

Eastern Polymer Industry Co., Ltd
ALP Aeroflex India Private Limited
Microbiological Analysis Report

Project #: 4253
Date Received: 1/23/2012
Date of Analysis: 1/24/2012

Subject:

Bacteria Resistance Test in Hydrophobic or Polymeric Surfaces using the ASTM E 2180 test method

Protocol

ASTM E 2180-01, Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) in Polymeric or Hydrophobic Materials, was strictly followed. Specifics of the test method applied to this project are described below.

Overview of Test Protocol

This test method is designed to evaluate (quantitatively) the antimicrobial effectiveness of agent(s) incorporated or bound into or onto mainly flat (two dimensional) hydrophobic or polymeric surfaces.

Specifics of the Test

Laboratory
Identification

4253

Sample Identification

Aeroflex/Aerocel

Test Organisms

Staphylococcus aureus ATCC # 6538

Klebsiella pneumoniae ATCC # 4352

Contact Time

0 and 24 hours

Neutralizing Broth

Formulation per Liter: Lecithin 3.43gr, Polysorbate (Tween 80) 10mL, 10% Aqueous Sodium Thiosulfate 5mL

Recovery Media

Tryptic Soy Agar

1/27/2012

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Test Protocol

Testing of each sample was conducted in triplicate. Samples were inoculated with 1.0 mL of a standardized culture of the test organism(s) in a molten agar slurry. The samples were incubated at $36 \pm 2^\circ\text{C}$ for 24 hours. Surviving microorganisms were recovered via elution of the agar slurry inoculum from the test sample into 10 mL neutralizing broth. Microbial counts of the samples were determined and the percent reduction of microorganisms was calculated according to the following equation:

$$\text{Percent reduction} = \frac{\text{Average Bacteria (Untreated Control Sample at T=24)} - \text{Avg. Bacteria (Test Sample at T=24hr)}}{\text{Avg. Bacteria (Untreated Control Sample at T=24)}} \times 100$$

Results

Tables 1-8 show the Bacteria Resistance Test results.

Conclusions:

The triplicate test samples [Aeroflex/Aerocel] showed 99.95% reduction after twenty-four hours contact time against *Staphylococcus aureus* and 96.29% reduction against *Klebsiella pneumoniae* in the ASTM E 2180-01 Test.

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Table 1: *Staphylococcus aureus* ATCC # 6538 inoculum per sample (1.0 milliliter)

Inoculum (2.5 mL added to 100 mL Agar Slurry)	Replicate cfu/mL recovered				Average cfu/sample
	cfu at 10 ⁻⁷ dil		cfu at 10 ⁻⁶ dil		
	13	14	183	160	3.83 x 10 ⁶

Table 2. *Staphylococcus aureus* ATCC #6538 vs. Untreated Control (glass cover slide)

Contact Time		<i>Staphylococcus aureus</i> ATCC #6538			
		Replicate cfu/sample recovered			
		cfu 10 ⁻⁵ dil	cfu 10 ⁻⁶ dil	Average per replicate	Average
T= 0	UTC A	37	5	4.35 x 10 ⁶	3.48 x 10 ⁶
		32	2	2.60 x 10 ⁶	
	UTC B	47	8	6.35 x 10 ⁶	5.03 x 10 ⁶
		34	4	3.70 x 10 ⁶	
	UTC C	35	7	5.25 x 10 ⁶	4.83 x 10 ⁶
		38	5	4.40 x 10 ⁶	
					4.45 x 10 ⁶
		cfu 10 ⁻⁵ dil	cfu 10 ⁻⁶ dil	Average per replicate	Average
T= 24 hours	UTC A	68	5	5.90 x 10 ⁶	6.18 x 10 ⁶
		58	7	6.45 x 10 ⁶	
	UTC B	60	8	7.00 x 10 ⁶	7.15 x 10 ⁶
		76	7	7.30 x 10 ⁶	
	UTC C	64	11	8.70 x 10 ⁶	7.95 x 10 ⁶
		74	7	7.20 x 10 ⁶	
					7.09 x 10 ⁶

