BiOGLOVE®

MICROBIAL DEFENSE SYSTEMS

Technical Summary
BioGlove™
Hand Sanitizer

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A. Introduction:

Antiseptic Hand Sanitizer: BioGlove™ Hand Sanitizer is a unique antimicrobial technology that destroys on contact. Additionally, its persistent residual activity designed to help prevent infections that are associated with hospitals, clinics and nursing homes. Other industry segments where hand transmission of infectious diseases can occur includes: food services, money handling, sports and fitness centers, first responders, schools, the transport industry, and public amusement attractions. The proprietary blend of QAT forms a film resulting in a highly effective antimicrobial solution. The antimicrobial agents are compliant with FDA regulations and CDC guidelines for non-alcohol hand sanitizer products. Safe, water-based, and alcohol-free, it is faster acting and longer lasting than traditional hand sanitizers. BioGlove™ is unsurpassed in its ability to kill germs using a new technology with a unique mode of action. BioGlove™ is available as a fine mist hand sanitizer spray or hospital grade disinfectant all-purpose spray.

B. Efficacy Testing:

Rapid Broad-Spectrum Activity

BioGlove™ hand sanitizer offers rapid, broad-spectrum kill and persistence. Within 15 seconds, the product killed more than 99.99% FDA-specified organisms (See Table 1). Shown in this table is time kill test data (Quantitative Suspension Test) as specified in FDA 1994 for the 25 Microbes listed in the OTC Topical Antimicrobial Drug Monograph as well as additional strains and species of public health significance. Test methods include ASTM 2315, European EN 1276 and EN 1040 for Bacteria as well as EN 1275 for Fungi.

Methods: The products were evaluated according to standard Time-Kill protocol and against 45 different microorganisms, listed in the section below, 25 of which are specified in the FDA Tentative Final Monograph. Each test product was evaluated at a 99% concentration, and the percent (%) and log reductions were determined following exposure times of 15, 30, and 60 seconds. All agar-plating was performed in duplicate. A combination of clinical isolates and lab strains were used in the testing. Neutralization studies of each test product were performed to ensure the neutralizing solution employed (Butterfield's Phosphate Buffer solution with product neutralizers) was effective and non-toxic to each of the representative challenge species.

Rapid Broad Spectrum Antimicrobial Activity:

Table 1: Time-Kill Testing Results: BioGlove™ Hand Sanitizer
Meets or Exceeds Efficacy Standards in Applicable Regulations

Micro-organism	Time (Seconds)	log10 (%) Reductions
1.Acinetobacter baumannii (ATCC# 25285) [5]	15 Sec	>6.02 (99.9999%)
	30 Sec	>6.02 (99.9999%)
	60 Sec	>6.02 (99.9999%)
2.Bacteroides fragilis (ATCC# 25285) [5]	15 Sec	6.03 (99.9999%)
	30 Sec	6.03 (99.9999%)
	60 Sec	6.03 (99.9999%)
3.Haemophilus influenzae (ATCC #33930) [5]	15 Sec	>6.12 (99.9999%)
	30 Sec	>6.12 (99.9999%)
	60 Sec	>6.12 (99.9999%)
4. Enterobacter aerogenes (ATCC #13048) [5]	15 Sec	>6.02 (99.9999%)
	30 Sec	>6.02 (99.9999%)
	60 Sec	>6.02 (99.9999%)
5. Escherichia coli (ATCC #11229) 3, 5	15 Sec	>6.01 (99.9999%)
	30 Sec	>6.01 (99.9999%)
	60 Sec	>6.01 (99.9999%)
6. Escherichia coli (ATCC #10536) [5]	15 Sec	>6.78 (99.9999%)
	30 Sec	>6.78 (99.9999%)
	60 Sec	>6.78 (99.9999%)
7. Escherichia coli (ATCC #25922) [4]	30 Sec	>5.0 (99.999%)
	60 Sec	>5.0 (99.999%)
	15 Sec	>4.0 (99.99%)
8. Escherichia coli (O157:H7) [1]	15 Sec	>6.03 (99.9999%)
	30 Sec	>6.03 (99.9999%)
	60 Sec	>6.03 (99.9999%)
9. Klebsiella oxytoca (ATCC #13182) [5]	15 Sec	>5.00 (99.999%)
	30 Sec	>5.00 (99.999%)
	60 Sec	>5.00 (99.999%)
9. Klebsiella oxytoca (ATCC #13182) 5	15 Sec	>6.04 (99.9999%)
	30 Sec	>6.04 (99.9999%)
	60 Sec	>6.04 (99.9999%)
10. Klebsiella pneumoniae (ATCC #51504) [4]	15 Sec	>5.00 (99.999%)
	30 Sec	>5.00 (99.999%)
	60 Sec	>5.00 (99.999%)
11. Klebsiella pneumoniae (ATCC #4352) [5]	15 Sec	>6.04 (99.9999%)
	30 Sec	>6.04 (99.9999%)
	60 Sec	>6.04 (99.9999%)

