THE SCIENCE BEHIND

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DEFENSE AGAINST BACTERIA
Hand Sanitizer + Protectant

THE SCIENCE BEHIND DAB

DAB eradicates 99.99% of bacteria, including some strains now shown to be more tolerant to alcohol-based hand sanitizers. The active ingredient is benzalkonium chloride, shortened to BZK, an organic salt classified as a quaternary ammonium compound. Along with BZK, DAB utilizes a proprietary ratio of specific components.

INDEPENDENTLY TESTED AND CLINICALLY PROVEN.

Tested against 22 challenge organisms, DAB killed 99.99% of them within 15 seconds and showed persistent, hours-long protection compared to just minutes for alcohol-based products.

But don't take our word for it. DAB was the hand sanitizer studied in two recent AJIC articles that proved its persistence on the skin as an antibacterial and its effectiveness against Staph germs.

SCIENTIST-CREATED FOR ULTIMATE PROTECTION.

DAB's founder and his team of pharmacists created DAB to prevent cross-contamination. The benzalkonium chloride (BZK) ingredient and the DAB formula was so effective, they started bottling and selling it.

Dab®
DEFENSE AGAINST BACTERIA
Hand Sanitizer + Protectant

TIME KILL PROCEDURE

EVALUATION OF ANTIMICROBIAL ACTIVITY OF DAB FORMULATION

Performed by Q Laboratories, Inc.

DAB was tested for antimicrobial efficacy against a wide variety of bacterial species as well as yeast (Candida) by Q Laboratories, Inc. (Cincinnati, OH) (February 2013) using a modification of ASTM Committee E35.15 standard method E2315.03 "The assessment of antimicrobial activity using a Time Kill procedure."

The following table is a list of the organisms tested in the Time Kill Study.

Challenge Microorganisms : 15 Second Time Kill

Acinetobacter baumanii ATCC 19606
Staphylococcus haemolyticus ATCC 29970
Bacteroides fragilis ATCC 25285
Staphylococcus saprophyticus ATCC 15305
Haemophilus influenza ATCC 19418
Staphylococcus hominis ATCC 27844
Enterobacter aerogenes ATCC 35029
Streptococcus pyogenes ATCC 19615
Escherichia coli ATCC 25922
Enterococcus faecalis ATCC 29212
Escherichia coli O157:H7 ATCC 43895
Enterococcus faecium ATCC 19434
Klebsiella oxytoca ATCC 43165
Streptococcus pneumoniae ATCC 6302
Klebsiella pneumonia ATCC 4352
Pseudomonas aeruginosa ATCC 27853
Proteus mirabilis ATCC 7002
Pseudomonas aeruginosa ATCC 15442
Serratia marcescens ATCC 14756
Candida glabrata ATCC 26512
Staphylococcus aureus ATCC 29213
Candida albicans ATCC 10231
Staphylococcus aureus MRSA ATCC 33592
Micrococcus luteus ATCC 7468
Staphylococcus epidermidis ATCC 12228

dab®
DEFENSE AGAINST BACTERIA
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EVALUATION OF THE RESIDUAL ANTIMICROBIAL ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS*

Performed by BioScience Laboratories, Inc.

DAB keeps on killing bacteria for at least

4 hours

by creating a glove-like barrier of protection.

Staphylococcus aureus is the leading cause of staph infections.

PRODUCT	TIME	BACTERIAL REMOVAL
DAB Sanitizer + Protectant	1-HOUR	99.9924%
	4-HOUR	99.9826%
Alcohol-Based Hand Sanitizer	1-HOUR	80.0474%
	4-HOUR	53.2265%

DEFENSE AGAINST BACTERIA
Hand Sanitizer + Protectant

WHAT IS GLOVE JUICE?

Glove Juice

noun

1 a : Bacteria-rich moisture that forms inside rubber work gloves when proper hand hygiene procedures are not followed. Encased in the rubber glove, the skin gets warm and produces sweat. This warm, wet juice is the perfect environment for bacteria to multiply.

THE ISSUES WITH EMPLOYEES AND THE SPREAD OF GLOVE JUICE

How many times have you seen someone take their gloves off nearby or over your food? Ever think about how that glove snapping off is probably spraying micro bits of that person's sweat and glove powder into your food? And if a worker's glove is torn or nicked while working, an ultra-concentrated colony of germs is released.

It may come as a shock that wearing gloves may be one of the most effective ways to spread pathogens in a food service system.

CENTERS FOR DISEASE CONTROL, ENVIRONMENTAL HEALTH SERVICES JUNE 18, 2019 FOOD WORKER HANDWASHING & FOOD PREPARATION STUDY

EHS-Net is a federally funded collaboration of federal, state, and local environmental health specialists and epidemiologists working to better understand the environmental causes of foodborne illness.

WHY THE STUDY WAS DONE

The spread of germs from the hands of food workers to food is an important cause of foodborne illness outbreaks in restaurants. In fact, it caused 89% of outbreaks in which food was contaminated by food workers.

WHAT THE STUDY DESCRIBED

This study described restaurant food workers' handwashing practices and focused on when workers washed their hands.

WHAT THE STUDY FOUND

Overall, workers engaged in about 9 activities an hour that should have involved handwashing. Workers washed their hands in only 27% of activities in which they should have. Handwashing rates differed by activity.

Workers were more likely to wash their hands at the right time when they were not wearing gloves than when they were.

HOW TO AVOID GLOVE JUICE

The key to reducing the risk presented by glove juice is to reduce the number of pathogens on the hands before gloving. Correct hand washing is a good start, but why stop there when the goal is to **maximize pathogen reduction** and minimize risk?

Sanitizing after handwashing will further reduce the colony forming units (CFUs) making it more difficult for the bacteria to recolonize.

DAB's 4 hours of clinically proven protection will not only insure workers hands are sanitized for a longer period of time than other brands but the alcohol-free formula will cause less irritation to gloved hands.

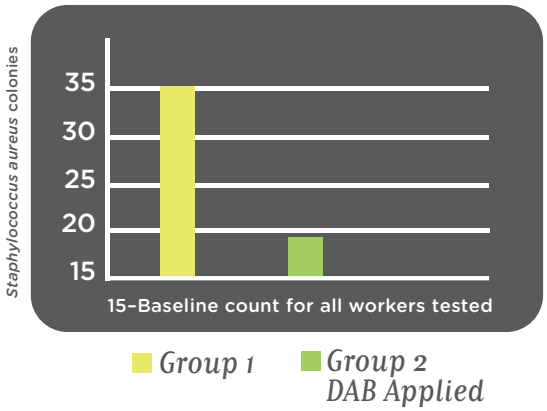
1 *Wash hands* **2** *Sanitize hands* **3** *Change gloves often*

PROOF THAT DAB REDUCES BACTERIA ON A GLOVED HAND

DAB was evaluated among restaurant workers during food preparation to measure bacterial colonization while using gloves.

All workers fingertips and thumbtips were cultured to look for Staph germs at the beginning of their shift and at the end of their shift.

- *Group 1* followed their usual routine of washing hands then putting on gloves before preparing food.
- *Group 2* washed their hands, applied DAB to both hands, then put on gloves before preparing food.



Group 1 had twice as many *Staphylococcus aureus* germs as Group 2 (DAB applied).

Two medical journal papers that evaluated DAB found that DAB persisted in killing Staph in the lab for at least four hours and had prolonged persistence on the hands of health care workers.

This study further demonstrates DAB's residual effectiveness among restaurant workers as previously proven in labs and in clinics.

DEFENSE AGAINST BACTERIA
Hand Sanitizer + Protectant

EAS CONSULTING GROUP ARTICLE

Background on the study of the efficacy and persistent activity of the benzalkonium chloride-based DAB hand sanitizer

DEFENSE AGAINST BACTERIA
Hand Sanitizer + Protectant

November 18, 2019

Background on the study of the efficacy and persistent activity of the benzalkonium chloride-based DAB hand sanitizer

The prevention of nosocomial infections has been a goal for the medical community since the elucidation of the germ theory of disease. Modern approaches include extensive facilities sanitation programs and multiple personal hygiene practices[1]. Of the latter, regular hand washing and the use of hand sanitizer products are now routine[2]. Hand sanitizer formulations have traditionally contained ethanol (60 to 70%) as the active ingredient responsible for the antibacterial action. Ethanol works through desiccation of the target organisms. Applied to the skin, the ethanol-based sanitizers are effective in reducing the bioburden of many types of microbes. However, the active ingredient is highly volatile and can quickly evaporate from the skin's surface so the residual antibacterial activity may be limited. This study examined the efficacy and persistent antibacterial activity of DAB hand sanitizer which uses 0.12% Benzalkonium Chloride as the active antibacterial ingredient.

The published manuscript contains most of the details of the study so those will not be recapitulated in this document. The focus here will be to provide a background on the regulatory framework and background on how the study was designed. Certain data are also provided which were beyond the scope of the clinical publication.

FDA Guidance:

Products such as DAB hand sanitizer are categorized as Antiseptic Rubs (in contrast to washes which are removed with water) as they are meant to be applied to the skin without subsequent rinsing with water. The USFDA proposed major changes to the OTC guidance for defining and testing Antiseptic Rubs in 2016 and this rule was finalized in August 2019. While the study was designed under the proposed rule, it is consistent with the guidance of the final rule.

