SAMM 1016

Test Report No.: TR-23-0143

Determination of the Bactericidal Efficacy of **PENTANANO ANTIMICROBIAL NITRILE EXAMINATION GLOVES** according to ASTM D7907-14 (2019)

Test Method
ASTM D7907-14 (2019)
Standard test methods for determination of bactericidal efficacy on the surface of medical examination gloves

Client
Pentavest Holdings Sdn. Bhd.
No. 9574-9578, Jalan PTB 2
Kawasan Perindustrian Tangga Batu
76400 Melaka
Malaysia

Testing Laboratory
TECOLAB Sdn. Bhd.
J-2-6, Pusat Komersial Jalan Kuching
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51200 Kuala Lumpur
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Kuala Lumpur, 8 March 2023

Dr Marven Lee Cheng Shoou
Managing Director

IDENTIFICATION OF TESTING LABORATORY

TECOLAB Sdn. Bhd.
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IDENTIFICATION OF CLIENT

Pentavest Holdings Sdn. Bhd.
No. 9574-9578, Jalan PTB 2
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76400 Melaka
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IDENTIFICATION OF TEST ITEM

Test item name:	Pentanano Antimicrobial Nitrile Examination Gloves
Test item lab ID:	P003-23-005
Test item batch no.:	012212-1L-INC
Specimen control name:	Latex Examination Glove
Specimen control lab ID:	P003-22-008
Specimen control batch no.:	Not specified
Expiry date:	November 2025
Manufacturer:	Pentavest Holdings Sdn. Bhd.
Receipt date:	10 February 2023
Storage conditions:	Room temperature away from sunlight
Active substances:	Not specified
Product appearance:	Green, nitrile gloves

TEST METHOD & VALIDATION

Test method:	ASTM D7907-14 (2019) Standard test methods for determination of bactericidal efficacy on the surface of medical examination gloves
Soiling condition:	Without organic load (Method A)
Challenge delivery method:	Direct inoculation onto specimen surface (Option 1)
Inactivation method:	Neutralization assay with recovery on solid medium according to ASTM E1054-02 (Standard test methods for evaluation of inactivators of antimicrobial agents)

Inactivator:	30 g/L Tween 80 3 g/L Lecithin

EXPERIMENTAL CONDITIONS

Date of test:	24 February 2023
Contact time:	0, 5, 10, 20, and 30 minutes
Number of replicates:	3
Sampling site:	Outer surface of fingertips
Test organism:	*Staphylococcus aureus* (Methicillin-resistant) ATCC 43300
Incubation temperature:	(37 ± 1) °C
Incubation period:	24 hours

CONTROLS AND VALIDATION

Test Organism	Neutralizer Effectiveness	Neutralizer Toxicity	Test Organism Viability	Extraction Control
S. aureus (MR) ATCC 43300	A: 46.3 p: 0.3216	B: 46.0 p: 0.2403	C: 47.5	EC: 5.53 x 10^6 80% CI: 4.34 x 10^6

The control and validation tests A, B, C, and EC were within the basic limits:

- A must not be significantly different from C ($p > 0.05$) to verify the neutralizer's ability to inactivate, neutralize, or quench the microbiocidal properties of an antimicrobial agent,
- B must not be significantly different from C ($p > 0.05$) to verify the absence of any inhibitory effects the neutralizer may have on the survival of a microbial population,
- The population or viability of the challenge microorganism, C, must be between 30 to 100, and
- EC must be ≥80% of the challenge inoculum CI to verify the efficiency of the extraction method.

TEST RESULT

For each contact time, the log reduction (lg R_T and lg R_C) is calculated using the formula lg R_T = lg CI – lg TS and lg R_C = lg SC – lg TS, respectively, in which:

