethylene film (cut from a sterile Stomacher bag; optical transmittance of > 85%) for up to 24 hours at $25 \pm 5^\circ\text{C}$.

Per species, per treatment, 6 replicate surfaces were incubated under a Tungsten Halogen lamp (correlated colour temperature: 2930k; E Vis: 2103.1 Lx, mW/m²/nm @ 670nm: 47,53220) and 6 replicate surfaces were incubated in the dark.

After a contact interval of 1 and 24 hours, the size of the surviving population on 3 replicate test pieces was determined using the method described in ISO 17094 : 2014. The viable cells in the suspension were enumerated by spiral dilution and by the pour plate method described in ISO 17097 : 2014 using Trypcase Soya Agar.

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.

These plates were then incubated at 35°C for 24 hours and then counted.

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 ($P = 0.05$).

Statistical significance of any effects in the residual data set were detected was tested by analysis of variance (ANOVA, $P = 0.05$).

4 Results / Discussion

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

**Table 1: Activity Against *Escherichia coli*
(Geometric Mean of Replicates as Colony Forming Units Surface⁻¹)**

Sample	Contact Time			Reduction from Control (RL)				Log Reduction from Dark Equivalent (R)	
	0 hours	1 hour ‡	24 hours ‡	1 hour		24 hours		1 hour	24 hours
				Log ₁₀	%	Log ₁₀	%		
Polypropylene Control (Light)	1.9 x 10 ⁵ (A)	1.7 x 10 ⁵ (BL)	2.9 x 10 ⁶ (BL)	-	-	-	-	-	-
Polypropylene Control (Dark)	1.9 x 10 ⁵	1.6 x 10 ⁵ (BD)	1.6 x 10 ⁶ (BD)	-	-	-	-	-	-
Singlotex - Lot 004-33A (Light)	1.9 x 10 ⁵	1.4 x 10 ⁵ (CL)	1.4 x 10 ⁴ (CL)	0.1	14.88	2.3	99.52	-	2.2
Singlotex - Lot 004-33A (Dark)	1.9 x 10 ⁵	1.5 x 10 ⁵ (CD)	1.1 x 10 ⁶ (CD)	-	-	-	-	-	-

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

Validation:

- The number of viable bacteria recovered from the non-treated test piece immediately after inoculation shall be between 1.0 x 10⁵ - 4.0 x 10⁵ (A)
- The viable bacteria on the non-treated test piece after light exposure shall be more than 1.0 x 10³
- After being kept in the dark, the viable bacteria on the non-treated test piece shall be more than 1.0 x 10³

$$RL = (\text{Log}_{10} \text{ BL}) - (\text{Log}_{10} \text{ CL})$$

$$R = ((\text{Log}_{10} \text{ BL}) - (\text{Log}_{10} \text{ CL})) - ((\text{Log}_{10} \text{ BD}) - (\text{Log}_{10} \text{ CD}))$$

It can be seen from the results above that the population of *Escherichia coli* exposed to the IMSL Polypropylene Control incubated under dark conditions remained constant after 1 hour and increased by 0.7 orders of magnitude after 24 hours compared to the initial population. This is considered a normal response for this species on an inert surface under the conditions imposed by ISO 17074.

Similarly, the population of *Escherichia coli* exposed to the IMSL Polypropylene Control incubated under light conditions remained constant after 1 hour and increased by 0.9 orders of magnitude after 24 hours compared to the initial population.

The populations of *Escherichia coli* exposed to the surfaces of Singlotex - Lot 004-33A incubated under dark conditions remained constant after 1 hour and increased by 0.8 orders of magnitude after 24 hours compared to the initial population.

The data in Table 3 shows the populations of *Escherichia coli* recovered from the surfaces of Singlotex - Lot 004-33A (Dark) were not statistically significantly smaller than the populations recovered from the IMSL Polypropylene Control (Dark).