Micro-organism	Time (Seconds)	log10 (%) Reductions
12. Pseudomonas aeruginosa) (ATCC #9027) [7]	15 Sec	>6.78 (99.9999%)
	30 Sec	>6.78 (99.9999%)
	60 Sec	>6.78 (99.9999%)
13. Pseudomonas aeruginosa (ATCC #27853) [5]	15 Sec	>6.23 (99.9999%)
	30 Sec	>6.23 (99.9999%)
	60 Sec	>6.23 (99.9999%)
14. Pseudomonas aeruginosa (ATCC #27853) [5]	15 Sec	>5.00 (99.999%)
	30 Sec	>5.00 (99.999%)
	60 Sec	>5.00 (99.999%)
15. Pseudomonas aeruginosa ((ATCC #15442) [2, 3]	15 Sec	>5.00 (99.999%)
	30 Sec	>5.00 (99.999%)
	60 Sec	>5.00 (99.999%)
16. Proteus mirabilis (ATCC #7002) [5]	15 Sec	>6.12 (99.9999%)
	30 Sec	>6.12 (99.9999%)
	60 Sec	>6.12 (99.9999%)
17. Serratia marcescens (ATCC #14756) [5]	15 Sec	>6.12 (99.9999%)
	30 Sec	>6.12 (99.9999%)
	60 Sec	>6.12 (99.9999%)
18. Salmonella enterica (ATCC #10398) [3]	30 Sec	>5.0 (99.999%)
	60 Sec	>4.0 (99.99%)
	15 Sec	>6.76 (99.9999%)
19. Salmonella typhimurium [1]	15 Sec	>4.0 (99.99%)
20. Staphylococcus aureus (ATCC #6538) [3, 7]	15 Sec	>6.76 (99.9999%)
	30 Sec	>6.76 (99.9999%)
	60 Sec	>6.76 (99.9999%)
21. Staphylococcus aureus (ATCC #29213) [4, 5]	15 Sec	>6.11 (99.9999%)
	30 Sec	>6.11 (99.9999%)
	60 Sec	>6.11 (99.9999%)
22. Staphylococcus epidermidis (ATCC #12228) [5]	15 Sec	>6.22 (99.9999%)
	30 Sec	>6.22 (99.9999%)
	60 Sec	>6.22 (99.9999%)
23. Staphylococcus hominis (ATCC #27844) [5]	15 Sec	>6.22 (99.9999%)
	30 Sec	>6.22 (99.9999%)
	60 Sec	>6.22 (99.9999%)
24. Staphylococcus haemolyticus (ATCC #43253) [4]	15 Sec	>5.00 (99.999%)
	30 Sec	>5.00 (99.999%)
	60 Sec	>5.00 (99.999%)