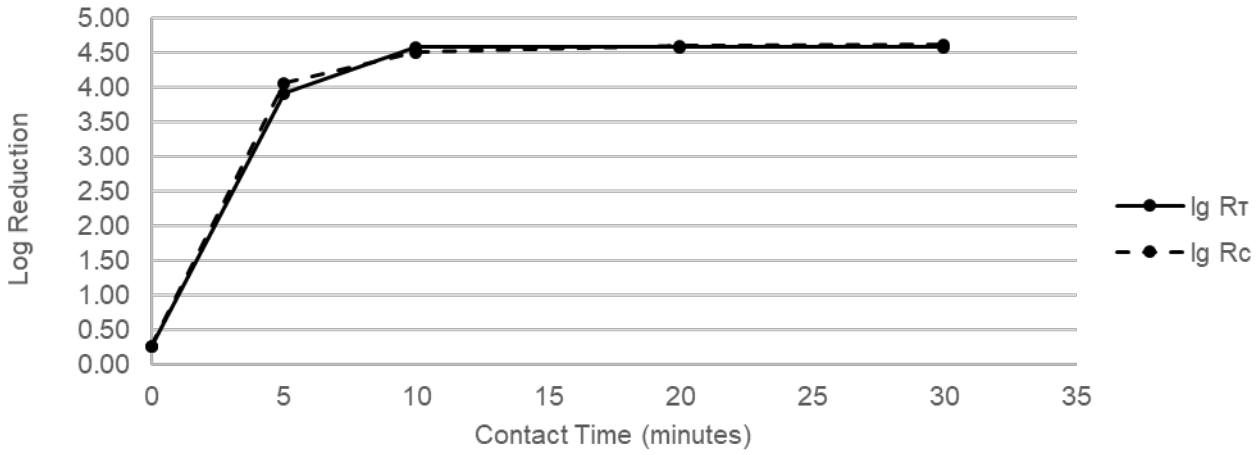
- CI is the number of cells in the challenge inoculum (average between challenge inoculum titre at the beginning and the end of test session) inoculated onto the specimen,
- TS is the number of cells extracted from the test specimen at the end of the contact time and before neutralization, and
- SC is the number of cells extracted from the control specimen at the end of the contact time and before neutralization.

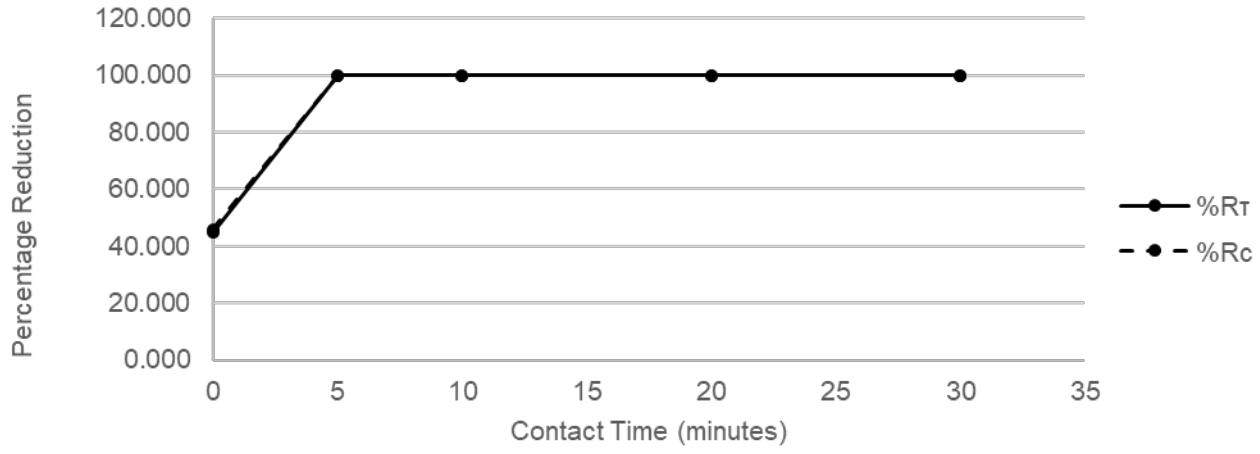
Test organism: *Staphylococcus aureus* (MR) ATCC 43300

Challenge Inoculum, CI	CI: 5.43 x 10^6 lg CI: 6.73

Contact Time (minutes)	Specimen Control Replicates	Average Specimen Control, SC
0	SC_1: 6.75 x 10^6 SC_2: 5.05 x 10^6 SC_3: 4.80 x 10^6	SC: 5.53 x 10^6 lg SC: 6.74
5	SC_1: 4.80 x 10^6 SC_2: 8.15 x 10^6 SC_3: 1.01 x 10^7	SC: 7.68 x 10^6 lg SC: 6.89
10	SC_1: 4.55 x 10^6 SC_2: 4.65 x 10^6 SC_3: 4.55 x 10^6	SC: 4.58 x 10^6 lg SC: 6.66
20	SC_1: 5.95 x 10^5 SC_2: 6.55 x 10^6 SC_3: 4.50 x 10^6	SC: 5.67 x 10^6 lg SC: 6.75
30	SC_1: 7.10 x 10^6 SC_2: 6.20 x 10^6 SC_3: 4.25 x 10^6	SC: 5.85 x 10^6 lg SC: 6.77

