FINAL REPORT

PROTOCOL
ISO Method 27447 – Test for Antimicrobial Activity
of Photocatalytic Materials

PRODUCT TESTED
Antimicrobial Photocatalytic Coating on Tiles

EMSL ORDER NUMBER
152006572

TESTING LABORATORY
EMSL Analytical, Inc.
5950 Fairbanks North Houston Rd.
Houston TX 77040
Phone: (713) 686-3635
Web: www.emsl.com

SPONSOR
REair Global
468 N. Camden Drive
Beverly Hills, CA 90210

STUDY START DATE
October 6, 2020

STUDY COMPLETION DATE
November 9, 2020

Certificate of Analysis

Client: REair Global

Contact: Matteo Enea

Project: Efficacy Testing of Photocatalytic coating using 370nm UV-Light at 0.25 mW/cm^2 (simulates UV intensity beside a window in the daytime)

EMSL NO: 152006572

Product: Antimicrobial coating applied to ceramic tiles

Samples received: 10/06/2020

Report date: 11/09/2020

Challenge Bacteria:
Staphylococcus aureus (ATCC 6538)
Escherichia coli (ATCC 25922)
Salmonella enterica (ATCC 1045)

Experimental Summary:
The testing procedure follows ISO 27447 as requested by the client to determine the antibacterial activity of their coating material.

Test Method:
Culture preparation: Pure stock cultures of *S. aureus*, *E. coli,* and *S. enterica* were plated, separately, onto tryptic soy agar supplemented with sheep blood (TSAB) and incubated at 35°C for 24 hours. A well -isolated colony was transferred into tryptic soy broth (TSB) and incubated for 20 hours at 35°C. One loop of the test bacteria was transferred and plated onto TSAB for 24 hours at 35°C. Two loops of well-isolated colonies were then harvested, suspended in 20 mL of 1/500 TSB. This suspension was diluted with 1/500 TSB and adjusted to ~$10X10^6$ CFU/mL.

Inoculation of test material: REair Global submitted 3x3 inch ceramic tile samples with their coating applied as well as untreated control samples without the coating. Each test sample was inoculated with 0.5 mL of bacterial suspension as prepared above. Polystyrene film was cut to fit on the slides and spread the inoculum across the surface. All test samples were incubated at 25°C with 8 hours with UV light on or with no lighting. All tests were performed in triplicate.

To determine the starting population, a 0.5 mL aliquot of the bacterial suspension was placed into 9.5 mL of sterile dilution water. A 1-mL aliquot of this solution was then taken and serially diluted. Dilutions were plated onto APC Petrifilm plates to determine starting population of the inoculum.

Recovery of test organism: The following exposure time points were evaluated for the coated and uncoated test samples: 0 (instantaneous) and 8 hours. After treatment, both test and the control samples were removed and placed into sterile Whirl-pak bags and diluted with 20 mL of sterile D/E neutralizing broth. Samples were shaken for 60 seconds to dislodge any remaining bacteria into suspension. The suspensions were then serially diluted and plated onto APC Petrifilm plates. These plates were incubated at 35ºC for 24-48 hours before colonies were counted.

Experimental Results:

Table 1. Antibacterial efficacy of the photocatalytic coating against *S. aureus.*

Material & Treatment	Bacterial Recovery (average CFU/sample)	Log	Log Reduction	%Kill
Lab Control T=0	5,800,000	6.76		
Uncoated - No UV	7,230,000	6.86	None	None
Coated - 8h UV	<100	>2.00	>4.76	>99.998
Lab Control T=0	30,000,000	7.48		
Uncoated - 8h UV	32,600,000	7.51	None	None
Coated - No UV	1,620,000	6.21	1.27	94.60

Log Reduction = difference between Log of Control (untreated) and Log of treatment. Detection limit on culture plates was 10 CFU/Test surface.

Table 2. Antibacterial efficacy of the photocatalytic coating against *E. coli.*

Material & Treatment	Bacterial Recovery (average CFU/sample)	Log	Log Reduction	%Kill
Lab Control T=0	21,433,000	7.33		
Uncoated - No UV	22,630,000	7.35	None	None
Coated - 8h UV	<100	>2.0	>5.33	>99.9995
Lab Control T=0	52,700,000	7.72		
Uncoated - 8h UV	6,620,000	6.82	0.90	87.4
Coated - No UV	48,000	4.68	3.04	99.91

Log Reduction = difference between Log of Control (untreated) and Log of treatment. Detection limit on culture plates was 10 CFU/Test surface.

Table 3. Antibacterial efficacy of the photocatalytic coating against *S. enterica.*

Material & Treatment	Bacterial Recovery (average CFU/sample)	Log	Log Reduction	%Kill
Lab Control T=0	10,700,000	7.03		
Uncoated - No UV	8,700,000	6.94	0.09	18.7
Coated - 8h UV	70	1.85	5.18	99.999
Lab Control T=0	82,300,000	7.92		
Uncoated - 8h UV	59,300,000	7.77	0.14	27.9
Coated - No UV	171,000	5.23	2.68	99.79

Log Reduction = difference between Log of Control (untreated) and Log of treatment. Detection limit on culture plates was 10 CFU/Test surface.

Antibacterial activity calculation:

$$R_L = \left[\log(B_L/A) - \log(C_L/A)\right] = \log\left[B_L/C_L\right] \quad (4)$$

where

R_L is the photocatalyst antibacterial activity value, after UV irradiation of intensity L;

L is the UV irradiation intensity (mW/cm^2);

A is the average number of viable bacteria of non-treated specimens, just after inoculation;

B_L is the average number of viable bacteria of non-treated specimens, after UV irradiation of intensity L;

C_L is the average number of viable bacteria of photocatalytic treated specimens, after UV irradiation of intensity L.

Antibacterial activity results:

Antibacterial activity against *S. aureus* = 4.8
Antibacterial activity against *E. coli* = 4.4
Antibacterial activity against *S. enterica* = 5.0

Conclusions:

The photocatalytic coating demonstrated strong antimicrobial properties against the three test bacteria; *S. aureus*, *E. coli,* and *S. enterica.*

Signatures:

Study Performed by:

Mona Ramadi, Ph.D.
Microbiologist

Date 11/09/2020

Report Issued by:

Jason Dobranic, Ph.D.
Vice President of Microbiology & Life Sciences
Study Director

Date 11/09/2020