Micro-organism	Time (Seconds)	log10 (%) Reductions
25. Staphylococcus haemolyticus(ATCC #29970) [5]	15 Sec	>6.11 (99.9999%)
	30 Sec	>6.11 (99.9999%)
	60 Sec	>6.11 (99.9999%)
26. Staphylococcus saprophyticus (ATCC #35552) [5]	15 Sec	>6.11 (99.9999%)
	30 Sec	>6.11 (99.9999%)
	60 Sec	>6.11 (99.9999%)
27. Micrococcus luteus (ATCC #7468) [5]	15 Sec	>6.22 (99.9999%)
	30 Sec	>6.22 (99.9999%)
	60 Sec	>6.22 (99.9999%)
28. Streptococcus pyogenes (ATCC #19615) [3, 7]	15 Sec	>6.03 (99.9999%)
	30 Sec	>6.03 (99.9999%)
	60 Sec	>6.03 (99.9999%)
29. Enterococcus faecalis (ATCC# 29212) [5]	15 Sec	>6.01 (99.9999%)
	30 Sec	>6.01 (99.9999%)
	60 Sec	>6.01 (99.9999%)
30. Enterococcus hirae (ATCC #6057) [7]	15 Sec	>6.76 (99.9999%)
	30 Sec	>6.76 (99.9999%)
	60 Sec	>6.76 (99.9999%)
31. Streptococcus pneumoniae (ATCC #6303) [5]	15 Sec	>6.06 (99.9999%)
	30 Sec	>6.06 (99.9999%)
	60 Sec	>6.06 (99.9999%)
32. Candida albicans (ATCC# 10231) [6]	15 Sec	>5.12 (99.999%)
	30 Sec	>5.12 (99.999%)
	60 Sec	>5.12 (99.999%)
33. Aspergillus niger (ATCC# 16404) [6]	15 Sec	>5.44 (99.9995%)
	30 Sec	>5.44 (99.9995%)
	60 Sec	>5.44 (99.9995%)

2. Burkholderia Cepacia_Assessment Of Time Kil Activity Using ASTM E2315-16

Burkholderia cepacia is a bacterial species that has been found by the FDA to contaminate health care products during the manufacturing process. Burkholderia cepacia showed growth to 57,000 cfu in 2.0% Chlorhexidine gluconate hand sanitizer wipes. Testing BioGlove™ Powered by PBG Hand Sanitizer in a Time Kill Study using ASTM E 2315 showed a steady decrease in activity over time negating any threat of growth.

Results: B. cepacia ATCC#25416								
Lab#	Sample ID	Lot#	Exposure Time	Replicate	Cfu/ml Average (cfu/ml)		% Reduction	Log Reduction Sample
-	Control	-	0 min	1	$1.10x10^8$	$1.06x10^8$	-	-
				2	$1.02x10^8$			
129332	BioGlove - PBG Hand Sanitizer	38L17 Ex 09/19 Jan 22.2018	2 min	1	$6.2x10^6$	$6.4x10^6$	93.96%	1.2192
				2	$6.6x10^6$			
			5 min	1	$2.8x10^6$	$3.4x10^6$	96.79%	1.4939
				2	$4.0x10^6$			
			10 min	1	$4.4x10^4$	$4.8x10^4$	99.95%	3.3441
				2	$5.2x10^4$			

CONCLUSION: Sample ID: BioGlove™ Powered by PBG Lot# 38L17 Ex 09/19 Jan 22, 2018 showed 93.96% anti-microbial activity against B. cepacia at 2 min, 96.79% at 5 min and 99.95% reduction at 10 min of contact time against Burkholderia
cepacia ATCC# 25416.

3. Healthcare Hand Rub Efficacy Testing (EN 1500)

EN 1500 testing is used as the European standard method for qualifying hand rubs that are to be used in healthcare settings. 20 test subjects are used in each test group and performance of test product is compared to a standard reference alcohol rub. In this case Propan-2-ol. 60% (v/v) as per test method was used. Hands were contaminated with 2 x 108 of Escherichia coli strain # K13 NCTC 10538. For both reference alcohol and test product, 6ml applied -3.0mL for 30 seconds, a further 3.0mL for another 30 seconds totaling 60 seconds rubbing time and hands are sampled and compared to control inoculate levels. To be EN 1500 compliant test product must surpass reference alcohol by a convincing margin or via statistical analysis of results. Data from this testing is presented in Table 2 below.

Table 2: BioGlove™ Powered by PBG Hand Sanitizer antimicrobial activity on hands using BS EN 1500:2013 test method (EN 1500 Compliant)

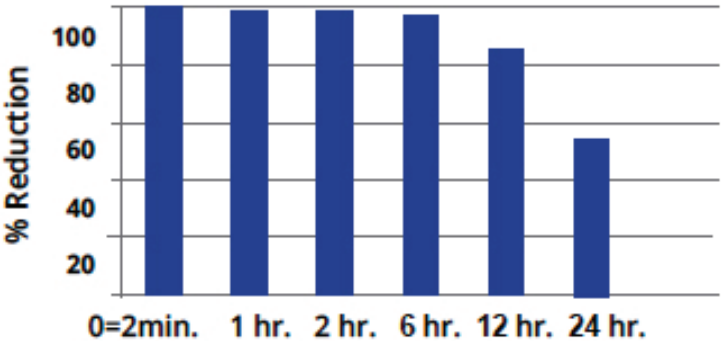
Healthcare Hand Rub Testing[8]	Reference Alcohol	BioGlove Hand Sanitizer
Pre log10 Inoculum Values (SD)	6.43 (0.69)	6.62 (0.73)
Post Treatment log10 Values (SD)	2.53 (0.35)	2.38 (0.23)
log10 Reduction Values	3.90	4.23