Contact Time (minutes)	Test Specimen Replicates	Average Test Specimen, TS	Reduction from Challenge Inoculum, R_T	Reduction from Specimen Control, R_C
0	TS_1: 2.02 x 10^5 TS_2: 1.17 x 10^6 TS_3: 7.60 x 10^6	TS: 2.99 x 10^6 lg TS: 6.48	lg R_T: 0.26 ± 0.14 %R_T: 44.873%	lg R_C: 0.27 ± 0.18 %R_C: 45.952%
5	TS_1: 6.60 x 10^2 TS_2: 5.70 x 10^2 TS_3: 7.30 x 10^2	TS: 6.53 x 10^2 lg TS: 2.82	lg R_T: 3.92 ± 0.14 %R_T: 99.988%	lg R_C: 4.07 ± 0.18 %R_C: 99.991%
10	TS_1: <1.40 x 10^2 TS_2: <1.40 x 10^2 TS_3: <1.40 x 10^2	TS: <1.40 x 10^2 lg TS: <2.15	lg R_T: >4.59 ± 0.14 %R_T: >99.997%	lg R_C: >4.52 ± 0.18 %R_C: >99.997%
20	TS_1: <1.40 x 10^2 TS_2: <1.40 x 10^2 TS_3: <1.40 x 10^2	TS: <1.40 x 10^2 lg TS: <2.15	lg R_T: >4.59 ± 0.14 %R_T: >99.997%	lg R_C: >4.61 ± 0.18 %R_C: >99.998%
30	TS_1: <1.40 x 10^2 TS_2: <1.40 x 10^2 TS_3: <1.40 x 10^2	TS: <1.40 x 10^2 lg TS: <2.15	lg R_T: >4.59 ± 0.14 %R_T: >99.997%	lg R_C: >4.62 ± 0.18 %R_C: >99.998%





CONCLUSION

The test item achieved a highest log reduction (R_T) of >4.59 (>99.997%) after 10 minutes against the test organism *Staphylococcus aureus* (MR) ATCC 43300 when compared to the challenge inoculum under the tested conditions. The highest log reduction (R_C) of >4.62 (>99.998%) was achieved at 30 minutes when compared to the specimen control.

Therefore, **Pentanano Antimicrobial Nitrile Examination Gloves** has demonstrated a bactericidal efficacy against *Staphylococcus aureus* (MR) ATCC 43300 according to ASTM D7907-14 (2019) under the following conditions:

Contact Time	Soiling	lg R_T	lg R_C
0 minute	Without soiling	0.26 (44.873%)	0.27 (45.952%)
5 minutes	Without soiling	3.92 (99.988%)	4.07 (99.991%)
10 minutes	Without soiling	>4.59 (>99.997%)	>4.52 (>99.997%)
20 minutes	Without soiling	>4.59 (>99.997%)	>4.61 (>99.998%)
30 minutes	Without soiling	>4.59 (>99.997%)	>4.62 (>99.998%)

Kuala Lumpur, 8 March 2023

Neni Iffanida Ismail
Microbiologist

EXPERT OPINION

This expert opinion is based on the test report TR-23-0143 dated 8 March 2023. Opinions and interpretations expressed herein are outside the scope of the Laboratory Accreditation Scheme of Malaysia (SAMM).

The product **Pentanano Antimicrobial Nitrile Examination Gloves** was tested according to ASTM D7907-14 (2019) against *Staphylococcus aureus* (Methicillin-resistant) ATCC 43300. This organism is chosen as a surrogate species for the clinical strains of MRSA, taking into account its relative resistance, relevance to practical use, handling properties, and microbiological safety.

Bactericidal activity is defined as a capability of a product or active substance to produce a reduction in the number of viable bacterial cells of relevant test organisms under defined conditions. According to ASTM D7907-14, a medical examination glove is considered to possess a bactericidal activity if it demonstrates the required performance criteria under the tested conditions specified by the regulatory agencies and/or manufacturer.

When tested under the following conditions, **Pentanano Antimicrobial Nitrile Examination Gloves** achieved a highest log reduction (R_T) of >4.59 (>99.997%) after 10 minutes when compared to the challenge inoculum, i.e., the viable microorganism used to contaminate the test specimen. The highest log reduction (R_C) of >4.62 (>99.998%) was achieved at 30 minutes when compared to the specimen control, i.e., the same glove formulation as the test specimen in every aspect, but without the addition of the antimicrobial agent(s). The results for each condition are summarized as follows:

Contact Time	Soiling	lg R_T	lg R_C
0 minute	Without soiling	0.26 (44.873%)	0.27 (45.952%)
5 minutes	Without soiling	3.92 (99.988%)	4.07 (99.991%)
10 minutes	Without soiling	>4.59 (>99.997%)	>4.52 (>99.997%)
20 minutes	Without soiling	>4.59 (>99.997%)	>4.61 (>99.998%)
30 minutes	Without soiling	>4.59 (>99.997%)	>4.62 (>99.998%)

Therefore, **Pentanano Antimicrobial Nitrile Examination Gloves** has demonstrated a bactericidal activity against *Staphylococcus aureus* (MR) ATCC 43300 according to ASTM D7907-14 (2019) under the aforementioned conditions.