The populations of *Escherichia coli* exposed to the surfaces of Singlotex - Lot 004-33A incubated under light conditions remained constant after 1 hour and declined by 1.1 orders of magnitude after 24 hours compared to the initial population giving an RL value of 2.3 and a R Value of 2.2.

The size of the populations of *Escherichia coli* recovered from the surfaces of Singlotex - Lot 004-33A (Light) were shown to be statistically significantly smaller than the populations recovered from IMSL Polypropylene Control (Light) and the surfaces of Singlotex - Lot 004-33A (Dark) (Table 3).

**Table 2: Activity Against *Staphylococcus aureus*
(Geometric Mean of Replicates as Colony Forming Units Surface⁻¹)**

Sample	Contact Time			Reduction from Control (RL)				Log Reduction from Dark Equivalent (R)	
	0 hours	1 hour ‡	24 hours ‡	1 hour		24 hours		1 hour	24 hours
				Log ₁₀	%	Log ₁₀	%		
Polypropylene Control (Light)	2.0 x 10 ⁵ (A)	1.6 x 10 ⁵ (BL)	2.2 x 10 ⁵ (BL)	-	-	-	-	-	-
Polypropylene Control (Dark)	2.0 x 10 ⁵	1.7 x 10 ⁵ (BD)	1.9 x 10 ⁵ (BD)	-	-	-	-	-	-
Singlotex - Lot 004-33A (Light)	2.0 x 10 ⁵	1.7 x 10 ⁵ (CL)	3.1 x 10 ³ (CL)	-	-	1.9	98.60	0.1	1.8
Singlotex - Lot 004-33A (Dark)	2.0 x 10 ⁵	2.1 x 10 ⁵ (CD)	1.6 x 10 ⁵ (CD)	-	-	0.1	14.32	-	-

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

Validation:

- a) The number of viable bacteria recovered from the non-treated test piece immediately after inoculation shall be between 1.0 x 10⁵ - 4.0 x 10⁵ (A)
- b) The viable bacteria on the non-treated test piece after light exposure shall be more than 1.0 x 10³ (BL)
- c) After being kept in the dark, the viable bacteria on the non-treated test piece shall be more than 1.0 x 10³ (BD)

$$RL = (\text{Log}_{10} \text{ BL}) - (\text{Log}_{10} \text{ CL})$$

$$R = ((\text{Log}_{10} \text{ BL}) - (\text{Log}_{10} \text{ CL})) - ((\text{Log}_{10} \text{ BD}) - (\text{Log}_{10} \text{ CD}))$$

It can be seen from the results above that the population of *Staphylococcus aureus* exposed to the IMSL Polypropylene Control incubated under dark conditions remained relatively constant during the 24 hour contact interval compared to the initial population. This is again considered a normal response for this species on an inert surface under the conditions imposed by ISO 17074.

Similarly, the population of *Staphylococcus aureus* exposed to the IMSL Polypropylene Control incubated under light conditions remained constant over the 24 hour contact interval compared to the initial population.

The populations of *Staphylococcus aureus* exposed to the surfaces of Singlotex - Lot 004-33A incubated under dark conditions also remained constant after 24 hours compared to the initial population.