Several points are important in the new rule. Previous rules (i.e. 1994) established certain ingredients as Generally Recognized as Safe and Effective (GRAS/GRAE). That changed in the new guidance as reflected in the table below[3]:

[1] Dixon, R.E., Control of health-care-associated infections, 1961-2011. Centers for Disease Control and Prevention Morbidity and Mortality Weekly Report, Supplement, 2011. 60: p. 58-63.
[2] Anonymous, WHO Guidelines on Hand Hygiene in Health Care. World Health Organization, 2009.
[3] 21 CFR Part 310 Safety and Effectiveness of Consumer Antiseptic Rubs; Topical Antimicrobial Drug Products for Over-the-Counter Human Use Federal Register /Vol. 84, No. 71 / Friday, April 12, 2019

Table 1 Copy of table 3 from the 21 CFR Part 310

TABLE 3—CLASSIFICATION OF OTC CONSUMER ANTISEPTIC RUB ACTIVE INGREDIENTS IN THE 1994 TFM AND IN THE 2016 PROPOSED RULE

Active ingredient	1994 TFM proposal [1]	2016 Proposed rule
Alcohol 60 to 95 percent	I [2]	IIISE [3].
Isopropyl alcohol 70 to 91.3 percent	IIIE	IIISE.
Benzalkonium chloride	IIISE	IIISE.

[1] Because the 1994 TFM did not describe antiseptic hand washes and rubs separately, the 1994 TFM classification was for use as an antiseptic hand wash or healthcare antiseptic hand wash.
[2] "I" denotes a classification that an active ingredient is GRAS/GRAE and not misbranded.
[3] "III" denotes a classification that the available data are insufficient to classify the active ingredient as GRAS/GRAE. "S" denotes safety data needed. "E" denotes effectiveness data needed.

These changes put the ethanol, isopropyl and benzalkonium chloride active ingredients on the same footing and set guidance for testing. It is also important to note that the remaining active ingredients proposed for this application were rated as ineligible active ingredients.

The following paragraph outlines the expectations for an efficacy study[4]:

"D. Updated Statistical Analysis for Efficacy
In the 1994 TFM, FDA recommended that the general effectiveness of antiseptics be assessed in several ways, including by conducting clinical simulation studies with the surrogate endpoint of the number of bacteria removed from the skin. In the 2015 Health Care Antiseptic proposed rule and the 2016 Consumer Antiseptic Rub proposed rule, FDA made revisions to the effectiveness criteria proposed in the 1994 TFM, while continuing to recommend that bacterial log reduction studies be used to demonstrate that an active ingredient is GRAE for use in a consumer antiseptic rub product. FDA recommended that these bacterial log reduction studies: (1) Include both a negative control (test product vehicle or saline solution) and an active control (an FDA-approved product); (2) have an adequate sample size to show that the test product is superior to its negative control; (3) incorporate the use of an appropriate neutralizer and a demonstration of neutralizer validation; and (4) include an analysis of the proportion of subjects who meet the recommended log reduction criteria based on a two-sided statistical test for superiority to negative control and a 95 percent confidence interval approach (81 FR 42912 at 42921 to 42922). FDA also recommended that the success rate or responder rate of the test product be significantly higher than 70 percent."

The study published as "Bondurant, S.W., Duley, C.M., Harbell, J.W. (2019) Demonstrating the persistent antibacterial efficacy of a hand sanitizer containing benzalkonium chloride on human

[4] Ibid footnote 3

skin at 1, 2, and 4 hours after application. American Journal of Infection Control 47(8):928-932" was intended to address these points.

Background

The project began in January of 2018. The goal was to determine the persistence of the antibacterial action of the benzalkonium chloride (BK) product compared to an alcohol (ethanol)-based sanitizer product. The BK product had been tested for antimicrobial efficacy against a wide range of bacterial species as well as yeast (Candida) by Q-Laboratories, Inc. (Cincinnati, OH) (February 2013) using a modification of ASTM Committee E35.15 standard method E2315.03 "The assessment of antimicrobial activity using a Time Kill procedure". Table 2 lists the bacterial and fungi used in this study.

Table 2 List of organisms tested in the Time Kill Study

Challenge Microorganisms:
Acinetobacter baumanii ATCC 19606
Staphylococcus haemolyticus ATCC 29970
Bacteroides fragilis ATCC 25285
Staphylococcus saprophyticus ATCC 15305
Haemophilus influenza ATCC 19418
Staphylococcus hominis ATCC 27844
Enterobacter aerogenes ATCC 35029
Streptococcus pyogenes ATCC 19615
Escherichia coli ATCC 25922
Enterococcus faecalis ATCC 29212
Escherichia coli O157:H7 ATCC 43895
Enterococcus faecium ATCC 19434
Klebsiella oxytoca ATCC 43165
Streptococcus pneumoniae ATCC 6302
Klebsiella pneumonia ATCC 4352
Pseudomonas aeruginosa ATCC 27853
Proteus mirabilis ATCC 7002
Pseudomonas aeruginosa ATCC 15442
Serratia marcescens ATCC 14756
Candida glabrata ATCC 26512
Staphylococcus aureus ATCC 29213
Candida albicans ATCC 10231
Staphylococcus aureus MRSA ATCC 33592
Micrococcus luteus ATCC 7468
Staphylococcus epidermidis ATCC 12228

Exposures for this study were 15, 30, 60 and 120 seconds. At the appropriate time points, samples of the treated bacterial suspension were removed, added to the neutralizer (Letheen) and serially diluted and plated to determine the number of viable colony forming units. This value was compared to the starting concentration of the challenge organism to derive a log kill value. Most organisms exhibited a >5 log kill at 15 seconds and all of the bacteria showed a > 5 log kill by 30 seconds. The yeast species (Candida) showed a 2.8 log kill at 30 seconds. From this study, it was concluded that the BK product had wide ranging antibacterial activity in this *in vitro* study.

Design of the efficacy and persistence study

The clinical study to show clinical efficacy and persistence of that efficacy as well as provide a comparison with a more traditional ethanol-based product was designed in collaboration with the scientific staff at Biosciences Laboratories, Inc. who then performed the study. The protocol was based on ASTM, (2015) E2752-10 Standard Guide for Evaluation of Residual Effectiveness of Antibacterial Personal Cleaning Products. This protocol is based on application to the volar forearms so that each panelist becomes his or her own control. The treatment groups were negative control (clean skin), the BK product (0.12%) and the ethanol product (63% ethanol). The clean skin negative control was selected since the vehicle for each of the two test products was different. Thus point one of the FDA guidance for a negative control (maximum viability), a traditional product (ethanol-based) and the new product (BK product) was satisfied. While the ethanol-based product is expected to be effective in killing bacteria on contact, it is not expected to show persistence once the ethanol has evaporated.

This study was conducted under the Good Laboratory Practices guideline promulgated by the USFDA and USEPA for studies intended for use in the regulatory decision-making process. This is important because it means that the study is fully auditable.

Staphylococcus aureus (ATCC strain# 6538) was selected as the test organism for this study as it is one of the most common skin pathogens encountered in the clinical setting and responsible for many if not most skin infections.

The determination of the number of panelists required provide statistical power for the study was done with the guidance of Daryl S. Paulson, PhD, President and CEO of BioScience Laboratories, Inc. and a recognized expert in statistics for such studies. Twenty four panelists were used and each received each treatment (control and experimental) so that the log kill could be determined against the negative control colony yield from that panelists (see Table 3 for an example).

For this study, the same product neutralizer was selected for both the test (BK) and comparator (ethanol) products. Before the study began, the effectiveness of the product neutralizer was confirmed using ASTM E1054 (2013), Standard Test Method for Evaluation of Inactivators of Antibacterial Agents. Four replicate samples with two exposure periods each (one and 30 minutes) were tested for each treatment condition; untreated control, test product, comparator product. The results of the product neutralizer testing showed the efficacy of the neutralization formulation. In all cases, there was no significant difference between the mean untreated control $\log_{10}$ colony counts (n=4) and the mean treated $\log_{10}$ colony counts (n=4) indicating that there was no significant residual antibacterial activity.

Additional Data

The absolute efficacy (log kill compared to the negative control) and relative efficacy (relative to the ethanol-based product) was measured at three time points after application of the BK/ethanol products to the skin; one hour, two hours and four hours. For the published manuscript, summary data were provided as the raw data were too voluminous for the journal. The raw data are provided here as Table 3 (one hour post application), Table 4 (two hours post application) and Table 5 (four hours post application). These tables provide the statistical data for mean, standard deviation and f statistics (p value for significance) for comparison of the viable colony forming units recovered (BK-treated vs negative control and BK-treated vs alcohol-treated). Statistical difference between the recovery of colony forming units (viable bacteria) from the BK-treated sites compared to the recovery from the negative control or alcohol-treated site was highly significant. These p values are listed as $p << 0.001$ but are actually $p << 10^{-10}$ or less. They also show that the vast majority of the BK-treated areas on each panelist showed a least a three log decrease in viable bacteria relative to the untreated control (Log_{10} Differences) which are bolded in each table. These tables then address the requirements in point four of the FDA guidance.

Table 3 Recovery of viable bacterial (colony forming units) from the treated and control sites one hour after application of the test materials.

