April 16, 2016

Testing of Study #160296-311, entitled, **"PILOT STUDY FOR NON-GLP CLINICAL EVALUTATION TO COMPARE PRUKLENZ AND PURSAN VERSUS THREE TOPICAL SKIN PREPARATIONS AND WATER (SALINE)",** has been completed. GIRB approval was given on *03115/16,* with recruitment of study subjects beginning on 03115/16 and concluding on 03/16/16. Eight subjects were consented, ages 18-65. Five subjects completed testing. Three subjects were dismissed from testing with Study Full status. There were no amendments made to the Work Instructions and no Adverse Events occurred during the course of the study.

The purpose of this pilot NON-GLP study was to compare the efficacy of the six test materials, specifically the efficacy of Test Product #1 and #2 (PurKlenz and PurSan, respectively),when compared to the remaining products. Testing was performed on both volar aspects of each subject's forearms. Each arm had 4 test sites, 1.5 inch by 1.5 inch, for a total of eight test sites per subject. Each subject had two baseline samples (one from each volar forearm) collected from Sitel (left) and Site 5 (right). The remaining six test sites were randomized for product application. Also randomized was the product exposure time per subject; three subjects having samples collected at least one minute post product application and two subjects having samples collected at least twenty minutes post product application. Each subject admitted into testing had every test material applied. All products were applied per study Work Instructions. The sterile water inadvertently had two instructions for application that were very similar; the sterile water was applied by thoroughly wetting a sterile gauze pad. The sterile water impregnated gauze was scrubbed on the forearm for 30 seconds± 5 seconds. A second sterile gauze was used to wipe the area dry.

The test materials were as follows:

Test Material	Descriotion	Lot Number	Exoiration Date
Test Product# 1	PurKlenz; 3% Chloroxylenol; tall clear plastic bottle with white cap containing clear thick liquid; Manufacture Date: April 2014	4D1	April 2016
Test Product #2	PurSan; 0.45% Chloroxylenol; small clear plastic bottle with white flip-cap containing thick white substance; Manufacture Date: Julv 2015	4M1	December 2017
Test Product #3	Microsan; 1.75% Chloroxylenol; white plastic bottle with pump containing fluid; Manufacture Date: *NIP*	141204	December 31, 2017
Test Product #4	Aplicare Swabsticks; 10% Povidone-lodine, 1% Iodine; white box with black and orange print; Manufacture Date: *NIP*	57430	June 30, 2016
Test Product #5	Alcohol Pads; Isopropyl Alcohol, 70% vlv; small blue box containing pads; Manufacture Date: *NIP*	67015080020	***NIP***
Test Product #6	Sterile Water; Isotonic solution, 0.9% Sodium Chloride; small clear plastic bottle containing clear liquid; Manufacture Date: ***NI***A	Gl 16251	January 31, 2018

All sampling was performed using the Cup Scrub Technique, at either at least 1 minute post product application or at least 20 minutes post product application. Samples were plated on TSA+, and counted. Bacterial counts were converted into colony-forming units per square centimeter (CFU/cm^2) and the logia of that value used. Reductions from baseline were calculated for each product, and the data used for the statistical analysis.

Statistical Results:

A two-factor ANOVA was performed comparing the logl0 Reduction from Baseline to the time of the sample and the product used for each sample. The results are presented in Table I.

A Tukey Pairwise Differences Test was performed on the sampling times confirm if there was a difference in the results based on the times the samples were taken – either 1 minute after product application, or 20 minutes after product application.

A Dunnett's Multiple Comparison Test was performed for both Test Product #1 (PurKlenz) and Test Product #2 (PurSan) results against the other four test materials.

Descriptive Statistics were performed.

Table 1. General Linear Model

Factor	Type	Levels	Values
Time	Fixed	2	1, 2
Product	Fixed	6	1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value[1]	*P*-Value[2]	Significance[3]
Time	1	0.7069	0.4069	2.99	0.101	Not Significant
Product	5	6.5324	1.3065	5.52	0.003	Significant
Time * Product	5	1.1122	0.2224	0.94	0.479	Not Significant
Error	18	4.2605	0.2367			
Total	29	12.9025				

$s = 0.486511$

[1] $F = \frac{Adjusted\ Mean\ Square\ Source}{Adjusted\ Mean\ Square\ Error}$; F is the adjusted mean square values divided by adjusted mean square error. The MS_E is s^2, which is 0.2367, and the standard deviation was $\sqrt{s^2} = s = 0.4865$.