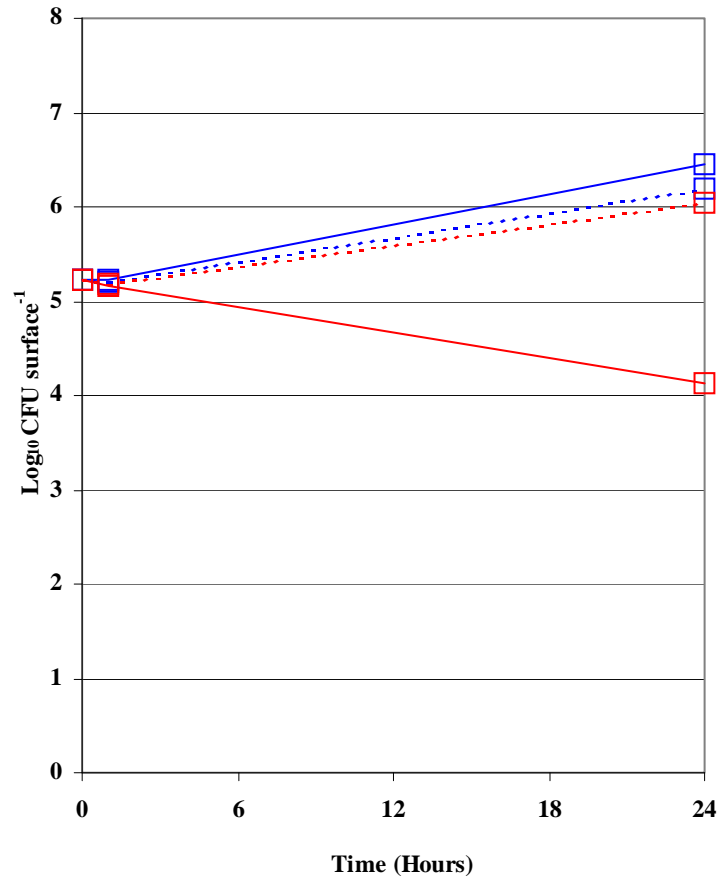
Table 4 shows that the populations of *Staphylococcus aureus* recovered from the surfaces of Singlotex - Lot 004-33A (Dark) were not statistically significantly smaller than the populations recovered from the IMSL Polypropylene Control (Dark).

In contrast, the populations of *Staphylococcus aureus* exposed to the surfaces of Singlotex - Lot 004-33A incubated under light conditions remained constant after 1 hour and declined by 1.7 orders of magnitude after 24 hours compared to the initial population giving an RL value of 1.9 and a R Value of 1.8.

The size of the populations of *Staphylococcus aureus* recovered from the surfaces of Singlotex - Lot 004-33A (Light) were shown to be statistically significantly smaller than the populations recovered from IMSL Polypropylene Control (Light) and the surfaces of Singlotex - Lot 004-33A (Dark) (Table 4).

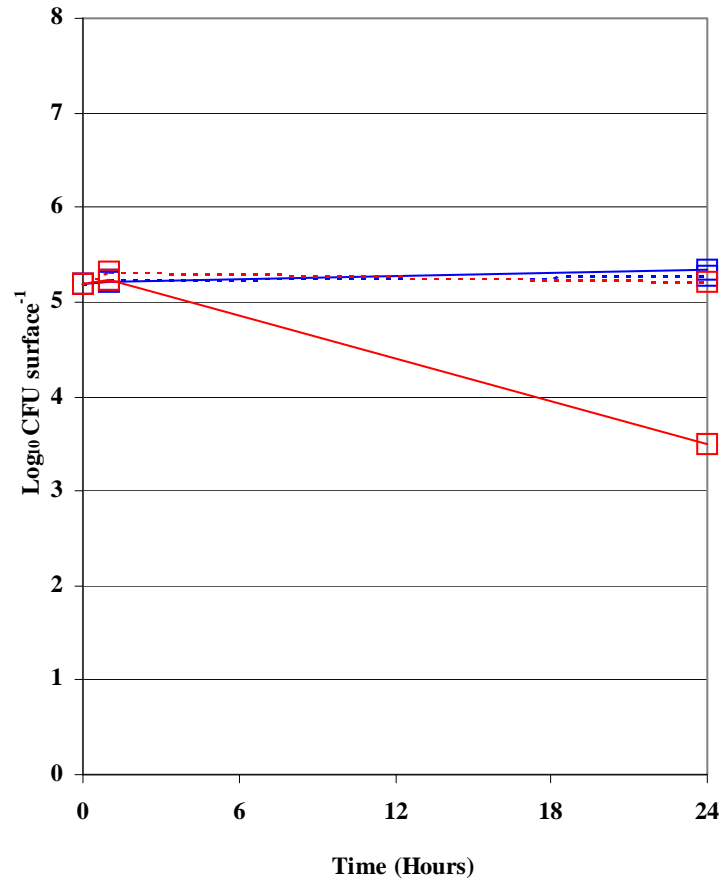
Figure 1: Results as Log₁₀ CFU cm⁻²

Escherichia coli



- IMSL Control Polypropylene (Light)
- IMSL Control Polypropylene (Dark)
- SingloTex - Lot: 004-33A (Light)
- SingloTex - Lot: 004-33A (Dark)

