

ANTIMICROBIAL TEST LABORATORIES

Microbiology Study Report NG4529-A1

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Client Information

Company Name:	<u>MicroShield 360</u>	Sponsor:	<u>Phil Kubec</u>
Phone Number:	<u>(216) 393-3945</u>	E-mail:	<u>Phil.kubec@microshield360.com</u>

Test Information

Test(s) Performed:	<u>AATCC 100 (Study ID NG4529-A1)</u>		
SOP Followed:	<u>Testing Facility Operation 01 0.1</u>	Performed by:	<u>M. Sawyer</u>

Test Substance Information

Test Substances:	<u>MicroShield 360 Full Strength, 1:5, 1:10, and 1:20</u>		
Date Received:	<u>19 SEP 2013</u>	Number Received:	<u>4</u>

Parameters

Microorganisms):	<u><i>S. epidermidis</i> ATCC 12228</u>	Type of Carrier:	<u>4.8 cm diameter swatches/41ayers</u>
Subculture#:	<u>1</u>	#of Replicates:	<u>2</u>
Growth Medium:	<u>Tryptic Soy Broth (TSB)</u>	Exposure Temp.:	<u>36.0 ± 1°C</u>
Dilution Medium:	<u>Phosphate Buffered Saline (PBS)</u>	Diluent Supplement:	<u>0.196 Triton</u>
Enumeration Medium:	<u>Tryptic Soy Agar (TSA)</u>	Contact Time:	<u>10 Minutes, 1 Hour, and 24 Hours</u>
Inoculum Volume:	<u>1.0 ml</u>	Neutralizer Used:	<u>Dey/Engley (D/E) Broth, 10 ml</u>
Plate Inc. Time:	<u>48 Hours</u>	Plate Incubation:	<u>36.0 ± 1°C</u>

Controls

Neutralized:	<u>Passed (D/E Broth)</u>	Growth Control:	<u>Passed</u>
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Test Results

Test(s) Valid?:	<u>See Below</u>	Confirmation:	<u>Morphology on TSA</u>
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Notes: Test samples were cut to 4.8 cm diameter swatches, 4 swatches were placed, per carrier, into sterile Petri dishes. An overnight culture of test microorganism was diluted directly into PBS, supplemented with 0.196 Triton, to create the test inoculum. A 1.0 ml volume of the test inoculum was added to each carrier at a target concentration of $\sim 1.5 \times 10^5$ CFU/ml. Carriers were incubated for 10 minutes, 1 hour, or 24 hours then harvested in 10.0 mL of D/E broth. Standard dilution and plating techniques were used for enumeration. Calculations were compared to an ATL provided Control at time zero as the Study-Sponsor Untreated Control showed antimicrobial activity. Sample sterility controls showed slight bacterial growth on all test samples at time zero. Counts were performed with careful attention to morphology and this is not thought to have affected study results.

Tests Completed:	<u>26 SEP 2013</u>	Report Sent:	<u>27 SEP 2013</u>
		Amended Report Sent:	<u>01 OCT 2013</u>

