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ACCUGEN LABORATORIES INC.

FINAL REPORT

TIME KILL STUDIES ASTM E 2315

Assessment of Antimicrobial Activity Using a Time-Kill Procedure E2315-16

TEST AGENT

Sample ID: Colloidal detergent solution, CTAB, Alkylphenol ethoxylate, Lot#: NA

TEST TYPE

Liquid

Order #: 4843, **Sample #:**43187

PO#

NA

DATA REQUIREMENTS

US FDA CFR21 Part 58 (GLP)

TEST ORDERED

ASTM E 2315-16

SPONSOR

295 W. Foothill Blvd Suite C439

Claremont, CA 91711

Contact: Eric Weber. PhD

Phone: 970-218-6269

E-mail: eric.weber@ecofuelsaver.com

TESTING LABORATORY

Accugen Laboratories, Inc.

2121 W Army Trail Road

Addison, IL 60101

Tel: 630-789-8105, Toll free: 800-282-7102, Fax: 630-812-2219

Web: www.accugenlabs.com

E-mail: info@accugenlabs.com

DATE TEST STARTED: 05/27/2021 **DATE TEST COMPLETED:** 06-15-2021

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COMPLIANCE STATEMENT

This study meets the requirements for US FDA CFR21 Part 58 (GLP)

with the following exceptions:

- Information on the identity, strength, purity, stability, uniformity, dose solution and analysis of the test agent resides with the sponsor of the study.

The following technical personnel participated in this study:

Shamim Rizvi BS, Zafar Mirza M.D.



Study Director: _____
Tehseen Naqvi, M.S Microbiology, M (ASCP)

Date: 06/15/2021

QUALITY ASSURANCE STATEMENT

Title of Study: Assessment of Antimicrobial Activity Using a Time-Kill Procedure E2315-16

The Quality Assurance Unit has inspected the study and found in compliance with 21 CFR Part 58.

The dates that inspections were made and the dates that findings were reported to management and to the study director are listed below.

Phase Inspected	Date of Inspection	Date Reported to Study Director	Date Reported to Management
Protocol Approval	-	NA	
In Process	05/27/2021	06/01/2021	06/01/2021
In Process	06/04/2021	06/04/2021	06/04/2021
In Process	06/07/2021	06/07/2021	06/07/2021
In Process	06/08/2021	06/08/2021	06/08/2021
In Process	06/10/2021	06/10/2021	06/10/2021
In Process	06/14/2021	06/14/2021	06/14/2021
Final Report	06/15/2021	06/15/2021	06/15/2021



Quality Assurance

Date: 06/15/2021

TITLE:

Time Kill ASTM E 2315-16

TEST AGENT:Colloidal detergent solution, CTAB,
Alkylphenol ethoxylate,**SAMPLE TYPE:**

Liquid

SAMPLE PREPERATION:

Ready to use.

INOCULUM AMOUNT:

≤ 5% of the total volume of the test material.

SOLID MEDIA & DILUENT USED

- Tryptic soy agar
Lot# US112879A -052821-01
Exp:08/28/2021
- Tryptic soy agar
Lot#: US112879A -060221-01
Exp: 09-02-2021
- Phosphate buffer
Lot# 052421-PBS-01Exp:08/24/21
Lot# 060121-PBS-01Exp:09/01/21

NEUTRALIZER:

- D/E Neutralizing Broth
Lot# US112097A-051921-01
Exp: 08-19-2021
Lot# US112097A-060921-01
Exp: 09-09-2021

CHALLENGE MICROORGANISMS:

- Escherichia coli (0157:H7) ATCC#48390
- Staphylococcus aureus ATCC# 6538
- Shigella sonnei ATCC# 9290
- Salmonella typhimurium ATCC#14028
- MRSA ATCC#33591
- Streptococcus pyogenes ATCC#19615
- Candida albicans ATCC#10231

REAGENTS:

- Gram stain kit
- Biochemicals (catalase, coagulase, indole, & Oxidase), API kit

CONTACT TIME:

15 ,30, & 60 seconds

CONTACT TEMPERATURE:

25 ± 2° C

INCUBATION CONDITION:

32.5 °C ± 2.5 Aerobically

STERILITY CONTROL(S):

Pass

PURITY CONTROL(S):

Pass

GROWTH PROMOTION TEST:

Pass

PERSONNEL PARTICIPATED IN STUDY:

Shamim Rizvi B.S., Zafar Mirza M.D.

