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**PERFORMANCE OF A NOVEL BENZALKONIUM CHLORIDE HAND
CLEANSER FORMULA FOR PERIOPERATIVE AND GENERAL USE**

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BACKGROUND: Nosocomial infection mortality increased more than fivefold (19,000-150,000 deaths) from 1992 to 1996, and continues to rise. Centers for Disease Control officials estimate that one-third of nosocomial infections are caused by poor adherence to infection control practices, like handwashing. Contributing factors include: 1) high-volume patient contacts; 2) skin dryness and micro-abrasions from repeated handwashing necessary with large patient loads; and 3) short recovery time from washing between patients to re-establish the hands' natural protective factors. A cleanser formulation of surfactants, allantoin and benzalkonium chloride (SAB) was developed to address the need for a broad-spectrum antimicrobial healthcare personnel handwash that is well tolerated with high-frequency use, and demonstrates persistence of antimicrobial activity with repeated use.

METHODS: SAB cleanser performance was assessed examining antimicrobial spectrum via in-vitro time kill against yeast, Gram-negative and Gram-positive bacteria, and glove juice assays of *S. marcescens* bacterial load reduction, following methods published in "21CFR/333: Health Care Antiseptic Drug Products; Proposed Rule/470: Effectiveness testing of an antiseptic handwash."

RESULTS: The SAB cleanser killed 99.99%-99.999% of bacterial and fungal species in 30 seconds, including: *E. fecalis* (VRE), *S. aureus* (MRSA), *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. enteritidis*, *S. marcescens*, *S. saprophyticus*, *S. typhimurium* and *C. albicans*. Bacterial load reduction results with *S. marcescens* showed that the SAB cleanser exceeded FDA performance standards by 75% and 167% after the first and 10th handwashes, respectively. In contrast, a branded 4% chlorhexidine gluconate (CHG) healthcare personnel handwash did not. Evaluation of subject's hands revealed that frequent use of the SAB cleanser did not irritate the skin.

CONCLUSIONS: The SAB cleanser outperformed the 4% CHG cleanser by means of persistence of activity with repeated use. Moreover, frequent use of the SAB cleanser did not irritate the skin.

ANTIMICROBIAL PERFORMANCE OF A NOVEL SURFACTANT FOR USE IN PERIOPERATIVE AND GENERAL

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PURPOSE:

Nosocomial infection mortality increased over five fold (19000-150,000 deaths) between 1992 and 1996, and continues to increase. CDC officials estimate that one third of all nosocomial infections are caused by poor adherence to established infection control practices, such as handwashing. Although there are many contributing factors to this problem, primary causes include: 1) high volume patient contacts; 2) skin dryness and microabrasions from the repeated handwashing that is necessary with large numbers of patients and 3) short recovery time from washing between patients to re-establish the hands' natural protective factors. Many soap formulations used in patient care settings either possess a limited antimicrobial spectrum, do not meet FDA performance standards for healthcare personnel handwashes, or contain active ingredients that irritate and sensitize the skin at high concentrations and high-frequency use. A cleanser formulation containing the skin emollient allantoin and benzalkonium chloride (SAB cleanser) was developed to address the need for a broad-spectrum antimicrobial healthcare personnel handwash that is well tolerated with high frequency use.

METHODS:

SAB cleanser performance assessment included examining antimicrobial spectrum via *in vitro* time kill against yeast, Gram-negative and Gram-positive bacteria, and glove juice assays of *Serratia marcescens* bacterial load reduction (10 contamination/10 handwash glove juice protocol). This study followed methods published in 21CFR333: Health care antiseptic drug products proposed rule. §470: Effectiveness testing of healthcare antiseptics. (after 21 CFR Parts 333.470 and 1994 Fed Reg 59: 31448 to 31450)

Inoculum Preparation:

The microorganisms used in the study were obtained from the American Type Culture Collection, Rockville, Maryland (see table for name and ATCC identification number). The stock was prepared by inoculating Tryptic Soy Broth (TSB) with the organism and plating a small amount on TSA plate. Appropriate growth media were used for fungal cultures. After an incubation period of 24 hours at 37°C, the plate was stored at 4°C until two days before the study. Following this approximately 5 colonies (or a 1 cm streak of the bacterial lawn, if applicable) was inoculated into 5 ml of TSB and allowed to grow at 37°C for 24 hours. The bacterial suspension was then passaged and incubated an additional 24 hours before use in the study. On the day of the study, the bacterial suspension was diluted to a McFarland 0.5 density in Mueller Hinton Broth. This solution was then used as the inoculum in both the MIC and Time Kill assays.