4. Long Term Sustained Activity when Needed

A. BioGlove™ Powered by PBG Hand Sanitizer: Sustained kill against Gram-Positive & Gram-negative bacteria when not removed from the skin.

As shown in Figure 1 (with data in Table 3B), in an ex-vivo test using a pigskin model (using ASTME2897-12 & ASTM WK36911), BioGlove™ Hand Sanitizer was applied to the skin. After the specified amount of time, the skin samples were challenged with S. aureus (ATCC #12600). From the data presented in Table 3A, BioGlove Powered by PBG killed more than 98% of both the gram-positive and gram-negative antibiotic resistant bacteria introduced at 1 hour after application, and around 90% at 4 hours. Activity against all three antibiotic resistant strains MRSA, VRE and CRE (Figure 2) is consistent with testing on S. aureus that showed continued killing even after 24 hours after application. BioGlove™ Hand Sanitizer testing was conducted at an independent laboratory. It should be noted that the CRE strain used in this testing, Klebsiella pneumoniae is also known as Klebsiella pneumoniae Carbapenemase (KPC).

Figure 1. BioGlove™ Sustained Kill Shown up to 24 Hours on the skin



Time Period Post Skin Treatment w/BioGlove™

C. Sustained Activity of BioGlove™ on Hands and when Gloves are Worn (ASTM E1115 11)

To assess persistent activity on hands when used in clinical settings incorporating periods of glove use, ASTM Test method E1115-11 was used with 20 human volunteers. This method is typically used to evaluate surgical hand scrub formulations. When tested in accordance with ASTM E1115-11, BioGlove™ Hand Sanitizer possesses Immediate Activity and Persistent Activity. Immediate activity results demonstrated that bacterial reduction of hand flora after using the product was greater than a 5.8 log10 reduction factor. Persistent activity suppressing regrowth of skin bacteria was shown to be in the same order of magnitude (5.8 log10) at 3 hours and 6 hours post treatment. Even at 12 hours after product application with hands kept occluded within surgical gloves, only a minimal antibacterial activity (suppression of growth) was observed. Persistent activity at 3 and 6 hours was > 99.999% (>5 log10) while geometric mean persistent antibacterial reduction value at 12 hours was seen to be 99.99% (4.0 log10). This is consistent with data obtained in the ex-vivo studies demonstrating 24-hour activity and persistence against antibiotic resistant strains.

1. Challenge Testing as Further Proof of Wide Spectrum Activity

Challenge testing of products regulated by FDA as over-the-counter, antimicrobial drugs is the ultimate test of effectiveness of individual formulations. Instead of just killing germs on hands or in suspension tests, multiple high count inoculation and long term incubation is used. Thus both biocidal (bactericidal and fungicidal) activity and persistence effectiveness is determined.

Table 4 provides results of testing in accordance with both ASTM E640-0 6 (Re-approved 2012), USP 35-NF30 51, and EP7.0-5.1.3. As a variation of the ASTM E640 testing a wide range of microbial strains and types were selected. As shown in Table 4A & 4B, BioGlove™ Hand Sanitizer possesses bactericidal and fungicidal reduction of microbial species in double challenge testing as well as testing to normal US and EU standards, thus satisfying all applicable test standards including ASTM E640-06 (2012).