PROTOCOL #: N/A

TITLE: Assessment of Antimicrobial Activity Using a Time-Kill Procedure ASTM E2315-16

STUDY DESIGN:

This study was designed to assess the antimicrobial activity of a test agent to kill Specified organisms within a given contact time.

SUMMARY:

The test material was brought into contact with a known population of microorganisms for a specified exposure time at a specified temperature. The activity of the test material was quenched by appropriate neutralization technique and the surviving organisms were enumerated. The percent reduction/ log reduction was calculated from initial population.

DEFINITIONS:

N/A- Not Applicable **TNTC-** Too Numerous To Count **CFU-** Colony Forming Units
OC: Organism Control Number **PO#-** Purchase Order Number **LOG-** Logarithm

TEST MATERIALS:

APPARATUS

- Sterile Test Tubes.
- Sterile Pipettes
- Sterile Cotton Applicator Swabs.
- Plating System

Equipment:

- Calibrated Timer (Stop-clock) that displays minutes and seconds. (NIST standard).
- Sterilizer
- Vortex Mixer/ Magnetic Stirrer
- Water Bath
- Colony Counter
- Incubator set at appropriate temperature.

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DISCLOSURE

Information on the identity, strength, stability, Method uniformity and validity, and dose solution analysis of the test agent resides with the sponsor of the study. The results apply to the sample as received and tested.

TESTING FACILITY:

Studies were conducted by Accugen Laboratories, Inc located at 2121 W Army Trail Road, Addison IL 60101

METHOD REFERENCE:

- ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, ASTM E 2315 -16
- Jeng, D. K., "Testing Methodology of Preoperative Skin Preparation and Surgical Scrub as Over-the-Counter Drugs," *Handbook of Topical Antimicrobials*, Paulson, D. S., Ed., Marcel Dekker, Chapter 25, 2003, pp. 377-393.
- The Food and Drug Administration (FDA), *Tentative Final Monograph for Health-Care Antiseptic Drug Products*; Proposed Rule 59 Federal Register 31402-31451, June 17, 1994.

RECORDS TO BE MAINTAINED:

All testing data, test material records, final reports, and any correspondence will be stored in the archives, including but not limited to the following.

- Data for Growth Promotion and Media Qualification
- Data for Microbial Enumeration and activity results form
- Data for Media, Reagents and Equipment Use

TEST METHOD

Organism Preparation: Culture(s) from stock cultures were transferred into appropriate growth media. The organisms were sub-cultured onto the growth media twice.

Inoculum Suspension Preparation and Determination of the Microbial Population:

The inoculum suspensions were enumerated in duplicate by standard microbiological procedures at the initiation and completion of testing. Appropriate dilutions were prepared and enumerated by standard microbiological procedures

- To prepare inoculum suspension directly from broth growth medium, dilution was performed in same growth media or in appropriate diluent.

or

- To prepare the inoculum suspension from an agar plate, microbial growth from the agar surface was washed with appropriate diluent.
- 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} dilutions of organism was prepared.
- 1.0 ml of each dilution was plated onto the growth media. The plates were incubated at appropriate temperature and time.
- The plates were then observed for growth after incubation.
- Colonies were counted.

The inoculum suspension carrying a minimum of 10^8 cfu/mL microbial population was selected to achieve a minimum population concentration of 10^6 cfu/mL. The final inoculum suspension was well mixed prior to addition to test materials.