[2] The P or P-value is $P(F \geq x^* | H_0\ true) < \alpha$. The level of significance is $\alpha = 0.05$. * $x = F$ value calculated.

[3] Significant/Not Significant at $\alpha = 0.05$. If $p < 0.05$, the test is significant. If $p \geq 0.05$, it is not significant.

The time the samples were taken was not significant. The ANOVA showed a statistically significant difference in product. A test for Equal Variances was performed. Table 2 presents the Test for Equal Variances.

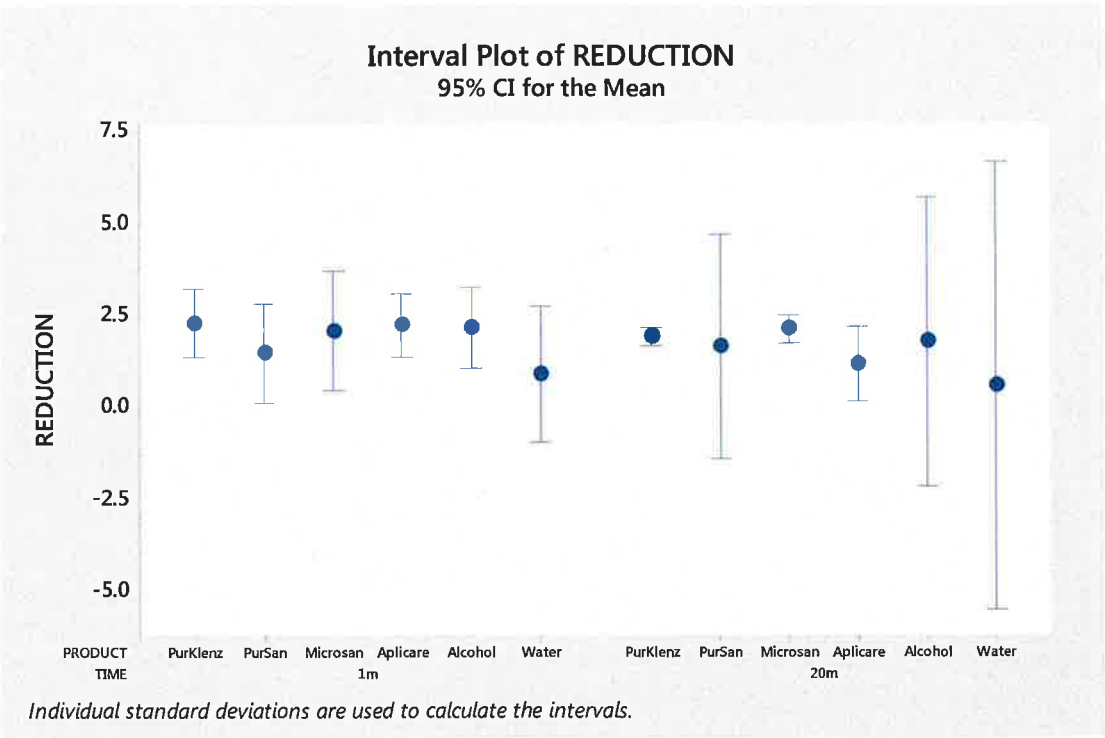
Table 2. Test for Equal Variances
(95% Bonferroni confidence intervals for standard deviations)

Time	Product	*N*	StDev	CI
1 Minute	PurKlenz	3	0.380044	(0.0000000, 1.78114E+12)
	PurSan	3	0.540648	(0.0000000, 2.53383E+12)
	Microsan	3	0.656074	(0.0000000, 3.07480E+12)
	Aplicare	3	0.347898	(0.0000000, 1.63048E+12)
	Alcohol	3	0.439356	(0.0000000, 2.05911E+12)
	Water	3	0.746458	(0.0000000, 3.49840E+12)
20 Minutes	PurKlenz	2	0.028284	(*, *)
	PurSan	2	0.339411	(*, *)
	Microsan	2	0.042426	(*, *)
	Aplicare	2	0.113137	(*, *)
	Alcohol	2	0.438406	(*, *)
	Water	2	0.678823	(*, *)

Note – No confidence intervals are noted for the 20 minute sample as there were only two samples.

Figure 1 presents the 95% Confidence Interval of the Log_{10} Reductions from Baseline of each Test Product at each time point.