Minimum inhibitory concentration assay:

Fifty microliter aliquots of the inoculum (1/25 dilution of the MF 0.5 density bacterial suspension) were exposed to 200 ul aliquots of the product (neat and diluted samples) at t= 0 seconds.

After a 30 second exposure period, a 100 ul aliquot of each sample was removed and neutralized in 1 ml of Lethen broth (LB). A positive bacterial control was obtained by directly dispensing 50 ul of the inoculum into 200 ul of LB. A negative bacterial control was also included in the assay to confirm the sterility of the LB medium. Following a 24 hour incubation period, the antimicrobial properties of the product were evaluated and compared at the specified concentrations. Note that each dilution factor was assayed in quadruplicate

Minimum bactericidal concentration assay

Following the MIC determination, the two lowest concentrations that visually showed no bacterial growth were used as the test samples in the MBC assay. A small portion (approximately 10 ul) of these solutions was obtained and plated out on TSA plates with a sterile loop. These plates were then incubated overnight at 37°C overnight and read thereafter. The dilution that showed no growth on the plates was taken to be the MBC.

Time kill protocol:

At time t= 0 seconds, a 100 ul aliquot of the inoculum (1/10 dilution of the MF 0.5 density bacterial suspension) was dispensed into an eppendorf containing 1 ml of the undiluted test substance. The mixture was vortexed for 7 to 10 seconds and held for the remainder of the specified exposure times (if applicable) at room temperature. The exposure times assayed were 30, 60 and 300 seconds. Immediately following each exposure period, 100 ul was removed and added to 1 ml of LB to stop the action of the test substance. A small amount (50 ul) of these solutions was then plated out on TSA plates. A negative sample control was obtained by performing the above steps except with the substitution of the test substance with Phosphate Buffered Saline (PBS). At the end of the assay, the plates were incubated overnight at 37°C to examine the antimicrobial activity of the test substance.

10-handwash glove juice protocol:

Serratia marcescens ATCC no. 14756 was obtained from American Type Culture Collections, Rockville MD. Butterfield's phosphate buffer (PBS) adjusted to pH 7.2 containing 0.5% lecithin and 4% polysorbate 20 served as both sampling and diluant solution. On the day prior to the test, three colonies of *S. marcescens* from a Trypticase Soy Agar (TSA) plate was inoculated into five milliliters of Trypticase Soy Broth (TSB) in a sterile culture bottle and incubated with agitation for 5 - 7 hours at room temperature (25°C). The starter culture was diluted to a standard concentration (approximately 10⁸ colony forming units (cfu) per milliliter). One (1) milliliter of this standardized, active culture was pipetted into 250 milliliters of sterile TSB and agitated overnight. On the day of the test, the *S. marcescens* /TSB starter culture was diluted (1:8) in TSB and mixed at room temperature (25°C). The suspension was shaken before aliquot were dispensed. The resulting inoculum density was approximately 10⁸ cfu/ml.

Subject preparation:

The participants refrained from the use of products for a period of seven days (i.e. antiperspirants, deodorants, shampoos, as materials such as acids bases and sol prior to the initiation of the test. Subject non-antimicrobial compositions for use period. Participants were asked to refrain from nail polish during the test period. Subject with false, or excessively long nails (> 0.5 cm) were excluded from the study.

