TECHNICAL REPORT

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

Antimicrobial Activity of treated sponges against Gram Negative Escherichia coli

Analysis performed by:

John Limbach
Microbiology Technical Director

Report Approved by:

Jeff Walsh
Laboratory Operations Manager

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1. Materials Submitted for Testing:

Three samples of kitchen sponges had been received by Sanders Laboratories for ASTM E2149 antimicrobial activity testing:
A. Sponges marked "A" treated with antimicrobial agent EV360™.
B. Sponges marked "B" also treated with antimicrobial agent EV360™ at a different concentration.
C. Sponges marked "R" containing the existing antibacterial agent.

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.

Escherichia coli (ATCC #8739) was grown on a Brain Heart Infusion Agar slant for 24 hours and collected in sterile buffer. 50 mL of the diluted culture, was added to a 125 mL control culture flask and to three additional 125 mL flasks each containing a sponge sample.

4. Preparation of the Test Specimen:

   A. Each sample was cut into a 1 1/2 inch by 1 1/2 inch square 3/8 inch thick.
   B. The three sponges were squeezed under tap water for 1 minute and then squeezed under DI water for 1 minute.

5. Testing Procedure:

   A. Each sponge sample was squeezed out and placed into a flask containing a dilute suspension of Escherichia coli. The bacterial load was 22,000,000 cfu/mL as in the previous test employing Staphylococcus aureus
   B. Each flask was placed on the shaker for 1 hour, 4 hours and 24 hours to allow the antimicrobial agents to kill the bacteria in the suspension.
   C. After 1 hour, 4 hours and 24 hours on the shaker, a 1 mL aliquot was taken from each of the flasks. Dilutions were made and plated on Standard Methods Agar for counts of the remaining unkilled Escherichia coli.

6. Evaluation of Results:

Sample ID	cfu/mL **Escherichia coli**
1. Control Culture – Initial	22,000,000
2. Control Culture – 1 hour shake	22,000,000
3. Sponge A – 1 hour shake	1,300
4. Sponge B – 1 hour shake	70,000
5. Sponge R – 1 hour shake	17,000,000
6. Control Culture – 4 hour shake	19,000,000
7. Sponge A - 4 hour shake	<1
8. Sponge B – 4 hour shake	<1
9. Sponge R - 4 hour shake	14,000,000
10.Control Culture – 24 hour shake	15,000,000
11.Sponge A – 24 hour shake	<1
12.Sponge B – 24 hour shake	<1
13.Sponge R – 24 hour shake	2,400,000

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 1 hour, 4 hours and 24 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 1 hour, 4 hours and 24 hours of contact.

	Antibacterial Activity	% Kill
1. Control Culture – Initial		
2. Control Culture – 1 hour shake		
3. Sponge A – 1 hour shake	4.23	99.99%
4. Sponge B – 1 hour shake	2.49	99.68%
5. Sponge R – 1 hour shake	0.11	22.73%
6. Control Culture – 4 hour shake		
7. Sponge A - 4 hour shake	7.28	100.00%
8. Sponge B – 4 hour shake	7.28	100.00%
9. Sponge R - 4 hour shake	0.13	26.32%
10. Control Culture – 24 hour shake		
11. Sponge A – 24 hour shake	7.18	100.00%
12. Sponge B – 24 hour shake	7.18	100.00%
13. Sponge R – 24 hour shake	0.80	84.00%

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

7. Conclusions:

A. Sponges "A" and "B" work very rapidly against the Gram Negative Escherichia coli bacteria.
B. Sponge "A" might be slightly better than sponge "B" at killing the Gram Negative Escherichia coli bacteria.
C. Sponge R worked very poorly against the Gram Negative Escherichia coli bacteria.
D. Sponges "A" and "B" turn the pH indicator Bromothymol Blue a blue color. The pH indicator Bromothymol Blue remains green when contacting Sponge "R".