Figure 1. Interval Plot



All reductions from all products at both times were equivalent, because all confidence intervals overlapped. The large confidence intervals were because of the small number of replicate samples taken. The dot above indicates the average for each product. You can see that water alone had the smallest reduction in bacteria, at both time points.

A Tukey Test was applied to the data to compare the times. It was only used for the times. The products were all embedded in the model. Table 3 evaluates the times individually, to see if the times are significantly different. Table 4 evaluated the difference of the means.

Table 3. Tukey Pairwise Comparisons: Response = Reduction, Term = Time
Grouping Information Using the Tukey Method And 95% Confidence

Time	*N*	Mean	Grouping
1 Minute	18	1.86667	A
20 Minutes	12	1.55333	A

Note - Means That Do Not Share A Letter Are Significantly Different.

The average of all samples taken at each time point, regardless of product, were not statistically significant.

Table 4. Tukey Simultaneous Tests for Differences of Means

Difference of Inoculum Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	*T*-Value	*P*-Value	Significance
20 Minutes – 1 Minute	-0.313	0.181	(-0.694, 0.068)	-1.73	0.101	Not Significant

Table 4 shows the difference of the means was not significantly different, meaning the two times were not different at $\alpha = 0.05$.

Two Dunnett's Tests were performed; the first to compare PurKlenz to the other products, and the second, to compare PurSan to the other products.

The first Dunnett's Test was applied to the data to compare all test products to PurKlenz with the two times embedded in the model. The test hypotheses are:

H_0: PurKlenz = Other Products OR H_A: PurKlenz ≠ Other Products

Table 5 presents the Log_{10} Reductions from Baseline results of the Dunnett's Test for PurKlenz.

Table 5. Dunnett Multiple Comparisons with a Control:
Response = Reduction, Term = Product
Grouping Information Using the Dunnett Method and 95% Confidence

Product	*N*	Mean	Grouping
PurKlenz (control)	5	2.11667	A
Microsan	5	2.11833	A
Alcohol	5	1.99167	A
Aplicare	5	1.71333	A
PurSan	5	1.57500	A
Water	5	0.74500	

The Log_{10} Reductions from Baseline for the first five products (PurKlenz, Microsan, Alcohol, Aplicare and PurSan) were not statistically different.

Table 6 presents the Differences in Reductions from PurKlenz against the remaining test materials.

Table 6. Dunnett simultaneous Tests for Level Mean – Control Mean

Product	Difference of Means	SE of Difference	Simultaneous 95% CI	*T*-Value	Adjusted *P*-Value	Significance
PurSan – PurKlenz	-0.542	0.314	(-1.409, 0.326)	-1.72	0.323	Not Significant
Microsan – PurKlenz	0.002	0.314	(-0.866, 0.869)	0.01	1.000	Not Significant
Aplicare – PurKlenz	-0.403	0.314	(-1.271, 0.464)	-1.28	0.585	Not Significant
Alcohol – PurKlenz	-0.125	0.314	(-0.992, 0.742)	-0.40	0.993	Not Significant
Water – PurKlenz	-1.372	0.314	(-2.239, -0.504)	-4.37	0.002	Significant

Table 6 confirms that the PurKlenz product was statistically different from sterile water only.

The second Dunnett's Test compared PurSan to the other products with the two times embedded in the model. Table 7 presents the Log_{10} Reduction from baseline results of the Dunnett's Test for PurSan.

Table 7. Dunnett Multiple Comparisons with a Control:
Response = Reduction, Term = Product
Grouping Information Using the Dunnett Method and 95% Confidence

Product	*N*	Mean	Grouping
PurSan (control)	5	1.57500	A
Microsan	5	2.11833	A
PurKlenz	5	2.11667	A
Alcohol	5	1.99167	A
Aplicare	5	1.71333	A
Water	5	0.74500	A

The Log_{10} Reductions from Baseline for all products when compared to PurSan were not statistically significant. This is because the PurSan did not kill as well as PurKlenz.

Table 8. Dunnett Simultaneous Tests for Level Mean – Control Mean

Product	Difference of Means	SE of Difference	Simultaneous 95% CI	*T*-Value	Adjusted *P*-Value	Significance
PurKlenz – PurSan	0.542	0.314	(-0.326, 1.409)	1.72	0.323	Not Significant
Microsan – PurSan	0.543	0.314	(-0.324, 1.411)	1.73	0.321	Not Significant
Aplicare – PurSan	0.138	0.314	(-0.729, 1.006)	0.44	0.989	Not Significant
Alcohol – PurSan	0.417	0.314	(-0.451, 1.284)	1.33	0.557	Not Significant
Water – PurSan	-0.830	0.314	(-1.697, 0.037)	-2.64	0.063	Not Significant

Table 8 confirms that no differences could be detected when comparing all test materials to PurSan.