	Test Product One Hour Post Application			Comparator Product One Hour Post Application		
Subject	**Untreated Log10 Microbial Recovery**	**Treated Log10 Microbial Recovery**	**Log10 Difference**	**Untreated Log10 Microbial Recovery**	**Treated Log10 Microbial Recovery**	**Log10 Difference**
3	4.90	0.86	**4.04**	4.90	3.76	1.14
11	5.39	1.56	**3.83**	5.39	4.02	1.37
4	5.38	1.81	**3.57**	5.38	3.20	2.18
6	5.39	0.86	**4.53**	5.39	5.26	0.13
1	5.32	0.86	**4.46**	5.32	4.25	1.08
15	5.34	2.03	**3.31**	5.34	4.24	1.11
7	5.23	0.86	**4.37**	5.23	5.15	0.08
9	5.05	1.16	**3.89**	5.05	3.83	1.22
8	5.17	0.86	**4.31**	5.17	5.31	-0.14
10	5.22	1.16	**4.06**	5.22	4.75	0.47
17	5.12	0.86	**4.26**	5.12	5.30	-0.18
2	4.91	0.86	**4.05**	4.91	4.84	0.07
22	5.30	0.86	**4.44**	5.30	3.58	1.72
24	5.17	0.86	**4.31**	5.17	5.00	0.17
27	5.14	0.86	**4.28**	5.14	5.21	-0.07
12	5.29	0.86	**4.43**	5.29	3.38	1.90
23	4.86	0.86	**4.00**	4.86	3.46	1.40
20	4.75	0.86	**3.89**	4.75	3.50	1.25
34	5.40	2.14	**3.27**	5.40	5.41	0.00
32	5.44	0.86	**4.58**	5.44	4.85	0.59
37	5.34	1.16	**4.18**	5.34	5.00	0.34
36	5.14	1.16	**3.98**	5.14	5.05	0.09
33	5.31	0.86	**4.45**	5.31	4.77	0.54
35	5.21	0.86	**4.35**	5.21	4.86	0.35
Median	5.23	0.86	4.22	5.23	4.81	0.51
Mean	5.20	1.08	4.12	5.20	4.50	0.70
Standard deviation	0.189	0.395	0.36	0.189	0.727	0.70
Standard Error	0.434	0.629	0.599	0.434	0.853	0.838
t value	2.069	2.069		2.069	2.069	
95% upper	6.097	2.381		6.097	6.264	
95% Lower	4.300	-0.220		4.300	2.735	
BK vs negative control		p value (one tailed)	p<<0.001			
BK vs alcohol		p value (one tailed)	p<<0.001			

Table 4 Recovery of viable bacterial (colony forming units) from the treated and control sites two hour after application of the test materials (note that the ethanol product was not tested at two hours).

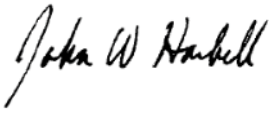
	Test Product Two Hours Post Application		
Subject	**Untreated Log10 Microbial Recovery**	**Treated Log10 Microbial Recovery**	**Log10 Difference**
3	5.08	0.86	**4.22**
11	5.34	1.34	**4**
4	5.26	0.86	**4.4**
6	5.34	0.86	**4.48**
1	5.12	0.86	**4.26**
15	5.25	0.86	**4.39**
7	5.1	0.86	**4.24**
9	5.16	0.86	**4.3**
8	lost	0.86	*
10	5.09	1.16	**3.93**
17	5.09	0.86	**4.23**
2	5.15	0.86	**4.29**
22	5.17	0.86	**4.31**
24	5.19	0.86	**4.33**
27	5.14	0.86	**4.28**
12	4.8	0.86	**3.94**
23	5.06	0.86	**4.2**
20	4.53	0.86	**3.67**
34	5.4	2.47	2.93
32	5.24	0.86	**4.38**
37	5.36	1.56	**3.8**
36	5.17	0.86	**4.31**
33	5.42	0.86	**4.56**
35	5.35	1.34	**4.01**
Median	5.17	0.86	4.26
Mean	5.17	1.01	4.15
Standard deviation	0.196	0.367	0.91
Standard Error	0.443	0.606	0.955
t value	2.074	2.074	
95% upper	6.085	2.266	
95% Lower	4.246	-0.248	
BK vs negative control		p value (one tailed)	p<<0.001

Table 5 Recovery of viable bacterial (colony forming units) from the treated and control sites four hour after application of the test materials.

	Test Product Four Hour Post Application			Comparator Product Four Hour Post Application		
Subject	Untreated Log10 Microbial Recovery	Treated Log10 Microbial Recovery	Log10 Difference	Untreated Log10 Microbial Recovery	Treated Log10 Microbial Recovery	Log10 Difference
3	3.69	0.86	2.83	3.69	4.23	-0.54
11	4.81	1.46	**3.35**	4.81	4.09	0.72
4	5.36	1.16	**4.20**	5.36	3.68	1.68
6	5.09	0.86	**4.24**	5.09	5.46	-0.36
1	5.05	0.86	**4.19**	5.05	5.24	-0.19
15	5.11	2.09	**3.02**	5.11	4.50	0.61
7	5.12	0.86	**4.26**	5.12	4.76	0.35
9	5.07	1.34	**3.74**	5.07	4.28	0.8
8	4.01	0.86	**3.15**	4.01	4.10	-0.09
10	4.77	0.86	**3.91**	4.77	4.46	0.31
17	5.03	0.86	**4.17**	5.03	5.12	-0.08
2	5.13	0.86	**4.27**	5.13	5.02	0.11
22	5.13	1.16	**3.97**	5.13	3.70	1.43
24	5.14	0.86	**4.28**	5.14	4.59	0.55
27	5.00	0.86	**4.14**	5.00	5.09	-0.1
12	4.77	0.86	**3.91**	4.77	4.56	0.21
23	4.34	0.86	**3.48**	4.34	4.38	-0.04
20	4.33	2.11	2.22	4.33	2.66	1.67
34	5.29	2.42	2.87	5.29	5.32	-0.03
32	5.32	0.86	**4.46**	5.32	5.09	0.23
37	5.24	2.16	**3.08**	5.24	5.12	0.12
36	4.96	0.86	**4.10**	4.96	5.18	-0.22
33	5.11	0.86	**4.25**	5.11	5.07	0.04
35	5.11	1.16	**3.95**	5.11	4.53	0.58
Median	5.08	0.86	3.96	5.08	4.58	0.17
Mean	4.92	1.17	3.75	4.92	4.59	0.32
Standard deviation	0.420	0.503	0.60	0.420	0.649	0.60
Standard Error	0.648	0.709	0.776	0.648	0.806	0.773
t value	2.069	2.069		2.069	2.069	
95% upper	6.256	2.633		6.256	6.260	
95% Lower	3.576	-0.303		3.576	2.926	
BK vs negative control		p value (one tailed)	p<<0.001			
BK vs alcohol		p value (one tailed)	p<<0.001			

These data show the efficacy and persistence of the antibacterial action of the DAB benzalkonium chloride-based product against the common skin pathogen S. aureus. This document is intended to be paired with the published report of Bondarant et al. 2019.

Respectfully yours,



John W. Harbell, Ph.D.
EAS Consulting Group

AJIC ARTICLE

The American Journal of Infection Control
Evaluating DAB Hand Sanitizer + Protectant

DEFENSE AGAINST BACTERIA
Hand Protectant + Sanitizer

American Journal of Infection Control 000 (2019) 1–5

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journal homepage: www.ajicjournal.org

Major Article

Demonstrating the persistent antibacterial efficacy of a hand sanitizer containing benzalkonium chloride on human skin at 1, 2, and 4 hours after application

Sidney W. Bondurant MD [a], Collette M. Duley BS [b], John W. Harbell PhD [c,*]

[a] University of Mississippi Medical Center, Jackson, MS
[b] BioSciences Laboratories, Inc., Bozeman, MN
[c] EAS Consulting Group, Dallas, TX

Key Words:
Antibacterial
Persistence
Ethanol
Staphylococcus aureus
ASTM E2752-10
Nosocomial infection

Background: Use of hand sanitizers has become a cornerstone in clinical practice for the prevention of disease transmission between practitioners and patients. Traditionally, these preparations have relied on ethanol (60%-70%) for bactericidal action.

Methods: This study was conducted to measure the persistence of antibacterial activity of 2 preparations. One was a non-alcohol-based formulation using benzalkonium chloride (BK) (0.12%) and the other was an ethanol-based formulation (63%) (comparator product). The persistence of antibacterial activity was measured against *Staphylococcus aureus* using a technique modification prescribed in American Society for Testing and Materials protocol E2752-10 at up to 4 hours after application.

Results: The test product (BK) produced a marked reduction in colony-forming units at each of the 3 time points tested (3.75-4.16-$\log_{10}$ reductions), whereas the comparator produced less than 1-$\log_{10}$ reduction over the same time. The differences were highly significant.

Discussion: In the course of patient care or examination, there are instances where opportunities exist for the practitioner's hands to become contaminated (eg, key boards and tables). Persistent antibacterial activity would reduce the chances of transfer to the patient.

Conclusions: These results show a major improvement in persistent antibacterial activity for the BK formulation compared to the comparator ethanol-based formulation.

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The prevention of nosocomial infections has been a goal for the medical community since the elucidation of the germ theory of disease. Modern approaches include extensive facilities sanitation programs and multiple personal hygiene practices.[1] Of the latter, regular hand washing and the use of hand sanitizer products are now routine.[2] Hand sanitizer formulations have traditionally contained ethanol or other short-chained alcohols (60%-70%) as the active ingredient responsible for the antibacterial action. Ethanol provides its antimicrobial action through desiccation of the target organisms. Applied to the skin, the ethanol-based sanitizers are effective in reducing the bioburden of many types of microbes.[3] However, alcohols are volatile and can evaporate from the skin's surface, so the residual antibacterial activity may be limited.[4] The importance of persistent antimicrobial activity has been increasingly recognized in the medical/surgical setting.[2,5] Recent reports have also shown that certain pathogen populations are becoming more tolerant to ethanol exposure.[6] These data suggest that the use of alternative antibacterial actives might be a benefit in the clinical setting.

