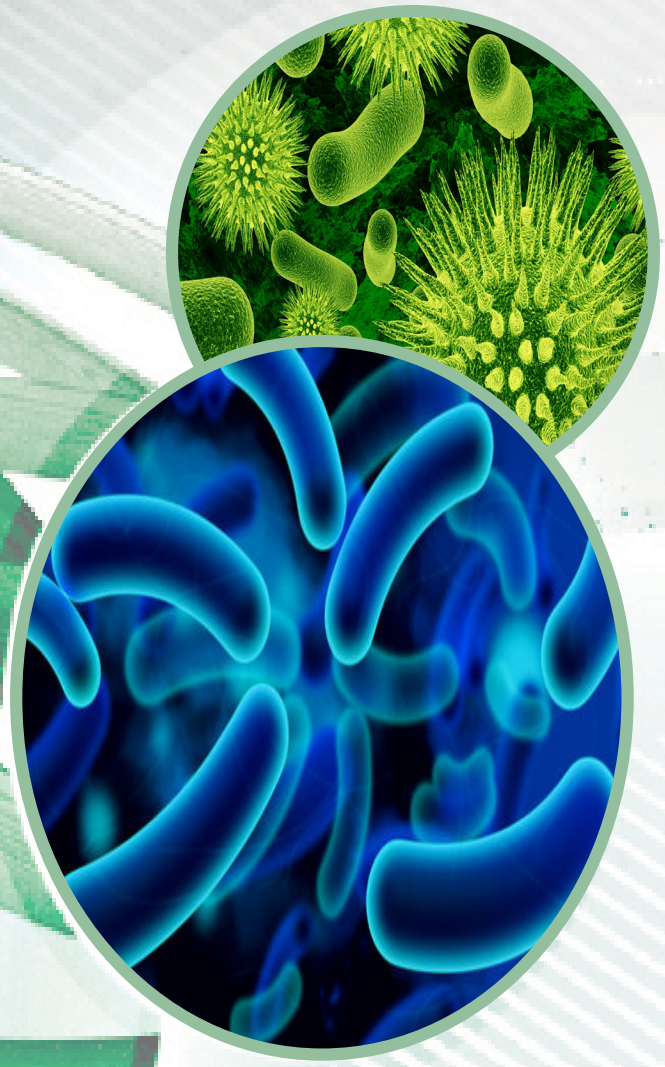
ACTIVE MICRO TECHNOLOGIES

Technical Dossier



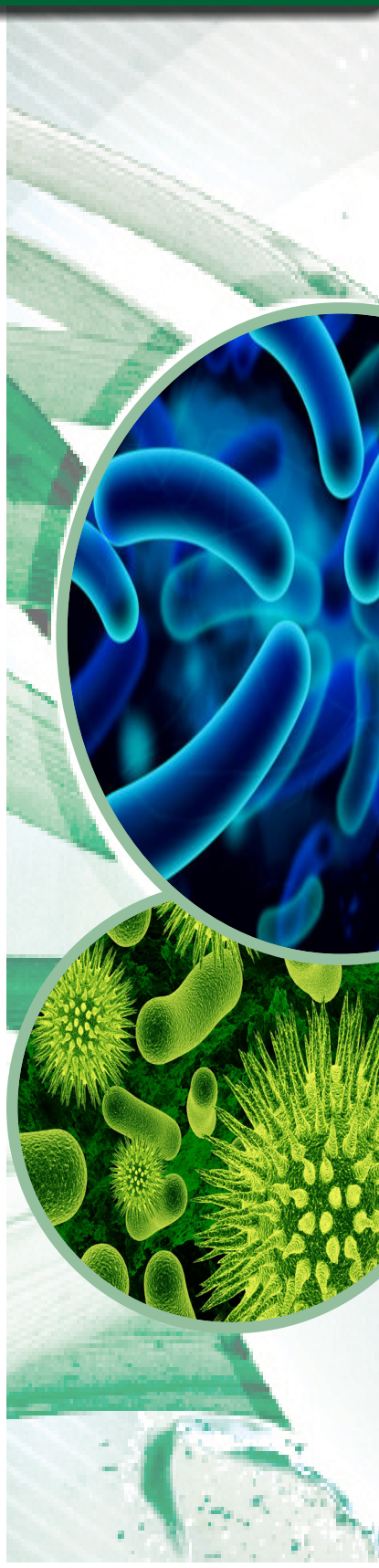
Leucidal® Liquid

Code Number: M15008

INCI Name: Leuconostoc/Radish Root Ferment Filtrate

Table of Contents

Click on the logo to return to the Table of Contents



Leucidal® Liquid Code Number: M15008

INCI Name: Leuconostoc/Radish Root Ferment Filtrate

Leucidal® Liquid

Patent Pending: Application Number 62/013,669

Technical Data Sheet

BACKGROUND

Over the past several decades there has been growing public pressure, increasingly strict chemical regulations, preservative sensitization issues, and the potential for developing microbial resistance to the chemical preservative products typically used in cosmetic and personal care formulations. These factors have resulted in numerous preservation chemicals being pulled from the marketplace, despite being the products of choice at one time. To offer a solution to this preservation paradigm, **Active Micro Technologies (AMT)** has developed a line of products based on naturally occurring compounds that provide active cosmetic properties, but by their very nature are also capable of providing product preservation. This antimicrobial capability is due to natural mechanisms developed by plants and microorganisms by which they protect themselves from their environment and other competing organisms.

Code Number: M15008
INCI Nomenclature:
Leuconostoc/Radish Root Ferment Filtrate
INCI Status: Approved
REACH Status: Fully Compliant
CAS Number: 1686112-10-6
EINECS Number: N/A
Origin: Biotechnology/Botanical:
Leuconostoc kimchii & *Raphanus Sativus*
Processing:
GMO Free
No Ethoxylation
No Irradiation
No Sulphonation
No Ethylene Oxide treatment
No Hydrogenation
Additives: None
-Preservatives: None
-Antioxidants: None
Other additives: None
Solvents used: Water
Appearance: Clear to Slightly Hazy Liquid
Soluble/Miscible: Aqueous Ferment Extract
Suggested Use Levels: 2.0 - 4.0%
