ACCUGEN LABORATORIES,INC.

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.

ACCUGEN LABORATORIES, INC.

FINAL REPORT

ISO 846
Plastics - Evaluation of the action of microorganisms- Resistance to Fungi & Bacteria Test method

TEST AGENT
Arcoplast Engineered Polymer 9.5mm Lot# Ref# 12262

LABORATORY NUMBER
130820

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TEST STARTED
04-20-18

TEST COMPLETED
06-04-18

TABLE OF CONTENTS

TITLE: Plastics - Evaluation of the action of microorganisms- Resistance to Fungi & Bacteria Test method

SCOPE: This International Standard ISO 846 is used to determine the deterioration of plastics due to action microorganisms.

SUMMARY: Test specimen was exposed to a mixed suspension of fungus spores (in case of Method A,) and bacteria (in case of method C) and cultured on an incomplete growth medium which was without carbon source.

TEST MATERIALS: Arcoplast Engineered Polymer 9.5mm Lot# Ref# 12262

TEST CONDITIONS:

Fungal Challenge Organisms: Aspergillus niger van Tieghem ATCC# 6275
Penicillium funiculosum thom, ATCC# 36839
Gliocladium virens Milleret al, ATCC# 9645
Chaetomium globosum Kunze:Fries ATCC# 6205
Aureobasidium pullulans (de Bary) Arnaud ATCC# 9348

Bacterial Challenge Organisms: Pseudomonas aeruginosa ATCC# 9027

Sample Dimensions: 50 x 50 mm
Contact time: 4 weeks
Incubation: At 28^0C±1 for fungi, at 29^0C±1 for bacteria and > 95 % humidity

Microbiocidal Solution Type: Ethanol and sterile water in proportion of 70:30

Reference: ISO 846: 1997 Plastics - Evaluation of the action of microorganisms- Resistance to Fungi & Bacteria Test method

Control: Batch 0: Control specimens stored under standard temperature and humidity
Test: Batch I: Test Samples inoculated with microorganisms stored under standard temperature and humidity.

Negative Control: Batch S: Sterile Specimens not inoculated but stored under same standard temperature and humidity.

Culture medium:

1 Mineral Salt Solution
2 Incomplete agar Medium-Mineral Salt Agar
3 Complete Agar Medium-Mineral Salt Agar with glucose
4 Sabouraud's dextrose agar plates and slants
5 Tryptic soy agar plates and slants
6 Phosphate buffer solution
7 Phosphate-buffered physiological saline
8 Distilled or deionized water
9 Microbiocidal solutions- Ethanol-water mixture in ratio of 70:30

Supplies:

1 Sterile petri dishes 15 x 100 mm
2 Sterile petri dishes 50 x 200 mm
3 1 mL, 5 mL and 10 mL sterile pipettes
4 Sterile bottles
5 Sterile 16 x 100 mm glass test tubes with screw caps
6 Pipetters, sterile, with 1 000 µl tips.
7 Inoculating loops
8 Routine Microbiological test materials for doing plate count determinations
9 Gauze or absorbent cotton.
10 1000 ml volumetric flask.
11 Stoppered Erlenmeyer flasks or media bottles, as required for preparation of media.

Equipment:

1 Vortex mixer
2 Weighing balance capable of weighing to ±□0,01g.
3 pH meter sensitive to 0.1 pH unit
4 Water bath capable of 45^{0}C
5 Refrigerator 2-8^{0}C
6 Steam sterilizer capable of 1210C and 15 lbs pressure

7 Dry-heat sterilizer, capable of maintaining the temperature at a value between 160°C and 180°C
8 Incubators 20-35^{0}C at relative humidity of 90 % or more
9 Hotplate with stirrer, or hot-water bath.
10 Microscope

STUDY DATES AND FACILITIES:

The laboratory phase of this test was performed at ACCUGEN LABORATORIES, INC, 2121 W Army Trail Rd, Addison Il 60101

RECORDS TO BE MAINTAINED:

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

PROCEDURE:

Pre-culture of Microorganisms:

Transferred fungi and bacteria from the stock culture to the slant culture medium by using inoculating loop. Fungal cultures were incubated at 28 ±1°C for 5 to 7 days and bacterial at 35°C ±1 for 2-3 days.

Used a sterile inoculating loop to transfer organisms onto fresh slant culture medium and incubated fungal cultures at 28 ±1°C for 5 to 7 days and bacterial at 35°C ±1 for 2-3 days.