Alcohol-free formulations have been developed, with the surfactant benzalkonium chloride (BK) as the active antibacterial agent. This active ingredient acts by disrupting the cell membranes of the target organisms and is active at relatively low concentrations (0.12%-0.13%).[7] Since this surfactant is not volatile, it is expected to remain on the skin as the product dries. Although this report focuses only on the antibacterial action of BK against *Staphylococcus aureus*, this surfactant has also been studied for virucidal activity against influenza, Newcastle disease, and avian infectious bronchitis viruses.[8]

* Address correspondence to John W. Harbell, PhD, Toxicology Consultant, EAS Consulting Group, 16334 Sunset Valley Dr, Dallas, TX 75248
E-mail address: jharbell@easconsultinggroup.com (J.W. Harbell).
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This study was performed to measure the residual antibacterial activity of 2 hand sanitizer products using the standard method prescribed in the American Society for Testing and Materials protocol E2752-10.[9] The test product was a surfactant-based product using BK (0.12%) as its active antibacterial agent, and the second product was a standard commercial ethanol-based formulation (with 63% ethanol but no other antibacterial actives), which served as the comparator product. The comparator product's ethanol concentration falls within the recognized effective concentration range for effective immediate contact antimicrobial activity.[3] Persistence of antibacterial activity was measured as a function of $\log_{10}$ kill of reference bacteria versus time after application of the hand sanitizer. The antibacterial activity was measured from 1-4 hours after application of the products. The test product was evaluated at 1, 2, and 4 hours after application, whereas the comparator product was evaluated at 1 and 4 hours after application.

METHODS

For this study of residual antibacterial activity on the skin, 2 products were compared. The commercial brand DAB hand sanitizer (active ingredient 0.12% BK) and a comparator hand sanitizer, containing 63% ethyl alcohol), were provided by Best Sanitizers (Walton, KY) to the testing laboratory, Biosciences Laboratories, Inc. (Bozeman, MN).[10] The DAB brand is produced by Best Sanitizer under contract to Three Kings Inc. (Corinth, MS). The study was conducted in compliance with good laboratory practices for nonclinical studies (21CFR58). As stated in the study protocol, "The purpose of this study was to evaluate the residual antibacterial efficacy of 1 test product verses a comparator ethanol-based product, as determined by the difference between the number of challenge bacteria species recovered following exposure to the test materials and the number recovered from the untreated (negative control) test sites."

Panelists and skin preparation

The study was performed on 24 subjects (19-63 years old) with healthy skin (16 men and 8 women). The study protocol and informed consent form were approved by the Gallatin Internal Review Board. The volar forearms were used, and the test sites were marked for the test product, comparator product, and negative control. The volar forearm was chosen to provide multiple replicate test sites on each arm, which would not be possible using the hands. The sites and arms were randomized among the treatment groups to prevent anatomical bias. The arms were washed with nonmedicated soap to remove surface dirt and oil, dried, and finally decontaminated with 70% isopropyl alcohol and allowed to air dry. The test sites and control sites were marked with a surgical marker as rectangles (2 × 6 inch [5.08 × 15.24 cm]) for the test product on 1 arm and as rectangles (2 × 4 inch [5.08 × 10.16 cm]) for the comparator product on the other arm. An area for the untreated control skin (no further treatment) was also marked. The areas for the test and comparator products were randomized between arms across the test panel. Within the test sites, 3 circles (2 cm in diameter) were marked with a surgical marker. Only 2 circles were marked in the 2 × 4-inch box for the comparator product, as only 2 time points were to be assessed. These were the sites to which the bacteria were to be applied.

Challenge bacteria

The challenge bacterial strain for this study was *S aureus* (ATCC 6538). *S aureus* is a common skin contaminant and therefore provides an appropriate test organism.[11] Fresh, active stocks were prepared in broth medium daily. The day before testing, a sample of the broth culture was applied to and spread over the surface of a tryptic soy agar plate and incubated for 24 hours. Just before beginning the study, a portion of the bacteria on the surface of the agar plate was transferred to phosphate buffered saline. After mixing the bacteria into the saline to form a uniform suspension, the turbidity of the suspension was measured and the sample diluted to approximately 1.0×10^8 colony-forming units (CFU) per mL of suspension. Ten microliters of this suspension (approximately 10^6 CFU) were applied to and spread over the 2-cm circles at the appropriate times.

Product neutralizer

It is essential that once the bacteria are removed from the treated skin that residual skin sanitizer not continue to act on the bacteria as they are being prepared (diluted and plated). To this end, a product neutralizer was prepared and added to the dilution liquids. For this study, the same product neutralizer was selected for both the test and comparator products. Before the study began, the effectiveness of the product neutralizer was confirmed using American Society for Testing and Materials E1054 (2013), Standard Test Method for Evaluation of Inactivators of Antibacterial Agents.[12] Four replicate samples for each of the 2 exposure periods (1 and 30 minutes) were tested for each treatment condition: untreated control, test product, comparator product, Butterfield's Phosphate Buffer (BPB++), and Stripping Suspension Fluid (SSF++). The "++" refers to the presence of the product neutralizer. In addition, the antibacterial efficacy of the test and comparator products without neutralization were verified.

Evaluation of antibacterial efficacy

Application of the test and comparator products

Each product was applied to the skin at a rate of 0.25 mL per square inch (0.039 mL/cm^2) (3 mL for the 2 × 6-inch test rectangle and 2 mL for the 2 × 4-inch comparator product rectangle). In both cases, the liquid was applied in stages, spread over the whole area, and allowed to dry for 1-2 minutes between each application. Once all of the applications were made, the subjects were sequestered and monitored at the test facility to ensure test site integrity.

The persistent efficacy of the test product was evaluated at 1, 2, and 4 hours after application of the product to the skin. The comparator product was evaluated at only 1 and 4 hours after application. At each time point, 10 μL of the bacterial suspension were applied to 1 of the 2-cm circles in the test product treatment area and spread over the surface with a sterile glass rod. The procedure was repeated on the comparator product treatment area (except for the 2-hour time point) and on the negative control area. Each inoculation was allowed to dry in place for at least 20 but not for more than 25 minutes. At the end of this exposure period, a 2-step procedure known as the cup scrub technique was used to remove the bacteria for determination of viability. A sterile stainless steel cylinder with an interior area of 3.46 cm^2 was held against the skin within the 2-cm circle. A volume of 2.5 mL of sterile SSF was dispensed into the cylinder. The fluid contained the specific product neutralizer (SFF++) to stop the action of the test and comparator products. A sterile rod was used to massage the skin for 1 minute to lift the bacteria from the skin into the fluid. This fluid was transferred to a sterile tube, and a second 2.5 mL volume of SSF++ was dispensed into the cylinder. Again, the skin was massaged for 1 minute, and the second fluid sample was combined with the first. This process was repeated for each exposure condition at that time point. For example, at the 1-hour postexposure time point, 3 bacterial suspensions were collected from each of the 24 subjects; 1 from the test product-treated skin, 1 from the comparator product-treated skin, and 1 from the negative control-treated skin. To determine the number of viable bacteria (number of CFU) in each sample, serial 10-fold dilutions of each bacterial suspension sample were prepared in BPB solution again containing the product neutralizer (BPB++). Samples from each dilution were spread onto 2

Table 1
Mean log_{10} microbial recoveries and reductions from the untreated control of *Staphylococcus aureus* (ATCC 6538), 1 hour following application of the test product or comparator product

		Test product 1 h after application		Comparator product 1 h after application	
Measure	Untreated log_{10} microbial recovery	Treated log_{10} microbial recovery	Log_{10} difference	Treated log_{10} microbial recovery	Log_{10} difference
Median	5.23	0.86	4.22	4.81	0.51
Mean	5.20	1.08	4.12	4.50	0.70
SD	0.189	0.395	0.359	0.727	0.703
		P value (1 tailed)	*P* <.001		

individual mannitol salt agar plates, which were incubated at 35±2°C for 48 hours. On mannitol salt agar, *S aureus* produce golden-yellow colonies, and only those colonies were counted.

Calculation of the recovery of viable CFU of bacteria

By definition, a CFU is 1 bacterium that is capable of continued replication to produce a large number of bacteria to form a colony. Each inoculum to the skin contained approximately 10^6 CFU. Each sample from the skin was serially diluted and samples plated. Knowing the area of the skin sampled (3.46 cm^2), the volume of SSF (5 mL), the dilution of the sample producing the counted plate, and volume of the sample added to the plate, the number of CFU per unit area on the skin could be calculated.

The number of CFU from each site at each postapplication time was converted to a log_{10} value. The residual antibacterial activity was calculated by comparing the log_{10} value from the negative control site (time matched) to the log_{10} value from the test and comparator product-treated sites to determine the log_{10} difference (antibacterial effectiveness) for each treatment. The relative values were internally controlled for each subject. For the 1- and 4-hour postexposure times, the statistical significance between the log_{10} difference for the test and comparator values for the 24 subjects was evaluated using a paired Student t test (Excel).

RESULTS

The results of the product neutralizer testing showed the efficacy of the neutralization formulation. In all cases, there was no significant difference between the mean untreated control log_{10} colony counts (n = 4) and the mean treated log_{10} colony counts (n = 4), indicating that there was no significant residual antibacterial activity.