Suggested Applications:
Moisturization, Skin/Scalp Conditioning, Antimicrobial

SCIENCE

Leucidal® Liquid is based on an antimicrobial peptide originally derived from the lactic acid bacteria, *Leuconostoc kimchii*. *L. kimchii* is one of 15 species of microorganisms that make up the mixed culture used for producing the Korean dietary staple known as kimchi, a type of fermented cabbage.

Like many lactic acid bacteria, *L. kimchii* is capable of restricting the growth of other microorganisms by acidifying its environment, but as is common in nature, it is not content to limit itself to a single mechanism of defense. In addition to acidifying its environment, it also produces a novel antimicrobial peptide. Using modern fermentation and bioprocessing technology, **AMT** has commercialized this antimicrobial peptide to produce **Leucidal® Liquid.**

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Version 17- 08.02.16

Leucidal® Liquid

Patent Pending: Application Number 62/013,669

BENEFITS

A skin moisturization study was performed using an untreated control, generic cream base, and an experimental with the same cream base containing 2.0% **Leucidal® Liquid**. Comparative moisturization results from this study are shown in Figure 1. As demonstrated by the results of this study, the addition of 2.0% **Leucidal® Liquid** improved moisture levels by 42.01% after 24 hours and by 62.33% after four weeks when compared to the untreated control. When compared to the base cream **Leucidal® Liquid** improved moisturization by 14.38% and after 24 hours and by 24.13% after four weeks. Based on these results, adding this innovative product provides the formulator the opportunity to capitalize on both the natural antimicrobial properties of **Leucidal® Liquid**, as well as its ability to provide potent moisturizing benefits to the cosmetic formulation. These properties make it ideal for applications addressing numerous skin and scalp conditions.

