Sanders Laboratories, Inc.
Environmental Testing Services

TECHNICAL REPORT

For

Jon Whittaker
Vexall Industries

ASTM E2149 - 2001
Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions – "Shaker Test"

Antimicrobial Activity of Treated Granite Tile against
Gram Positive *Staphylococcus aureus*

Analysis performed by:

John Limbach
Microbiology Technical Director

Report Approved by:

Jeff Walsh
Laboratory Operations Manager

Initiation Date: October 27, 2014
Completion Date: October 31, 2014
Sanders Laboratory Number 1101-2430

1. Materials Submitted for Testing:

Two samples of granite tile had been received by Sanders Laboratories for ASTM E2149 antimicrobial activity testing:

1. Untreated Granite Tile
2. Treated Granite Tile

2. Significance and Use:

The ASTM E2149 test method is designed to evaluate the resistance of non-leaching antimicrobial treated specimens to the growth of microbes under dynamic contact conditions. This dynamic shake flask test was developed for routine quality control and screening tests in order to overcome difficulties in using classical antimicrobial test methods to evaluate substrate-bound antimicrobials.

This test also allows for the versatility of testing contamination due to such things as hard water, proteins, blood, serum, various chemicals and other contaminates or physical/chemical stresses or manipulations of the specimens of interest. The antimicrobial activity of a substrate bound antimicrobial agent is dependent upon direct contact of microbes with the active chemical agent. This test determines the antimicrobial activity of treated specimens by shaking samples of surface bound materials in a concentrated bacterial suspension for a time period of 1 to 24 hours as specified by the supplier of the samples.

2.1 Surface antimicrobial activity is determined by comparing results from the test sample to simultaneously run controls.

2.2 The presence of a leaching antimicrobial is both pre and post-determined by the presence of a zone of inhibition. Vexall Industries chose to eliminate the leaching test.

2.3 Stresses may include laundry, wear and abrasion, radiation and steam sterilization, UV exposure, solvent manipulation, temperature susceptibility, dish washing soaps or similar physical or chemical manipulation.

3. Preparation of Bacterial Inoculum.

Staphylococcus aureus (ATCC #33591) was grown on a Brain Heart Infusion Agar slant for 24 hours and collected in sterile buffer. 200 mL of the diluted culture, was added to each of three 500 mL square plastic containers. One contained the control culture only. The second contained the untreated granite tile. The third contained the treated granite tile.

4. Preparation of the Test Specimen:

Each of the 4 inch by 4 inch tiles was tested with bromothymol blue before testing. The bromothymol blue washed out of the untreated granite sample and permanently stained the treated granite sample.

5. Testing Procedure:

A. Each tile sample was placed into a square 500 mL plastic container with a 200 mL dilute suspension of *Staphylococcus aureus.*
B. Each container was placed on the shaker for 24 hours, 36 hours and 72 hours to allow the antimicrobial agents to kill the bacteria in the suspension.
C. After 24 hours, 36 hours and 72 hours on the shaker, a 1 mL aliquot was taken from each of the containers. Dilutions were made and plated on Standard Methods Agar for counts of the remaining unkilled *Staphylococcus aureus.*

6. Evaluation of Results:

	Sample ID	cfu/mL *Staphylococcus aureus*
1.	Control Culture – **Initial Count**	1,100,000
	24 hours on Shaker	
2.	Control Culture	1,250,000
3.	Untreated Granite Tile	720,000
4.	Treated Granite Tile	<1
	36 hours on Shaker	
5.	Control Culture	1,500,000
6.	Untreated Granite Tile	600,000
7.	Treated Granite Tile	<1
	72 hours on Shaker	
8.	Control Culture	1,800,000
9.	Untreated Granite Tile	56,000
10.	Treated Granite Tile	<1

Calculation of the "Antibacterial Activity": This is the difference in the logarithm of the viable cell count found on an antimicrobial-treated product and a control culture after inoculation with, and incubation of, the bacteria. The following equation is used: $R = (U_t - U_o) - (A_t - U_o) = U_t - A_t$

Where: R = the "Antibacterial Activity"
U_o = the average of the common logarithm of the number of viable bacteria (bacteria/mL) recovered from the control test specimens immediately after inoculation.

U_t = the average of the common logarithm of the number of viable bacteria (bacteria/mL) recovered from the untreated test specimens after 24, 36 and 72 hours of contact.
A_t = the average of the common logarithm of the number of viable bacteria (bacteria/mL) recovered from the treated test specimens after 24, 36 and 72 hours of contact.

		Antibacterial Activity	% Kill
	24 hours on Shaker		
3.	Untreated Granite Tile	0.24	42.40%
4.	Treated Granite Tile	6.10	99.99%
	36 hours on Shaker		
6.	Untreated Granite Tile	0.40	60.00%
7.	Treated Granite Tile	6.18	99.99%
	72 hours on Shaker		
9.	Untreated Granite Tile	1.51	96.90%
10	Treated Granite Tile	6.26	99.99%

Antibacterial Activity	%Kill compared to control	Comment
<1.5	<96.8	poor
1.5 to 2.0	96.8 to 99.0	borderline
2.0 to 3.0	99.0 to 99.9	good
>3.0	>99.9	excellent

4. Conclusions:
Antibacterial testing under dynamic contact conditions on treated, unwashed Granite tile samples demonstrated excellent antibacterial activity against Gram Positive Staphylococccus aureus over a 24 hour to 72 hour period.