



ACCUGEN LABORATORIES, INC.

FINAL REPORT

ASTM E 2180

Standard Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Material Designation: E 2180 – 07 (2012)

TEST AGENT

Marmoleum with Topshield2

SAMPLE TYPE

3x3 cm square pcs greenish/golden color with shiny surface

Laboratory Number

118913

Testing Laboratory

Accugen Laboratories, Inc.
50 West 75th street, Ste 209
Willowbrook, IL 60527
Tel: 630-789-8105
Toll free: 800-282-7102
Fax: 630-789-8104
Web address: www.accugenlabs.com
E-mail: info@accugenlabs.com

Sponsor

Forbo Flooring Systems
8 Maplewood Dr
Hazleton, PA 18202
Contact: Becky Hudock
Phone: 570-450-0316
Fax: 570-450-0330
E-mail: becky.hudock@forbo.com

Test Reported

03-28-17

TABLE OF CONTENTS

FINAL REPORT - COVER PAGE	1- 2
TABLE OF CONTENTS	3
SCOPE.....	4
TEST SUMMARY.....	4
TEST CONDITIONS.....	4-5
STUDY DATES AND FACILITIES.....	6
RECORDS TO BE MAINTAINED.....	6
TEST METHOD.....	6-7
RESULTS.....	8-16
CONCLUSIONS.....	16

TITLE: ASTM E 2180- Standard Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Material

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

SUMMARY: Samples were tested following ASTM E2180 test method. A thin layer of the inoculated agar slurry was pipetted onto the test and untreated control material in triplicate. After the specified contact, surviving microorganisms were recovered into neutralizing broth. Serial dilutions were made, and colonies from each dilution series were counted and recorded. Percent reduction of organism from treated and untreated samples was calculated.

TEST MATERIALS: Marmoleum with Topshield 2



TEST CONDITIONS:

Challenge Organisms: Escherichia coli ATCC# BAA 2452-CRE
 Methicillin Resistant Staphylococcus aureus (MRSA) ATCC#3359

Staphylococcus aureus ATCC# 6538
Klebsiella pneumoniae ATCC# 4352
Pseudomonas aeruginosa ATCC# 9027
Salmonella cholerasuis ATCC # 10708
Bacillus cereus ATCC# 11778
Stachybotrys chartarum ATCC# 9182
Escherichia coli ATCC# 8739
Proteus vulgaris ATCC# 33420
Bacillus subtilis ATCC# 6633
Trichophyton mentagrophyte ATCC# 9533
Vancomycin resistant enterococcus faecalis ATCC#51299
Sacharomyces cerevisiae ATCC# 9763
Clostridium difficle ATCC# 43598
Aspergillus brasiliensis ATCC# 16404

Neutralizer used: Dey Engley (DE)
Contact time: 24 hours
Contact temperature: 35-± 2°C aerobically for aerobic bacteria, anaerobically for anaerobic bacteria and 25°C for yeast and mold
Negative Control: Buffer without organism.

Reference:

ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, ASTM E 2180-07 (2012)

Media and reagents:

- Sabaroud Dextrose Agar
- Tryptic Soy Agar
- Neutralizing Broth- DE neutralizing broth
- Agar-agar.
- NaCl.
- Sterile Deionized Water.
- Phosphate buffer
- Columbia Agar

- Reinforced clostridium media
- MSA
- Cetrimide
- MacConkey Agar
- MacConkey broth
- XLD
- Tryptic soy broth
- Rappaport Vasiallades Salmonella enrichment broth
- Potato dextrose agar
- biochemical reagents
- Gram stain Reagents

Apparatus:

- Erlenmeyer Flask, 250 mL.
- Petri Dishes, (15 X100 mm), sterile.
- Colony Counter.
- Pipetters, (1000 µL)
- Pipette Tips, sterile.
- Test Tubes, 16 x 100 mm.
- Incubator, set at required temperature (35± 2°C and 25 °C).
- Autoclave.
- Water Bath, capable of maintaining water at 45 ± 2°C.
- Sterile Cotton Swabs.
- Vortex Mixer.
- pH Meter.
- Hot Plate, with stirrer.
- Spectrophotometer, set at 600 nm.

STUDY DATES AND FACILITIES:

The laboratory phase of this test was performed at ACCUGEN LABORATORIES, INC, 50 West 75th Street, Willow brook, IL 60527. Study was initiated on 11/18/16. The study completion date is the date the study director signed the final report which is 03/28/2017

All analytical data and reports are client confidential and available only to the client. Authorization for publication of excerpt, statements, or conclusions regarding our reports is reserved pending written approval from Accugen, Laboratories, Inc.

RECORDS TO BE MAINTAINED:

All testing data, test material records, the final report, and correspondence will be stored in the archives.

