



VIVO
CLINICAL TESTING

**Non-GLP 1004 ASTM E2315 Suspension Time-Kill Test
Final Study Report**

Study Title

Quantitative Suspension Time Kill Test of Five Quality Logo Products Test Articles against *S. aureus* ATCC 6538

Study Identification Number

NG1004

Test Microorganism

S. aureus ATCC 6538

Study Sponsor

Alyssa Mertes with Quality Logo Products, LLC

Testing Facility

Vivo Clinical Testing, LLC

Study Completion

10NOV2017

Study Objective

The purpose of this study was to measure the ability of each of the test substances to kill *S. aureus* ATCC 6538 in suspension at ambient temperature and in the time appropriate to each test article's use instructions.



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Procedure and Parameters

Preparation of Bacterial Culture

- The test microorganism was prepared by growth in a liquid culture medium, Tryptic Soy Broth. An overnight culture was prepared and used to prepare the final test inoculum at a concentration of approximately 1.0×10^6 CFU/ml.

Preparation of Test Substance

- Test substance arrived ready to use from the sponsor.

Initial Numbers Control

- The test culture was assayed to determine the original number of CFU in the reaction tube. 0.100 ml of *S. aureus* ATCC 6538 inoculum, at a concentration of approximately 1.0×10^7 CFU/ml, was added to 1.00 ml of PBS and mixed on a vortex mixer. 1.00 ml was then transferred to 9.00 ml of Dey Engley Broth (D/E) to simulate the neutralization step.
- A serial dilution series was initiated with 0.1 ml removed from the solution and added to 0.9 ml of PBS. Appropriate dilutions were plated on Tryptic Soy Agar (TSA) (Test 1) or Mannitol Salt Agar (MSA) (Test 2), and incubated at $36 \pm 1^\circ\text{C}$ for 48 ± 4 hours.

Test Procedures

- 0.100 ml of *S. aureus* ATCC 6538 inoculum, at a concentration of approximately 1.0×10^7 CFU/ml, was added to 1.00 ml of each test article and mixed on a vortex mixer. 1.00 ml was then transferred to 10.00 ml of Dey Engley Broth (D/E) at each appropriate contact time. See results table for corresponding contact time per test substance.
- Dilutions were plated on Tryptic Soy Agar (Test 1) or Mannitol Salt Agar (Test 2), and incubated at $36 \pm 1^\circ\text{C}$ for 48 ± 4 hours.

Neutralization Verification

- A neutralization validation was performed, modeled after the testing procedure, to determine that the neutralizer selected for this study, Dey Engley Broth, was sufficient to quench the antimicrobial at the end of the contact time and allow for adequate evaluation of the test article after its appropriate contact time.



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Study Controls

- Sterility controls were plated for all media used during each day of the study (Test 1 and Test 2), including D/E neutralizer, PBS, Tryptic Soy Agar and Mannitol Salt Agar.
- A viability control was plated on TSA and MSA to confirm both the viability of the culture and to confirm that each media type used was appropriate for growth.

Notes

- A total anaerobic plate count was performed on samples “Alcohol-free Antibacterial Hand Sanitizer Gel (Lot # Q51542)” and test article “Alcohol Free Sprayer (Lot # Q51520)” to determine sterility of test articles prior to testing. This was performed after a first round of testing (Test 1) where microbial contamination was observed. The contaminant was identified as a contaminant due to its significantly different morphology relative to the target microorganisms, *S. aureus* ATCC 6538. It was confirmed that both test articles contained a high level of contaminants. In order to mediate this issue and successfully evaluate the test articles’ efficacy against the target microorganism, *S. aureus* ATCC 6538, Mannitol Salt Agar was used during test 2 to select for the target microorganism only, during evaluation and enumeration of the test articles efficacy.

