Customer Name	Maeda-Kougyou Japan 218-2 Norimatsu Yahatanishi ward Kitakyushu City Japan
Contact	Dr. Khaled Hussein
Customer PO no.	N/A
Test Requested	BS ISO 27447: 2009 Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials (Test method modified due to client's requests)
Sample Description	Textile: non-coated and coated with Miracle Titanium (Primary and MVX)
Date of Receipt	27th April 2012
Project Number	ASCR0092009
Report Date	5th July 2012

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Description of Test Items

The following items were tested. The samples to be tested all measured 50 x 50 mm.

Test Item	Product Code
Treated Textile	ASC002166
Untreated Textile	ASC002167

Introduction

The purpose of the project was to ascertain the effect of the MVX coating on *Pseudomonas aeruginosa* viability when applied to a textile following exposure to UV and incandescent light as per the client's request. The test was agreed to be performed in accordance with BS ISO 27447: 2009 Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials. As the client's requests included alterations in the bacterial to be used, some alterations were made, however these have been subsequently noted.

Procedure

The experimental procedure was performed in accordance with BS ISO 27447: 2009 Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials, with alterations made to accommodate the client's sample and microbiological challenge specifications. The method for analysis was as follows:

Textile Samples (Glass adhesion method)

1. Prior to inoculation all samples and coverslips were sterilised by autoclaving at 121°C for 15 minutes.
2. *P. aeruginosa* was grown in Tryptic Soy Broth under aerobic conditions at 37°C for 18 hours.
3. The concentration of bacteria was adjusted to a target concentration of 1 x 10^5 cells ml^{-1} using 1/500 nutrient broth.
4. Specimens were individually placed in sterile petri dishes. In order to preserve moisture a sterile filter paper was moistened with sterile water with the specimens to be tested separated by a glass slide.

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5. A 150 µl aliquot of the adjusted bacterial suspension was placed on the surface of the textile samples and a glass slide was placed on top to press the bacterial suspension uniformly under the glass.
6. Treated and untreated samples were kept in a dark place or exposed to the light source as specified in the standard with the additional incandescent light source as per the client's request.
7. Three untreated samples were immediately washed in 20 ml PBS. A 2 ml aliquot of this washout was serially diluted in sterile phosphate buffered saline (PBS).
8. A 500 µl aliquot of the neat washout and the serially diluted samples was plated in duplicate on sterile petri-dishes.
9. Approximately 15 – 20 mls of Tryptic Soy Agar was placed into each petri dish in order to enumerate viable colony forming units by the pour plate method following incubation at 37°C for 24 – 48 hours. Where the number of cfu exceeded 300 the plates were recorded as TNTC (Too numerous to count).

Satisfaction of criteria for a valid test and calculations

The test requirement fulfilment validation follows the raw data in the results section (see below). In addition the results expressing photocatalyst antibacterial activity value on textiles (S_L) and the photocatalyst antibacterial activity value with UV and incandescent light irradiation on textiles (ΔS).

Glass adhesion method

$M = P \times 20$

M is the number of cells of viable bacteria

P is the bacteria concentration (cells/ml)

20 is the quantity of PBS used for washout (ml)

Propagation values for validation of conditions for a valid test

$$F_{BL} = M_{BL} - M_{BA}$$

F_{BL} is the growth value after light exposure

M_{BL} is the average logarithmic value of the number of bacteria for 3 non treated specimens after light exposure

M_{BA} is the average logarithmic value of the number of viable bacteria for three non treated specimens just after inoculation

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$F_{BD} = M_{BD} - M_{BA}$

F_{BD} is the growth value after being kept in a dark place

M_{BD} is the average logarithmic value of the number of viable bacteria for three non treated specimens after being kept in a dark place

Photocatalyst antibacterial activity value calculation

$S_L = M_{BL} - M_L$

S_L is the photocatalyst antibacterial activity value after light exposure

M_L is the average logarithmic value of the number of viable bacteria for 3 photocatalytic treated specimens after light exposure