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AATCC 100 Results

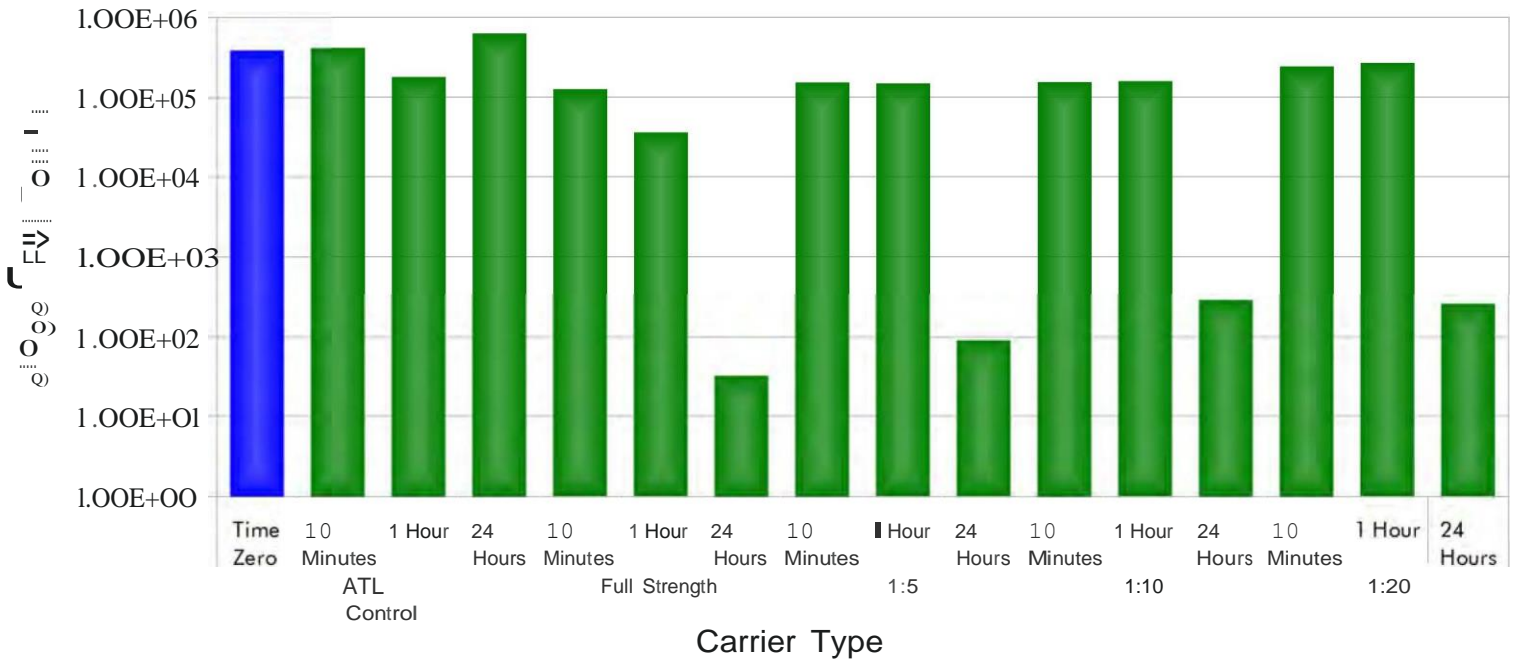
Test Microorganism	Carrier Type	Time Point	Replicate	Replicate CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to ATL Control at Time Zero	Log Reduction Compared to ATL Control at Time Zero		
<i>S. epidermidis</i> ATCC 12228	ATL Control	Time Zero	1	4.05E+05	3.85E+05	N/A			
			2	3.65E+05					
		10 Minutes	1	4.90E+05	4.10E+05				
			2	3.30E+05					
		1 Hour	1	2.20E+05	1.78E+05				
			2	1.35E+05					
		24 Hours	1	6.10E+05	6.25E+05				
			2	6.40E+05					
	Full Strength	10 Minutes	1	1.33E+05	1.25E+05	67.66%	0.49		
			2	1.16E+05					
		1 Hour	1	1.65E+04	3.58E+04	90.71%	1.03		
			2	5.50E+04					
		24 Hours	1	1.50E+01	3.25E+01	99.992%	4.07		
			2	5.00E+01					
	1:5	10 Minutes	1	1.50E+05	1.52E+05	60.52%	0.40		
			2	1.54E+05					
		1 Hour	1	1.55E+05	1.47E+05				
			2	1.38E+05					
		24 Hours	1	8.00E+01	9.00E+01			99.98%	3.63
			2	1.00E+02					
	1:10	10 Minutes	1	1.42E+05	1.53E+05	60.26%	0.40		
			2	1.64E+05					
		1 Hour	1	1.42E+05	1.57E+05			59.22%	0.39
			2	1.72E+05					
24 Hours		1	2.80E+02	2.90E+02	99.92%			3.12	
		2	3.00E+02						
1:20	10 Minutes	1	2.16E+05	2.40E+05	37.66%	0.21			
		2	2.64E+05						
	1 Hour	1	2.66E+05	2.65E+05			31.17%	0.16	
		2	2.64E+05						
	24 Hours	1	4.50E+01	2.58E+02			99.93%	3.17	
		2	4.70E+02						

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AATCC 100 Results



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Results

Test Microorganism	Carrier Type	Time Point	Replicate	Replicate CFU/Carrier	Average CFU/Carrier	Percent Change Compared to ATL Control at Time Zero	Log Change Compared to ATL Control at Time Zero
<i>S. epidermidis</i> ATCC 12228	ATL Control	Time Zero	1	4.05E+05	3.85E+05	N/A	
			2	3.65E+05			
		10 Minutes	1	4.90E+05	4.10E+05	6.49%	0.03
			2	3.30E+05			
		1 Hour	1	2.20E+05	1.78E+05	-53.90%	-0.34
			2	1.35E+05			
		24 Hours	1	6.10E+05	6.25E+05	62.34%	0.21
			2	6.40E+05			

Photographs



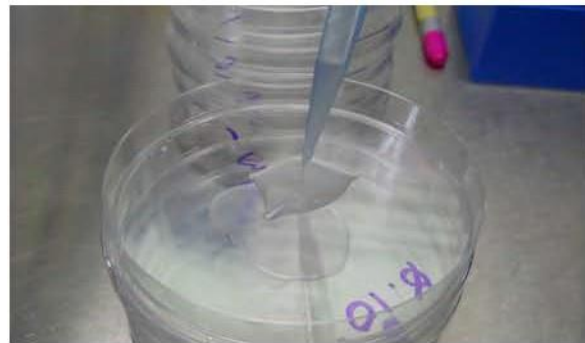
Left: Carriers being Inoculated with Test Microorganism



Right: Harvested Carrier in 10.0 ml of D/E Broth (Neutralizer)



Left: Samples were still "wet" with Inoculum after contact time



Right: Samples were harvested using standard dilution and plating techniques

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Additional Information

Sample Calculations

$$\% \text{ Reduction} = 100 \times (A-B)/A$$

Where:

A = Number of bacteria on the control carrier at time zero (Immediately after inoculation)

B = Number of bacteria on the test carriers after the contact time

$$\text{Log Reduction} = \text{Log } (A/B)$$

Where:

A = Number of bacteria on the control carrier at time zero (Immediately after inoculation)

B = Number of bacteria on the test carriers after the contact time

$$\% \text{ Change} = 100 \times (B-A)/A$$

Where:

A = Number of bacteria on the control carrier at time zero (Immediately after inoculation)

B = Number of bacteria on the test carriers after the contact time

$$\text{Log Change} = \text{Log } (B/A)$$

Where:

A = Number of bacteria on the control carrier at time zero (Immediately after inoculation)

B = Number of bacteria on the test carriers after the contact time