PROCEDURE:

- 1 The test samples were added to sterile glass tubes in duplicate.
- 2 Each recovery sample was plated in duplicate.
- 3 Contact times were as specified by the sponsor.
- 4 The microbial population inoculum was a minimum of 10^6 cfu / mL.
- 5 The organism suspension was added directly onto the test sample tubes and control tubes containing sterile phosphate buffer. Tubes were vortexed thoroughly to mix the organisms.
- 6 At 0 hour (for control) and after contact time as specified by sponsor, aliquot of the sample and control were taken out and added into sterile neutralizing broth tubes. Suspensions were vortexed and 10-fold serial dilutions were prepared.

- 7 Aliquots from each dilution were plated in duplicate and plates were incubated at appropriate temperature and time for organism.
- 8 Colonies were counted, and the concentration of viable cells was calculated.

STUDY CONTROLS:**PURITY CONTROL:**

Test organism was streaked on growth media, incubated at appropriate temperature and time. Observed to confirm the presence of a pure culture. Characteristics like Gram stain reaction and colony morphology were confirmed to assure the purity of test microorganisms. Test organisms was found pure.

STERILITY CONTROLS / NEGATIVE CONTROLS:

Sterility of all reagents including diluents, growth media, and neutralizer were checked alongside the test. No test agent and no test organism were added. No growth was observed.

ENVIRONMENT CONTROL:

Growth media plate was left open during the test to monitor the test environment. No organism was isolated.

GROWTH PROMOTION TEST:

To determine that the media used will support the growth of test organisms, growth promotion tests were carried out. Less than 100 cfu of culture was inoculated into growth media. Media which passed the growth promotion tests were used to carry out the test.

POSITIVE CONTROLS

Same concentration of test organisms as in case of test sample were added to same volume of phosphate buffer without adding any test agent. Suspensions were plated and incubated along the test.

Neutralization Effectiveness Control:

One ml of the test sample was added in 9 ml of neutralizer. Two tenfold serial dilutions of the test agent were made using neutralizing broth. 0.1 mL of organism suspension

containing approximately 100-1000 cfu/mL to make it ≤ 100 CFU/ml was added to the neutralized samples and vortexed to mix. Control was also carried out by adding 0.1 mL of the same organism suspension to 9 ml of the same neutralizer. Plated in duplicate and incubated at appropriate temperature and time for organism.

CALCULATIONS:

1. The measured initial microbial population of each replicate control Blank was transformed into the \log_{10} scale.
2. The mean and variance associated with each control replicate for each of the \log_{10} values.
3. The measured surviving microbial population, per replicate, per contact time, was transformed into the \log_{10} scale.
4. The mean and variance associated with each test replicate for each of the \log_{10} values.

The log reduction was calculated following the formula:

$$\text{Log}_{10} \text{ reduction (LR)} = \text{mean } \log_{10}(\text{measured initial microbial population}) - \text{mean } \log_{10}(\text{surviving microbial test population})$$

5. The Percent Reduction was calculated using the log reduction.

$$\text{Percent Reduction} = 100 * (1 - 10^{-LR})$$

TABLE 1: NEUTRALIZER EFFECTIVENESS TEST RESULTS									
Test Organism	CFU/ml						Log Difference	Percent recovered	Result Passed (≥70 %) @ Dilution
	Control		Growth on 10 ⁻¹ dilution of Test agent		Growth on 10 ⁻² dilution of Test agent				
	Plate 1	Average	Plate 1	Average	Plate 1	Average			
	Plate 2		Plate 2		Plate 2				
MRSA ATCC# 33591	34	37	44	41	34	31.5	-0.0445	110.8%	10 ⁻¹
	40		38		29				
E.coli (0157:H7) ATCC# 48390	86	88	81	76.5	68	71.5	0.061	115.03%	10 ⁻¹
	90		72		75				
Streptococcus pyogenes ATCC#19615	98	95.5	98	96	97	94	0.423	97.96%	10 ⁻¹
	93		94		91				
Shigella sonnei ATCC # 9290	87	86	84	88	83	81	-0.9015	102.33%	10 ⁻¹
	85		92		79				
Salmonella typhimurium ATCC # 14028	155	160	123	124	115	119	0.19	77.50%	10 ⁻¹
	165		125		123				
Candida. albicans ATCC # 10231	73	75	72	74	80	79	0.586	98.67%	10 ⁻¹
	77		76		78				
Streptococcus mutans ATCC# 25175	94	96	92	91	76	82	0.0232	94.79%	10 ⁻¹
	98		90		88				

