

201125 - Lab Comments:

Hi Jose,

Unfortunately, little to no dissolution was achieved overnight (please see attached photos). Many flocs are still visible when agitated and settle into clumps when still. This was prepared yesterday afternoon and has been in the refrigerator since about 5pm yesterday. I haven't yet filtered the solution since it didn't appear to be soluble.

Please let me know how you would choose to proceed. Thanks for your help

Danielle Goveia | Quality Assurance Specialist

201125 - Reply to Lab Comments:

Hi Danielle

This was expected, so let's go ahead and filter as planned, and if needed 2X filtration to remove the particulates that did not dissolve. Also, if you can retain some of the filtrate in case we see an effect on the bacteria, we would like to run an assay. Use a sealed vial to keep the filtrate for further testing. We still feel that some dissolution has occurred, even with what is being observed.

Thanks and again, call me if you have any questions...

Jose

TABLE 1: Time-kill Results

Cutibacterium acnes (ATCC #6919) - Benzoyl Peroxide (75%), Lot #2JI0263 evaluated at 0.075% (w/v)

Initial Population		Numbers Control		Exposure	Replicate	Post-Exposure Population		Log ₁₀ Reduction	Mean log ₁₀ Reduction	Percent Reduction
(CFU/mL)	Log ₁₀	(CFU/mL)	Log ₁₀			CFU/mL	Log ₁₀			
1.80 x 10 ⁸	8.26	1.40 x 10 ⁶	6.15	1 minute	1	1.56 x 10 ⁶	6.19	0.00	0.02	0.00%
					2	1.25 x 10 ⁶	6.10	0.05		10.71%
					3	1.62 x 10 ⁶	6.21	0.00		0.00%
				3 minutes	1	1.05 x 10 ⁶	6.02	0.13	0.06	25.00%
					2	1.24 x 10 ⁶	6.09	0.06		11.43%
					3	1.56 x 10 ⁶	6.19	0.00		0.00%
				30 minutes	1	8.35 x 10 ⁵	5.92	0.23	0.15	40.36%
					2	1.04 x 10 ⁶	6.02	0.13		25.71%
					3	1.18 x 10 ⁶	6.07	0.08		15.71%

TABLE 2: Neutralization Results

Cutibacterium acnes (ATCC #6919) - Benzoyl Peroxide (75%), Lot #2JI0263 evaluated at 0.075% (w/v)

Test	Log ₁₀ Population	Results (Pass/Fail)
Test C: Organism Viability	2.27	N/A
Test B: Neutralizer Toxicity	2.19	Pass ①
Test A: Neutralizer Effectiveness	2.26	Pass ①

① If log₁₀ populations from Test A and Test B were no more than 0.2 log₁₀ lower than that of Test C, neutralization of the test product was considered effective and the neutralizing formulation was considered non-toxic to the challenge species.

Bioscience Labs Inc., an independent testing organization used the In-Vitro Kinetic Time-Kill Method to evaluate the properties of Irrisept solution containing 0.05% Chlorhexidine Gluconate when challenged with several different microorganism species. CHG acts as a preservative to inhibit microbial growth in the solution. All testing was performed in accordance with Good Laboratory Practices, as specified in FDA 21 CFR Part 58.



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Chlorhexidine Gluconate 0.05% in sterile water, USP (99.95%)					
Bacteria	Challenge Suspension* (CFU/ml)	Exposure Time	Post-Exposure Population (CFU/ml)	Log ₁₀ Reduction	Percent Reduction
<i>Achromobacter xylosoxidans</i> (ATCC# 27061) Report# 1605248-201	5.40 x 10 ⁸	1 minute	3.49 x 10 ⁷	1.19	93.54%
		5 minutes	3.00 x 10 ⁵	3.26	99.94%
		30 minutes	1.03 x 10 ³	5.72	99.99%
<i>Acinetobacter baumannii</i> (ATCC# 19606) Report# 130377-201	1.60 x 10 ⁹	1 minute	1.36 x 10 ⁷	2.07	99.15%
		5 minutes	3.35 x 10 ⁵	3.68	99.98%
		30 minutes	< 1.00 x 10 ³	6.20	99.99%
<i>Acinetobacter baumannii</i> (BSLI# 092216Asp1) Report# 1705193-201	2.46 x 10 ⁷	1 minute	2.74 x 10 ⁶	0.97	88.85%
		5 minutes	1.58 x 10 ⁴	3.19	99.94%
		30 minutes	<5.92 x 10 ³	5.31	99.98%
<i>Acinetobacter baumannii</i> MDR (ATCC# BAA-1605) Report #130377-201	4.25 x 10 ⁹	1 minute	1.20 x 10 ⁸	1.55	97.19%
		5 minutes	8.50 x 10 ³	5.70	99.99%
		30 minutes	< 1.00 x 10 ³	6.63	99.99%

