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TESTING LABORATORY

ACCUGEN LABORATORIES,INC.

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Accugen Laboratories, Inc., founded in 1997, is a FDA registered, independent contract microbiology laboratory. We offer full microbiological testing and analyze products from a wide variety of industries. Our microbiological testing laboratory is comprised of a highly experienced team of microbiologists who are experts in testing ASTM, AOAC, AATCC, FDA, EPA, USDA, USP, CTFA, JIS, ISO and other methods of analysis. Our competent professionals have decades of experience in routine microbiological analysis, special microbiology, research microbiology, and a variety of other microbiological testing. We are considered leading authorities in microbial testing.

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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 – 18

TEST AGENT

Order #:12187**,**

Sample #:73732**, Sample Test Id-** 212220 **Sample Name:** Wood panel Coating with UV Coating (1) – (Epoxy acrylate with antimicrobial additive)

Sample #:73733 **Sample Test Id-** 212221 **Sample Name:** Wood panel Coating with UV Coating (2)- Polyester acrylate without antimicrobial additive.

STUDY REQUIREMENTS

Research Purpose

TEST ORDERED

ASTM E 2180-18

SPONSOR

Mid-America Protective Coatings

85 W Industrial Rd

Addison, IL 60131

Contact: Len Borozin

Phone: (630) 628-4501

E-mail: lborozin@midamcoat.com

TESTING LABORATORY

Accugen Laboratories, Inc.

2121 W Army Trail Road

Addison, IL 60101

Tel: 630-789-8105, Toll free: 800-282-7102

Web: www.accugenlabs.com E-mail: info@accugenlabs.com

DATE SAMPLE RECEIVED:03/06/2023

DATE TEST STARTED: 03/06/2023.

TEST COMPLETED: 03/14/2023.

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SUMMARY

TITLE:

ASTM E 2180-18

TEST AGENT:

1. Wood panel Coating with UV Coating (1) – (Epoxy acrylate with antimicrobial additive)
2. Wood panel Coating with UV Coating (2)- Polyester acrylate without antimicrobial additive

SAMPLE TYPE:

Wood 3 x 3 cm Pieces

SAMPLE PREPERATION:

Ready to use

INOCULATED AGAR SLURRY AMOUNT:

1 mL

SOLID MEDIA & DILUENT USED:

- Phosphate buffer

Lot# 022823-PBS-01**Exp:** 07/28/2023

- TSA

Lot#US114187A-022823-01**Exp:**07/28/2023

- Slurry

Lot#030623-slurry-01**Exp:**08/06/2023

NEUTRILIZER:

- D/E Neutralizing Broth

Lot#US113842A-022823-01Exp:03/28/2023

CHALLENGE MICROORGANISMS:

- Staphylococcus aureus ATCC 6538

REAGENTS:

- Gram stain kit
- Biochemicals (catalase, coagulase, indole, & Oxidase)

CONTACT TIME:

24 hours

CONTACT TEMPERATURE:

35^0C± 2

INCUBATION CONDITION:

35^0C± 2 Aerobically

GROWTH PROMOTION TEST: Pass

PERSONNEL PARTICIPATED IN STUDY:

Zafar Mirza. MD Shamim Rizvi B.S

RESULTS:

Antimicrobial Activity against

Test Id- 212220 =S. aureus ≥ 99.99%

Test Id 212221= S. aureus 82.0239%

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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 bacterial colonies from each dilution series were counted and recorded. Percent reduction of bacteria from treated versus untreated samples was calculated.

DEFINITIONS:

N/A- Not Applicable **TNTC**- Too Numerous To Count **CFU**- Colony Forming Units
PO#- Purchase Order Number **LOG**- Logarithm

Apparatus:

- Sterile Glass Bottles, 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
- 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.

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- Aerobic System.

DISCLOSURE

Information on the identity, strength, stability, Method uniformity and validity, and dose solution analysis of the test agent resides with the sponsor of the study. The results apply to the sample as received and tested.

TESTING FACILITY:

Studies were conducted by Accugen Laboratories, Inc located at 2121 W Army Trail Road, Addison IL 60101

METHOD REFERENCE:

ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, ASTM E 2180-18

RECORDS:

All testing data, test material records, final reports, and any correspondence will be stored in the archives, including but not limited to the following.