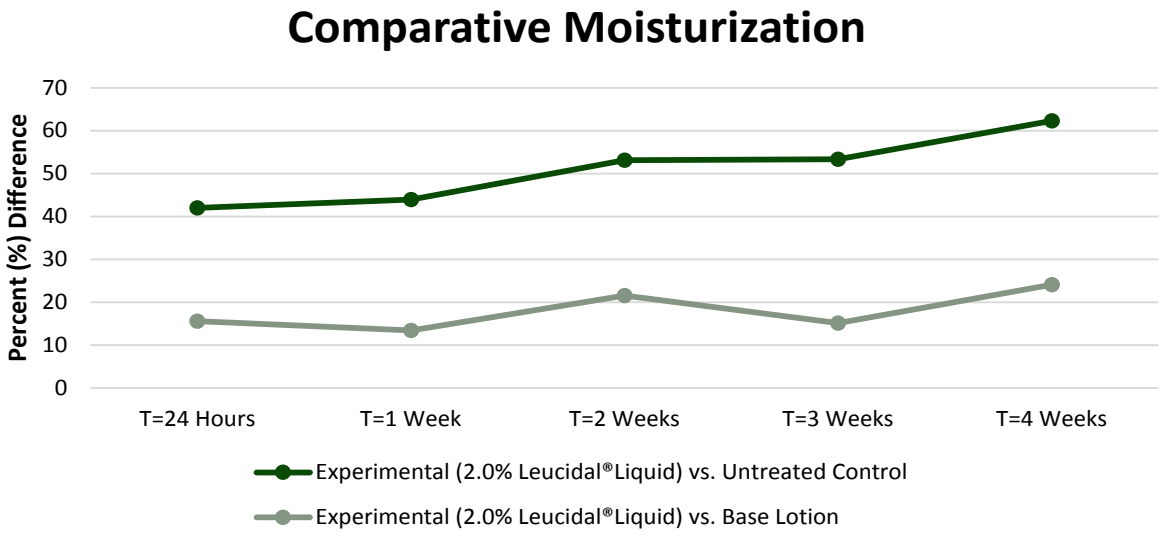

Figure 1. Percent Difference in Moisturization for **Leucidal® Liquid**.

One of the first steps in the development of this product was to determine the peptide's potential ability to inhibit the growth of a variety of bacteria and fungi. Using standard serial dilution protocols in growth media, the Minimum Inhibitory Concentrations (MICs) for **Leucidal® Liquid** were determined for a variety of both bacterial and fungal organisms. The results of these tests are shown in Figure 2.

Microorganism Tested	MIC (%)
E. coli	1.60
P. aeruginosa	0.80
S. aureus	1.60
C. albicans	0.80
A. niger	2.40

Figure 2. MIC Data for **Leucidal® Liquid**.

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Leucidal® Liquid

Patent Pending: Application Number 62/013,669

The positive MIC screening results warranted further testing to confirm its ability to provide product preservation. Double Challenge Tests were completed using either 2.0% or 4.0% **Leucidal® Liquid** in a generic cream base formulation at pH values of 3, 5, and 7. Samples were inoculated with *E. coli, P. aeruginosa*, *S. aureus*, *C. albicans*, and *A. brasiliensis*. During the first 28-day incubation period, samples were periodically collected and tested for the presence of these microorganisms. Following this initial 28 days of incubation, the cream samples were then re-inoculated with the microbial cultures and sampled over an additional 28-day period. Figure 3 shows the positive preservation results for 4.0% **Leucidal® Liquid** in a cream formula at pH 5.