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TABLE 2

**Organism Name: MRSA ATCC#33591 (15 seconds, 30 seconds, 60 seconds)
O.C #496-82-1-Sa-02-P3-083121**

Lab#	Replicate	CFU/Plate	Dilution	Recovered Organisms in CFU/mL	Mean in CFU/mL	Mean CFU/mL	Log of Mean CFU/mL	Geom. Mean of Log	Variance	Standard Error	Log Reduction*	Standard Error of the mean	% Reduction
Initial Control Count	1	33	1.00E+05	3.30E+06	3.35E+06	3.48E+06	6.53	6.54	0.00	0.00	--	--	--
		34	1.00E+05	3.40E+06									
	2	33	1.00E+05	3.30E+06	3.60E+06	3.48E+06	6.56	6.54	0.00	0.00	--	--	--
		39	1.00E+05	3.90E+06									
Final Control Count at Longest Specified contact time: 60 sec	1	38	1.00E+05	3.80E+06	3.65E+06	3.58E+06	6.56	6.55	0.00	0.00	0.013	--	--
		35	1.00E+05	3.50E+06									
	2	39	1.00E+05	3.90E+06	3.50E+06	3.58E+06	6.54	6.55	0.00	0.00	Valid	--	--
		31	1.00E+05	3.10E+06									
Test agent Count at Specified contact time: 15 sec	1	46	1.00E+05	4.60E+06	3.90E+06	4.10E+06	6.59	6.61	0.00	0.00	-0.07	0.03	-17.926538
		32	1.00E+05	3.20E+06									
	2	47	1.00E+05	4.70E+06	4.30E+06	4.10E+06	6.63	6.61	0.00	0.00	-0.07	0.03	-17.926538
		39	1.00E+05	3.90E+06									
Test agent Count at Specified contact time: 30 sec	1	48	1.00E+05	4.80E+06	4.65E+06	4.20E+06	6.67	6.62	0.00	0.00	-0.08	0.05	-20.204949
		45	1.00E+05	4.50E+06									
	2	38	1.00E+05	3.80E+06	3.75E+06	4.20E+06	6.57	6.62	0.00	0.00	-0.08	0.05	-20.204949
		37	1.00E+05	3.70E+06									
Test agent Count at Specified contact time: 60 sec	1	37	1.00E+05	3.70E+06	3.60E+06	4.00E+06	6.56	6.60	0.00	0.00	-0.06	0.05	-14.610025
		35	1.00E+05	3.50E+06									
	2	46	1.00E+05	4.60E+06	4.40E+06	4.00E+06	6.64	6.60	0.00	0.00	-0.06	0.05	-14.610025
		42	1.00E+05	4.20E+06									

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TABLE 3

Organism Name: Escherichia coli (0157:H7) ATCC# 43890 15 Seconds, 30 Seconds, 60 seconds)

