

# ISO 22196: 2011(E)

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

*FINAL REPORT: R2020-330*

Prepared for:

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## **Objective:**

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

## **Test Sample Identification:**

1. CCI ARM 065 85
2. CCI ARMI 065 85 w/Surfaseal
3. CCI ANXT 075 8318
4. CCI LTR 090 85 w/Surfaseal

## **Test Procedure Summary:**

The test organism was adjusted and diluted to obtain the starting inoculum concentration of 2.5-10 x 10<sup>5</sup> CFU/mL. The control was tested in triplicate at Time = 0 and the Contact Time. The test samples were tested in triplicate at the Contact Time. 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 2-7°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 48-72 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

<b>Test Organism:</b>	<i>Listeria monocytogenes</i> ATCC #19115
<b>Sample Size:</b>	50 mm x 50 mm
<b>Method of Sterilization /Pre-Cleaning:</b>	None
<b>Control Sample:</b>	Untreated plastic control supplied by MicroStar
<b>Film Used:</b>	40 mm x 40 mm x 0.05 mm plastic pieces cut from sterile Whirlpak™ bags
<b>Dilution Medium Used:</b>	Sterile dilute nutrient broth per standard
<b>Neutralizing Broth Used:</b>	D/E Neutralizing Broth
<b>Amount of Neutralizing Broth:</b>	10 mL
<b>Starting Inoculum Concentration:</b>	<i>L. monocytogenes</i> ATCC #19115: 1.0 x 10 <sup>6</sup>
<b>Amount of Inoculum:</b>	0.4 mL
<b>Contact Time:</b>	2 hours, 24 hours, 48 hours, 72 hours and 168 hours
<b>Deviations from Standard Test Method:</b>	Test organism used is listed above. Samples were incubated at 2-7°C for additional contact times (2, 48, 72, 168 hours)



## **Test Results:**

### **Results after 2 hours:**

Untreated Control	$U_0$ : Average of logarithm numbers of viable bacteria at Time = 0	4.41
Untreated Control	$U_t$ : Average of logarithm numbers of viable bacteria at Time = 2 h	4.44
CCI ARM 065 85	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 2 h	4.43
CCI ARMI 065 85 w/Surfaseal	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 2 h	4.30
CCI ANXT 075 8318	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 2 h	4.42
CCI LTR 090 85 w/Surfaseal	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 2 h	4.33

<u>Sample</u>	<u>Value of Antimicrobial Activity (R)</u>	<u>Percent Reduction</u>
CCI ARM 065 85	0.01	1%
CCI ARMI 065 85 w/Surfaseal	0.14	27%
CCI ANXT 075 8318	0.02	4%
CCI LTR 090 85 w/Surfaseal	0.11	22%



Results after 24 hours:

Untreated Control	$U_0$ : Average of logarithm numbers of viable bacteria at Time = 0	4.41
Untreated Control	$U_t$ : Average of logarithm numbers of viable bacteria at Time = 24 h	4.34
CCI ARM 065 85	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 24 h	4.31
CCI ARMI 065 85 w/Surfaseal	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 24 h	4.27
CCI ANXT 075 8318	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 24 h	4.26
CCI LTR 090 85 w/Surfaseal	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 24 h	4.35

<u>Sample</u>	<u>Value of Antimicrobial Activity (R)</u>	<u>Percent Reduction</u>
CCI ARM 065 85	0.03	8%
CCI ARMI 065 85 w/Surfaseal	0.07	15%
CCI ANXT 075 8318	0.08	17%
CCI LTR 090 85 w/Surfaseal	-0.01	No Reduction



Results after 48 hours:

Untreated Control	$U_0$ : Average of logarithm numbers of viable bacteria at Time = 0	4.41
Untreated Control	$U_t$ : Average of logarithm numbers of viable bacteria at Time = 48 h	4.16
CCI ARM 065 85	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 48 h	4.07
CCI ARMI 065 85 w/Surfaseal	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 48 h	4.15
CCI ANXT 075 8318	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 48 h	4.13
CCI LTR 090 85 w/Surfaseal	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 48 h	4.06

<u>Sample</u>	<u>Value of Antimicrobial Activity (R)</u>	<u>Percent Reduction</u>
CCI ARM 065 85	0.09	19%
CCI ARMI 065 85 w/Surfaseal	0.01	2%
CCI ANXT 075 8318	0.03	8%
CCI LTR 090 85 w/Surfaseal	0.10	21%



Results after 72 hours:

Untreated Control	$U_0$ : Average of logarithm numbers of viable bacteria at Time = 0	4.41
Untreated Control	$U_t$ : Average of logarithm numbers of viable bacteria at Time = 72 h	4.16
CCI ARM 065 85	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 72 h	3.98
CCI ARMI 065 85 w/Surfaseal	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 72 h	4.18
CCI ANXT 075 8318	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 72 h	4.19
CCI LTR 090 85 w/Surfaseal	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 72 h	4.28

<u>Sample</u>	<u>Value of Antimicrobial Activity (R)</u>	<u>Percent Reduction</u>
CCI ARM 065 85	0.18	34%
CCI ARMI 065 85 w/Surfaseal	-0.02	No Reduction
CCI ANXT 075 8318	-0.03	No Reduction
CCI LTR 090 85 w/Surfaseal	-0.12	No Reduction



Results after 168 hours:

Untreated Control	$U_0$ : Average of logarithm numbers of viable bacteria at Time = 0	4.41
Untreated Control	$U_t$ : Average of logarithm numbers of viable bacteria at Time = 168 h	3.88
CCI ARM 065 85	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 168 h	3.91
CCI ARMI 065 85 w/Surfaseal	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 168 h	3.86
CCI ANXT 075 8318	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 168 h	4.07
CCI LTR 090 85 w/Surfaseal	$A_t$ : Average of logarithm numbers of viable bacteria at Time = 168 h	3.85

<u>Sample</u>	<u>Value of Antimicrobial Activity (R)</u>	<u>Percent Reduction</u>
CCI ARM 065 85	-0.03	No Reduction
CCI ARMI 065 85 w/Surfaseal	0.02	6%
CCI ANXT 075 8318	-0.19	No Reduction
CCI LTR 090 85 w/Surfaseal	0.03	6%



### 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<sub>o</sub>*: average of logarithm numbers of viable bacteria from untreated plastic control at Time = 0 hour

*U<sub>t</sub>*: average of logarithm numbers of viable bacteria from untreated plastic control at contact time

*A<sub>t</sub>*: average of logarithm numbers of viable bacteria from test sample at contact time

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 untreated plastic control 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