Kuala Lumpur, 8 March 2023

Dr Marven Lee Cheng Shoou
Managing Director

INFORMATION ON MEASUREMENT UNCERTAINTY & DECISION RULE

The statement of conformity given by ASTM D7907-14 (2019) states that the test item shall be considered to have passed ASTM D7907-14 if it demonstrates the required performance criteria under the tested conditions specified by the regulatory agencies and/or manufacturer.

The laboratory employs the simple acceptance decision rule to account for the measurement uncertainty when stating the statement of conformity. Since no performance criteria was specified by the client, the conformance and conformance probability were not applicable. The measurement uncertainty is summarized as follows:

Test Organism	Contact Time	Log Reduction (R_T)	Log Reduction (R_C)	Conformance	Conformance Probability†
S. aureus (MR) ATCC 43300	0 minute	0.26 ± 0.14	0.27 ± 0.18	N/A	N/A
	5 minutes	3.92 ± 0.14	4.07 ± 0.18	N/A	N/A
	10 minutes	>4.59 ± 0.14	>4.52 ± 0.18	N/A	N/A
	20 minutes	>4.59 ± 0.14	>4.61 ± 0.18	N/A	N/A
	30 minutes	>4.59 ± 0.14	>4.62 ± 0.18	N/A	N/A

† The conformance probability follows a normal distribution. Therefore, the percentage of conformance can never be zero or 100% due to the asymptotic tails.

RAW DATA

Test Method:	ASTM D7907-14 (2019)				
Test Specimen (TS):	Pentanano Antimicrobial Nitrile Examination Gloves			Lab ID (TS):	P003-23-005
Sampling Site (TS):	Outer surface of fingertips	Batch No. (TS):	012212-1L-INC		
Specimen Control (SC):	Latex Examination Glove			Lab ID (SC):	P003-22-008
Sampling Site (SC):	Outer surface of fingertips	Batch No. (SC):	-		
Inactivation:	Standard neutralization and enumeration			Plating:	Pour plate
Neutralizer:	30 g/L Tween 80, 3 g/L Lecithin				
Soiling Condition:	Without organic load (Method A)			Inoculum Delivery Method:	Option 1
Test Organism:	Staphylococcus aureus (MR) ATCC 43300			Incubation Temperature (°C):	37
Passing Criteria (lg):	-	Measurement Uncertainty for lg R_T (±):	0.14	Measurement Uncertainty for lg R_C (±):	0.18
Testing Period:	24/02/2023	Tested By:	AZZ	Verified By:	CSE

Validation & Controls

Extraction Control (EC)	EC = 5.53E+06	
	80% x CI = 4.34E+06	Limit: EC ≥ 80% x CI
Neutralizer Effectiveness (A)	A = 46.3	lg A = 1.66
	p = 0.3216	Limit: p (A:C) > 0.05
Neutralizer Toxicity (B)	B = 46.0	lg B = 1.66
	p = 0.2403	Limit: p (B:C) > 0.05
Test Organism Viability (C)	C = 47.5	lg C = 1.68
	Limit: 30 ≤ C ≤ 100	

Test Suspension & Procedure

Challenge Inoculum (CI)	CI_0 = 5.63E+06	CI_t = 5.22E+06
	$(CI_0 + CI_t)/2$ = CI = 5.43E+06	
	lg CI = 6.73	Limit: lg CI ≥ 6.15
	%ΔCI = 92.60	Limit: %ΔCI ≥ 90%

Contact Time (minutes)	Reduction from Challenge Inoculum (R_T)				
	Test Specimen (TS)	lg TS	lg R_T = lg CI - lg TS	%R_T = (CI - TS) x 100 % / CI	Conformance Probability (lg R_T)
0	2.99E+06	6.48	0.26 ± 0.14	44.873%	
5	6.53E+02	2.82	3.92 ± 0.14	99.988%	
10	<1.40E+02	<2.15	>4.59 ± 0.14	>99.997%	
20	<1.40E+02	<2.15	>4.59 ± 0.14	>99.997%	
30	<1.40E+02	<2.15	>4.59 ± 0.14	>99.997%	

Contact Time (minutes)	Reduction from Specimen Control (R_C)				
	Specimen Control (SC)	lg SC	lg R_C = lg SC - lg TS	%R_C = (SC - TS) x 100 % / SC	Conformance Probability (lg R_C)
0	5.53E+06	6.74	0.27 ± 0.18	45.952%	
5	7.68E+06	6.89	4.07 ± 0.18	99.991%	
10	4.58E+06	6.66	>4.52 ± 0.18	>99.997%	
20	5.67E+06	6.75	>4.61 ± 0.18	>99.998%	
30	5.85E+06	6.77	>4.62 ± 0.18	>99.998%	