The results of the study are expressed as log_{10} mean recovery of CFU of *S aureus* from the untreated control site, the test product, and the comparator product sites for each postapplication time point. The mean values from the individual postapplication time point values for the test and the comparator products are provided (Tables 1-3).

DISCUSSION

This study was performed to measure the antibacterial efficacy of a benzalkonium-based test product in comparison with a comparator product containing 63% ethanol as a function of time after application of the individual products to human skin. *S aureus* was used as the test organism since it is a known skin pathogen.[11] The test and comparator products were applied to defined areas of opposing forearms at 0.039 mL/cm^2. Within those areas, 2-cm diameter circles were marked, to which the bacterial suspension would be applied at the specific times after application of the products. For the test product treatment, bacteria were applied at 1, 2, and 4 hours after product application and for the comparator product treatment, bacteria were applied at 1 and 4 hours after product application. Bacteria were applied to untreated skin at each time point to provide the baseline bacterial recovery. The difference in the recovery between the test and comparator products was striking. Although the test product reduced bacterial viability by 3-4 log_{10} at each time point, the comparator product did not reduce bacterial viability by even 1 log_{10}. The differences in efficacy were statistically significant at $P < .001$. These data suggest that the active ingredient BK (0.12%) can provide a marked improvement in persistent antibacterial activity over the 63% ethanol-based product.

The effectiveness of BK as an antibacterial agent on skin has been evaluated in the past. Dyer et al (1998) compared the efficacy of 3 hand sanitizer preparations containing either ethanol (63% or 70%) or BK (0.13%) against *Serratia marcescens* applied to the hands.[7] In this study, the hands were contaminated with 5 mL of *S marcescens*, spread over the hands, and allowed to dry for 45 seconds. Five grams of test product were used to "wash" the hands, and then the remaining bacteria were recovered using the "glove juice sampling method." Polyethylene gloves with 50 mL of recovery fluid were placed, and the hands and the fluid massaged for 1 minute to recover the bacteria. The bacterial suspension was diluted and plated to obtain the number of CFU recovered. This process was

Table 2
Mean log_{10} microbial recoveries and reductions from the untreated control of *Staphylococcus aureus* (ATCC 6538), 2 hours following application of the test product

Sample	Sample size	Mean (log_{10})	SD
Untreated log_{10} microbial recovery (2 h)	23*	5.17	0.20
Treated log_{10} microbial recovery (2 h)	24	1.01	0.37
Log_{10} difference (2 h)	23	4.16	0.35

*One untreated control sample lost.

Table 3
Mean log_{10} microbial recoveries and reductions from the untreated control of *Staphylococcus aureus* (ATCC 6538), 4 hours following application of the test product or the comparator product

		Test product 4 h after application		Comparator product 4 h after application	
Measure	Untreated log_{10} microbial recovery	Treated log_{10} microbial recovery	Log_{10} difference	Treated log_{10} microbial recovery	Log_{10} difference
Median	5.08	0.86	3.96	4.58	0.17
Mean	4.92	1.17	3.75	4.59	0.32
SD	0.420	0.503	0.602	0.649	0.597
		P value (1-tailed)	*P* <.001		

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repeated 10 times for each treatment condition, and the reduction factors were calculated. The process took approximately 10 minutes per cycle. Only the BK formulation produced a progressive increase in effectiveness (increased reduction factor) over the 10 cycles. The ethanol formulations showed declines in effectiveness relative to the first cycle for each.

The concentration of ethanol in the hand sanitizer formulation can have a marked impact on antibacterial activity. Kampf (2008) compared 4 ethanol-based formulations (85%, 62%, 61%, and 60%) and 2 application volumes of 2.4 and 3.6 mL (total both hands) were evaluated.[13] Again, *S marcescens* was used as the test bacterium. Approximately 5 mL of bacterial suspension were rubbed over the hands and allowed to dry. The viable bacteria were recovered using the glove juice sampling method described in the preceding text. The bacterial suspension was diluted and plated to obtain the number of CFU recovered. The untreated recovery values were compared to the treated conditions where either 2.4 or 3.6 mL were provided to rub over the hands (covering all skin). Both volumes were sufficient to cover the hands of most of the 16 subjects in each test group. The mean $\log_{10}$ reductions for each treatment were statistically compared by an analysis of variance analysis. Although all of the preparations reduced the number of viable bacteria, the larger volume was more effective at all ethanol concentrations and the 85% ethanol formulation was statistically more effective than the other 3 concentrations. For the 3.6 mL application volume, the mean $\log_{10}$ reduction for the treatment groups were 3.04 ± 0.81 (85%), 2.85 ± 0.51 (62%), 2.63 ± 0.59 (61%), and 2.53 ± 0.60 (60%). However, 85% ethanol is much higher than what is normally contained in current commercial hand sanitizer formulations.

Although *S aureus* accounts for a large fraction of the hospital-acquired infections, other bacteria are a concern. *Enterococcus faecium* is a gram-positive bacterium, which has become a leading antibiotic-resistant pathogen (bloodstream, urinary tract, and surgical wounds).[14] Hospital strains can be resistant to multiple antibiotics, which make them particularly difficult to treat once the infection is established.[15] The rise in incidents of nosocomial infections has raised concerns that preventive measures, such as the use of ethanol-based hand sanitizers, have applied selection pressure on the populations to select for more tolerant strains. Pidot et al (2018) have examined the resistance to isopropyl alcohol in 139 strains of hospital-associated *E faecium* isolated from 2 major Australian hospitals over 17 years.[6] These hospitals have active hand sanitation programs based on alcohol-based hand disinfectants. To measure resistance, bacterial suspensions were exposed to 23% isopropyl alcohol for 5 minutes and the number of remaining CFU determined. The concentration of isopropanol and time of exposure were selected to maximize resolution among the strains. Breaking the isolates into groups by date of isolation (1997-2003, 2004-2009, and 2010-2015), there was a high statistically significant decrease in mean sensitivity (based on mean $\log_{10}$ reduction) for the 2010-2015 isolates compared to the 1997-2003 and to the 2004-2009 isolates. These data suggest that there has been a population selection, which has reduced the overall sensitivity to the alcohol-based infection control measures.

Selection for increased tolerance to other disinfectants as a function of repeated use/exposure has been examined under various environmental exposure conditions. Holah et al (2002)[16] compared *Listeria monocytogenes* and *Escherichia coli* populations found in cannery processing lines where quaternary anomia disinfectants were routinely used. These isolates were compared to isolates from sites not routinely subjected to disinfectant use. They concluded that the persistent populations on the cannery lines were not inherently more tolerant to the disinfectant but that other factors (ie, surface attachment, biofilm formation, and growth rate) were likely responsible for their ability to persist in the disinfectant-treated environment. Kim et al (2018)[17] examined the impact of continuous exposure to BK on bacterial populations isolated from contaminated river sludge. The sediment samples were maintained for extended periods (3 years) in bioreactors containing nutrient medium and increasing concentrations of BK or nutrient medium alone. Changes in benzalkonium tolerance were measured using the minimal inhibitory concentration assay on nutrient agar. Certain species (ie, *Pseudomonas aeruginosa*) showed increased tolerance to BK (200 vs 50 mg/L), whereas others did not (ie, *Klebsiella michiganensis*). The basis for the difference in the selected strains with increased tolerance was a small change in the antibiotic efflux gene sequence.

It is not surprising that disinfectants can provide some selective pressure on bacterial populations. This pressure is most effective at sublethal concentrations of the disinfectant, which allow the more tolerant subpopulations to thrive and predominate. Lethal concentrations are less likely to select for tolerant clones where the surviving fraction of the population is very low.[18,19] The current study was not designed to measure selection pressure on the *S aureus* population. It was designed to measure persistence of antibacterial efficacy. The persistence of high antibacterial efficacy from the BK-containing test product may reduce the chances for selection of more tolerant clones.

Normal clinical infection control protocols specify use of hand sanitizers between patients to prevent patient-to-patient microbial transfer. That is not expected to change with the use of a persistent antimicrobial agent. However, in the course of patient care or examination, there are instances where there are opportunities for the practitioner's hands to become contaminated. Various surfaces such as key boards, tables, chairs, bed frames and other fixtures will need to be touched or handled. Use of a persistent antimicrobial hand sanitizer would be expected to reduce the opportunity for microbial transfer to the patient.

This study was undertaken to measure the absolute and relative persistence of antibacterial activity under very controlled test conditions. Having demonstrated persistent activity, the logical next step would be a clinical use study. As a first evaluation, a study is planned that will compare a 70% ethanol product and the test product from this study. Subjects will be medical clinic personnel, who will use both products in a cross-over study design.

In the United States, hand sanitizers (both medical professional and consumer) fall under the purview of the U.S. Food and Drug Administration, the 1994 tentative final monograph or proposed rule (the 1994 TFM) for over-the-counter antiseptic drug products (Federal Register of June 17, 1994 [59 FR 31402]). These rules are in the process of being revised to separate the professional and consumer products, and the agency is seeking additional data on active ingredients, including ethanol and BK. One factor to consider is the persistence of the antibacterial activity on the skin. This study provides quantitative data on the persistence of BK-induced antibacterial action, which could be a marked benefit in the prevention of nosocomial infections.

CONCLUSIONS

These results show a major improvement in persistent antibacterial activity for the BK formulation compared to the comparator ethanol-based formulation. Persistent antibacterial activity may be beneficial in the patient care setting to reduce the chances of incidental contamination of the hands and subsequent transfer to the patient.

