FINAL REPORT

Biotecta 60 Antimicrobial Activity Efficacy Testing

PROTOCOL
ASTM E2315

ORDER Number
371001930

PREPARED FOR:

Graboplast Floor Producing Company

Jason Dobranic, Ph.D.
EMSL Analytical, Inc.
107 Haddon Avenue, Westmont, NJ 08108
Phone: (856) 858-4800 Fax: (856) 858-0648 Web: http://www.emsl.com

EMSL Analytical, Inc.

Certificate of Analysis

Client: Graboplast Floor Producing Company

Contact: Ildikó Horváth
Project: Photocatalytic antibacterial flooring

Product : Silver Knight
EMSL NO: 371001930

Sample received: 2/25/2010
Start date: 4/5/2010
Report date: 4/12/2010
Challenge Bacteria: Gram Positive - *Staphylococcus aureus* (MRSA)
Gram Positive – *Enterococcus faecalis* (VRE)
Gram Negative – *Klebsiella pneumoniae* (ESBL)

Experimental Summary:

The testing procedure was designed after discussions between EMSL Analytical, the testing company, and the client, Graboplast Floor Producing Company. The testing procedure is based on ISO 27447, with the assessment of antimicrobial activity in photocatalyitic materials. The testing was conducted in our Westmont Microbiology Laboratory.

Procedure:

Three species of bacteria (MRSA, VRE and *K. pneumoniae* ESBL) were individually exposed to the photocatalytic material, Silver Knight. The film adhesion method was then employed as per ISO 27447. Each Petri dish consisted of a moisture control paper filter, a glass rod to avoid contact between test piece and filter paper and the photocatalytic test piece. The test material was then inoculated with 0.15 ml of a 10^6 cell suspension. This was followed by placing a clear adhesive film on the pre-inoculated flooring to spread the bacteria evenly over the surface. Finally a glass cover was placed over the test microcosm. Each test species was then split up into three groups: 1) Post-inoculated specimen (non-treated control), 2) Kept in darkness for 8 h (non-treated and treated materials), and 3) UV irradiation exposure for 8 h (non-treated and treated materials). The UV irradiation intensity was set to 0.1 wM/cm^2. After the specified exposure time the film was aseptically removed from the material and both were placed into 10 ml of tryptic soy broth. This was done for both non-treated control and treated test specimens. A 1ml aliquot was then taken and placed into 9ml of phosphate buffer and repeated to create serial dilutions. Dilutions were then plated onto nutrient agar and plates were incubated at 35ºC for 48h before colonies were counted. All tests were done in triplicate and statistics were calculated for the results.

Experimental Results:

Table 1.1 Test Validations

Species	1	2	3	4
MRSA	0.05	$3.20x10^5$ $3.90x10^5$ $2.00x10^5$	$1.99x10^4$ $6.90x10^4$ $4.80x10^4$	$5.50x10^3$ $3.60x10^4$ $4.00x10^4$
VRE	0.07	$2.30x10^5$ $1.70x10^5$ $1.00x10^5$	$4.50x10^4$ $1.66x10^4$ $3.10x10^4$	$4.70x10^4$ $3.50x10^4$ $3.10x10^4$
ESBL	0.04	$4.60x10^5$ $3.70x10^5$ $2.90x10^5$	$5.40x10^4$ $3.00x10^4$ $3.80x10^4$	$1.27x10^5$ $9.00x10^4$ $4.60x10^4$

1) The logarithmic value of the number of viable bacteria of non-treated cells in To shall be ≤ 0.2
2) The logarithmic value of viable bacteria of non-treated cells after inoculation shall be within 1.0E+05 to 4.0E+05
3) The viable bacteria of non-treated specimens after light exposure shall be more than 1.0E+03
4) The viable bacteria of non-treated specimens kept in a dark place shall be more than 1.0E+03

Table 1.2 Photocatalyst Antibacterial Activity

MRSA	$R_{0.10}$	ΔR	Log Reduction	% Reduction
Test Material	2.3	2.0	2.39	99.59
Control	-	-	0.06	12.79
VRE				
Test Material	2.6	2.4	2.47	99.66
Control	-	-	0.11	23.14
ESBL				
Test Material	1.7	1.7	2.01	99.02
Control	-	-	0.31	51.08

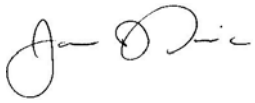
UV irradiation intensity = 0.10 mW/cm^2
Log reduction = (mean viable cells 8 h darkness) - (mean viable cells 8 h UV irradiation)

Conclusions/Observations:

As shown in Table 1.1 all tests were performed within the specified test validations, as given by ISO 27447. When comparing the test material to the control it was observed that the Silver Knight floor tiles demonstrated antimicrobial properties by reducing bacterial populations after 8 h exposure to UV irradiation intensity 0.10 mW/cm^2. Photocatalytic floor tiles were observed to reduce MRSA by 99.59%, VRE by 99.66% and *K. pneumoniae* ESBL by 99.02%. Furthermore the $R_{0.10}$ and ΔR were calculated; a higher number suggests an increased efficacy. The $R_{0.10}$ is calculated to determine the efficacy of the test material at UV irradiation intensity 0.10 mW/cm^2. As shown in table 1.2 the $R_{0.10}$ was observed to be 2.3, 2.6, and 1.7 for MRSA, VRE and ESBL respectively. The ΔR is used to compare the difference in reduction of bacterial populations on the test material when exposed to darkness and UV irradiation. As shown in Table 1.2 ΔR was observed to be 2.0, 2.4, and 1.7 for MRSA, VRE and ESBL

respectively. These calculations demonstrate that there was a significant reduction of bacterial populations by the test material after exposure to UV irradiation as opposed to darkness for 8 h.

In conclusion it has been demonstrated that the Silver Knight photocatalytic test material provided by Graboplast Floor Producing Company exhibits antibacterial activity when exposed to UV irradiation intensity 0.10 mW/cm^2 for 8 h. After such exposure a greater than 99% reduction in bacterial population is expected.



Jason Dobranic, Ph.D.
National Director of Microbiology

107 Haddon Avenue, Westmont, NJ 08108
Phone: (856) 858-4800 Fax: (856) 858-0648