Table 9 presents the sample size (*n*), means ($\bar{x}$), standard deviations, the minimum and maximum, Log_{10} Reductions from Baseline for all test materials at 1 minute.

Table 9. Descriptive Statistics at 1 minute

Results for Time = 1 (1 minute)

Variable	Product	*N*	*N**	Mean	StDev	Minimum	Maximum
Reduction	PurKlenz	3	0	2.303	0.380	1.920	2.680
	PurSan	3	0	1.480	0.541	1.060	2.090
	Microsan	3	0	2.097	0.656	1.420	2.730
	Aplicare	3	0	2.237	0.348	1.840	2.490
	Alcohol	3	0	2.183	0.439	1.680	2.490
	Water	3	0	0.900	0.746	0.080	1.540

Table 10 presents the sample size (*n*), means ($\bar{x}$), standard deviations, the minimum and maximum, of the Log_{10} Reductions from Baseline for all test materials at 20 minutes.

Table 10. Descriptive Statistics at 20 minutes

Results for Time = 2 (20 minute)

Variable	Product	*N*	*N**	Mean	StDev	Minimum	Maximum
Reduction	PurKlenz	2	0	1.9300	0.0283	1.9100	1.9500
	PurSan	2	0	1.670	0.339	1.430	1.910
	Microsan	2	0	2.1400	0.0424	2.1100	2.1700
	Aplicare	2	0	1.1900	0.1131	1.1100	1.2700
	Alcohol	2	0	1.800	0.438	1.490	2.110
	Water	2	0	0.590	0.679	0.110	1.070

Conclusion:

PurKlenz and Microsan were the most effective at removing bacterial from the forearm over both sample times. PurSan was slightly lower for the immediate sample but was higher at the 20 minute sample time. This is expected, as PurKlenz and Microsan have higher percentages of the same active ingredient as PurSan. Aplicare did very well at one minute but decreased in effectiveness at the 20 minute sample time. Alcohol was very effective at one minute but went down at 20 minutes. Water (which shows mechanical removal of bacterial only) was the least effective at each of the two sample times.

While this study was small in sample size, it did show that the two test materials PurKlenz and PurSans, when applied according to the directions given in the Work Instructions, are comparable to what is currently on the market.

Future pivotal studies should employ a larger sample size, be performed on an area of the body that can present higher baseline recoveries and subsequent reductions in bacteria than the forearm, and have surface area that would allow for additional samples at each site. The abdomen is suggested.

Please feel free to contact me if you have any questions regarding the data. I can be reached at abogert@biosciencelabs.com or telephone (406) 782-5498 ext. 203 or on my cell (970) 420-4933.

We appreciate your business and look forward to serving you in the near future with any of your testing needs. Please contact our Sales and Marketing Department at (877) 858-2754, toll-free, for assistance, or directly to John Dyba, (406)587-5735 ext. 119.

Sincerely,

Alicia G. Bogert
Principal Investigator

Duanna J. Field for Daryl S. Paulson
Daryl S. Paulson, Ph.D.
President & CEO

PurKlenz BioBurden Challenge Report

Date: June 2013
Lot: 2J1

Science Option Laboratories Inc. submitted a partially used bottle of PurKlenz to the Lab for a Bioburden (microbial count) evaluation using the same OTC Validation analysis and criteria required for all OTC Drugs during initial manufacture. The successful Bioburden results below confirm that PurKlenz remains free of microbiological organisms during repeated use in a bulk format.

Microbial Quality Assurance Form

Date: June 5, 2013

Customer: SOLABS

Product: Purklenz Antiseptic Skin Cleanser 44c-244-1

Size: 30 OZ/900mL Shade:

Batch Number: 7461158 Filled as Lot Number: 2J1

Microbiology Control Number: R5-23

Bulk Sample: ☐ Plac: ☐ Filled Sample: ☒

This product has been cultured (K.A.P. Number 15) and found to be free of:

Pseudomonas aeruginosa
Burkholderia cepacia
Escherichia coli
Enteric pathogens
Coagulase positive *Staphylococcus aureus*
β-haemolytic *Streptococcus* spp.
Pathogenic fungi
Candida albicans

The level of innocuous organisms is within the following acceptable ranges as per SOP M-004 *Product Limit Acceptability Guideline*:

		Dry Products	Liquid Products
Bacteria	☒	Max. 100 cfu per g	Max. 100 cfu per mL
Yeasts & Mold	☒	Max. 100 cfu per g	Max. 100 cfu per mL

The above tests have been performed on the product as indicated.
This does not guarantee against any contamination which may be incurred by your further handling of the product.