OC: 1204-07-8-Ec-01-P3-063022

Lab#	Replicate	CFU/Plate	Dilution	Recovered Organisms in CFU/mL	Mean in CFU/mL	Mean CFU/mL	Log of Mean CFU/mL	Geom. Mean of Log	Variance	Standard Error	Log Reduction*	Standard Error of the mean	% Reduction
Initial Control Count	1	30	1.00E+05	3.00E+06	3.45E+06	3.85E+06	6.54	6.58	0.00	0.00	--	--	--
		39	1.00E+05	3.90E+06									
	2	45	1.00E+05	4.50E+06	4.25E+06		6.63						
		40	1.00E+05	4.00E+06									
Final Control Count at Longest Specified contact time: 60 sec	1	57	1.00E+05	5.70E+06	4.90E+06	4.75E+06	6.69	6.68	0.00	0.00	0.094	--	--
		41	1.00E+05	4.10E+06									
	2	37	1.00E+05	3.70E+06	4.60E+06		6.66				Valid		
		55	1.00E+05	5.50E+06									
Test agent Count at Specified contact time: 15 sec	1	71	1.00E+04	7.10E+05	7.20E+05	6.80E+05	5.86	5.83	0.00	0.00	0.75	0.05	82.265948
		73	1.00E+04	7.30E+05									
	2	74	1.00E+04	7.40E+05	6.40E+05		5.81						
		54	1.00E+04	5.40E+05									
Test agent Count at Specified contact time: 30 sec	1	70	1.00E+04	7.00E+05	8.20E+05	6.58E+05	5.91	5.80	0.02	0.01	0.78	0.12	83.395480
		94	1.00E+04	9.40E+05									
	2	42	1.00E+04	4.20E+05	4.95E+05		5.69						
		57	1.00E+04	5.70E+05									
Test agent Count at Specified contact time: 60 sec	1	53	1.00E+04	5.30E+05	5.10E+05	6.15E+05	5.71	5.78	0.01	0.01	0.80	0.09	84.169192
		49	1.00E+04	4.90E+05									
	2	65	1.00E+04	6.50E+05	7.20E+05		5.86						
		79	1.00E+04	7.90E+05									

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TABLE 4

Organism Name: Streptococcus pyogenes ATCC#19615 (15 Seconds, 30 Seconds, 60 seconds)

O.C #: 385-187-5-Sp-02-P3-093021

Lab#	Replicate	CFU/Plate	Dilution	Recovered Organisms in CFU/mL	Mean in CFU/mL	Mean CFU/mL	Log of Mean CFU/mL	Geom. Mean of Log	Variance	Standard Error	Log Reduction*	Standard Error of the mean	% Reduction
Initial Control Count	1	64	1.00E+06	6.40E+07	7.45E+07	7.08E+07	7.87	7.85	0.00	0.00	--	--	--
		85	1.00E+06	8.50E+07			7.83						
	2	68	1.00E+06	6.80E+07	6.70E+07		7.83						
		66	1.00E+06	6.60E+07									
Final Control Count at Longest Specified contact time:60 sec	1	22	1.00E+06	2.20E+07	2.40E+07	3.08E+07	7.38	7.48	0.02	0.01	0.373	--	--
		26	1.00E+06	2.60E+07			7.57						
	2	35	1.00E+06	3.50E+07	3.75E+07		7.57						
		40	1.00E+06	4.00E+07									
Test agent Count at Specified contact time: 15 sec	1	26	1.00E+03	2.60E+04	2.85E+04	1.49E+04	4.45	3.78	0.92	0.46	4.07	0.68	99.991551
		31	1.00E+03	3.10E+04			3.10						
	2	10	1.00E+02	1.00E+03	1.25E+03		3.10						
		15	1.00E+02	1.50E+03									
Test agent Count at Specified contact time: 30 sec	1	4	1.00E+01	4.00E+01	3.50E+01	1.75E+01	1.54	1.24	0.15	0.07	6.61	0.27	99.999975
		3	1.00E+01	3.00E+01			< 1.00						
	2	0	1.00E+01	<10	<10		< 1.00						
		0	1.00E+01	<10									
Test agent Count at Specified contact time: 60 sec	1	0	1.00E+01	<10	<10	<10	< 1.00	< 1.00	0.00	0.00	> 6.85	0.02	> 99.999986
		0	1.00E+01	<10			< 1.00						
	2	0	1.00E+01	<10	<10		< 1.00						
		0	1.00E+01	<10									