Bacteria	Initial Population (CFU/ml)	Exposure Time	Mean Post-Exposure Population (CFU/ml)	Mean Log ₁₀ Reduction	Mean Percent Reduction
<i>Bacteroides fragilis</i>** (BSLI #080916Bf1) Report # 1710439-201.01	1.67 x 10 ¹⁰	1 minute	2.43 x 10 ⁴	3.31	99.95%
		5 minutes	<1.00 x 10 ¹	6.68	99.99%
		30 minutes	<1.00 x 10 ¹	6.68	99.99%
<i>Clostridium difficile</i>** Spore suspension (ATCC #43598) Report # 1710439-201.01	2.33 x 10 ⁹	1 minute	2.23 x 10 ⁷	0.03	6.55%
		5 minutes	2.18 x 10 ⁷	0.05	10.04%
		30 minutes	2.06 x 10 ⁷	0.07	13.81%
<i>Clostridium difficile</i>** Vegetative cells (ATCC #43598) Report # 1710439-201.01	6.05 x 10 ⁷	1 minute	7.72 x 10 ⁵	0.14	27.88%
		5 minutes	7.83 x 10 ⁵	0.14	26.79%
		30 minutes	7.25 x 10 ⁵	0.17	32.24%
<i>Cutibacterium acnes</i> (formerly <i>Propionibacterium acnes</i> ***) (ATCC# 6919) Report# 140946-201	2.23 X 10 ⁹	1 minute	2.10 x 10 ⁷	1.55	96.62%
		3 minutes	2.09 x 10 ⁷	2.25	99.39%
		30 minutes	2.14 x 10 ⁷	4.30	99.99%

*Reference Sections in Study Protocols for the calculations of reductions from the challenge suspensions.

**Testing was based upon recommendations outlined in ASTM E2783-11 (2016).

***Testing was based upon recommendations outlined in ASTM E2783-11, using a numbers control per the method at each time point because of the fastidious nature of *P. acnes*. This avoids the possible attribution of the product efficacy to die-off of the organism due to the length of the exposure time to environmental conditions, allowing for a more accurate and actual assessment of the inoculum level.

MDR = Multi-Drug Resistant.

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Chlorhexidine Gluconate 0.05% in sterile water, USP (99.95%)					
Bacteria	Challenge Suspension* (CFU/ml)	Exposure Time	Post-Exposure Population (CFU/ml)	Log ₁₀ Reduction	Percent Reduction
<i>Enterobacter cloacae</i> MDR¹ (ATCC# BAA-2468) Report# 130377-201	3.80 x 10 ⁹	1 minute	4.55 x 10 ⁵	3.92	99.99%
		5 minutes	< 1.00 x 10 ³	6.58	99.99%
		30 minutes	< 1.00 x 10 ³	6.58	99.99%
<i>Enterococcus faecalis</i> (BSLI# 092216Efs7) Report# 1708328-201	6.68 x 10 ⁷	1 minute	> 2.99 x 10 ⁷	0.35	55.24%
		5 minutes	9.10 x 10 ⁶	0.87	86.38%
		30 minutes	1.88 x 10 ⁴	3.71	99.97%
<i>Enterococcus faecium</i> VRE (BSLI #060613VRE9) Report #1705193-201	3.61 x 10 ⁷	1 minute	3.04 x 10 ⁷	0.08	16.02%
		5 minutes	1.95 x 10 ⁷	0.27	46.00%
		30 minutes	1.65 x 10 ⁵	3.17	99.54%
<i>Enterococcus faecium</i> VSE (BSLI #112613VSEfm10) Report #1705193-201	4.75 x 10 ⁷	1 minute	3.89 x 10 ⁷	0.09	18.08%
		5 minutes	1.32 x 10 ⁷	0.99	72.22%
		30 minutes	7.32 x 10 ³	3.86	99.98%
<i>Escherichia coli</i> (ATCC #BAA-2469) ^{1,2,3,4} Report #1605248-201	2.95 x 10 ⁷	1 minute	2.06 x 10 ⁴	3.16	99.93%
		5 minutes	2.50 x 10 ²	5.07	99.99%
		30 minutes	<1.00 x 10 ¹	6.47	99.99%
<i>Escherichia coli</i> 0157:H7 (ATCC# 43888) Report# 130377-201	2.70 x 10 ⁹	1 minute	2.35 x 10 ⁴	5.06	99.99%
		5 minutes	< 1.00 x 10 ³	6.43	99.99%
		30 minutes	< 1.00 x 10 ³	6.43	99.99%
<i>Escherichia coli</i> (BSLI# 083116Ec2) Report# 1705193-201	1.34 x 10 ⁷	1 minute	<9.150 x 10 ²	5.31	99.99%
		5 minutes	<5.83 x 10 ¹	5.73	99.99%
		30 minutes	<9.00 x 10 ¹	5.66	99.99%
<i>Klebsiella pneumoniae</i> (BSLI# 030116Kpn2) Report# 1705193-201	1.09 x 10 ⁷	1 minute	1.47 x 10 ²	4.91	99.99%
		5 minutes	<1.00 x 10 ¹	6.04	99.99%
		30 minutes	<1.00 x 10 ¹	6.04	99.99%
<i>Klebsiella pneumoniae pneumoniae</i> (ATCC# BAA-2146) ^{1,2,3,4} Report# 1605248-201	3.60 x 10 ⁷	1 minute	1.10 x 10 ²	5.51	99.99%
		5 minutes	<1.00 x 10 ¹	6.56	99.99%
		30 minutes	<1.00 x 10 ¹	6.56	99.99%