RAW DATA

Replicate 1

Validation & Controls

Extraction Control (EC)	EC	V_{C1}	V_{C2}	$\bar{x}_{wm}$ x 10 = EC = 6.75E+06
	10^{-3}	>330	>330	
	10^{-4}	63	72	
Neutralizer Effectiveness (A)	A	V_{C1}	V_{C2}	A = 46.3 lg A = 1.67
	A_0	40	46	
	A_t	48	51	
Neutralizer Toxicity (B)	B	V_{C1}	V_{C2}	B = 46.0 lg B = 1.66
	B_0	43	44	
	B_t	47	50	
Test Organism Viability (C)	C	V_{C1}	V_{C2}	C = 47.5 lg C = 1.68
	C_0	39	46	
	C_t	52	53	

Test Suspension & Procedure

Initial Challenge Inoculum (Cl_0)	Cl_0	V_{C1}	V_{C2}	$\bar{x}_{wm}$ x 10 = Cl_0 = 5.80E+06
	10^{-3}	>330	>330	
	10^{-4}	56	60	
Final Challenge Inoculum (Cl_t)	Cl_t	V_{C1}	V_{C2}	$\bar{x}_{wm}$ x 10 = Cl_t = 5.05E+06
	10^{-3}	>330	>330	
	10^{-4}	50	51	

Contact Time (minutes)	Test Specimen (TS) Dilution	V_{C1}	V_{C2}	TS = $\bar{x}$ or $\bar{x}_{wm}$ x 10	lg TS	Specimen Control (SC) Dilution	V_{C1}	V_{C2}	SC = $\bar{x}$ or $\bar{x}_{wm}$ x 10	lg SC
0	10^{-2}	185	218	2.02E+05	5.31	10^{-2}	>330	>330	6.75E+06	6.83
	10^{-3}	27	<14			10^{-3}	>330	>330		
	10^{-4}	<14	<14			10^{-4}	63	72		
5	10^{0}	70	62	6.60E+02	2.82	10^{-2}	>330	>330	4.80E+06	6.68
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	49	47		
10	10^{0}	<14	<14	<1.40E+02	<2.15	10^{-2}	>330	>330	4.55E+06	6.66
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	51	40		
20	10^{0}	<14	<14	<1.40E+02	<2.15	10^{-2}	>330	>330	5.95E+06	6.77
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	56	63		
30	10^{0}	<14	<14	<1.40E+02	<2.15	10^{-2}	>330	>330	7.10E+06	6.85
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	70	72		

Raw Data of Colony Count

	EC $^{-3}$	EC $^{-4}$	A_0	A_t	B_0	B_t	C_0	C_t	Cl_0 $^{-3}$	Cl_0 $^{-4}$	Cl_t $^{-3}$	Cl_t $^{-4}$
V_{C1}	>330	63	40	48	43	47	39	52	>330	56	>330	50
V_{C2}	>330	72	46	51	44	50	46	53	>330	60	>330	51

	TS_1 $^{-2}$	TS_1 $^{-3}$	TS_1 $^{-4}$	TS_2 ^{0}	TS_2 $^{-1}$	TS_2 $^{-2}$	TS_3 ^{0}	TS_3 $^{-1}$	TS_3 $^{-2}$	TS_4 ^{0}	TS_4 $^{-1}$	TS_4 $^{-2}$	TS_5 ^{0}	TS_5 $^{-1}$	TS_5 $^{-2}$
V_{C1}	185	27	3	70	9	0	4	0	0	0	0	0	0	0	0
V_{C2}	218	13	0	62	8	0	3	0	0	0	0	0	0	0	0

	SC_1 $^{-2}$	SC_1 $^{-3}$	SC_1 $^{-4}$	SC_2 $^{-2}$	SC_2 $^{-3}$	SC_2 $^{-4}$	SC_3 $^{-2}$	SC_3 $^{-3}$	SC_3 $^{-4}$	SC_4 $^{-2}$	SC_4 $^{-3}$	SC_4 $^{-4}$	SC_5 $^{-2}$	SC_5 $^{-3}$	SC_5 $^{-4}$
V_{C1}	>330	>330	63	>330	>330	49	>330	>330	51	>330	>330	56	>330	>330	70
V_{C2}	>330	>330	72	>330	>330	47	>330	>330	40	>330	>330	63	>330	>330	72

RAW DATA

Replicate 2

Validation & Controls

Extraction Control (EC)	EC	V_{C1}	V_{C2}	$\bar{x}_{wm}$ x 10 = EC = 5.05E+06
	10^{-3}	>330	>330	
	10^{-4}	58	43	
Neutralizer Effectiveness (A)	A	V_{C1}	V_{C2}	A = 50.5 lg A = 1.70
	A_0	49	51	
	A_t	58	44	
Neutralizer Toxicity (B)	B	V_{C1}	V_{C2}	B = 49.0 lg B = 1.69
	B_0	53	49	
	B_t	44	50	
Test Organism Viability (C)	C	V_{C1}	V_{C2}	C = 48.8 lg C = 1.69
	C_0	41	43	
	C_t	56	55	

