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## TECHNICAL REPORT

For

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Vexall Industries

ASTM E2149 - 2001

Determining the Antimicrobial Activity of Immobilized Antimicrobial  
Agents Under Dynamic Contact Conditions – “Shaker Test”

Antimicrobial Activity of treated carpet and tile against  
Gram Positive *Staphylococcus aureus*

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Sanders Laboratory Number 1101-2300 and 1101-2301

1. Materials Submitted for Testing:

Six samples of carpet and two samples of tile had been received by Sanders Laboratories for ASTM E2149 antimicrobial activity testing:

1. Treated Beige Carpet
2. Treated Brown Carpet
3. Treated Blue Carpet
4. Treated Tile
5. Untreated Beige Carpet
6. Untreated Brown Carpet
7. Untreated Blue Carpet
8. Untreated 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 contaminants 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 #6538 –MRSA) was grown on a Brain Heart Infusion Agar slant for 24 hours and collected in sterile buffer. 100 mL of the diluted culture, was added to a 500 mL control culture beaker and to eight additional 500 mL beakers each containing a carpet or tile sample.

4. Preparation of the Test Specimen:

Each sample was cut into a 2 inch by 2 inch square.

5. Testing Procedure:

- A. Each carpet or tile sample was placed upside down into a 500 mL beaker containing a 100 mL dilute suspension of *Staphylococcus aureus*.
- B. Each beaker 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 four 24 hours on the shaker, a 1 mL aliquot was taken from each of the beakers. Dilutions were made and plated on Standard Methods Agar for counts of the remaining unkilld *Staphylococcus aureus*.
- D. After 36 hours and 72 hours on the shaker, additional 1 mL aliquots were taken from each beaker and plated with Standard Methods Agar for counts of the remaining viable *Staphylococcus aureus*.

6. Evaluation of Results:

<u>Sample ID</u>	<u>cfu/mL <i>Staphylococcus aureus</i></u>
1. Control Culture – <b>Initial Count</b>	580,000
<b>24 hours on Shaker</b>	
2. Control Culture	290,000
3. Treated Beige Carpet	<1
4. Treated Brown Carpet	<1
5. Treated Blue Carpet	<1
6. Treated Tile	<1
7. Untreated Beige Carpet	450,000
8. Untreated Brown Carpet	1,300,000
9. Untreated Blue Carpet at	420,000
10. Untreated Tile	2,100,000

Sample ID	cfu/mL <i>Staphylococcus aureus</i>
<b>36 hours on Shaker</b>	
11. Control Culture	560,000
12. Treated Beige Carpet	110
13. Treated Brown Carpet	<1
14. Treated Blue Carpet	24
15. Treated Tile	4,100
16. Untreated Beige Carpet	170,000
17. Untreated Brown Carpet	15,000,000
18. Untreated Blue Carpet	5,200,000
19. Untreated Tile	2,600,000
<b>72 hours on Shaker</b>	
20. Control Culture	330,000
21. Treated Beige Carpet	52
22. Treated Brown Carpet	<1
23. Treated Blue Carpet	<1
24. Treated Tile	525
25. Untreated Beige Carpet	180,000
26. Untreated Brown Carpet	13,000,000
27. Untreated Blue Carpet	12,000,000
28. Untreated Tile	1,900,000

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.

	<u>Antibacterial Activity</u>	<u>% Kill</u>
<b>24 hours on Shaker</b>		
3. Treated Beige Carpet	5.76	99.99%
4. Treated Brown Carpet	5.76	99.99%
5. Treated Blue Carpet	5.76	99.99%
6. Treated Tile	5.76	99.99%
<b>36 hours on Shaker</b>		
12. Treated Beige Carpet	3.71	99.98%
13. Treated Brown Carpet	5.75	99.99%
14. Treated Blue Carpet	4.37	99.99%
15. Treated Tile	2.14	99.27%
<b>72 hours on Shaker</b>		
21. Treated Beige Carpet	3.80	99.98%
22. Treated Brown Carpet	5.52	99.99%
23. Treated Blue Carpet	5.52	99.99%
24. Treated Tile	2.80	99.84%

<u>Antibacterial Activity</u>	<u>%Kill compared to control</u>	<u>Comment</u>
<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

7. Conclusions:

Antibacterial testing under dynamic contact conditions on treated carpet and tile samples demonstrated excellent antibacterial activity against Gram Positive *Staphylococcus aureus* over a 24 hour to 72 hour period.