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TABLE 5**Organism Name: Shigella sonnei ATCC # 9290 (15 Seconds, 30 Seconds, 60 seconds)****OC # 446-80-9-Ss -S4-012821-01**

Lab#	Replicate	CFU/ Plate	Dilution	Recovered Organisms in CFU/mL	Mean in CFU/mL	Mean CFU/mL	Log of Mean CFU/ mL	Geom. Mean of Log	Variance	Standard Error	Log Reduction *	Standard Error of the mean	% Reduction
Initial Control Count	1	24	1.00E+06	2.40E+07	2.90E+07	2.75E+07	7.46	7.44	0.00	0.00	--	--	--
		34	1.00E+06	3.40E+07									
	2	30	1.00E+06	3.00E+07	2.60E+07		7.41						
		22	1.00E+06	2.20E+07									
Final Control Count at Longest Specified contact time:60 sec	1	61	1.00E+06	6.10E+07	6.40E+07	6.05E+07	7.81	7.78	0.00	0.00	0.342	--	--
		67	1.00E+06	6.70E+07									
	2	55	1.00E+06	5.50E+07	5.70E+07		7.76						
		59	1.00E+06	5.90E+07									
Test agent Count at Specified contact time: 15 sec	1	48	1.00E+06	4.80E+07	4.45E+07	4.63E+07	7.65	7.66	0.00	0.00	-0.23	0.03	-68.326598
		41	1.00E+06	4.10E+07									
	2	53	1.00E+06	5.30E+07	4.80E+07		7.68						
		43	1.00E+06	4.30E+07									
Test agent Count at Specified contact time: 30 sec	1	51	1.00E+06	5.10E+07	5.30E+07	5.13E+07	7.72	7.71	0.00	0.00	-0.27	0.03	-86.542753
		55	1.00E+06	5.50E+07									
	2	50	1.00E+06	5.00E+07	4.95E+07		7.69						
		49	1.00E+06	4.90E+07									
Test agent Count at Specified contact time: 60 sec	1	77	1.00E+06	7.70E+07	7.45E+07	7.65E+07	7.87	7.88	0.00	0.00	-0.44	0.03	-178.525579
		72	1.00E+06	7.20E+07									
	2	86	1.00E+06	8.60E+07	7.85E+07		7.89						
		71	1.00E+06	7.10E+07									

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TABLE# 6**Organism Name: Salmonella typhimurium ATCC#14028****(15 Seconds, 30 Seconds, 60 seconds)****O.C # 363-520-2-St-01-P3-053122**

Lab#	Replicate	CFU/Plate	Dilution	Recovered Organisms in CFU/mL	Mean in CFU/mL	Mean CFU/mL	Log of Mean CFU/mL	Geom. Mean of Log	Variance	Standard Error	Log Reduction*	Standard Error of the mean	% Reduction
Initial Control Count	1	33	1.00E+05	3.30E+06	3.40E+06	3.80E+06	6.53	6.58	0.00	0.00	--	--	--
		35	1.00E+05	3.50E+06									
	2	40	1.00E+05	4.00E+06	4.20E+06		6.62						
		44	1.00E+05	4.40E+06									
Final Control Count at Longest Specified contact time:60 sec	1	33	1.00E+05	3.30E+06	3.95E+06	4.33E+06	6.60	6.63	0.00	0.00	0.057	--	--
		46	1.00E+05	4.60E+06									
	2	48	1.00E+05	4.80E+06	4.70E+06		6.67				Valid		
		46	1.00E+05	4.60E+06									
Test agent Count at Specified contact time: 15 sec	1	81	1.00E+05	8.10E+06	7.75E+06	7.25E+06	6.89	6.86	0.00	0.00	-0.28	0.05	-91.469097
		74	1.00E+05	7.40E+06									
	2	70	1.00E+05	7.00E+06	6.75E+06		6.83						
		65	1.00E+05	6.50E+06									
Test agent Count at Specified contact time: 30 sec	1	108	1.00E+05	1.08E+07	9.90E+06	1.05E+07	7.00	7.02	0.00	0.00	-0.44	0.05	-176.856117
		90	1.00E+05	9.00E+06									
	2	103	1.00E+05	1.03E+07	1.11E+07		7.04						
		118	1.00E+05	1.18E+07									
Test agent Count at Specified contact time: 60 sec	1	120	1.00E+05	1.20E+07	1.34E+07	1.19E+07	7.13	7.07	0.01	0.00	-0.49	0.07	-211.004707
		148	1.00E+05	1.48E+07									
	2	98	1.00E+05	9.80E+06	1.03E+07		7.01						
		108	1.00E+05	1.08E+07									