Test Suspension & Procedure

Initial Challenge Inoculum (Cl_0)	Cl_0	V_{C1}	V_{C2}	$\bar{x}_{wm}$ x 10 = Cl_0 = 5.35E+06
	10^{-3}	>330	>330	
	10^{-4}	54	53	
Final Challenge Inoculum (Cl_t)	Cl_t	V_{C1}	V_{C2}	$\bar{x}_{wm}$ x 10 = Cl_t = 5.45E+06
	10^{-3}	>330	>330	
	10^{-4}	57	52	

Contact Time (minutes)	Test Specimen (TS)			TS = $\bar{x}$ or $\bar{x}_{wm}$ x 10	lg TS	Specimen Control (SC)			SC = $\bar{x}$ or $\bar{x}_{wm}$ x 10	lg SC
	Dilution	V_{C1}	V_{C2}			Dilution	V_{C1}	V_{C2}		
0	10^{-2}	>330	>330	1.17E+06	6.07	10^{-2}	>330	>330	5.05E+06	6.70
	10^{-3}	178	42			10^{-3}	>330	>330		
	10^{-4}	20	17			10^{-4}	58	43		
5	10^{0}	71	43	5.70E+02	2.76	10^{-2}	>330	>330	8.15E+06	6.91
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	72	91		
10	10^{0}	<14	<14	<1.40E+02	<2.15	10^{-2}	>330	>330	4.65E+06	6.67
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	42	51		
20	10^{0}	<14	<14	<1.40E+02	<2.15	10^{-2}	>330	>330	6.55E+06	6.82
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	61	70		
30	10^{0}	<14	<14	<1.40E+02	<2.15	10^{-2}	>330	>330	6.20E+06	6.79
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	63	61		

Raw Data of Colony Count

	EC $^{-3}$	EC $^{-4}$	A_0	A_t	B_0	B_t	C_0	C_t	Cl_0 $^{-3}$	Cl_0 $^{-4}$	Cl_t $^{-3}$	Cl_t $^{-4}$
V_{C1}	>330	58	49	58	53	44	41	56	>330	54	>330	57
V_{C2}	>330	43	51	44	49	50	43	55	>330	53	>330	52

	TS_1 $^{-2}$	TS_1 $^{-3}$	TS_1 $^{-4}$	TS_2 ^{0}	TS_2 $^{-1}$	TS_2 $^{-2}$	TS_3 ^{0}	TS_3 $^{-1}$	TS_3 $^{-2}$	TS_4 ^{0}	TS_4 $^{-1}$	TS_4 $^{-2}$	TS_5 ^{0}	TS_5 $^{-1}$	TS_5 $^{-2}$
V_{C1}	>330	178	20	71	0	0	0	0	0	0	0	0	0	0	0
V_{C2}	>330	42	17	43	0	0	0	0	0	0	0	0	0	0	0

	SC_1 $^{-2}$	SC_1 $^{-3}$	SC_1 $^{-4}$	SC_2 $^{-2}$	SC_2 $^{-3}$	SC_2 $^{-4}$	SC_3 $^{-2}$	SC_3 $^{-3}$	SC_3 $^{-4}$	SC_4 $^{-2}$	SC_4 $^{-3}$	SC_4 $^{-4}$	SC_5 $^{-2}$	SC_5 $^{-3}$	SC_5 $^{-4}$
V_{C1}	>330	>330	58	>330	>330	72	>330	>330	42	>330	>330	61	>330	>330	63
V_{C2}	>330	>330	43	>330	>330	91	>330	>330	51	>330	>330	70	>330	>330	61

RAW DATA

Replicate 3

Validation & Controls

Extraction Control (EC)	EC	V_{C1}	V_{C2}	$\bar{x}_{wm}$ x 10 = EC = 4.80E+06
	10^{-3}	>330	>330	
	10^{-4}	42	54	
Neutralizer Effectiveness (A)	A	V_{C1}	V_{C2}	A = 42.0 lg A = 1.62
	A_0	39	33	
	A_t	49	47	
Neutralizer Toxicity (B)	B	V_{C1}	V_{C2}	B = 43.0 lg B = 1.63
	B_0	35	37	
	B_t	53	47	
Test Organism Viability (C)	C	V_{C1}	V_{C2}	C = 46.3 lg C = 1.67
	C_0	51	35	
	C_t	48	51	

Test Suspension & Procedure

Initial Challenge Inoculum (Cl_0)	Cl_0	V_{C1}	V_{C2}	$\bar{x}_{wm}$ x 10 = Cl_0 = 5.75E+06
	10^{-3}	>330	>330	
	10^{-4}	61	54	
Final Challenge Inoculum (Cl_t)	Cl_t	V_{C1}	V_{C2}	$\bar{x}_{wm}$ x 10 = Cl_t = 5.15E+06
	10^{-3}	>330	>330	
	10^{-4}	54	49	