*Reference Sections in Study Protocols for the calculations of reductions from the challenge suspensions

1-New Delhi metallo-beta-lactamase (NDM-1) positive

2-*blaKPC* negative by PCR

3-*blaNDM* positive by PCR

4-Carbapenem-resistant (Imipenem and Ertapenem)

MDR = Multi-Drug Resistant

VRE = Vancomycin Resistant *Enterococcus*

VSE = Vancomycin Susceptible *Enterococcus*

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Chlorhexidine Gluconate 0.05% in sterile water, USP (99.95%)					
Bacteria	Initial Population (CFU/ml)	Exposure Time	Mean Post-Exposure Population (CFU/ml)	Mean Log ₁₀ Reduction	Mean Percent Reduction
<i>Prevotella intermedia</i> (ATCC# 25611) Report# 1710439-201.01	4.25 x 10 ⁸	1 minute	1.00 x 10 ⁴	2.51	99.42%
		5 minutes	1.35 x 10 ²	5.07	99.99%
		30 minutes	<1.00 x 10 ¹	5.39	99.99%
<i>Ralstonia pickettii</i> (ATCC# 27512) Report# 1710439-201.01	1.19 x 10 ¹⁰	1 minute	>4.56 x 10 ⁷	<0.30	<49.58%
		5 minutes	>4.81 x 10 ⁷	<0.28	<46.85%
		30 minutes	>4.32 x 10 ⁷	<0.33	<52.23%

Bacteria	Challenge Suspension* (CFU/ml)	Exposure Time	Post-Exposure Population (CFU/ml)	Log ₁₀ Reduction	Percent Reduction
<i>Pseudomonas aeruginosa</i> (BSLI# 083116Pa18) Report# 1708328-201	9.75 x 10 ⁶	1 minute	<1.00 x 10 ¹	5.99	99.99%
		5 minutes	<1.00 x 10 ¹	5.99	99.99%
		30 minutes	<1.00 x 10 ¹	5.99	99.99%
<i>Staphylococcus aureus</i> MRSA^{CI} (BSLI# 042511MRSA) Report# 130417-201	1.89 x 10 ⁹	1 minute	1.03 x 10 ⁸	1.26	94.56%
		3 minutes	5.80 x 10 ⁶	2.51	99.69%
		15 minutes	1.17 x 10 ⁵	4.21	99.99%
<i>Staphylococcus aureus</i> MRSA^{CI} (BSLI# 092211SaMRSA1) Report# 130417-201	2.01 x 10 ⁹	1 minute	5.80 x 10 ⁸	0.54	71.07%
		3 minutes	6.40 x 10 ⁷	1.50	96.81%
		15 minutes	3.80 x 10 ⁴	4.72	99.99%
<i>Staphylococcus epidermidis</i> (BSLI# 080916Se1) Report# 1705193-201	2.27 x 10 ⁷	1 minute	1.57 x 10 ³	4.19	99.99%
		5 minutes	<1.00 x 10 ¹	6.36	99.99%
		30 minutes	<1.00 x 10 ¹	6.36	99.99%
<i>Staphylococcus epidermidis</i> (BSLI# 092216Se1) Report# 1705193-201	2.06 x 10 ⁷	1 minute	2.68 x 10 ²	4.89	99.99%
		5 minutes	<1.00 x 10 ¹	6.31	99.99%
		30 minutes	<1.00 x 10 ¹	6.31	99.99%
<i>Streptococcus pyogenes</i> (BSLI# 092216Spy1) Report# 1705193-201	2.93 x 10 ⁶	1 minute	8.20 x 10 ⁵	0.55	71.97%
		5 minutes	7.77 x 10 ³	2.59	99.73%
		30 minutes	3.25 x 10 ²	4.03	99.99%