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TABLE # 7**Organism Name: Candida albicans ATCC#10231, (15 Seconds, 30 Seconds, 60 seconds) O.C #443-118-1-Ca-01-P3-073122**

Lab#	Replicate	CFU/Plate	Dilution	Recovered Organisms in CFU/mL	Mean in CFU/mL	Mean CFU/mL	Log of Mean CFU/mL	Geom. Mean of Log	Variance	Standard Error	Log Reduction*	Standard Error of the mean	% Reduction
Initial Control Count	1	25	1.00E+05	2.50E+06	2.60E+06	2.53E+06	6.41	6.40	0.00	0.00	--	--	--
		27	1.00E+05	2.70E+06			6.39						
	2	23	1.00E+05	2.30E+06	2.45E+06		6.39						
		26	1.00E+05	2.60E+06			6.39						
Final Control Count at Longest Specified contact time:60 sec	1	21	1.00E+05	2.10E+06	2.40E+06	2.58E+06	6.38	6.41	0.00	0.00	0.008	--	--
		27	1.00E+05	2.70E+06			6.44						
	2	26	1.00E+05	2.60E+06	2.75E+06		6.44						
		29	1.00E+05	2.90E+06			6.44						
Test agent Count at Specified contact time: 15 sec	1	28	1.00E+05	2.80E+06	3.05E+06	3.20E+06	6.48	6.50	0.00	0.00	-0.10	0.02	-26.653038
		33	1.00E+05	3.30E+06			6.53						
	2	32	1.00E+05	3.20E+06	3.35E+06		6.53						
		35	1.00E+05	3.50E+06			6.53						
Test agent Count at Specified contact time: 30 sec	1	61	1.00E+05	6.10E+06	6.20E+06	6.08E+06	6.79	6.78	0.00	0.00	-0.38	0.02	-140.653248
		63	1.00E+05	6.30E+06			6.77						
	2	57	1.00E+05	5.70E+06	5.95E+06		6.77						
		62	1.00E+05	6.20E+06			6.77						
Test agent Count at Specified contact time: 60 sec	1	56	1.00E+05	5.60E+06	4.85E+06	4.83E+06	6.69	6.68	0.00	0.00	-0.28	0.01	-91.176619
		41	1.00E+05	4.10E+06			6.68						
	2	49	1.00E+05	4.90E+06	4.80E+06		6.68						
		47	1.00E+05	4.70E+06			6.68						

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TABLE # 8

Organism Name: Streptococcus mutans ATCC# 25175, (15 Seconds, 30 Seconds, 60 seconds)