TEST METHOD:

After three bacterial and fungal subcultures, 24 hr bacterial culture and 96 hr fungal culture was grown on growth media. The agar slurry was prepared by dissolving 0.85 g NaCl and 0.3 g agar-agar in 100 mL of deionized water. The treated and control test samples were cut into 3x3 cm squares and placed into sterile petri dishes in triplicate and sterilized with 70% alcohol. Organism broth cultures were adjusted to a concentration of 1-5 x 10⁸ cells/mL. Surface of sample was pre-wet with a cotton swab dipped in sterile 0.85 % saline to disperse the agar slurry evenly on the sample. One mL of standardized culture (1-5x10⁸ cells/mL) was added into the 100-mL agar slurry equilibrated at 45 °C. That made final concentration of 1-5 x10⁶cells/mL in the molten agar slurry. 1 mL of inoculated agar slurry was placed onto the test and control samples. The agar slurry inoculum was allowed to gel and then placed the samples in an incubator at 35⁰C for 24 hours. Serial dilutions of the agar slurry were made immediately from “0” h control samples and each dilution was plated to determine cfu/mL recoverable at time “0 h.”

Following the specified contact time, the incubation period control samples and incubation period treated samples were aseptically removed from the petri dishes to 120 mL container containing a sufficient volume of neutralizing broth to form an initial 1:10 dilution of the original inoculum. Samples were vortexed for 1 min of vigorous mechanical vortexing. The test surface was imprint cultured onto tryptic soy agar following vortexing to determine release efficiency of the inoculum from the treated surface. Serial dilutions were made from recovered slurry, plated and incubated at 35± 2°C aerobically for aerobic bacteria, anaerobically for anaerobic bacteria for 48 hours and 25±2°C for fungus for 5 days.

CALCULATION:

The geometric mean of the number of organisms recovered from the triplicate incubation period control and incubation period treated samples were calculated and percent reduction was determined by following formula.

Percent Reduction: % reduction = $\frac{a-b}{A} \times 100$

a = the antilog geometric mean of the number of organisms recovered from the incubation period control samples

b = geometric mean of the number of organisms recovered from the incubation period treated samples.

RESULTS:

Colony forming units (CFU) – after control and test sample were exposed to organisms.

Colony forming units are based on the average of three plate counts.

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	MRSA ATCC# 3359 *(Avg)	Percent Reduction
Lab Control	0Hr	-	1	3.0X10 ⁶	2.96x10 ⁶	-
			2	2.7X10 ⁶		
			3	3.2x10 ⁶		
Lab Control	24Hr	-	1	1.03X10 ⁷	1.29x10 ⁷	-
			2	1.46X10 ⁷		
			3	1.38x10 ⁷		
11891 3	24Hr	Marmoleum with Topshield 2	1	1.29x10 ⁵	1.656x10 ⁵	98.7162%
			2	2.14x10 ⁵		
			3	1.54x10 ⁵		

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Staphylococcus aureus ATCC# 6538 *(Avg)	Percent Reduction
Lab Control	0Hr	-	1	2.3X10 ⁶	2.23x10 ⁶	-
			2	2.0X10 ⁶		
			3	2.4x10 ⁶		
Lab Control	24Hr	-	1	5.1X10 ⁶	5.2x10 ⁶	-
			2	6.3X10 ⁶		
			3	4.2x10 ⁶		
118913	24Hr	Marmoleum with Topshield2	1	5.6x10 ⁵	5.46x10 ⁵	89.5%
			2	6.1x10 ⁵		
			3	4.7x10 ⁵		

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Escherichia coli ATCC# BAA 2452	Percent Reduction
Lab Control	0Hr	-	1	3.9X10 ⁶	3.7x10 ⁶	-
			2	3.5X10 ⁶		
			3	3.7x10 ⁶		
Lab Control	24Hr	-	1	4.5X10 ⁷	4.56x10 ⁷	-
			2	4.8X10 ⁷		
			3	4.4x10 ⁷		
118913	24Hr	Marmoleum with Topshield2	1	0	0	≥99.99%
			2	0		
			3	0		

All analytical data and reports are client confidential and available only to the client. Authorization for publication of excerpt, statements, or conclusions regarding our reports is reserved pending written approval from Accugen, Laboratories, Inc.