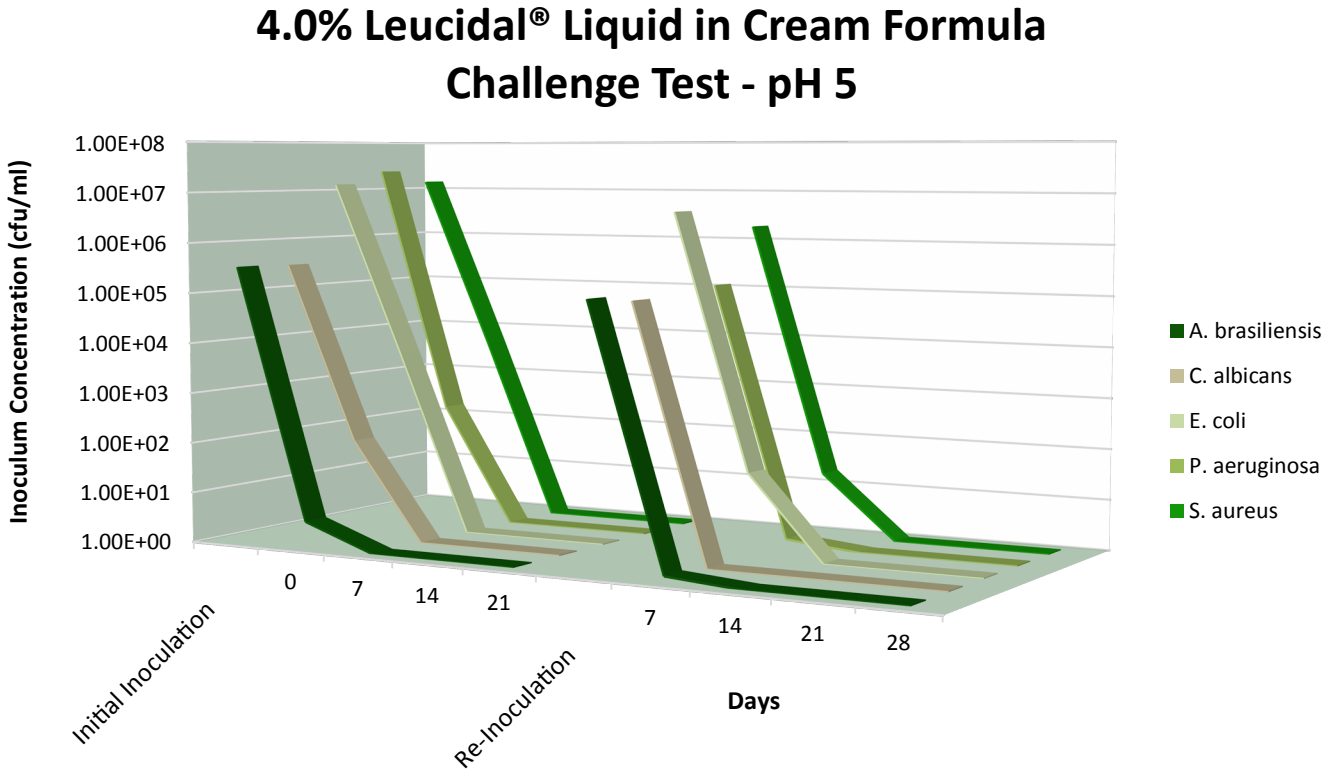

Figure 3. Challenge Test results for Generic Cream Formula pH 5 with 4.0% **Leucidal® Liquid** inoculated on Day 0 and re-inoculated on Day 28. Results show log reduction in viable organisms.

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Leucidal® Liquid

Patent Pending: Application Number 62/013,669

A Time Kill Test was performed to determine the change in population of aerobic microorganisms within a specified sampling time when tested against 4.0% **Leucidal® Liquid** solution. The activity of the test material inoculated was evaluated at determine time intervals of 30 seconds, 1, 5, 10 and 30 minutes after the inoculation to determine quantitatively the number of viable microorganisms remaining after the incubation time. As shown in Figure 5, the Gram-positive and Gram-negative bacteria as well as the yeast and mold were reduced by 99.9% within 30 seconds interval of the test after the inoculation.