$\Delta S = (M_{BL} - M_L) - (M_{BD} - M_D)$

ΔS is the photocatalyst antibacterial value with light exposure

M_D is the average logarithmic value of the number of viable bacteria for three photocatalytic treated specimens after being kept in a dark place

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Results

Pseudomonas aeuruginosa Textile

Sample description	Dilution	Colony count	Number of viable bacteria recovered per specimen	Log values
Untreated 1 t = 0	1×10^{0}	TNTC*	7600	3.88081359
	1×10^{-1}	17, 21		
Untreated 2 t=0	1×10^{0}	TNTC	7000	3.84509804
	1×10^{-1}	16, 19		
Untreated 3 t=0	1×10^{0}	TNTC	8200	3.91381385
	1×10^{-1}	21, 20		
Light untreated 1	1×10^{0}	TNTC	9400	3.97312785
	1×10^{-1}	19, 28		
Light untreated 2	1×10^{0}	TNTC	7800	3.8920946
	1×10^{-1}	20, 19		
Light untreated 3	1×10^{0}	TNTC	7400	3.86923172
	1×10^{-1}	19, 18		
Light treated 1	1×10^{0}	1, 0	40	1.60205999
	1×10^{-1}	0, 0		
Light treated 2	1×10^{0}	0, 0	0	0
	1×10^{-1}	0, 0		
Light treated 3	1×10^{0}	0, 0	0	0
	1×10^{-1}	0, 0		
Dark untreated 1	1×10^{0}	TNTC	4600	3.66275783
	1×10^{-1}	10, 13		
Dark untreated 2	1×10^{0}	TNTC	8200	3.91381385
	1×10^{-1}	17, 24		
Dark untreated 3	1×10^{0}	TNTC	13200	4.12057393
	1×10^{-1}	16, 17		

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Dark treated 1	1×10^{0}	TNTC	3200	3.50514998
	1×10^{-1}	8, 8		
Dark treated 2	1×10^{0}	TNTC	4000	3.60205999
	1×10^{-1}	8, 12		
Dark treated 3	1×10^{0}	TNTC	1000	3
	1×10^{-1}	2, 3		

*TNTC: Too Numerous To Count

Test requirement fulfilment validation

1. $F_{BL} = 3.911 - 3.873 = 0.038$
F_{BL} is greater than 0 therefore parameter is validated

2. $F_{BD} = 3.898 - 3.873 = 0.025$
F_{BD} is greater than 0 therefore parameter is validated

S_L = 3.911 – 0.533 = 3.378
ΔS = (3.911 – 0.533) – (3.898 – 3.369)
= 3.378 – 0.529
=2.849

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Discussion and Conclusion

In accordance with the wishes of the client the ISO 22447:2009 procedure was modified slightly as the surface to be tested consisted of textile samples. In addition, as agreed with the client *Pseudomonas aeruginosa* was used for analysing the effect of the coatings against viability of the bacteria of interest when exposed to light.

In understanding the data it must be noted that S_L values account for the reduction of viability caused by the exposure of the treated surfaces to light on textiles. In contrast, ΔS values address the reduction of bacterial viability caused by the coating becoming light activated while accounting for the reduction in viability caused by the same coatings in a dark environment. From the data presented here it is clear that in all cases the coatings resulted in a reduction in bacterial viability even when stored in a dark place. It is therefore clear that the coatings in the absence of light have a bactericidal effect.

As per the client's request *P. aeruginosa* NCTC10622 was tested on the textile surfaces using the glass adhesion method. The data demonstrated that the coatings were highly effective against *P. aeruginosa* demonstrating a ΔS value of 2.849 with the criteria for a valid test fulfilled.

In conclusion the coatings appear to be very effective at reducing the viability of *P. aeruginosa* on textile.

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*** End of Report***

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