Conclusion

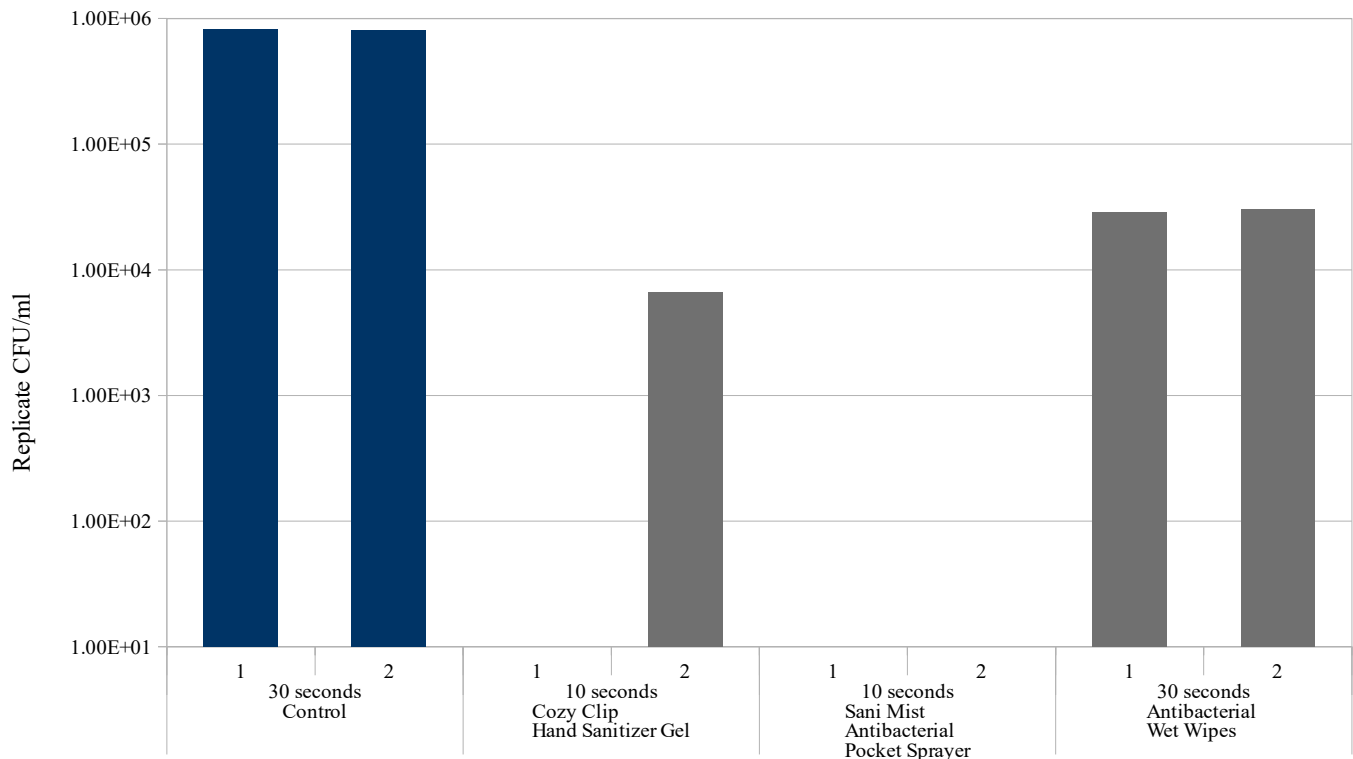
- Test Article “**Cozy Clip Hand Sanitizer Gel (Lot # Q39627)**” demonstrated a $\geq 99.59\%$ and $\geq 94.36\%$ reduction against *S. aureus* ATCC 6538, when evaluated at 10 seconds. This test article was tested twice due to presumed reduced observed reduction during evaluation due to its viscous properties. This variability in performance between replicates was observed during both evaluations.
- Test Article “**Sani Mist Antibacterial Pocket Sprayer (Lot # Q29956)**” demonstrated a $\geq 99.994\%$ reduction against *S. aureus* ATCC 6538, when evaluated at 10 seconds.
- Test Article “**Antibacterial Wet Wipes (Lot # Q56128)**” demonstrated a **96.40%** reduction against *S. aureus* ATCC 6538, when evaluated at 30 seconds.
- Test Article “**Alcohol-free Antibacterial Hand Sanitizer Gel (Lot # Q51542)**” demonstrated a $\geq 99.70\%$ reduction against *S. aureus* ATCC 6538, when evaluated at 15 seconds.
- Test Article “**Alcohol Free Sprayer (Lot # Q51520)**” demonstrated a **98.14%** reduction against *S. aureus* ATCC 6538, evaluated at 15 seconds.



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Results

Test Microorganism	Test Substance	Contact Time	Replicate	CFU/ml	Average CFU/ml	Percent Reduction versus control at Contact Time	Log Reduction versus control at Contact Time
<i>S. aureus</i> ATCC 6538	Control	30 seconds	1	8.28E+05	8.20E+05	N/A	N/A
			2	8.12E+05			
	Cozy Clip Hand Sanitizer Gel (Lot # Q39627)	10 seconds	1	<5.00E+01	<3.33E+03	≥99.59%	≥2.39
			2	6.60E+03			
	Sani Mist Antibacterial Pocket Sprayer (Lot # Q29956)	10 seconds	1	<5.00E+01	<5.00E+01	≥99.994%	≥4.21
			2	<5.00E+01			
	Antibacterial Wet Wipes (Lot # Q56128)	30 seconds	1	2.86E+04	2.95E+04	96.40%	1.44
			2	3.04E+04			