*Reference Sections in Study Protocols for the calculations of reductions from the challenge suspensions.

CI- Clinical Isolate

MRSA = Methicillin Resistant *Staphylococcus aureus*

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Chlorhexidine Gluconate 0.05% in sterile water, USP (99.95%)					
Fungi	Challenge Suspension* (CFU/ml)	Exposure Time	Post-Exposure Population (CFU/ml)	Log ₁₀ Reduction	Percent Reduction
<i>Aspergillus niger van Tiegham</i> (ATCC# 6275) Report# 130377-201	2.75 x 10 ⁹	10 minutes	3.90 x 10 ⁸	0.85	85.82%
		30 minutes	4.20 x 10 ⁸	0.82	84.73%
		60 minutes	3.05 x 10 ⁸	0.96	88.91%
<i>Candida albicans</i> (ATCC# 10231) Report# 130377-201	4.05 x 10 ⁹	1 minute	7.30 x 10 ⁵	3.74	99.98%
		5 minutes	1.65 x 10 ⁴	5.39	99.99%
		30 minutes	< 1.00 x 10 ³	6.61	99.99%
<i>Candida auris</i> (AR-BANK# 0381) Report# 1605248-201	3.80 x 10 ⁷	1 minute	5.80 x 10 ⁵	1.82	98.47%
		5 minutes	1.56 x 10 ³	4.39	99.99%
		30 minutes	<1.00 x 10 ¹	6.58	99.99%
<i>Candida auris</i> (AR-BANK# 0382) Report# 1605248-201	5.10 x 10 ⁷	1 minute	3.30 x 10 ⁶	1.19	93.53%
		5 minutes	1.21 x 10 ⁵	2.63	99.76%
		30 minutes	<1.00 x 10 ¹	6.71	99.99%
<i>Candida auris</i> (AR-BANK# 0383) Report# 1605248-201	6.30 x 10 ⁷	1 minute	1.84 x 10 ⁷	0.54	70.87%
		5 minutes	4.25 x 10 ⁴	3.17	99.93%
		30 minutes	<1.00 x 10 ¹	6.80	99.99%
<i>Candida glabrata</i> (ATCC# 2001) Report# 130377-201	1.16 x 10 ¹⁰	1 minute	4.03 x 10 ⁹	0.46	65.11%
		5 minutes	6.40 x 10 ⁷	2.26	99.45%
		30 minutes	< 1.00 x 10 ³	7.06	99.99%

Chlorhexidine Gluconate 0.05% in sterile water, USP (99.95%)				
Virus	Exposure Time	TCID ₅₀ (Log ₁₀) Post-Exposure Infectivity	Log ₁₀ Reduction	Percent Reduction
Hepatitis B Virus Surrogate: Duck Hepatitis B Virus (DHBV) Report# 130378-402	1 minute	5.00	1.00	90.00%
	5 minutes	4.75	1.25	94.38%
	30 minutes	4.00	2.00	99.00%
Hepatitis C Virus Surrogate: Bovine Viral Diarrhea Virus (BVDV) Report# 130378-402	1 minute	5.50	0.75	82.22%
	5 minutes	5.00	1.25	94.38%
	30 minutes	4.50	1.75	98.22%
Human Immunodeficiency Virus Type 1 (HIV-1) Report# 130378-402	1 minute	4.25	1.75	98.22%
	5 minutes	3.50	2.50	99.68%
	30 minutes	2.00	4.00	99.99%

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