

ISO 22196: 2011(E)

PLASTICS – MEASUREMENT OF ANTIBACTERIAL ACTIVITY ON PLASTICS AND OTHER NON-POROUS SURFACES

*FINAL REPORT: R2020-626-2
AMENDMENT TO R2020-626*

Prepared for:
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Testing Completed: October 26, 2020
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Objective:

To evaluate the antibacterial activity on the surface of two samples as demonstrated by the ISO 22196:2011(E) test method.

Test Sample Identification:

1. 007-3
2. 007-5
3. Control

Test Procedure Summary:

The test organism was adjusted and diluted to obtain the starting inoculum concentration of $2.5-10 \times 10^5$ CFU/mL. The control was tested in triplicate at Time = 0 and Time = 24 hours. The test samples were tested in triplicate at Time = 24 hours. Each sample piece was placed in a sterile Petri dish, inoculated and then covered with the sterile plastic in order to spread the inoculum evenly over the sample surface and hold it in place. The samples were incubated at 35°C and a relative humidity of at least 90%. At the appropriate time the neutralizing broth was added to each sample, placed onto a shaker and mixed thoroughly to facilitate the release of the inoculum from the sample surface. Serial dilutions of the neutralizing broth containing the inoculum were plated. All plates were incubated at 35°C for 24-48 hours. After incubation, bacterial colonies were counted and recorded. The results are found in the "Test Results" section below. These results pertain only to the samples tested.

Test Variables

Test Organism:	<i>Staphylococcus aureus</i> ATCC 6538P <i>Escherichia coli</i> ATCC 8739
Sample Size:	50 mm x 50 mm
Method of Sterilization /Pre-Cleaning:	Precleaned with 70% isopropyl alcohol and air dry overnight prior testing
Control Sample:	Control sample submitted by customer
Film Used:	40 mm x 40 mm x 0.05 mm plastic pieces cut from sterile Whirlpak™ bags
Dilution Medium Used:	Sterile dilute nutrient broth per standard
Neutralizing Broth Used:	D/E Neutralizing Broth
Amount of Neutralizing Broth:	10 mL
Starting Inoculum Concentration:	<i>S. aureus</i> ATCC#6538P: 3.2×10^5 <i>E. coli</i> ATCC#8739: 3.6×10^5
Amount of Inoculum:	0.4 mL
Contact Time:	24 hours
Deviations from Standard Test Method:	None, testing performed per ISO 22196 without deviation.



Test Results:

Results against *S. aureus* ATCC#6538P:

Control	<i>U</i> ₀ : Average of logarithm numbers of viable bacteria at Time = 0	4.16
Control	<i>U</i> _t : Average of logarithm numbers of viable bacteria at Time = 24 h	4.18
007-3	<i>A</i> _t : Average of logarithm numbers of viable bacteria at Time = 24 h	1.52
007-5	<i>A</i> _t : Average of logarithm numbers of viable bacteria at Time = 24 h	0.81

<u>Sample</u>	<u>Value of Antimicrobial Activity (R)</u>	<u>Percent Reduction</u>
007-3	2.66	99.8
007-5	3.37	99.96

Results against *E. coli* ATCC#8739:

Control	<i>U</i> ₀ : Average of logarithm numbers of viable bacteria at Time = 0	4.15
Control	<i>U</i> _t : Average of logarithm numbers of viable bacteria at Time = 24 h	5.98
007-3	<i>A</i> _t : Average of logarithm numbers of viable bacteria at Time = 24 h	0.84
007-5	<i>A</i> _t : Average of logarithm numbers of viable bacteria at Time = 24 h	0.60

<u>Sample</u>	<u>Value of Antimicrobial Activity (R)</u>	<u>Percent Reduction</u>
007-3	5.14	99.9993
007-5	5.38	99.9996



Test Results Interpretation:

The value of the antimicrobial activity was calculated according to the formula listed below and recorded as log reduction.

$$R = (U_t - U_o) - (A_t - U_o) = U_t - A_t$$

Where,

R: antimicrobial activity

U_o: average of logarithm numbers of viable bacteria from control sample
at Time = 0 hour

U_t: average of logarithm numbers of viable bacteria from control sample
at Time = 24 hour

A_t: average of logarithm numbers of viable bacteria from test sample at Time = 24 hour

According to the standard, an antibacterial product is determined to have antibacterial effectiveness when the antibacterial activity (R) is 2.0 or more.

Percent reductions are determined by comparing the sample after the contact time to the control sample after the contact. Reporting of percent reduction is not indicated by the test method but is provided by MicroStar as additional information.

Percent reduction is translated into log reduction by the following:

90% reduction = 1 log reduction; i.e. 1,000,000 reduced to 100,000 is a 1 log reduction

99% reduction = 2 log reduction; i.e. 1,000,000 reduced to 10,000 is a 2 log reduction

99.9% reduction = 3 log reduction; i.e. 1,000,000 reduced to 1,000 is a 3 log reduction

99.99% reduction = 4 log reduction; i.e. 1,000,000 reduced to 100 is a 4 log reduction

99.999% reduction = 5 log reduction; i.e. 1,000,000 reduced to 10 is a 5 log reduction