Procedures:

Following the seven day preparation period, subjects performed a thirty second handwash with non-antimicrobial control soap to remove any residual soap. Subjects were then familiarized with the test substance. Subjects removed all jewelry and adornments from their hands and trimmed nails to 1-2 mm if necessary. Subjects were then given a baseline skin hydration measurement and test washes as follows: Five milliliters of marker organism were dispensed into a 100 ml beaker and rubbed over surfaces of both hands, not for forty-five seconds. Hands were allowed to dry for five minutes. Five milliliters of test solution were dispensed into a 100 ml beaker and rubbed over all surfaces, with solution loss. Bacteria remaining on hands was removed by bathing hands in poly-ethylene gloves containing fluid with neutralizers for 60 seconds. Bacteria was then removed by surface inoculation of TSA plates. Control plates were incubated overnight at room temperature before use.

Impedance-based Skin Capacity Measurements:

The Nova DPM 9003 was used to assess skin hydration following exposure to test formulations. Baseline readings were taken before exposure to test formulations. Following exposure, the right forearm of each volunteer was sanitized, or the 4% CHG formulation was applied and lightly blotted afterward, and left to air dry. The left forearm served as a control, and was left to air dry. Readings were repeated 30, 90, and 180 minutes after exposure. Volunteers were asked to refrain from handwashing during the experiment.

Results:

The SAB sanitizer killed 99.99-99.9999% of test organisms in 30 seconds, including: *E. fecalis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. saprophyticus*, and *S. typhimurium*, and *S. marcescens* (see Table 1).

Bacterial load reduction results with *S. marcescens*

ANT ALLANTOIN AND BENZALKONIUM CHLORIDE CLEANSER GENERAL - USE HAND SANITIZATION SETTINGS

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...m the use of all topical anti-microbial
...1 days (including antimicrobial
...shampoos, lotions, and soaps) as well
...and solvents and chlorinated pools
...st. Subjects were provided alternative
...ns for use during the preparation
...ed to refrain from the use of nail
... Subject exclusion criteria included
... (> 0.5 centimeters) and open cuts or
...ms.

...eration period, upon arrival for testing
...thirty second "practice" wash with
...p to remove oil and dirt and to
...h the washing technique. Subjects
...ments from hands and wrists and
...cessary. Subjects then performed the
...llows: Five milliliters of inoculum
...dispensed on subjects hands, and
...hands, not reaching above the wrists,
...were allowed to air-dry for two
...it solution were applied to subject's
...faces, with care taken to minimize
...ing on hands were recovered by
...e gloves containing PBS sampling
...seconds. Bacterial count was assessed
...plates. Colonies were grown
...e before counting.