Preparation of test specimens:

1. Testing was performed on three specimens from each treated test material.
2. Samples were dipped into ethanol-water mixture for 1 min and were dried at 45 ^{0}C for 4 hours
3. When preparing specimens, precautions were taken to avoid contamination with microorganisms or extraneous organic debris.
4. Samples were stored in sterile petri dishes and petri dishes were labeled with sample ID.

Preparation of Fungal test inoculums (Spore Suspension):

Harvesting:

Five ml of Mineral salt solution with wetting solution was added to each culture tube of microorganisms.

With the sterile inoculating loop, the surface of the sporulating culture was scraped. Tubes were gently stirred three times to mix the spores in the liquid.

Spore suspensions were then mixed using sterile glass beads and filtered through a thin layer of sterile cotton to remove the mycelial fragments.

Filtered suspensions were centrifuged and supernatants were discarded.

Sediments were suspended in 25 ml of mineral salt solution and centrifuged again.

Washed sediments were suspended in 50 ml of stock mineral solution.

Spores were counted under microscope using counting chamber for each fungal suspension and concentrations were adjusted to about 10^8 spores/ml.

To check the spore viability, one drop of each spore suspension was cultured on two petri dishes of complete agar medium (Mineral salt with glucose) and incubated at 28^0C for 3 to 4 days.

Equal volume of each suspension containing same number of spores was mixed to prepare the inoculum.

Preparation of Bacterial test inoculum:

A tube of Tryptic Soy broth was inoculated with bacteria and incubated at 29°C ±1 for 24 hours. The 24 hours broth culture was inoculated to 10 ml of sterile buffer solution with one loop full of sterile inoculating loop. The suspension was diluted to achieve cell suspension containing about 10^6cfu/ml.

To check the viability, added few drops of the suspension on two petri dishes of tryptic soy agar and complete agar medium (Mineral salt with glucose) and incubated for 24 to 48 hours.

Inoculation of specimens:

Method A:

Pipetted 0.1 ml of the spore suspension evenly on the surface of three test sample (Batch I) and Incomplete agar medium (Mineral Salt Agar).

Pipetted 3 ml of microbiocidal solution on the surface of three sterile negative control samples (Batch S) cultured on incomplete agar medium.

Both inoculated and sterile negative control samples were incubated at 28^0C for 4 weeks to determine the fungistatic effect.

Method B:

Pipetted 0.1 ml of the spore suspension on the surface of three test sample and Complete agar medium (Mineral Salt Agar with Glucose).

Pipetted 3 ml of microbiocidal solution on the surface of three sterile negative control samples (Batch S) cultured on Complete agar medium

Method B':

Pipetted 0.1 ml of the spore suspension on the surface of three Complete agar medium (Mineral Salt Agar with Glucose). Incubated at 28^0C for 2 to 3 days and then placed each of the three test samples and inoculated 0.1 ml onto the surface of each sample.

Pipetted 3 ml of microbiocidal solution on the surface of three sterile negative control samples (Batch S) cultured on complete agar medium

Pipetted 0.1 ml of the spore suspension on the surface of three test sample and each placed in empty sterile petri dish.

All were incubated at 28^0 C and 95% humidity for 4 weeks.

Method C:

Batch I-Molten Mineral salt agar was inoculated with sufficient amount of bacterial cell suspension to obtain a concentration of about 50,000 cells per ml of agar. The inoculated agar was mixed, and plates were poured to make 5mm deep agar layer and solidified. One specimen was placed on the surface of each agar layer. Poured sufficient inoculated agar onto the top of specimen to cover it to a depth of 1 mm.

Batch S (Sterile Control). Uninoculated Mineral salt agar was poured into sterile petri dish. Specimens were disinfected by dipping into 70% alcohol, air dried and placed onto the solidified agar. Same solution was added to disinfect the agar. Uninoculated agar was poured to cover the specimens. Samples were incubated at 29°C ±1 and 90 % relative humidity for 4 weeks.

Assessment Criteria:

Table 1: The Following Criteria was Used:

Intensity of Growth	Evaluation
0	No growth seen under the microscope.
1	No growth was seen by naked eye but was visible under the microscope.
2	Growth Visible to the naked eye.25 % of the test sample surface was covered with fungal & bacteria growth
3	Growth Visible to the naked eye.50 % of the test sample surface was covered with fungal & bacteria growth
4	Growth Visible to the naked eye more than 50 % of the test sample surface was covered with fungal & bacteria growth
5	Heavy growth, covering the entire surface of the test sample

TEST RESULTS:

Test sample was found to be nonnutritive medium for fungal & bacteria growth. There was no fungal & bacteria growth observed by naked eye or under the microscope. There was heavy growth of fungi observed on the growth media, but not on sample in case of positive controls (B and B') and no growth of bacteria observed (C). There was no growth observed in cases of samples inoculated with microbiocidal solutions. See Table 2 & 3.