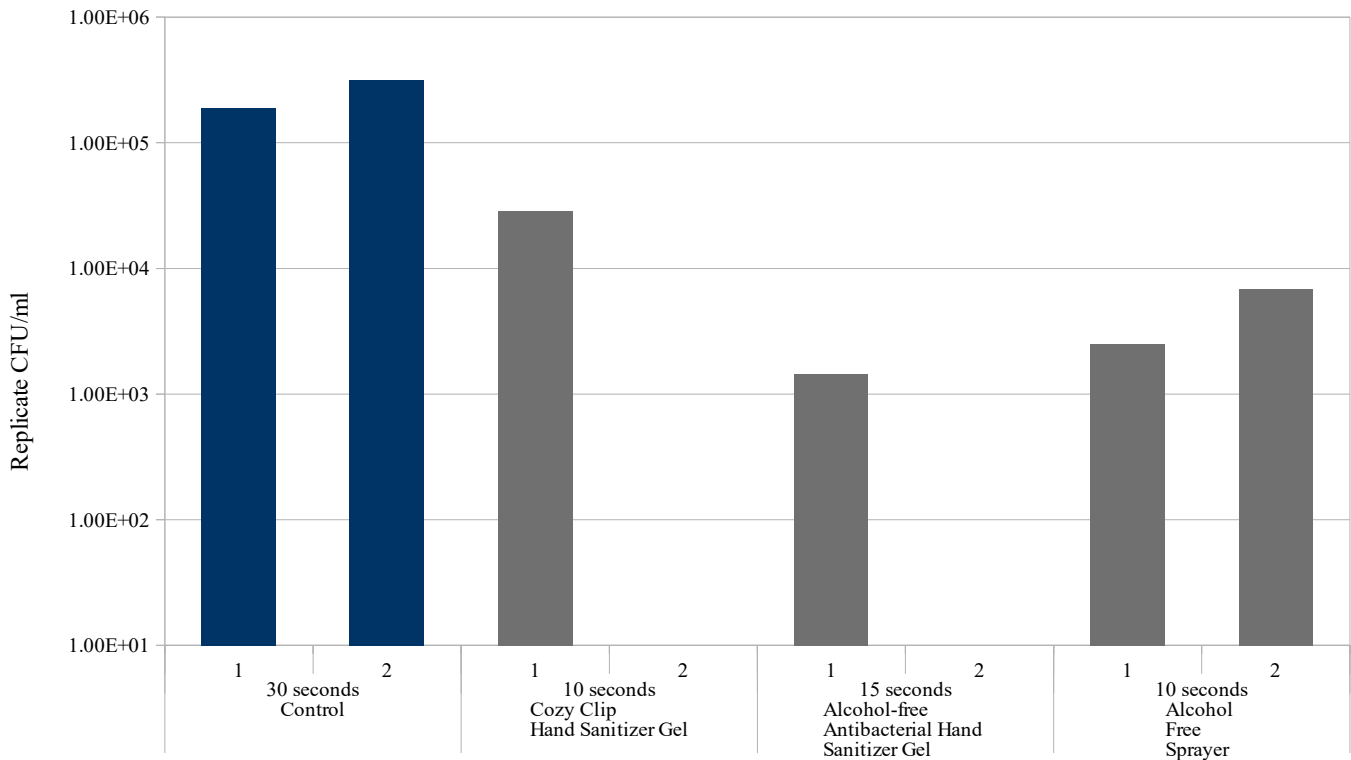
Samples presented as zero in the graph above were observed to be under the limit of detection, 5.00E+01 CFU/ml



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Results (Continued)

Test Microorganism	Test Substance	Contact Time	Replicate	CFU/ml	Average CFU/ml	Percent Reduction versus control at Contact Time	Log Reduction versus control at Contact Time
<i>S. aureus</i> ATCC 6538	Control	30 seconds	1	1.90E+05	2.53E+05	N/A	N/A
			2	3.15E+05			
	Cozy Clip Hand Sanitizer Gel (Lot # Q39627)	10 seconds	1	2.85E+04	<1.43E+04	≥94.36%	≥1.25
			2	<5.00E+01			
	Alcohol-free Antibacterial Hand Sanitizer Gel (Lot # Q51542)	15 seconds	1	1.45E+03	<7.50E+02	≥99.70%	≥2.53
			2	<5.00E+01			
	Alcohol Free Sprayer (Lot # Q51520)	15 seconds	1	2.50E+03	4.70E+03	98.14%	1.73
			2	6.90E+03			



Samples presented as zero in the graph above were observed to be under the limit of detection, 5.00E+01 CFU/ml