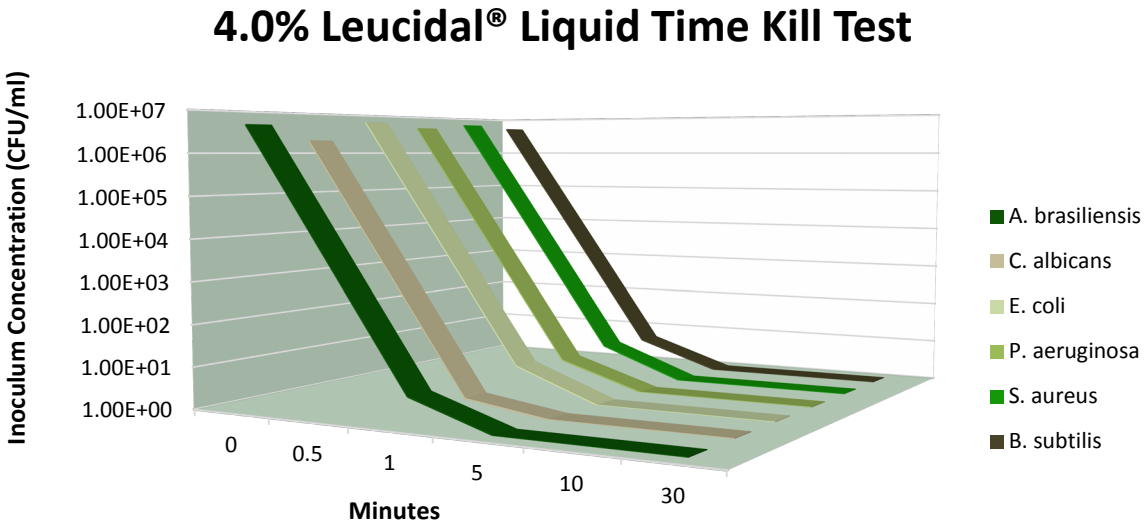

Figure 5. Time Kill Test results for 4.0% **Leucidal® Liquid**.

USE RECOMMENDATIONS

As with all biological materials, some attention must paid to the conditions under which **Leucidal® Liquid** is used. Based on bench-scale evaluations, as well as actual product applications, **Leucidal® Liquid** has been found to be effective over a wide range of typical cosmetic and personal care product manufacturing conditions. The product has been found to be heat stable up to 70°C and active under both acidic (pH 3) and basic conditions (pH 8).

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ACTIVE MICRO TECHNOLOGIES

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Patent Pending: Application Number 62/013,669

Specification

Product Name: Leucidal® Liquid
Code Number: M15008
CAS #'s: 1686112-10-6
EINECS #'s: N/A
INCI Name: Leuconostoc/Radish Root Ferment Filtrate

Specification	Parameter
Appearance	Clear to Slightly Hazy Liquid
Color	Yellow to Light Amber
Odor	Characteristic
Solids (1g-105°C-1hr)	48.0 – 52.0%
pH	4.0 – 6.0
Specific Gravity (25°C)	1.140 – 1.180
Ninhydrin	Positive
Phenolics (tested as Salicylic Acid)[1]	18.0 – 22.0%
Heavy Metals	< 20 ppm
Arsenic	< 2 ppm
Minimum Inhibitory Concentration[2].	
Organism (ATCC#)	
E. coli (#8739)	0.50 – 4.00%
S. aureus (#6538)	0.25 – 2.00%
P. aeruginosa (#9027)	1.00 – 4.00%
C. albicans (#10231)	0.25 – 2.00%
A. brasiliensis (#16404)	0.25 – 2.00%

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Patent Pending: Application Number 62/013,669

DO NOT FREEZE; Store at or near room temperature;
Mix well prior to use; May Sediment upon Standing

Note:

1) Phenolic compounds of natural origin, tested as Salicylic acid via USP HPLC method.
2) Refer to Inhibition Activity Data

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Compositional Breakdown

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Leucidal® Liquid
Code: M15008

Compositional Breakdown:

Ingredient	%
Water	48.00 - 52.00
Leuconostoc/Radish Root Ferment Filtrate	48.00 - 52.00

- **The above material is free of intact or viable Leuconostoc organisms and does not contain carry-over ingredients from manufacturing.**

- **To our knowledge the above material is free of the following list of heavy metals:**
  - **Heavy Metals < 20 ppm (Max.)**
  - **Lead < 10 ppm (Max.)**
  - **Antimony < 5 ppm (Max.)**
  - **Arsenic < 2 ppm (Max.)**
  - **Mercury < 1 ppm (Max.)**
  - **Cadmium < 1 ppm (Max.)**

Compositional Breakdown

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This is to certify that Leucidal® Liquid does not contain allergen levels exceeding the following (Gas Chromatography-Mass Spectrometer Coupled):