Contact Time (minutes)	Test Specimen (TS) Dilution	V_{C1}	V_{C2}	TS = $\bar{x}$ or $\bar{x}_{wm}$ x 10	lg TS	Specimen Control (SC) Dilution	V_{C1}	V_{C2}	SC = $\bar{x}$ or $\bar{x}_{wm}$ x 10	lg SC
0	10^{-2}	>330	>330	7.60E+06	6.88	10^{-2}	>330	>330	4.80E+06	6.68
	10^{-3}	>330	>330			10^{-3}	>330	>330		
	10^{-4}	80	72			10^{-4}	42	54		
5	10^{0}	76	70	7.30E+02	2.86	10^{-2}	>330	>330	1.01E+07	7.00
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	100	101		
10	10^{0}	<14	<14	<1.40E+02	<2.15	10^{-2}	>330	>330	4.55E+06	6.66
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	46	45		
20	10^{0}	<14	<14	<1.40E+02	<2.15	10^{-2}	>330	>330	4.50E+06	6.65
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	50	40		
30	10^{0}	<14	<14	<1.40E+02	<2.15	10^{-2}	>330	>330	4.25E+06	6.63
	10^{-1}	<14	<14			10^{-3}	>330	>330		
	10^{-2}	<14	<14			10^{-4}	42	43		

Raw Data of Colony Count

	EC^{-3}	EC^{-4}	A_0	A_t	B_0	B_t	C_0	C_t	Cl_0^{-3}	Cl_0^{-4}	Cl_t^{-3}	Cl_t^{-4}
V_{C1}	>330	42	39	49	35	53	51	48	>330	61	>330	54
V_{C2}	>330	54	33	47	37	47	35	51	>330	54	>330	49

	TS_1^{-2}	TS_1^{-3}	TS_1^{-4}	TS_2^{0}	TS_2^{-1}	TS_2^{-2}	TS_3^{0}	TS_3^{-1}	TS_3^{-2}	TS_4^{0}	TS_4^{-1}	TS_4^{-2}	TS_5^{0}	TS_5^{-1}	TS_5^{-2}
V_{C1}	>330	>330	80	76	10	0	8	0	0	0	0	0	0	0	0
V_{C2}	>330	>330	72	70	7	0	2	0	0	3	0	0	0	0	0

	SC_1^{-2}	SC_1^{-3}	SC_1^{-4}	SC_2^{-2}	SC_2^{-3}	SC_2^{-4}	SC_3^{-2}	SC_3^{-3}	SC_3^{-4}	SC_4^{-2}	SC_4^{-3}	SC_4^{-4}	SC_5^{-2}	SC_5^{-3}	SC_5^{-4}
V_{C1}	>330	>330	42	>330	>330	100	>330	>330	46	>330	>330	50	>330	>330	42
V_{C2}	>330	>330	54	>330	>330	101	>330	>330	45	>330	>330	40	>330	>330	43

TEST PROCEDURE

1. Test Specimen *TS*

   1.1 A test specimen with a surface area of (10.0 ± 1.0) cm^2 was cut from the glove and placed on a sterile Petri dish.

   1.2 A (20 ± 0.1) µL aliquot of the test suspension (~10^8 cfu/mL) prepared in standard saline was delivered directly onto the test specimen surface. With forceps, a sterile coverslip was immediately placed on top of the delivered challenge droplet.

   1.3 The test specimen was left in contact with the test suspension for the contact time *t*.

   1.4 Immediately at the end of *t*, the test specimen was inactivated and the remaining microorganism from the test specimen was extracted. A sterile or flamed and cooled forceps was used to immediately place the specimen with its coverslip into a 50 mL sterile conical centrifuge tube containing 10 mL of neutralizer. The mixture was vortexed for 15 seconds.

   1.5 A series of ten-fold dilutions was prepared in standard saline. Appropriate dilutions were taken in duplicate and plated out for enumeration using the pour or spread plate technique.

   1.6 If the procedure for *TS* was conducted in more than one replicate, the average of the replicates of *TS* was used in calculations.

2. Challenge Inoculum *CI*

   2.1 A (20 ± 0.1) µL aliquot of the test suspension was added to 10 mL of neutralizer.

   2.2 A series of ten-fold dilutions was prepared in standard saline. Appropriate dilutions were taken in duplicate and plated out for enumeration using the pour or spread plate technique.

   2.3 The challenge inoculum was done at the beginning and end of the testing session to ensure die-off or reproduction over that amount of time did not impact results. For the test to be valid, the microbial counts must be >90% similar.