- Data for Growth Promotion and Media Qualification
- Data for Microbial Enumeration and activity results form
- Data for Media, Reagents and Equipment Used

TEST METHOD:

- 18 hours old 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. One agar slurry was prepared for one organism tested.
- The treated and control test samples were measured and uniformed into size 3 x 3 cm.
- The treated and control test samples were placed into sterile petri dishes in triplicate.
- Organism culture suspensions was prepared in broth and adjusted to a concentration of 1-5 x 10^8 cells/mL with a spectrophotometer.
- 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.

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- One mL of standardized culture (1-5x10^8 cells/mL) was added into the 100-mL agar slurry equilibrated at 45 °C. That made final concentration of 1-5 x10^6cells/mL in the molten agar slurry.
- 0.5 - 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 contact temperature for contact time.
- Serial dilutions of the agar slurry were made immediately from “0” h control samples and each dilution were spread 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 on the media following vortexing to determine release efficiency of the inoculum from the treated surface.
- Serial dilutions were made from recovered slurry, plated, and incubated.

STUDY CONTROLS:

PURITY CONTROL:

Test organism was streaked on growth media, incubated at appropriate temperature and time. Observed to confirm the presence of a pure culture. Characteristics like Gram stain reaction and colony morphology were confirmed to assure the purity of test microorganisms. Test organisms was found pure.

STERILITY CONTROLS:

Sterility of all regents including diluents, growth media, and neutralizer were checked alongside the test. No growth was observed.

GROWTH PROMOTION TEST:

To determine that the media used will support the growth of test organisms, growth promotion tests were carried out. Less than 100 cfu of culture was inoculated into growth media. Media which passed the growth promotion tests were used to carry out the test.

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CALCULATION:

- Determine the geometric mean of the number of organisms recovered from the triplicate incubation period control and incubation period treated samples by the following equation:

$$\text{geometric mean} = \frac{(\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_3)}{3} \quad (1)$$

where:

X = number of organisms recovered from the incubation period control or incubation period treated samples.

- *Percent Reduction*—Use the following equation to calculate the percent reduction:

$$\% \text{ reduction} = \frac{(a - b) \times 100}{a} \quad (2)$$

where:

a = the antilog of the 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

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RESULTS:

Test Id#: 212220 Sample Name: Wood panel Coating with UV Coating (1) – (Epoxy acrylate with antimicrobial additive)

Test Id#212220	Replicate	Recovered Organisms CFU/mL	Log_{10} of Recovered Organisms	Geometric mean	Antilog of Geometric mean	% Reduction	Log Reduction
Control at 0 h (count immediately after inoculation from the untreated test specimens)	X_1	9.2×10^7	7.96	7.71	52018730.92	--	--
	X_2	5.1×10^7	7.70				
	X_3	3.0×10^7	7.47				
a-Control at 24 hours (Count from the untreated test specimens)	X_1	1.25×10^8	8.09	8.13	135339793.32	--	--
	X_2	1.34×10^8	8.12				
	X_3	1.48×10^8	8.17				
Test Id-212220 24 hours (after incubation period) Staphylococcus aureus ATCC# 6538	X_1	<10	1	1	10	≥99.99%	7.13
	X_2	<10	1				
	X_3	<10	1				

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Test Id#: 212221, **Sample Name:** Wood panel Coating with UV Coating (2)- Polyester acrylate without antimicrobial additive

Lab#212221	Replicate	Recovered Organisms CFU/mL	Log_{10} of Recovered Organisms	Geometric mean	Antilog of Geometric mean	% Reduction	Log Reduction
Control at 0 h (count immediately after inoculation from the untreated test specimens)	X_1	9.2×10^7	7.96	7.71	52018730.92	--	--
	X_2	5.1×10^7	7.70				
	X_3	3.0×10^7	7.47				
a-Control at 24 hours (count from the untreated test specimens)	X_1	1.25×10^8	8.09	8.13	135339793.32	--	--
	X_2	1.34×10^8	8.12				
	X_3	1.48×10^8	8.17				
Test Id-212221 24 hours (After incubation period) Staphylococcus aureus ATCC# 6538	X_1	3.0×10^7	7.47	7.38	24328807.98	82.0239%	0.75
	X_2	2.0×10^7	7.30				
	X_3	2.4×10^7	7.38				

CONCLUSION

Test Agent /Test Id-212220 showed 99.99% antimicrobial activity against Staphylococcus aureus ATCC#6538 at 24-hour contact time.

Test Agent/ Test Id-212221 showed 82.0239 % antimicrobial activity against Staphylococcus aureus ATCC#6538 at 24-hour contact time.

Approved
1:58 pm CT, Mar 15, 2023

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

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