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AJIC ARTICLE

Clinical Study of DAB Hand Sanitizer + Protectant vs 70% Alcohol-Based Hand Sanitizer

Reducing *Staphylococcus aureus* Skin Contamination in Health Care Workers

DEFENSE AGAINST BACTERIA
Hand Protectant + Sanitizer

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Major Article

Evaluation of a benzalkonium chloride hand sanitizer in reducing transient *Staphylococcus aureus* bacterial skin contamination in health care workers

Sidney Bondurant MD [a,*], Tanya McKinney PhD [b], Liza Bondurant PhD [b], Lisa Fitzpatrick MD, MPH, FIDSA [c]

[a] *Medical Spark Biologics, University of Mississippi Medical Center, Jackson, MS*
[b] *Department of Math and Sciences, Delta State University, Cleveland, MS*
[c] *George Washington University School of Public Health, George Washington University School of Medicine, Washington, DC*

Key Words:
Hand hygiene
Staphylococcus aureus
Benzalkonium chloride
Antibacterial persistence
Alcohol sanitizer
Nosocomial infection

Background: This study was performed to evaluate the effectiveness of a new commercially available hand sanitizer using 0.12% benzalkonium chloride (BZK) as the active ingredient in reducing transient skin contamination with *Staphylococcus aureus* in health care workers (HCWs), as compared with the effectiveness of a 70% ethanol-based hand sanitizer.

Methods: Fingertip touch culture plates were obtained from 40 HCWs in which all HCWs used antimicrobial soap containing 0.6% chloroxylenol for handwashing according to the Centers for Disease Control and Prevention guidelines for the entire study, while continuing to use the 70% ethanol-based hand sanitizer according to the Centers for Disease Control and Prevention guidelines for the first week. After the first week, the test subjects used the BZK hand sanitizer in place of the ethanol sanitizer. A paired sample t test was conducted to compare the mean bacterial colonies grown from HCWs fingertips during the use of the BZK and ethanol hand sanitizer.

Results: The results showed a significant reduction in total bacterial colony counts of *S aureus* during the week of BZK use as compared with the week of 70% ethanol sanitizer use.

Conclusions: There was a significant decrease in transient *S aureus* on the fingertips of HCWs in the BZK hand sanitizer use week as compared with the 70% ethanol hand sanitizer use week.

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A recent introduction to the consumer market of hand hygiene products, DAB (Three Kings Corporation, Corinth, MS), which contains 0.12% benzalkonium chloride (BZK) as its active ingredient was studied for persistence of antibacterial activity against *Staphylococcus aureus* on human skin as compared to a 63% ethanol-based hand sanitizer. That study showed significant killing of *S aureus* on the skin up to 4 hours postapplication for the BZK sanitizer, compared with essentially no persistent antibacterial activity of the ethanol sanitizer.[1]

In the March 8, 2019, *Morbidity and Mortality Weekly Report*, the Centers for Disease Control and Prevention (CDC) expressed concern about a failure of *S aureus* nosocomial infections to continue the downward rate trend that had been seen for several years. This statement was taken from that *Morbidity and Mortality Weekly Report*, "*S aureus* infections account for substantial morbidity in the United States. Despite significant reductions in health care–associated MRSA infections, progress is slowing. MSSA infections have not decreased as much in hospitals and might be increasing in the community. Adherence to CDC recommendations for preventing device- and procedure-associated infections and interrupting transmission, along with innovative interventions tailored to the needs of health care facilities (including decolonization) are needed to further prevent *S aureus* infections."[2]

Our study was designed to determine if the use of this new BZK-based hand sanitizer product was superior to, equal to, or inferior to a 70% ethanol-based hand sanitizer in the reduction of transient pathogenic staphylococci from the hands of health care workers (HCWs) in "real-world" conditions.

*Address correspondence to Sidney Bondurant, MD, Medical Spark Biologics, 4400 Old Canton Rd, Ste. 150, Jackson, MS 39211
E-mail address: sbondurant@medicalspark.com (S. Bondurant).

Conflicts of interest: The test product used in this study was furnished by Three Kings Corporation, Corinth, Mississippi. Sidney Bondurant and Lisa Fitzpatrick are paid consultants to Three Kings Corporation.

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Table 1
Descriptive statistics

Designation	Mean	N	Standard deviation	Standard error of the mean
Alcohol (total colony counts)	10.92	400	25.029	1.251
BZK (total colony counts)	6.63	400	14.931	0.747
Alcohol difference between AM and PM colony counts	0.385	200	29.9966	2.12108
BZK difference between AM and PM colony counts	0.325	200	19.05294	1.34725

BZK, benzalkonium chloride.

METHODS

Forty volunteer test subjects were recruited from HCWs at a cardiology clinic, a physical therapy clinic, a neurology/pain management clinic, a plastic surgery clinic, and a general medical clinic. Physicians, nurses, laboratory technicians, and physical therapists were all represented as test subjects and all were involved in direct patient care. There were 37 female test subjects and 3 male test subjects. All test subjects were already aware of current CDC recommendations for hand hygiene for HCWs. No attempt was made by the researchers to change the hand hygiene behavior of the test subjects during the study.

The study was designed to last 10 days (2 Monday through Friday workweeks), with all test subjects using the 70% ethanol hand sanitizer for the first week and then using the BZK product in place of the ethanol sanitizer for the second week. All test subjects continued to use 0.6% chloroxylenol antimicrobial hand soap for both weeks of the study. The BZK test product was provided by Three Kings Corporation.

The effect of each sanitizer on the staphylococcal population of test subject hands was assessed via the fingertip touch plate method. At the start of the workday, prior to use of any hand sanitizer or antimicrobial soap, microbial samples were collected by touching the fingertips of all 10 digits with gentle pressure to Mannitol Salt Agar plates (Hardy Diagnostics, Santa Maria, CA) for 5 seconds. This procedure was repeated at the end of the workday after determining that the test subject had not used hand sanitizer or antimicrobial soap for 15 minutes prior to collection of the touch plate. Plates were incubated for 48 hours at 35°C under aerobic conditions, and manual colony counts of *S aureus* colonies were conducted. The colony count for each determination was the total colony count from all 10 fingers.

The touch plate medium used was Mannitol Salt Agar. This medium was selected because it is selective and differential for the growth of staphylococci. It is selective for *S aureus* colonies because Mannitol Salt Agar plates allow growth of staphylococci while inhibiting the growth of most other bacterial species. It is differential in that *S aureus* colonies will be yellow surrounded by a yellow zone in otherwise light red colored media, whereas other staphylococci species will produce clear pink to red colonies with no color change in the media, and some micrococci that grow will produce large white to orange colonies with no color change in surrounding media.

The study protocol and informed consent document were approved by the Mississippi College institutional review board prior to the start of the study.

Data analysis

SPSS software (IBM Corporation, Armonk, NY) was used to conduct a paired sample t test. This analysis was conducted to compare the mean colony count on HCWs during the use of the BZK and ethanol hand sanitizer. The first analysis compared the difference in the number of bacterial colonies throughout the week of BZK and the week of ethanol hand sanitizer use. The second analysis compared the difference in the reduction of the number of bacterial colonies from the morning to the afternoon for the HCWs when using ethanol sanitizer versus when using BZK. Descriptive statistics for both comparisons are presented in Table 1, whereas the inferential statistics are presented in Table 2.

RESULTS

Our study showed a significant reduction in total bacterial colony counts (t_{399} = 2.898; $P < .01$) of *S aureus* during the week of BZK use as compared with the week of ethanol sanitizer use. Specifically, the total *S aureus* colony count for the alcohol week was 4,367 compared with a colony count of 2,653 for the BZK week. On average, BZK use among HCWs yielded 4.285 fewer bacterial colonies than ethanol sanitizer (95% confidence interval [1.378, 7.192]).

The mean colony count for the alcohol use week was 10.92. The mean colony count for the first day morning colony count of the BZK use week was 9.13. The mean colony count for the BZK use week was 6.63. The first day morning colony count for the BZK use week appears comparable to the mean number for the alcohol use week, which is what would be expected.

Figure 1 illustrates the cumulative graph of the daily colony counts for the week of BZK and alcohol use weeks. The graph demonstrates that users of alcohol had more bacteria on them than the users of BZK throughout the week. The line of best fit for the alcohol users had a rate of increase in cumulative colony counts of 11.785, whereas the BZK users rate of increase was 6.933. This indicates that the colony counts increased at a consistently higher rate on alcohol users than on the BZK users.

On average, HCWs who used ethanol sanitizer had 0.06 fewer bacterial colonies in the afternoon than they did in the morning

Table 2
Inferential statistics

Designation	Mean	Standard deviation	Standard error of the mean	95% confidence interval of the difference		t	*df*	Significance (2-tailed) *P* value
				Lower	Upper			
Alcohol vs BZK total colony counts	4.285	29.576	1.479	1.378	7.192	2.898	399	<.01
Alcohol vs BZK difference between AM and PM colony counts	0.06	32.98814	2.33261	−4.53981	4.65981	0.026	199	.98

BZK, benzalkonium chloride.