   2.4 If the procedure for *CI* was conducted in more than one replicate, the average of the replicates of *CI* was used in calculations.

3. Specimen Control *SC*

   3.1 The specimen control identical to the test specimen but without the antimicrobial agent was prepared in the same manner as *TS*.

   3.2 The specimen control *SC* was conducted in parallel with the test specimen *TS* at all the selected contact times. The inactivation and extraction methods were the same as chosen for *TS*.

   3.3 A series of ten-fold dilutions was prepared in standard saline. Appropriate dilutions were taken in duplicate and plated out for enumeration using the pour or spread plate technique.

   3.4 If the procedure for *SC* was conducted in more than one replicate, the average of the replicates of *SC* was used in calculations.

4. Extraction Control *EC*: Verification of the Efficiency of the Extraction Method

   4.1 The extraction control *EC* was performed by inoculating the specimen control using the same challenge inoculum delivery method chosen for *TS*.

4.2 The inoculated specimen was immediately placed into 10 mL of neutralizer and extracted using the same method chosen for *TS*.

4.3 A series of ten-fold dilutions was prepared in standard saline. Appropriate dilutions were taken in duplicate and plated out for enumeration using the pour or spread plate technique.

4.4 If the procedure for *EC* was conducted in more than one replicate, the average of the replicates of *EC* was used in calculations.

5. Neutralizer Effectiveness Control *A*: Verification of the Neutralizer's Ability to Inactivate, Neutralize, or Quench the Microbiocidal Properties of an Antimicrobial Agent

5.1 A test specimen as used in *TS* with a surface area of (10.0 ± 1.0) cm^2 was added to 10 mL of neutralizer.

5.2 Within 5 seconds, the specimen/neutralizer mixture was inoculated with (20 ± 0.1) µL aliquot of the control test suspension (1.5 – 5.0 x 10^4 cfu/mL) so that the resulting suspension contains 30 to 100 cfu/mL of the microorganism.

5.3 Within 1 minute, the mixture (containing the test specimen, neutralizer, and control test suspension) was taken in duplicate and plated out for enumeration using the pour or spread plate technique.

5.4 The mixture was allowed to stand for the longest contact time tested for *TS*.

5.5 After the hold time, the mixture was taken in duplicate and plated out for enumeration using the pour or spread plate technique.

5.6 If the procedure for *A* was conducted in more than one replicate, the average of the replicates of *A* was used in calculations.

6. Neutralizer Toxicity Control *B*: Verification of the Absence of Any Inhibitory Effects the Neutralizer May Have on the Survival of a Microbial Population

6.1 A specimen control as used in *SC* with a surface area of (10.0 ± 1.0) cm^2 was added to 10 mL of neutralizer.

6.2 Within 5 seconds, the specimen/neutralizer mixture was inoculated with (20 ± 0.1) µL aliquot of the control test suspension (1.5 – 5.0 x 10^4 cfu/mL) so that the resulting suspension contains 30 to 100 cfu/mL of the microorganism.

6.3 Within 1 minute, the mixture (containing the control specimen, neutralizer, and control test suspension) was taken in duplicate and plated out for enumeration using the pour or spread plate technique.

6.4 The mixture was allowed to stand for the longest contact time tested for *TS*.

6.5 After the hold time, the mixture was taken in duplicate and plated out for enumeration using the pour or spread plate technique.

6.6 If the procedure for *B* was conducted in more than one replicate, the average of the replicates of *B* was used in calculations.

7. Test Organism Viability Control *C*: Verification of the Population or Viability of the Challenge Microorganism

7.1 10 mL of standard saline was inoculated with a (20 ± 0.1) µL aliquot of the control test suspension (1.5 – 5.0 x 10^4 cfu/mL) so that the resulting suspension contains 30 to 100 cfu/mL of the microorganism.

7.2 Within 1 minute, the mixture (containing the control specimen and standard saline) was taken in duplicate and plated out for enumeration using the pour or spread plate technique.

7.3 The mixture was allowed to stand for the longest contact time tested for *TS*.

7.4 After the hold time, the mixture was taken in duplicate and plated out for enumeration using the pour or spread plate technique.

7.5 If the procedure for C was conducted in more than one replicate, the average of the replicates of C was used in calculations.

8. Incubation and Counting

8.1 The plates were incubated for 20 to 24 hours. The plates were counted to determine the number of cfu. Any plates which were not countable for any reason were discarded.

8.2 For each plate, the exact number of colonies were noted but any counts higher than 330 colonies were recorded as '>330'.

8.3 All experimental data were reported as V_C values, in which a V_C value is the number of cfu counted per 1.0 mL sample inoculated.

8.4 Only V_C values within the counting limits, i.e., 14 to 330 colonies, were taken into account for further calculation, except in the case of *TS* and *SC*.