Table 4A. Efficacy Testing as per ASTM E640-12 a Double 28 day Challenge 12

Micro-organism	ATCC Strain#	1st Inoculum Counts (28 day duration)	2nd Inoculum Counts (28 day duration)
5. Escherichia coli	11229	2×106	3×106
11. Klebsiella pneumoniae	4352	2×106	5×106
15. Pseudomonas aeruginosa	15442	2×106	5×106
20. Staphylococcus aureus	6538	2×106	3×106
32. Candida albicans	10231	2×106	5×106
38. Burkholderia cepacia capacia	25416	2×106	6×106
39. Bacillus subtilis	6051	2×106	5×106
40. Aspergillus niger	1015	2×106	5×106
41. Penicillium luteum	10466	2×106	1×106

Table 4B. Antimicrobial Effectiveness Testing as per USP35-NF30 51 & EP7.0

Micro-organism	ATCC Strain#	USP 35-NF3051	EP7.0-5.1.3
42. Escherichia coli	8739	X	
12. Pseudomonas aeruginosa	9027	X	X
20. Staphylococcus aureus	6538	X	X
32. Candida albicans	10231	X	X
40. Aspergillus niger	16404	X	X

2. Antiviral Activity

A. Norovirus Ex-Vivo Testing of BioGlove™ Hand Sanitizer

Norovirus is the leading cause of gastroenteritis, causing diarrhea, vomiting, and severe abdominal pain. Alcohol sanitizers have been shown to be somewhat ineffective against the Norovirus. In an ex-vivo test using porcine skin (using ASTM E2897-12 & ASTM WK3 6911) against a Norovirus surrogate, Murine norovirus (MNV-1), BioGlove™ hand sanitizer was demonstrated to reduce the Norovirus by 2.0 log10 (99%) during the time required for the product to dry on porcine skin (1-2 minutes).

B. BioGlove™ Hand Sanitizer Foam Demonstrated Effective Against other Viruses[17]

BioGlove™ Hand Sanitizer can be declared as virucidal against Rhinovirus, the predominate cause of the common cold (ATCC VR-482), Influenza virus (ATCC VR -1741) and Enterovirus 71 (Hand, Foot & Mouth Disease Virus). BioGlove™ Hand Sanitizer demonstrated effectiveness as dilute 80% solutions against Rhinovirus (common cold), Influenza virus and Enterovirus 71 a cause of Hand, Foot & Mouth Disease (ATCC VR-177 5) using BS EN 14476:205 with an expected 4 log10 reduction after a contact time of 60 minutes. Testing demonstrated activity meant to establish the 4 log10 benchmark allowing the disinfectant virucidal claim to be made. Additionally, BioGlove™ formulations have been shown to be effective against numerous virus family groups including the Human Corona virus family. For a full list of viruses that are deactivated by BioGlove™, please contact BioGlove.

C. R&D On Corona Virus, MERS CoV Surrogate, Feline Infectious Peritonitis Virus On Human Hands

A four person human hand study was conducted by The University of Arizona using BioGlove™ Hand Sanitizer FOAM after exposing the hands to Feline Infectious Peritonitis virus, a MERS surrogate. MERS CoV is a corona virus associated with camels and their barnyard environment. It has infected 2266 patients resulting in 804 deaths. Many health care providers have been infected. How the virus transferred is still undetermined. In this study hands were infected with the MERS surrogate, dried, and BioGlove™ Hand Sanitizer was applied and allowed to dry. The infected area was swabbed. Results were BioGlove™ Hand Sanitizer FOAM is effective at reducing feline infectious peritonitis virus on hands by at least 99.9% following manufacturer's instruction.

D. Clinical Trials

1. Hospital Associated Infection Rates - Clinical Trial

To test product practical value in healthcare a pilot clinical study was conducted over an 8-day period, in surgical and orthopedic wards of a US hospital. Microbial surveys of healthcare workers hands (~40) were obtained with 624 microbial cultures taken randomly for 4 days using standard alcohol hand sanitizer and for 4 days using BioGlove™ Hand Sanitizer. The use of BioGlove was demonstrated to reduce the frequency of commonly identified opportunistic pathogens responsible for hospital-associated infections on hands for each ward by around 50% when compared to standard alcohol hand sanitizer use. This efficacy is a direct result of the persistent activity demonstrated in testing described earlier.

E. Skin Irritation Tests-Safe for Repeated Application

At time of formula creation every precaution was taken to develop a product that did not irritate the skin with repeated use. Qualified skin care product experts reviewed every aspect of the formulation.
Now with several years successfully manufacturing and distributing these products, a clinical trial in a health care facility, low irritation potential of BioGlove™ is well established. The hospital trial also demonstrated skin compatibility in a well-monitored population of healthcare professionals. The various production runs of the product represent positive experiences by 70,000 to 90,000 individuals using these products in a wide variety of settings from households to schools, and healthcare to prisons.