ALLERGENS Dir 2003 15 CEE		
INCI NAME	CAS NUMBER	Limit (ppm)
Alpha-IsoMethyl Ionone	127-51-5	< 0.02
Amyl Cinnamal	122-40-7	< 0.10
Anise Alcohol	105-13-5	< 0.00
Benzyl Alcohol	100-51-69	< 0.01
Benzyl Benzoate	120-51-4	< 0.09
Benzyl Cinnamate	103-41-3	< 0.30
Benzyl Salicylate	118-58-1	< 0.06
Butylphenyl Methylpropional	80-54-6	< 0.50
Cinnamal	104-55-2	< 0.01
Cinnamyl Alcohol	104-54-1	< 0.30
Citral	5392-40-5	< 1.00
Citronellol	106-22-9	< 1.00
Coumarin	91-64-5	< 0.00
Eugenol	97-53-0	< 0.70
Farnesol	4602-84-0	< 0.04
Geraniol	106-24-1	< 0.08
Hexyl Cinnamal	101-86-0	< 0.40
Hydroxycitronellal	107-75-5	< 1.00
Hydroxymethylpentyl 3-Cyclohexene carboxaldehyde	31906-04-4	< 0.30
Isoeugenol	97-54-1	< 0.06
Limonene	5989-27-5	< 0.05
Linalool	78-70-6	< 0.00
Methyl 2 Octynoate	111-12-6	< 0.20
Evernia prunastri	90028-68-5	< 0.02
Evernia furfuracea	90028-67-4	< 0.00
Amylcinnamyl Alcohol	101-85-9	< 1.00

Compositional Breakdown

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This is to certify that Leucidal® Liquid does not contain pesticide levels exceeding the following (Reverse Phase High Performance Liquid Chromatography-Mass Spectrometer Coupled):

EPA Pesticide Levels	
INCI NAME	LIMIT (mg/kg)
Alachlor	< 0.02
Aldrin and Dieldrin	< 0 .05
Azinphos-methyl	< 1. 00
Bromopropylate	< 3.0 0
Chlordane(cis and trans)	< 0.05
Chlorfenvinphos	< 0.50
Chlorpyrifos	< 0.20
Chlorpyrifos-methyl	< 0.10
Cypermethrin	< 1.00
DDT	< 1.00
Deltamethrin	< 0.50
Diazinon	< 0.50
Dichlorvos	< 1.00
Dithiocarbamates	< 2.00
Endosulfan	< 3.00
Endrin	< 0.05
Ethion	< 2.00
Fenitrothion	< 0.50
Fenvalerate	< 1.50
Fonofos	< 0.05
Heptachlor	< 0.05
Hexachlorobenzene	< 0.10
Hexachlorocyclohexane	< 0.30
Lindane	< 0.60
Malathion	< 1.00
Methidathion	< 0.20
Parathion	< 0.50

Compositional Breakdown

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Parathion-methyl	< 0.20
Permethrin	< 1.00
Phosalone	< 0.10
Piperonyl butoxide	< 3.00
Pirimiphos-methyl	< 4.00
Pyrethrins	< 3.00
Quintozene(sum of 3 items)	< 1.00

Moisturization / Hydration Assay

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6

Test Request Form #: 1094

Lot #: 39359P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Moisturization/Hydration Assay

Introduction

An *in-vivo* study was conducted over a period of four weeks to evaluate the moisturization benefits **Leucidal® Liquid**. 10 M/F subjects between the ages of 23-45 participated in the study. Results indicate that this material is capable of significantly increasing moisturization compared to the control.

The moisturization assay was conducted to assess the moisturizing ability of **Leucidal® Liquid.**

Materials

A. Equipment: DermaLab Skin Combo (Hydration/ Moisture Pin Probe)

The moisture module provides information about the skin's hydration by measuring the conducting properties of the upper skin layers when subjected to an alternating voltage. The method is referred to as a conductance measurement and the output is presented in the unit of uSiemens (uS). A moisture pin probe is the tool used to gather hydration values.

10 volunteers M/F between the ages of 23 and 45 and who were known to be free of any skin pathologies participated in this study. A Dermalab Corneometer was used to measure the moisture levels on the subject's volar forearms. The Corneometer is an instrument that measures the amount of water within the skin. The presence of moisture in the skin improves conductance therefore results in higher readings than dry skin. Therefore the higher the levels of moisture, the higher the readings from the Corneometer will be. Baseline moisturization readings were taken on day one of the study.

Following initial measurements, all subjects were asked to apply 2 mg of each test material on their volar forearms. Measurements were taken immediately after application of test materials and then weekly for 4 weeks. The test material consisted of 2% **Leucidal® Liquid** in a base lotion.

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For added perspective, measurements of an untreated test site and a site treated with a base lotion (Cetaphil Moisturizing for All Skin Types) were recorded.