Table 2: Test Results with Fungal Exposure							
Method	A		B		B'		
Medium Used	Incomplete Agar Medium		Complete Agar Medium		None	Complete Agar Medium	
Batch	I	S	I	S	I	I	S
Solution Sprayed on Specimen	Spores inoculated	Microbiocidal soln inoculated	Spores inoculated	Microbiocidal soln inoculated	Spores inoculated	Spores inoculated	Microbiocidal soln inoculated
Growth Intensity	0	0	5	0	0	5	0
Comments	Sample is not nutritive for fungal growth		Sample does not have any fungistatic effect			Sample does not have any fungistatic effect	

Table 3: Test Results with Bacterial Exposure		
Method	C	
Medium Used	Mineral salt Agar	
Batch	I	S
Inoculum	Organisms inoculated into the media	Microbiocidal soln added onto uninoculated agar
Growth Intensity	0	0
Comments	Sample is non nutritive for bacterial growth	

CONCUSION:

The test sample does not support microbial growth and it resists the fungi and bacteria. The results obtained conclude that the test sample does not provide nutrition for bacteria and fungi.

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

Study Director

Figure 1: Method A- Fungal spore inoculated to the surface of test sample and Mineral salt agar © Accugen Labs

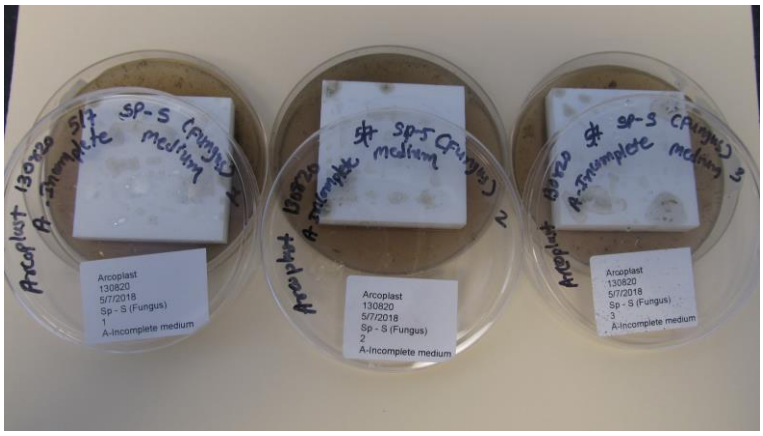

Figure 2: Method A- Microbiocidal solution added to the surface of test sample on Mineral salt Agar @Accugen Labs

Figure3: Method B. Fungal spore inoculated on the surface of test sample and Mineral salt agar with glucose © Accugen Labs

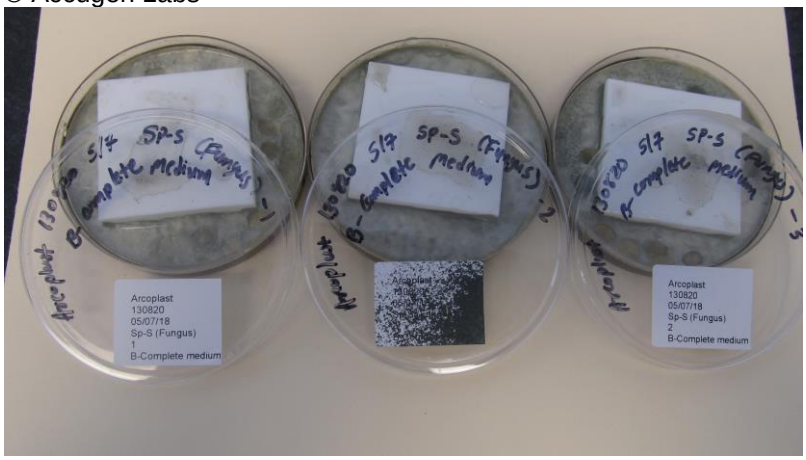

Figure 4: Method B Microbiocidal solution added to the surface of test sample on Mineral salt Agar with glucose @Accugen Labs