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Pseudomonas aeruginosa ATCC# 9027 *(Avg)	Percent Reduction
Lab Control	0Hr	-	1	9.4X10 ⁶	9.56x10 ⁶	-
			2	9.7X10 ⁶		
			3	9.6x10 ⁶		
Lab Control	24Hr	-	1	5.7X10 ⁷	5.8x10 ⁷	-
			2	5.9X10 ⁷		
			3	5.8x10 ⁷		
11891 3	24Hr	Marmoleum with Topshield2	1	9.6x10 ⁷	9.4x10 ⁷	0%
			2	9.2x10 ⁷		
			3	9.4x10 ⁷		

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Salmonella enterica ATCC# 10708 *(Avg)	Percent Reduction
Lab Control	0Hr	-	1	2.1X10 ⁷	2.13x10 ⁷	-
			2	2.4X10 ⁷		
			3	1.9x10 ⁷		
Lab Control	24Hr	-	1	3.0X10 ⁷	2.76x10 ⁷	-
			2	2.8X10 ⁷		
			3	2.5x10 ⁷		
11891 3	24Hr	Marmoleum with Topshield2	1	5.7x10 ⁸	5.16x10 ⁶	81.3043%
			2	4.8x10 ⁸		
			3	5.0x10 ⁸		

All analytical data and reports are client confidential and available only to the client. Authorization for publication of excerpt, statements, or conclusions regarding our reports is reserved pending written approval from Accugen, Laboratories, Inc.

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Bacillus cereus ATCC# 11778 *(Avg)	Percent Reduction
Lab Control	0Hr	-	1	7.0X10 ⁶	6.8x10 ⁶	-
			2	6.6X10 ⁶		
			3	6.8x10 ⁶		
Lab Control	24Hr	-	1	2.6X10 ⁶	1.69x10 ⁸	-
			2	1.9X10 ⁶		
			3	1.68x10 ⁶		
118913	24Hr	Marmoleum with Topshield2	1	0	0	≥99.99%
			2	0		
			3	0		

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Escherichia coli ATCC# 8739 *(Avg)	Percent Reduction
Lab Control	0Hr	-	1	1.20X10 ⁵	1.21x10 ⁵	-
			2	1.24 X10 ⁵		
			3	1.19x10 ⁵		
Lab Control	24Hr	-	1	2.13X10 ⁶	2.48x10 ⁶	-
			2	2.91X10 ⁶		
			3	2.40x10 ⁶		
118913	24Hr	Marmoleum with Topshield2	1	3.8x10 ⁶	3.5x10 ⁶	0%
			2	3.3x10 ⁶		
			3	3.4x10 ⁶		

All analytical data and reports are client confidential and available only to the client. Authorization for publication of excerpt, statements, or conclusions regarding our reports is reserved pending written approval from Accugen, Laboratories, Inc.

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Stachybotrys chartarum ATCC# 9182 *(Avg)	Percent Reduction
Lab Control	0Hr	-	1	1.9 x10 ⁵	2.1x10 ⁵	-
			2	2.3 x10 ⁵		
			3	2.1x10 ⁵		
Lab Control	24Hr	-	1	1.3 x10 ⁶	1.2x10 ⁶	-
			2	1.0x10 ⁶		
			3	1.3x10 ⁶		
11891 3	24Hr	Marmoleum with Topshield 2	1	3.8x10 ³	4.0x10 ³	99.666%
			2	4.1x10 ³		
			3	4.1x10 ³		

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Proteus vulgaris ATCC# 33420 *(Avg)	Percent Reduction
Lab Control	0Hr	-	1	6.8X10 ⁶	6.56x10 ⁶	-
			2	6.4X10 ⁶		
			3	6.5x10 ⁶		
Lab Control	24Hr	-	1	7.6X10 ⁷	7.66x10 ⁷	-
			2	7.8X10 ⁷		
			3	7.6x10 ⁷		
11891 3	24Hr	Marmoleum with Topshield2	1	4.5x10 ²	4.6x10 ²	99.99%
			2	4.7x10 ²		
			3	4.6x10 ²		

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Klebsiella pneumoniae ATCC# 4352*(Avg)	Percent Reduction
Lab Control	0Hr	-	1	3.5X10 ⁶	3.6x10 ⁶	-
			2	3.8X10 ⁶		
			3	3.5x10 ⁶		
Lab Control	24Hr	-	1	7.8X10 ⁷	7.6x10 ⁷	-
			2	7.4X10 ⁷		
			3	7.6x10 ⁷		
118913	24Hr	Marmoleum with Topshield2	1	5.7x10 ⁷	5.8x10 ⁷	23.68%
			2	5.9x10 ⁷		
			3	5.8x10 ⁷		

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Trichophyton mentagrophyte ATCC# 9533 *(Avg)	Percent Reduction
Lab Control	0Hr	-	1	2.7X10 ⁵	2.63x10 ⁵	-
			2	2.9X10 ⁵		
			3	2.3x10 ⁵		
Lab Control	24Hr	-	1	3.7X10 ⁵	3.2x10 ⁵	-
			2	1.9X10 ⁵		
			3	4.0x10 ⁵		
118913	24Hr	Marmoleum with Topshield2	1	1.4x10 ³	2.26x10 ³	99.29237%
			2	3.4x10 ³		
			3	2.0x10 ³		