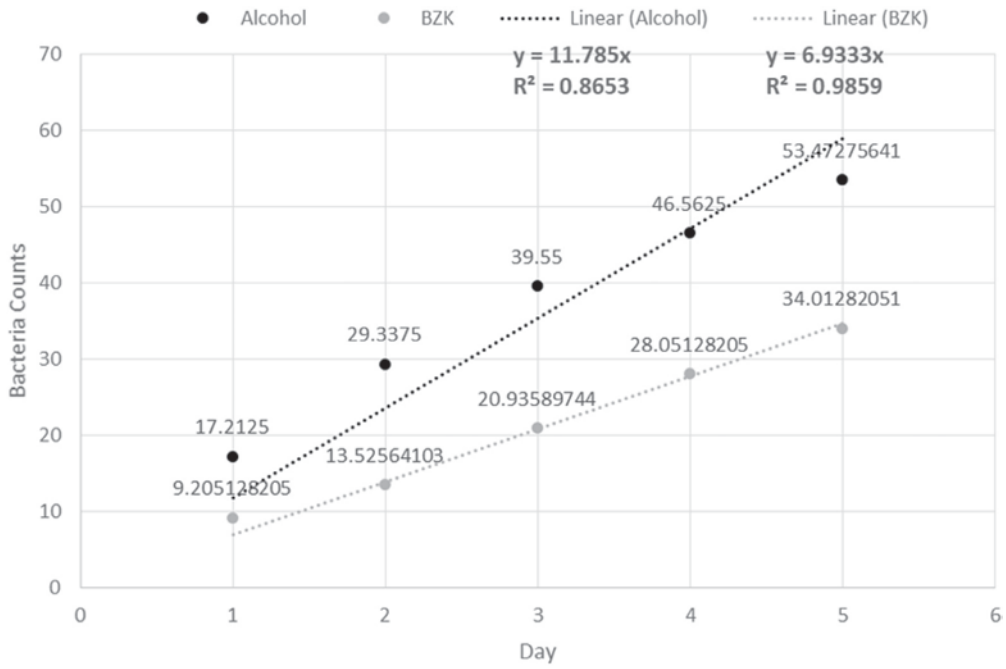

Fig 1. Cumulative bacteria counts. *BZK*, benzalkonium chloride.

compared with HCWs who used BZK (95% confidence interval [−4.53981, 4.65981]). The results did not show a significant difference between the morning and afternoon bacterial colony counts of *S aureus* of HCWs who used BZK compared with HCWs who used ethanol sanitizer ($t_{199} = 0.026$; $P > .05$).

DISCUSSION

Hand hygiene compliance is widely recognized as playing a major role in the prevention of hospital-acquired infections (HAI), and is incorporated in the CDC recommendations for preventing HAI. Despite this, compliance with the CDC guidelines is quite variable and, in some cases, very low. One recent review article of studies of hand hygiene compliance in hospital emergency departments showed that only 33% of the studies showed compliance of >50%.[3] Hand hygiene products that increase compliance should result in lower bacterial loads on the hands of HCWs.

Alcohol-based hand sanitizers (ABHS) and antiseptic hand soap for handwashing are 2 components of the current guidelines for hand hygiene for HCWs recommended by CDC.[4] The monograph used as the basis for these guidelines was published in 2002 and was an extensive review of the data available up to that time. In the section discussing BZK and other quaternary ammonium compounds the authors stated, "Further studies of such products are needed to determine if newer formulations are effective in health care settings."[5] Two areas of interest that would promote further reductions in the bacterial load on the hands would be the effect of persistent antibacterial activity of a hand sanitizer on the skin, and measures that would increase the likelihood of HCWs using the sanitizer as recommended by CDC.

BZK has been used as a hand hygiene antimicrobial for almost 90 years. It has a long history of use in both surface disinfectants used in the food industry and as a skin sanitizer. The mechanism of action for BZK is related to its ability to become adsorbed to and then penetrate the bacterial cell wall that leads to damage and loss of cell membrane structural integrity. This causes leakage of low molecular weight components of the cell and eventually cell wall lysis.[6] Alcohol is effective at killing bacteria by its ability to denature proteins. Concentrations between 60% and 95% are most effective, but higher concentrations actually lose potency because of the necessity to have water with the alcohol to be effective.[5]

Recent reports of increased tolerance to alcohol by certain pathogens have caused concern about the possibility of decreasing effectiveness of hand sanitizers.[7] Quaternary ammonium compounds such as BZK are widely used in the food industry as disinfectants, and have been studied in that context for findings of resistance to those compounds. Holah et al[8] compared *Listeria monocytogenes* and *Escherichia coli* populations taken from fish cannery lines in which 1 area was routinely disinfected with quaternary ammonium compounds and another area that had no exposure to those disinfectants. Their conclusion was that the persistent colonies found in disinfectant exposed areas were there because of factors other than tolerance to the disinfectant, primarily physical factors such as biofilm formation and surface attachment.[8] Another study found increased tolerance to BZK from some species (*Pseudomonas aeruginosa*) recovered from river sludge, whereas other species (*Klebsiella michiganensis*) from the same sample showed no increased tolerance. The basis for the difference was found to be a small change in the antibiotic efflux gene sequence.[9] Moreover, an additional study in the food industry of *L monocytogenes* found that at very low concentrations BZK did promote tolerance but at concentrations normally used the disinfectant was still very efficient at controlling this organism.[10] He et al[11] cultured inanimate objects from fitness centers and school dormitories for staphylococci. In areas where BZK antiseptics using different products with BZK concentrations ranging from 0.02%-0.12% were used, they found that 23.51% of the isolates were resistant to BZK.[11] These are not surprising findings. Sublethal concentrations of the disinfectant would allow the already tolerant subpopulation to thrive and then predominate. Lethal concentrations would kill effectively and leave the surviving fraction of the population only in low numbers.

The frequent use of ABHS can result in skin dryness and irritation, an irritant contact dermatitis. The addition of humectants and emollients to the ABHS products can help protect against this but even with these protections the use of ABHS can cause skin burning if

there is skin cracking or irritation already present on the user's hands. Both ABHS use and frequent handwashing with detergent/soap and water can cause skin cracking and irritation because of those agents' ability to denature skin proteins and to remove natural lipids on the skin that normally act to protect the skin. The effectiveness of the lipid dissolving property of alcohols is directly related to the alcohol concentration of the ABHS product.[12]

The BZK product used in this study is a new consumer product using a patent-pending formulation of BZK and inactive ingredients. The product is nonirritating, nonflammable, nonsticky, odorless, and is dispensed as a dose of 0.75 mL liquid that is converted to foam as it is dispensed. The manufacturing of BZK has changed over the years with improvements in ingredient purity. The sanitizer used in this study uses that improved purity ingredient. Previous studies have shown that HCWs using hand sanitizers prefer "fast absorption, soft/moisturized hand feel, not sticky, clean feel, and low smell" and that foam products are the preferred vehicle for delivery of the antimicrobial agent.[13]

The concentration of BZK found in the test product (0.12 %) makes it relatively nontoxic. The test product is also nondamaging to surfaces. According to the National Institute for Occupational Safety and Health, the lowest published oral toxic dose of BZK for a human is 266 mg/Kg.[14] For a 10 Kg child to ingest this amount would require drinking about 2.25 L of the test product. In contrast, alcohol hand sanitizers may be quite toxic to children in very small amounts. For the first 4 months of 2019 there were 5,829 exposure cases regarding hand sanitizers in children 12 years and younger managed by American poison control centers. Tiny amounts of alcohol hand sanitizer, such as licking a hand immediately after application of the sanitizer, would be unlikely to cause any illness but a child ingesting any amount more than just a taste would be at risk for alcohol poisoning. Alcohol poisoning may cause confusion, vomiting, drowsiness, respiratory depression, and in severe cases death. As little as 30 mL may be fatal in a small child.[15,16]

With the awareness of CDC concern about *S aureus* nosocomial infection rates at a plateau, and the problem of low compliance with hand hygiene protocols, we wanted to evaluate if replacing an ABHS with the test product would affect transient hand contamination with *S aureus*. Our results showed a mean colony count for *S aureus* of 10.92 during the medicated soap/alcohol use week. This count is consistent with the number of *S aureus* colony forming units found on the hands of HCWs in a previous study by Pittet et al,[17] therefore, we believed that our test subject population was representative. Because the BZK test product has a known persistence on human skin for up to 4 hours,[1] we theorized that there would be a decrease in the colony count on the afternoon plate from the morning plate as HCWs used BZK throughout the day. We also theorized that there would be a smaller decrease in the colony count in the ethanol week afternoon plate because of the known lack of persistent antimicrobial activity of ethanol. Neither theorized outcome was shown by the data in this study.

During the ethanol use week the morning colony count and the afternoon colony count showed no significant difference, and the BZK week showed the same result. We found the total colony count in the BZK week was significantly lower than the total colony count in the ethanol week. This may reflect the persistence of BZK on the skin for a longer time than has been previously documented. Ethanol sanitizer has an immediate kill effect on bacteria but then has no persistence. Repeated use of the ethanol sanitizer would kill bacteria present on the skin but would not prevent new bacteria from lodging on the skin surface when the test subject touched a contaminated object or person. The BZK test product is known to be effective at killing *S aureus* and maintains this killing effect for at least 4 hours, but the time the killing effect begins to wane is unknown. Another possibility could be that the "user friendly" BZK test product could have encouraged better hand hygiene compliance, and thus more killing of transient bacteria. Having fewer pathogenic transient bacteria on the hands of HCWs would provide less opportunity for the development of HAI.

Because of the many positive attributes of ABHS it is not expected that ABHS will be replaced anytime in the foreseeable future in the hand hygiene protocol recommended by the CDC. However, the negative dermatological and esthetic attributes of ABHS may be a significant factor contributing to low compliance with the CDC recommended hand hygiene protocol. Another study is planned in which the test product will be added as a "supplement" to the CDC recommended alcohol hand sanitizer plus medicated soap protocol. This planned study will add the BZK test product to the facility area where hand soap dispensers are located with recommendations for the user to apply the test product after drying the hands. This will be done in an inpatient facility to see if such use of the BZK test product can result in persistent decreased *S aureus* population on the hands of HCWs in a 2-week study, and in decreased nosocomial infection rates in a longer term study.