Results

Leucidal® Liquid showed high moisturizing capabilities at a 2.0% concentration. Please note that each value is an average of three consecutive readings per test site.

Moisturization		T = 0	T= 24 Hours	T = 1 Week	T = 2 Weeks	T= 3 Weeks	T= 4 Weeks
Panelist 1	Experimental	65	110	130	151	157	170
	Base Lotion	57	100	119	125	140	148
	Untreated	42	49	47	53	51	50
Panelist 2	Experimental	53	95	119	131	166	165
	Base Lotion	47	84	100	119	159	130
	Untreated	35	55	57	75	115	57
Panelist 3	Experimental	43	93	96	102	130	123
	Base Lotion	37	75	67	75	83	90
	Untreated	62	98	131	96	95	126
Panelist 4	Experimental	41	104	92	124	110	90
	Base Lotion	37	96	82	82	63	78
	Untreated	31	61	62	121	56	68
Panelist 5	Experimental	71	99	168	154	181	197
	Base Lotion	59	81	134	135	149	159
	Untreated	45	90	96	99	91	81
Panelist 6	Experimental	42	85	74	120	93	94
	Base Lotion	30	83	88	78	93	94
	Untreated	58	95	113	127	124	140
Panelist 7	Experimental	57	143	170	180	212	199
	Base Lotion	51	120	162	149	201	125
	Untreated	27	55	41	59	94	57
Panelist 8	Experimental	32	96	112	120	120	96
	Base Lotion	30	77	104	101	115	78
	Untreated	29	74	100	86	126	99
Panelist 9	Experimental	47	87	107	117	122	120
	Base Lotion	45	68	92	105	110	95
	Untreated	50	74	87	90	99	91
Panelist 10	Experimental	50	119	150	161	163	181
	Base Lotion	45	108	126	150	161	166
	Untreated	47	75	112	82	97	115
Number of Panelists		10	9	10	10	10	10

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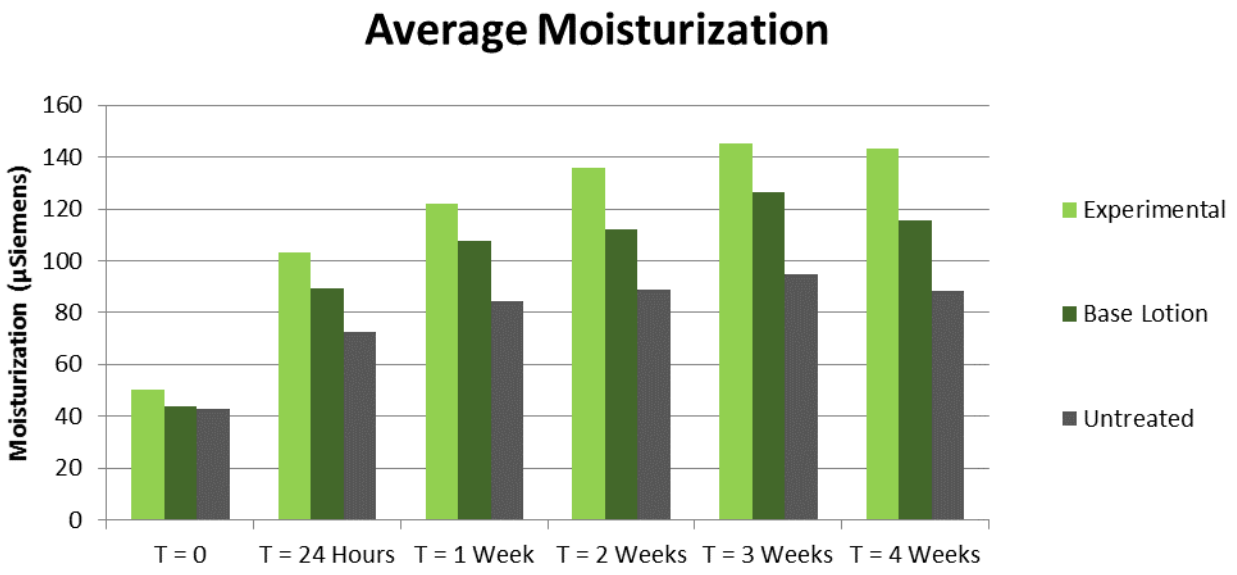
Moisturization / Hydration Assay