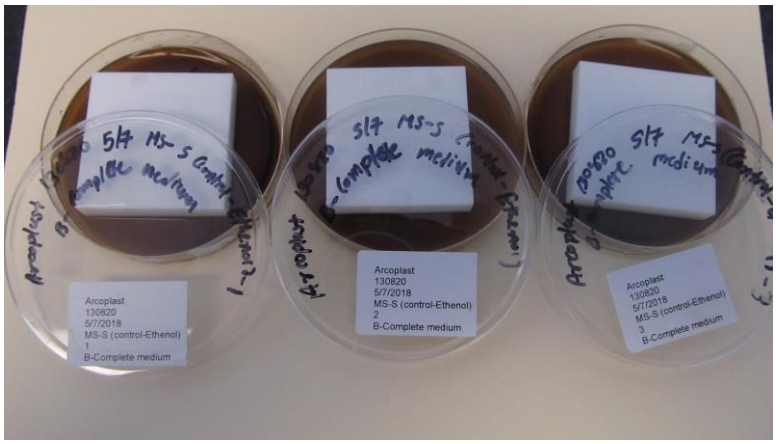

Figure5: Method B'- Fungal spore inoculated to the surface of test sample with none media© Accugen Labs

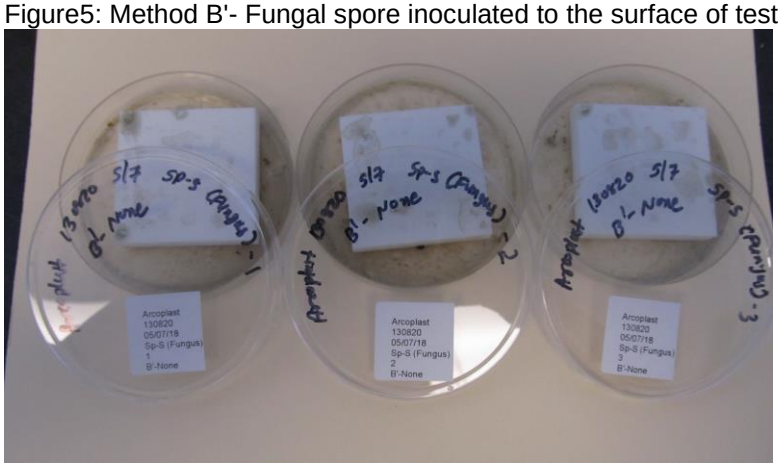

Figure 6: Method B'- Fungal spore inoculated to the surface of Mineral salt agar with glucose and test sample © Accugen Labs

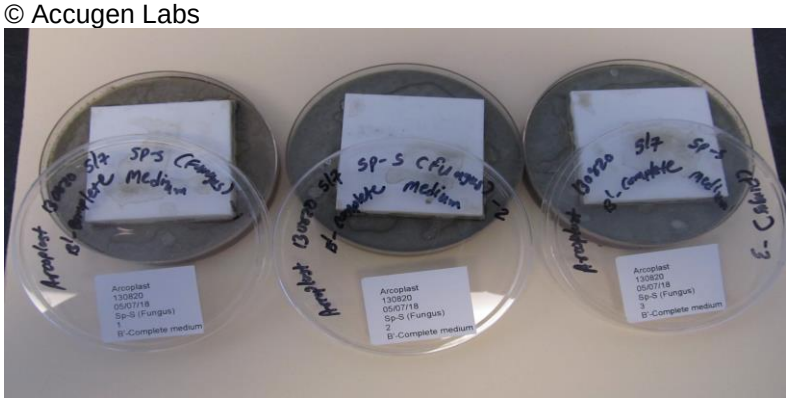

Figure 7: Method B' Microbiocidal solution added to the surface of Mineral salt Agar with glucose and test sample @Accugen

Figure8: Method C- bacterial cell suspension inoculated in to Mineral salt Agar with glucose © Accugen Labs

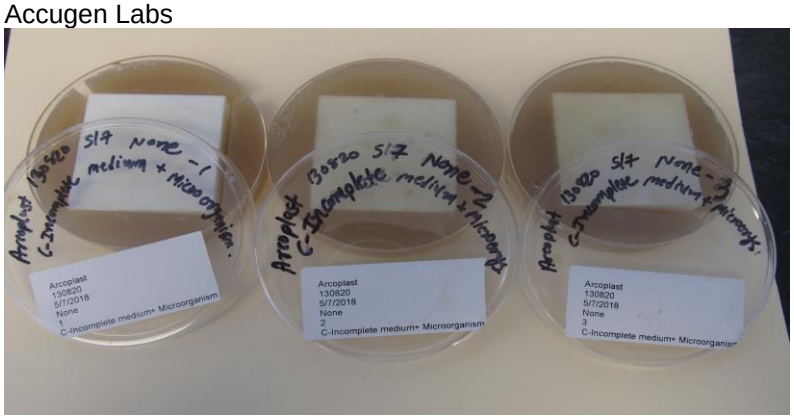

Figure 9: Method C- uninoculated Mineral salt Agar @Accugen

Figure10: Fungal Viability Control and Bacterial Viability Control