O.C #: 266-32-3-Sm-01-P3-083122

Lab#	Replicate	CFU/Plate	Dilution	Recovered Organisms in CFU/mL	Mean in CFU/mL	Mean CFU/mL	Log of Mean CFU/mL	Geom. Mean of Log	Variance	Standard Error	Log Reduction*	Standard Error of the mean	% Reduction							
Initial Control Count	1	159	1.00E+06	1.59E+08	1.33E+08	1.29E+08	8.12	8.11	0.00	0.00	--	--	--							
		107	1.00E+06	1.07E+08			8.10													
	2	123	1.00E+06	1.23E+08	1.26E+08		8.11							8.17	0.01	0.00	Valid	--	--	
		128	1.00E+06	1.28E+08																
Final Control Count at Longest Specified contact time: 60 sec	1	138	1.00E+06	1.38E+08	1.30E+08	1.51E+08		8.11	8.17	0.01	0.00	0.063	--							--
		122	1.00E+06	1.22E+08				8.24												
	2	180	1.00E+06	1.80E+08	1.72E+08		7.64	7.63						0.00	0.00	0.49	0.03	66.162953		
		164	1.00E+06	1.64E+08																
Test agent Count at Specified contact time: 15 sec	1	47	1.00E+06	4.70E+07	4.20E+07	4.38E+07			7.62	7.64	0.00	0.00	0.47						0.02	66.162953
		37	1.00E+06	3.70E+07					7.66											
	2	48	1.00E+06	4.80E+07	4.55E+07		7.60	7.63	0.00					0.00	0.49	0.03	67.345956			
		43	1.00E+06	4.30E+07																
Test agent Count at Specified contact time: 30 sec	1	43	1.00E+06	4.30E+07	4.00E+07	4.23E+07				7.60	7.63	0.00	0.00					0.49	0.03	67.345956
		37	1.00E+06	3.70E+07						7.65										
	2	41	1.00E+06	4.10E+07	4.45E+07		7.48	7.51	0.00	0.00				0.60	0.03	74.710162				
		48	1.00E+06	4.80E+07																
Test agent Count at Specified contact time: 60 sec	1	32	1.00E+06	3.20E+07	3.05E+07	3.28E+07					7.48	7.51	0.00				0.00	0.60	0.03	74.710162
		29	1.00E+06	2.90E+07							7.54									
	2	36	1.00E+06	3.60E+07	3.50E+07		7.54	7.54	0.00	0.00	0.60			0.03	74.710162					
		34	1.00E+06	3.40E+07																

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TABLE # 9: RESULTS SUMMARY

Microorganism ID.	Percent Reduction At 15 seconds	Logarithmic Reduction At 15 seconds	Percent Reduction At 30 seconds	Logarithmic Reduction At 30 seconds	Percent Reduction At 60 seconds	Logarithmic Reduction At 60 seconds
S. aureus (MRSA) ATCC# 33591	-17.926538	-0.07	-20.204949	-0.08	-14.610025	-0.06
E. coli (0157:H7) ATCC# 48390	82.268238	0.75	83.355863	0.78	84.169192	0.80
S. pyogenes ATCC#19615	99.991551	4.07	99.999975	6.61	>99.999986	>6.85
S. sonnei ATCC # 9290	-68.326598	-0.23	-86.542753	-0.27	-178.525579	-0.44
S. typhimurium ATCC # 14028	-91.469097	-0.28	-176.856117	-0.44	-211.004707	-0.49
C. albicans ATCC # 10231	-26.653038	-0.10	-140.653248	-0.38	-91.176619	-0.28
S. mutans ATCC#25175	66.162953	0.47	67.343314	0.49	74.713622	0.60

CONCLUSION:

Test agent showed 99.991551%, 99.999975% & >99.999986 % antimicrobial activity against Streptococcus pyogenes ATCC# 119615 at 15 seconds, 30 seconds, and 60 seconds contact times, respectively.

Test agent showed 82.268238, 83.355863 & 84.169192% antimicrobial activity against E. coli (0157:H7) ATCC#48390ATCC# 119615 at 15 seconds, 30 second 60 seconds contact times, respectively.

Test agent showed 66.162953, 67.343314 & 74.713622% antimicrobial activity against Streptococcus mutans ATCC#25175 at 15 seconds, 30 second 60 seconds contact times, respectively.



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



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