FINAL REPORT

Antibacterial activity of photocatalytic materials

PROTOCOL
ISO 27447

ORDER Number
371106892

PREPARED FOR:

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Certificate of Analysis

Client: Graboplast Floor Producing Company

Contact: Ildikó Horváth
Project: Photocatalytic antibacterial flooring

Product : Silver Knight
EMSL NO: 371106892

Sample received: 6/6/2011
Start date: 6/15/2011
Report date: 7/5/2011
Challenge Bacteria: Gram Positive – *Clostridium difficile* ATCC 70057

1. 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 Cinnaminson Microbiology Laboratory.

2. Procedure:

Clostridium difficile (*C. difficile*) was individually exposed to the photocatalytic material by adding 0.15 mL of a 10^6 cell suspension inoculum. The film adhesion method was then employed as per ISO 27447 and a 5 x 5 cm^2 piece of adhesive film was placed over the material to spread the inoculum evenly. The inoculated material was then placed in a Petri dish that consisted of a moisture control paper filter, and a glass rod to avoid contact between test piece and filter paper. Finally a glass cover was placed over the test microcosm. Each test and control material 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 then set to 0.1 wM/cm^2, and after the specified exposure time the film was aseptically removed from the material and both were placed into 10 mL of Tryptic soy broth (TSB). This was repeated for both the 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 Tryptic soy agar with 5%

sheep blood (TSAB) and plates were incubated at 35ºC for 72h at anaerobic conditions before colonies were counted. All tests were done in triplicate and statistics were calculated for the results.

3. Experimental Results:

Table 1.1 Test Validations

Species	1	2	3	4
C. difficile	0.05	$4.00x10^5$ $4.00x10^5$ $2.00x10^5$	$4.00x10^4$ $5.00x10^4$ $4.00x10^4$	$4.00x10^4$ $6.00x10^4$ $7.00x10^4$

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

C. difficile	$R_{0.10}$	ΔR	Log Reduction	% Reduction
Test Material	0.2	0.4	0.64	76.97
Control	-	-	0.11	21.91

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

4. Conclusions/Observations:

As shown in Table 1.1 all tests were performed within the specified test validations, as given by ISO 27447. While control materials resulted in a non-significant reduction of 0.11 log (Table 1.2); the photocatalytic floor tiles were observed to reduce *C. difficile* by 0.64 log. The $R_{0.10}$ was 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 0.2 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 0.4 respectively.

In conclusion it has been demonstrated that the photocatalytic test material provided by Graboplast Floor Producing Company was observed to reduce *C. difficile* populations by 76.97% after 8 h exposure to a UV irradiation intensity of 0.10 mW/cm^2.

Jason Dobranic, Ph.D.
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