Staphylococcus aureus



- IMSL Control Polypropylene (Light)
- IMSL Control Polypropylene (Dark)
- SingloTex - Lot: 004-33A (Light)
- SingloTex - Lot: 004-33A (Dark)

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

Log10 CFU g-1 by Treatment	n	Mean	SE	Pooled SE	SD
IMSL Control Polypropylene (Light)	3	6.460	0.1050	0.5284	0.182
IMSL Control Polypropylene (Dark)	3	6.194	0.1422	0.5284	0.246
SingloTex - Lot: 004-33A (Light)	3	4.139	0.9926	0.5284	1.719
SingloTex - Lot: 004-33A (Dark)	3	6.038	0.3164	0.5284	0.548

Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	10.114	3	3.371	4.03	0.0512
Residual	6.700	8	0.838		
Total	16.815	11			

LSD

Contrast	Difference	95% CI	
IMSL Control Polypropylene (Light) v IMSL Control Polypropylene (Dark)	0.266	-1.457 to 1.989	
IMSL Control Polypropylene (Light) v SingloTex - Lot: 004-33A (Light)	2.321	0.598 to 4.044	(significant)
IMSL Control Polypropylene (Light) v SingloTex - Lot: 004-33A (Dark)	0.422	-1.302 to 2.145	
IMSL Control Polypropylene (Dark) v SingloTex - Lot: 004-33A (Light)	2.055	0.332 to 3.778	(significant)
IMSL Control Polypropylene (Dark) v SingloTex - Lot: 004-33A (Dark)	0.156	-1.568 to 1.879	
SingloTex - Lot: 004-33A (Light) v SingloTex - Lot: 004-33A (Dark)	-1.899	-3.622 to -0.176	(significant)

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

Log10 CFU g-1 by Treatment	n	Mean	SE	Pooled SE	SD
IMSL Control Polypropylene (Light)	3	5.343	0.0063	0.4211	0.011
IMSL Control Polypropylene (Dark)	3	5.274	0.0254	0.4211	0.044
SingloTex - Lot: 004-33A (Light)	3	3.490	0.8404	0.4211	1.456
SingloTex - Lot: 004-33A (Dark)	3	5.207	0.0487	0.4211	0.084

Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	7.194	3	2.398	4.51	0.0393
Residual	4.256	8	0.532		
Total	11.450	11			

LSD

Contrast	Difference	95% CI	
IMSL Control Polypropylene (Light) v IMSL Control Polypropylene (Dark)	0.069	-1.305 to 1.442	
IMSL Control Polypropylene (Light) v SingloTex - Lot: 004-33A (Light)	1.853	0.480 to 3.226	(significant)
IMSL Control Polypropylene (Light) v SingloTex - Lot: 004-33A (Dark)	0.136	-1.237 to 1.509	
IMSL Control Polypropylene (Dark) v SingloTex - Lot: 004-33A (Light)	1.784	0.411 to 3.157	(significant)
IMSL Control Polypropylene (Dark) v SingloTex - Lot: 004-33A (Dark)	0.067	-1.306 to 1.440	
SingloTex - Lot: 004-33A (Light) v SingloTex - Lot: 004-33A (Dark)	-1.717	-3.090 to -0.344	(significant)

5 Raw Data

The raw data for this study will be held in file IMSL 2019/01/011.1 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 17094 : Test Method for Antibacterial Activity of Semiconducting Photocatalytic Materials Under Indoor Lighting Environment.

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.

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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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