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Clostridium difficile ATCC# 43598*(Avg)	Percent Reduction
Lab Control	0Hr	-	1	1.4x10 ⁶	1.8x10 ⁶	-
			2	1.8x10 ⁶		
			3	2.2x10 ⁶		
Lab Control	24Hr	-	1	4.6x10 ⁵	5.16x10 ⁵	-
			2	4.5x10 ⁵		
			3	6.4x10 ⁵		
11958 2	24Hr	Marmoleum with Topshield2	1	2.3x10 ⁸	2.4x10 ⁵	53.488%
			2	3.0x10 ⁸		
			3	1.9x10 ⁸		

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Sacharomyces cerevisiae ATCC# 9763*(Avg)	Percent Reduction
Lab Control	0Hr	-	1	3.7X10 ⁵	3.5x10 ⁵	-
			2	3.5X10 ⁵		
			3	3.2x10 ⁵		
Lab Control	24Hr	-	1	3.3X10 ⁵	3.33x10 ⁵	-
			2	3.1X10 ⁵		
			3	3.6x10 ⁵		
11891 3	24Hr	Marmoleum with Topshield2	1	2.7x10 ⁵	2.33x10 ⁵	30.03%
			2	2.5x10 ⁵		
			3	1.8x10 ⁵		

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Vancomycin resistant enterococcus faecalis ATCC#51299 (Avg)	Percent Reduction
Lab Control	0Hr	-	1	1.86X10 ⁶	1.876x10 ⁶	-
			2	1.92X10 ⁶		
			3	1.85x10 ⁶		
Lab Control	24Hr	-	1	4.9X10 ⁶	4.33x10 ⁶	-
			2	2.8X10 ⁶		
			3	5.3x10 ⁶		
11891 3	24Hr	Marmoleum with Topshield2	1	2.9x10 ⁴	3.1x10 ⁴	99.284%
			2	4.6x10 ⁴		
			3	1.8x10 ⁴		

Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Bacillus subtilis ATCC# 6633 *(Avg)	Percent Reduction
Lab Control	0Hr	-	1	2.7X10 ⁶	2.6x10 ⁶	-
			2	2.5X10 ⁶		
			3	2.6x10 ⁶		
Lab Control	24Hr	-	1	1.83X10 ⁸	1.83x10 ⁸	-
			2	1.85X10 ⁸		
			3	1.81x10 ⁸		
11891 3	24Hr	Marmoleum with Topshield2	1	9.1x10 ⁶	8.6x10 ⁶	95.30%
			2	9.1x10 ⁶		
			3	7.6x10 ⁶		

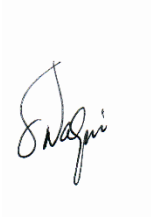
Lab #	Contact Time	Sample Identification	Replica	cfu/ml	Aspergillus brasiliensis ATCC# 16404*(Avg)	Percent Reduction
Lab Control	0Hr	-	1	4.0X10 ⁵	4.03x10 ⁵	-
			2	4.3X10 ⁵		
			3	3.8x10 ⁵		
Lab Control	24Hr	-	1	3.5X10 ⁵	3.33x10 ⁵	-
			2	3.5X10 ⁵		
			3	3.0x10 ⁵		
118913	24Hr	Marmoleum with Topshield2	1	2.9x10 ⁵	3.36x10 ⁵	0%
			2	3.6x10 ⁵		
			3	3.6x10 ⁵		

CONCLUSION:

Test agent Marmoleum with Topshield 2 showed **0% antimicrobial activity** against Escherichia coli ATCC#8739, Aspergillus brasiliensis ATCC# 16404 and Pseudomonas aeruginosa ATCC # 9027

Test agent Marmoleum with Topshield 2 showed **≥99.99% antimicrobial activity** against Bacillus cereus ATCC# 11778 and Escherichia coli ATCC# BAA 2452

Test agent Marmoleum with Topshield 2 showed **89.5%** antimicrobial activity against Staphylococcus aureus ATCC# 6538, **98.7162%** antimicrobial activity against MRSA ATCC# 3359, **89.5%** antimicrobial activity against Salmonella enterica ATCC# 10708, **95.30%** antimicrobial activity against Bacillus subtilis ATCC# 6633, **99.29237%** antimicrobial activity against Trichophyton mentagrophyte ATCC# 9533, **99.99%** antimicrobial activity against Proteus vulgaris ATCC# 33420, **23.68%** antimicrobial activity against Klebsiella pneumoniae ATCC# 4352, **30.03%** antimicrobial activity against Sacharomyces cerevisiae ATCC# 9763, **99.284%** antimicrobial activity against Vancomycin resistant enterococcus faecalis ATCC#51299, **53.488%** anti-microbial activity against Clostridium difficile ATCC# 43598 and **99.6666%** anti-microbial activity against Stachybotrys chartarum ATCC# 9182.



T. Naqvi M. S Microbiology, M (ASCP). Study Director.