Limitations

The limitations to our study were that the study population was small at 40 test subjects, there was no attempt to observe or document compliance with hand hygiene protocols, there was a predominance of female test subjects, the majority of test subjects were working in outpatient facilities only, and the study was limited to evaluation of only 1 pathogenic bacteria species.

CONCLUSIONS

Use of a new "user friendly" formulation of BZK hand sanitizer that also demonstrated persistence of the BZK on the skin reduced fingertip contamination by *S aureus* in HCWs significantly as measured by colony counts. Despite the limitations of the study, the results are promising and demonstrate significant reductions in *S aureus* hand contamination can be achieved relative to alcohol. Our study findings warrant consideration in modifying hand hygiene protocols to address the problem of nosocomial infections from *S aureus*.

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SAFETY DATA SHEET

SAFETY DATA SHEET

1. IDENTIFICATION

Product identifier

Product Name **Dab Hand Sanitizer**

Other means of identification

Synonyms **Hand Sanitizer, Soap**

Recommended use of the chemical and restrictions on use

Recommended use **Hand Clensing**

Distributor Address
Three Kings Corp.
3006 Hwy 72 West
Corith, MS 38834

Emergency telephone number

Emergency Phone Numbers For Transportation Emergencies,
call Chemtrec: 1-800-424-9300

2. HAZARDS IDENTIFICATION

Classification

This product is classified under 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200) as follows: Normal usage should not create hazardous conditions.

Appearance	**Aqueous solution**
Physical state	**Liquid**
Odor	**Mild Odor**

Precautionary Statements--Prevention	**Wash hand thoroughly after handling**
Precautionary Statements—Response	**IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.**
	IF SWALLOWED: Drink 1 or 2 glasses of water.
Precautionary Statements—Storage	**Store in dry area at normal temperatures.**
Precautionary Statements—Disposal	**Dispose to an approved waste disposal plant.**
Hazards not otherwise classified (HNOC)	**Not applicable**

This is a personal care or cosmetic product that is safe for users under normal and reasonably foreseeable use. While this material is not considered hazardous, the SDS contains valuable information critical to the safe handling and proper use of the product for industrial workplace conditions as well as unusual and unintended exposures such as large spills. This SDS should be retained and available for employees and other users of this product. For specific intended-use guidance, please refer to the information provided on the package or instruction sheet.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Chemical Name	CAS No.	Weight %	Trade Secret
1-Propanaminium, 3-amino-N-(carboxymethyl)-N, N-dimethyl-, N-coco acyl derivs., inner salts	61789-40-0	1 - 5	*
Quaternary ammonium compounds, alkybenzyldimethyl chlorides	8001-54-5	0.1-1.30	*

* The exact percentage (concentration) of composition has been withheld as a trade secret.

4. FIRST AID MEASURES

First aid measures

General Advice Show this safety data sheet to the doctor in attendance.

Eye Contact If in eyes, rinse slowly and gently with water for 15–20 minutes. If present, remove contact lenses. Call a poison control center or doctor for further treatment advice.

Skin Contact Not applicable.

Inhalation Move to fresh air. If breathing problems develop, call a doctor.

Ingestion Do not induce vomiting. Drink 1 or 2 glasses of water. Call a doctor or poison control center.

Most important symptoms and effects, both acute and delayed

Most Important Symptoms and Effects None under normal use conditions.

Indication of any immediate medical attention and special treatment needed

Notes to Physician Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable Extinguishing Media
Dry chemical, carbon dioxide (CO_2), foam, or water spray.

Specific Hazards Arising from the Chemical

None known.

Protective equipment and precautions for firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand. Keep containers cool with water spray.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions, protective equipment and emergency procedures

Personal Precautions Use personal protective equipment as required. Do not get into eyes.

Methods and material for containment and cleaning up

Methods for Containment Prevent further leakage or spillage if safe to do so.

Methods for Cleaning Up Eliminate all potential sources of ignition, and ventilate area. Absorb and containerize. Do not flush into surface water or sanitary sewer system.

7. HANDLING AND STORAGE

Precautions for safe handling

Handling Keep container closed when not in use. Never return spills to the original container.

Conditions for safe storage, including any incompatibilities

Storage Store in accordance all applicable regulations. Keep containers tightly closed in a cool, dry, well-ventilated place.

Incompatible Products None known.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Control parameters

Exposure Guidelines

Chemical Name	OSHA PEL
Glycerin (56-81-5	TWA: 15 mg/m^3 mist, total particulate TWA; 5 mg/m^3 mist, respirable fraction (vacated) TWA: 10 mg/m^3 mist, total particulate (vacated) TWA; 5 mg/m^3 mist, respirable fraction

ACGIH TLV: American Conference of Governmental Industrial Hygienists - Threshold Limit Value. OSHA PEL: Occupational Safety and Health Administration - Permissible Exposure Limits. NIOSH IDLH: Immediately Dangerous to Life or Health.

Appropriate engineering controls

Engineering Measures

Ensure adequate Ventilation systems

Individual protection measures, such as personal protective equipment

Eye/Face Protection	None required for consumer use. If splashes are likely to occur, wear safety glasses.
Skin and Body Protection	No special protective equipment required.
Respiratory Protection	No protective equipment is needed under normal use conditions. If exposure limits are exceeded or irritation is experienced, NIOSH/MSHA approved respiratory protection should be worn. Respiratory protection must be provided in accordance with current local regulations.
Hygiene Measures	Handle in accordance with good industrial hygiene and safety practice.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical and Chemical Properties

Physical State	Thin liquid		
Appearance	Clear	**Odor**	Alcohol
Color	colorless	**Odor Threshold**	No information available

Property	**Values**	**Remarks/ Method**
pH	5 - 7	None known
Melting/freezing point	No data available	None known
Boiling point / boiling range	No data available	None known
Flash Point	**> 93.3**°C (closed cup)	None known
Evaporation rate	No data available	None known
Flammability (solid, gas)	No data available	None known
Flammability Limits in Air		
Upper flammability limit	No data available	None known
Lower flammability limit	No data available	None known
Vapor pressure	No data available	None known
Vapor density	No data available	None known
Specific Gravity	No data available	None known
Water Solubility	Complete	None known
Solubility in other solvents	No data available	None known
Partition coefficient: n-octanol/water	No data available	None known
Autoignition temperature	No data available	None known
Decomposition temperature	No data available	None known
Kinematic viscosity	No data available	None known
Dynamic viscosity	No data available	None known

10. STABILITY AND REACTIVITY

Reactivity
Stable.

Chemical stability
Stable under recommended storage conditions.

Possibility of Hazardous Reactions
None known.

Conditions to avoid
None under normal processing.

Incompatible materials
None known.

Hazardous Decomposition Products
None known.

11. TOXICOLOGICAL INFORMATION

Information on likely routes of exposure

Product Information

Inhalation	Inhalation of high concentrations of vapor or mist may cause dizziness.
Eye Contact	May cause slight irritation.
Skin Contact	No known significant effects or critical hazards.
Ingestion	No known significant effects or critical hazards.

Component Information

Chemical Name	Inhalation LC50
Glycerin 56-81-5	>2.75 mg/L (Rat)

Information on toxicological effects

No known effect.

12. ECOLOGICAL INFORMATION

Ecotoxicity

The product is not expected to be hazardous to the environment.

Persistence and Degradability
No information available.

Bioaccumulation

Other adverse effects
No information available.

13. DISPOSAL CONSIDERATIONS

Disposal methods
Dispose of in accordance with all applicable federal, state, and local regulations.

Contaminated Packaging
Do not reuse empty containers. Dispose of in accordance with all applicable federal, state, and local regulations.

14. TRANSPORT INFORMATION

DOT

Not Regulated

15. REGULATORY INFORMATION

Chemical Inventories

TSCA	All components of this product are either on the TSCA 8(b) Inventory or otherwise exempt from listing.
DSL/NDSL	All components are on the DSL or NDSL.

TSCA - United States Toxic Substances Control Act Section 8(b) Inventory
DSL/NDSL - Canadian Domestic Substances List/Non-Domestic Substances List

U.S. Federal Regulations

SARA 313
Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product does not contain any chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372.

CWA (Clean Water Act)
This product does not contain any substances regulated as pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42).

CERCLA
This material, as supplied, does not contain any substances regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302) or the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 355). There may be specific reporting requirements at the local, regional, or state level pertaining to releases of this material.

US State Regulations

California Proposition 65
This product does not contain any Proposition 65 chemicals.

U.S. State Right-to-Know Regulations

Chemical Name	New Jersey	Massachusetts	Pennsylvania
Glycerin 56-81-5	X	X	X

16. OTHER INFORMATION

NFPA	**Health Hazard** 1	**Flammability** 1	**Instability** 0	**Physical and Chemical Hazards** -
HMIS	**Health Hazard 1**	**Flammability 1**	**Physical Hazard** 0	**Personal Protection** -

Prepared By Technical Department

Preparation/Revision Date August, 2018

Version 0

General Disclaimer

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information, and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal, and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

End of Safety Data Sheet

CONTACT

ANNETTE OUTLAN

OFFICE: 888-872-2350

CELL: 901-581-8129

annette@dabprotects.com

DABPROTECTS.COM

DEFENSE AGAINST BACTERIA
Hand Sanitizer + Protectant

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