Microbiology Test Method Validation Report and Conclusions

Product Validated: Purlenz Antiseptic Skin Cleanser at 1:50 dilution

Product Validated: Purlenz Antiseptic Skin Cleanser at 1:10 dilution

1 SCOPE

This scope applies to the validation of microbiological method for testing non-sterile cosmetic products This validation will establish documented evidence, with a high level of assurance, that Microbial Limit Testing Method will consistently perform at specification stated in success criteria 4.0 in accordance with the guidelines of USP monographs Microbial Limit Testing USP <61> Microbial Examination of Non-sterile Products: Microbial Enumeration Tests and <62> Microbial Examination of Non-sterile Products: Tests for Specified Microorganisms.

2 OBJECTIVES

This validation report demonstrates the following principles:

- Antimicrobial activity inherent in the test product does not adversely affect the reliability of the test. That is, the test method procedure must demonstrate the ability of the media without product and media with product to support low numbers of typical USP specified microorganism at <100 cfu.
- The neutralization method employed is effective in inhibiting the antimicrobial properties of the product without impairing the recovery of viable microorganisms. Neutralization must be achieved through chemical neutralizers and dilution.
- The reliability of counting, that is the ability of the media and test product to support low numbers of typical USP specified microorganism at <100 cfu.

In summary the objectives are to:

1. Provide data that demonstrates that neutralizer enrichment method recovers <100 cfu/g from media without produc and from media with product.
2. Establish documented evidence that Total Plate Count procedure accurately detects levels of bacteria, yeast and mold in test samples at less than 100 cfu in comparison to the control pour plates. Acceptable is defined as being an agreement within 0.5 log.

The test sample uses a representative test formula which demonstrates the worst case scenario selected from a range of products with similar physical and chemical characteristics.

ACCEPTANCE CRITERIA and CONCLUSIONS FOR TESTED PRODUCT FORMULATION

This validation is in compliance with acceptance criteria and considered a pass for: PURLENZ ANTISEPTIC SKIN CLEANSER AT 1:10 DILUTION

This validation is in compliance with acceptance criteria and considered a pass for: PURLENZ ANTISEPTIC SKIN CLEANSER AT 1:50 DILUTION

This product has been validated for the following methods:

KAP15 (aerobic plate count), KAP15 (yeast/mold testing), KAP15 Enrichment testing, and KAP (Microbial Limits Testing)

TABLE 6: TMI603 *Diagnostic Enrichment Test Results for Control Samples*

Organism	Organism Controls:	Logbook Page Ref	Growth or No Growth (+/-)	Pass/Fail
S. aureus	Mannitol Salt Agar	16	+	PASS
	TSALT	16	+	PASS
P. aeruginosa	Pseudomonas Agar	16	+	PASS
	TSALT	16	+	PASS
	MacConkey Agar	16	+	PASS
E. coli	MacConkey Agar	16	+	PASS
	TSALT	16	+	PASS
B. cepacia	Pseudomonas Agar	16	+	PASS
	TSALT	16	+	PASS
	MacConkey Agar	16	+	PASS
C. albicans	BiGGy Agar	16	+	PASS
	SDA	16	+	PASS
	TSALT	16	+	PASS
A. niger	Not applicable			

TABLE 7: *Diagnostic Enrichment Test Results for Validation Samples*

The test method is considered acceptable if it is able to recover each specific organism inoculated into the enrichment portion of the test.

Organism	TMV	Logbook Page Ref	Growth or No Growth (+/-)	Pass/Fail
S. aureus	Mannitol Salt Agar	16	+	PASS
	TSALT	16	+	PASS
P. aeruginosa	Pseudomonas Agar	16	+	PASS
	TSALT	16	+	PASS
	MacConkey Agar	16	+	PASS
E. coli	MacConkey Agar	16	+	PASS
	TSALT	16	+	PASS
B. cepacia	Pseudomonas Agar	16	+	PASS
	TSALT	16	+	PASS
	MacConkey Agar	16	+	PASS
C. albicans	BiGGy Agar	16	+	PASS
	SDA	16	+	PASS
	TSALT	16	+	PASS