BBI TEST LABS

Study Report #TR 23110714a

for

Kleen-Tex Polska Sp. Z.O.O.

c/o Roman Barcik
Fabryczna 5/12
26-130 Suchedniów
Poland

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Study Report:	ISO 22196: Measurement of antibacterial activity on plastics and other non-porous surfaces (2011)
Client:	Kleen-Tex
Report #:	23110714a
Study:	This study evaluated the antimicrobial activity of three nitrile rubber mats against *Escherichia coli* and *Staphylococcus aureus.* This data was compared to an untreated sample provided by the test lab to act as a control.
	The above study was conducted in the laboratories of Microban International at 11400 Vanstory Drive, Huntersville, NC 28078. This report represents a true and accurate account of the results obtained.
Study Start Date:	November 7th, 2023
Study Completion Date:	November 10th, 2023
Report Issued Date:	December 15th, 2023
Analyst: Person who conducts/reports test (Microbiologist)	Erin Kochy, Microbiologist II
Reviewer:	James Hanna, Microbiology Manager

Contents

1. Introduction

This report summarizes a study performed to assess the antibacterial performance of three nitrile rubber mat samples against *Escherichia coli* and *Staphylococcus aureus* using the ISO 22196:2011 (1). Laboratory-supplied untreated polypropylene plaques were used as the control to calculate antibacterial activity of the samples.

2. Test Materials

Test samples were supplied by Kleen-Tex.

- Sample 1: Kleen-Thru Plus (Nitrile Rubber Mat) – Standard
- Sample 2: Kleen-Thru Plus (Nitrile Rubber Mat) – 25 Washes
- Sample 3: Kleen-Thru Plus (Nitrile Rubber Mat) – 50 Washes

Validity control samples, untreated polypropylene plaques, were supplied by the BBI Test Labs.

3. Methods

Evaluate antimicrobial activity of three nitrile rubber mat samples against two (2) bacterial test organisms, *Escherichia coli* (ATCC #8739) and *Staphylococcus aureus* (ATCC #6538), using the method described in ISO 22196:2011. Each sample was tested in triplicate.

In summary:

- Samples were supplied in a clear bag and untouched for at least 24 hours prior to testing.
- Each test specimen was wiped with a 70% Isopropyl Alcohol wipe and placed in an individual sterile 120-mL specimen jar.
- Each specimen was inoculated with *Escherichia coli* (ATCC #8739) and *Staphylococcus aureus* (ATCC #6538) at a concentration of 2.5 - 10 x 10^5 CFU/mL.
- The inoculum was carefully covered with sterile PE film and incubated for 21 ± 3 hours at 36°C in a tightly closed specimen jar.
- Post-incubation, the test specimens were recovered using Letheen Broth as the antimicrobial neutralizer. The populations of viable organisms remaining on each specimen's surface were determined.

4. Results

Table 1 shows the geomean of the populations of viable organisms remaining on each sample surface immediately after inoculation and after 24-hour challenge.

Table 1: Geomean of microbial populations recovered from samples immediately after inoculation (T = 0h) and after bacterial challenge period (T=24h).

Sample Description	*E. coli* (8739) CFU[1]/sample		*S. aureus* (6538) CFU/sample	
	T = 0h (Log value)	T = 24h (Log value)	T = 0h (Log value)	T = 24h (Log value)
Sample 1: Kleen-Thru Plus (Nitrile Rubber Mat) – Standard	1.2×10^5 (5.1)	$< 1.0 \times 10^2$ (2.0)	1.7×10^5 (5.2)	1.0×10^2 (2.0)
Sample 2: Kleen-Thru Plus (Nitrile Rubber Mat) – 25 Washes	1.2×10^5 (5.1)	$< 1.0 \times 10^2$ (2.0)	1.7×10^5 (5.2)	1.0×10^2 (2.0)
Sample 3: Kleen-Thru Plus (Nitrile Rubber Mat) – 50 Washes	1.2×10^5 (5.1)	$< 1.0 \times 10^2$ (2.0)	1.7×10^5 (5.2)	$< 1.0 \times 10^2$ (2.0)
Lab Validity Control (Untreated Polypropylene plaques)	1.2×10^5 (5.1)	$> 4.9 \times 10^6$ (6.7)	1.7×10^5 (5.2)	1.0×10^6 (6.0)
Inoculum (CFU/sample)	1.2×10^5 (5.1)		1.7×10^5 (5.2)	

[1] CFU: Colony forming units

The antibacterial activity performance of each treated sample is shown in Table 2.

Table 2: Log reduction and antibacterial activity of treated samples versus untreated laboratory control sample.

Sample Description	Log Reduction[2] (T 0h -T 24h)		Antibacterial activity[3] T 24h – C 24h	
	E. coli (8739)	*S. aureus* (6538)	*E. coli* (8739)	*S. aureus* (6538)
Sample 1: Kleen-Thru Plus (Nitrile Rubber Mat) – Standard	3.1	3.2	4.7	4.0
Sample 2: Kleen-Thru Plus (Nitrile Rubber Mat) – 25 Washes	3.1	3.2	4.7	4.0
Sample 3: Kleen-Thru Plus (Nitrile Rubber Mat) – 50 Washes	3.1	3.2	4.7	4.0
Lab Validity Control (Untreated Polypropylene plaques)	-1.6	-0.8		

[2] Log reduction computations are based on the geomean of three replicates of each sample and determined at T = 0h versus the geomean of three replicates at T= 24h.

[3] Antibacterial activity computations are based on the log reductions of treated samples versus the log average of the growth value of the lab validity control samples.

5. Raw Data

The raw data for this study will be held in file BBI TEST LABS 23110714a in the Archive of BBI TEST LABS at 11400 Vanstory Drive for 6 years from the date of this report unless other specific instructions are given.

6. References

(1) ISO 22196: Measurement of antibacterial activity on plastics and other non-porous surfaces (2011)

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