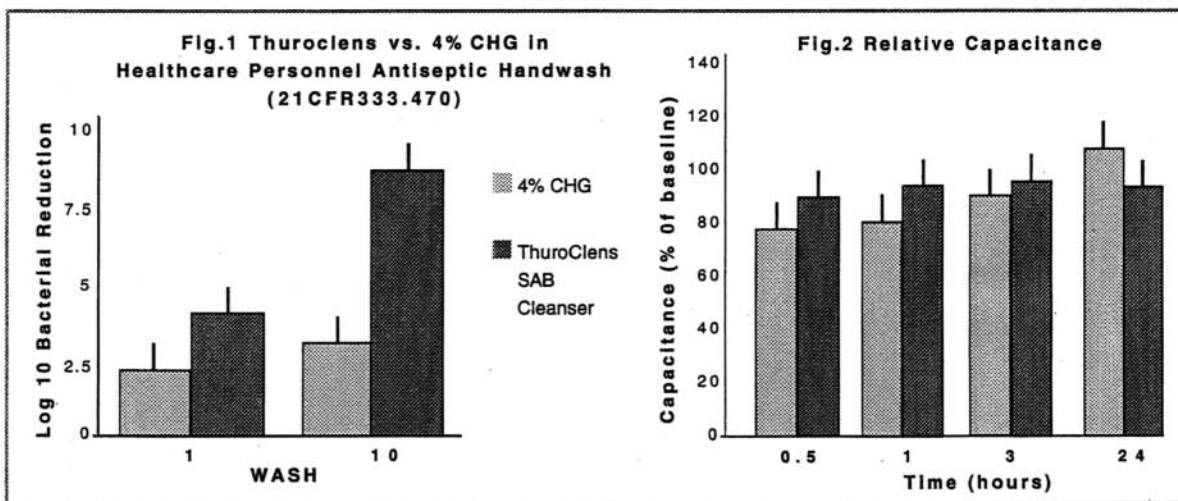
Capacitance

...l to assess skin capacitance as an
...lowing exposure to the test
...gs were made on five subjects prior to
...Following baseline measurements,
...nteer was bathed in either the SAB
...ulation for 10 minutes. Skin was
...left to air dry for 10 minutes. The left
...d was left untreated. Measurements
...minutes and at 24 hours afterward.
...in from washing the test sites during

...99.999% of bacterial and fungal
...g: *E. faecalis* (VRE), *S. aureus* (MRSA),
...*inosa*, *S. enteritidis*, *S. marcescens*, *S.*
...*ium*, and the yeast *C. albicans*

...with *S. marcescens* showed that the

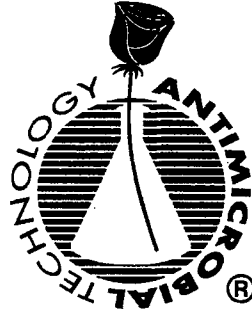
Organism	MIC		MBC		Log Reduction (30 sec.)
	parts per million	dilution factor (2 ^{Δn})	parts per million	dilution factor (2 ^{Δn})	
<i>C. albicans</i> (10231)	4	10	8	9	4.99
<i>E. coli</i> (11229)	125	5	125	5	6.68
<i>E. coli</i> (25922)	250	4	500	3	6.72
<i>E. faecalis</i> (49452)	8	9	8	9	6.53
<i>E. faecalis</i> (51575) VRE	16	8	16	8	6.09
<i>K. pneumonia</i> (9997)	31	7	31	7	6.11
<i>P. aeruginosa</i> (10145)	16	8	16	8	6.64
<i>P. aeruginosa</i> (15442)	125	5	250	4	5.97
<i>P. aeruginosa</i> (27853)	62	6	62	6	5.40
<i>P. aeruginosa</i> (9027)	31	7	62	6	6.18
<i>P. mirabilis</i> (12453)	125	5	125	5	6.42
<i>S. aureus</i> (29213)	31	7	31	7	6.34
<i>S. aureus</i> (33591) MRSA	31	7	62	6	6.19
<i>S. aureus</i> (6538)	16	8	16	8	6.24
<i>S. enteritidis</i> (13076)	16	8	31	7	6.53
<i>S. epidermidis</i> (12228)	16	8	16	8	5.40
<i>S. haemolyticus</i> (29970)	4	10	8	9	5.79
<i>S. marcescens</i> (14756)	125	5	125	5	5.39
<i>S. saprophyticus</i> (15305)	2	11	4	10	5.98
<i>S. typhimurium</i> (19585)	250	4	250	4	6.00



SAB cleanser exceeded FDA performance standards by 75% and 167% after the first and tenth handwashes, respectively. In contrast, a branded 4% chlorhexidine gluconate healthcare personnel handwash did not meet FDA standards (0.7% and 12.7% lower than required for the first and tenth handwashes respectively) (see Figure 1). Evaluation of subjects' hands revealed that frequent use of the SAB sanitizer did not irritate the skin. The SAB sanitizer was also assessed for tendency to dry skin with extended contact. Measurements of impedance-based skin capacitance indicated that volar forearm skin treated with the SAB sanitizer was not significantly different from skin left untreated, or treated with 4% CHG solution (Figure 1) at any of the time points tested.

CONCLUSIONS AND IMPLICATIONS:

Broad-spectrum fast acting microbicidal activity is necessary in healthcare settings. In addition, increased routine handwashing compliance is predicted by the compatibility of the cleansing formulation with the skin. The results indicate that the SAB cleanser is a fast acting broad-spectrum antiseptic handwash that exceeds Federal performance standards, but does not irritate or dry the skin. The SAB cleanser is therefore suitable for frequent use by healthcare personnel.



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