UTC=Untreated Control

Table 3. *Staphylococcus aureus* ATCC #6538 vs. Aeroflex/Aerocel

Contact Time		<i>Staphylococcus aureus</i> ATCC #6538			
		Replicate cfu/sample recovered			
		cfu 10 ⁻² dil	cfu 10 ⁻³ dil	Average per replicate	Average
T= 24 hours	4253-A	40	8	6.00 x 10 ³	5.88 x 10 ³
		15	10	5.75 x 10 ³	
	4253-B	32	2	2.60 x 10 ³	2.45 x 10 ³
		36	1	2.30 x 10 ³	
	4253-C	15	2	1.75 x 10 ³	2.80 x 10 ³
		27	5	3.85 x 10 ³	
					3.71 x 10 ³

Table 4. Percent Reduction

Sample Identification	<i>Staphylococcus aureus</i> vs Control Broth at T=24
4253 Aeroflex/Aerocel	99.95%

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Table 5: *Klebsiella pneumoniae* ATCC # 4352 inoculum per sample (1.0 milliliter)

Inoculum (2.5 mL added to 100 mL Agar Slurry)	Replicate cfu/mL recovered				Average cfu/sample
	cfu at 10 ⁻⁷ dil		cfu at 10 ⁻⁶ dil		
	6	4	41	49	1.19 x 10 ⁶

Table 6. *Klebsiella pneumoniae* ATCC #4352 vs. Untreated Control (glass cover slide)

Contact Time		<i>Klebsiella pneumoniae</i> ATCC #4352			
		Replicate cfu/sample recovered			
		cfu 10 ⁻⁴ dil	cfu 10 ⁻⁵ dil	Average per replicate	Average
T= 0	UTC A	41	3	3.55 x 10 ⁵	3.55 x 10 ⁵
		51	2	3.55 x 10 ⁵	
	UTC B	29	4	3.45 x 10 ⁵	3.98 x 10 ⁵
		40	5	4.50 x 10 ⁵	
	UTC C	30	4	3.50 x 10 ⁵	3.75 x 10 ⁵
		40	4	4.00 x 10 ⁵	
					3.76 x 10 ⁵
		cfu 10 ⁻⁶ dil	cfu 10 ⁻⁷ dil	Average per replicate	Average
T= 24 hours	UTC A	65	4	5.25 x 10 ⁷	6.40 x 10 ⁷
		71	8	7.55 x 10 ⁷	
	UTC B	76	7	7.30 x 10 ⁷	7.88 x 10 ⁷
		79	9	8.45 x 10 ⁷	
	UTC C	81	12	1.01 x 10 ⁸	7.78 x 10 ⁷
		89	2	5.45 x 10 ⁷	
					7.35 x 10 ⁷

UTC=Untreated Control

Table 7. *Klebsiella pneumoniae* ATCC #4352 vs. Aeroflex/Aerocel

Contact Time		<i>Klebsiella pneumoniae</i> ATCC #4352			
		Replicate cfu/sample recovered			
		cfu 10 ⁻⁴ dil	cfu 10 ⁻⁵ dil	Average per replicate	Average
T= 24 hours	4253-A	608	75	6.79 x 10 ⁶	6.97 x 10 ⁶
		648	78	7.14 x 10 ⁶	
	4253-B	110	17	1.40 x 10 ⁶	1.08 x 10 ⁶
		102	5	7.60 x 10 ⁵	
	4253-C	13	1	1.15 x 10 ⁵	1.10 x 10 ⁵
		11	1	1.05 x 10 ⁵	
					2.72 x 10 ⁶

Table 8. Percent Reduction

Sample Identification	<i>Klebsiella pneumoniae</i> vs Control Broth at T=24
4253 Aeroflex/Aerocel	96.29%