Averages	T = 0	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks
Experimental (2.0% Leucidal® Liquid) in Base Lotion	51.0	103.1	121.8	136	145.4	143.5
Base Lotion Control	43.8	89.2	107.4	111.9	126.3	115.6
Untreated Control	42.6	72.6	84.6	88.8	94.8	88.4

Percent (%) Change	T = 0	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks
Base Lotion vs. Untreated Control	2.82	22.86	26.95	26.01	33.23	30.77
Experimental (2.0% Leucidal® Liquid) vs. Untreated Control	17.61	42.01	43.97	53.15	53.37	62.33
Experimental (2.0% Leucidal® Liquid) vs. Base Lotion	14.38	15.58	13.40	21.53	15.12	24.13



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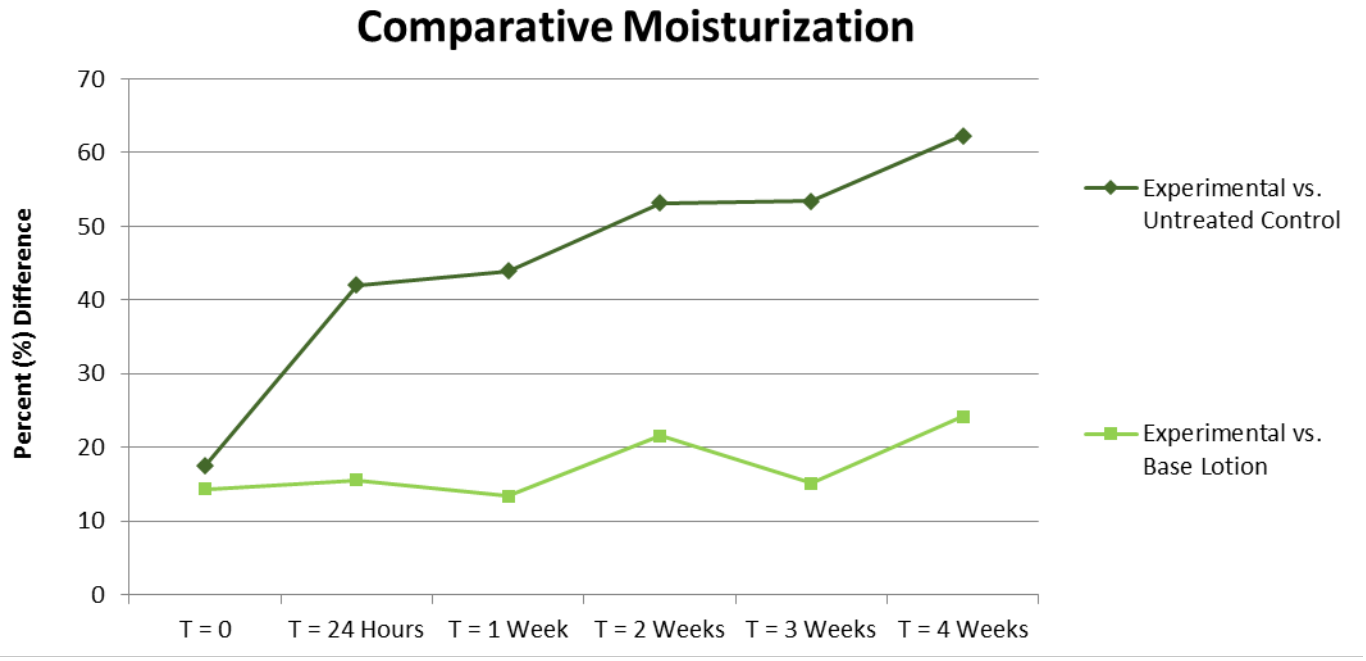

Discussion

As evidenced in a four week efficacy study of **Leucidal® Liquid** on skin, moisture levels were improved by 42.01% after 24 hours and by 62.33% after four weeks when compared to the untreated control. When compared to the base cream **Leucidal® Liquid** improved moisturization by 14.38% and after 24 hours and by 24.13% after four weeks. Results indicate that **Leucidal® Liquid** is capable of increasing moisturization when compared to both the untreated control as well as the base lotion.

The present study confirms that **Leucidal® Liquid** is capable of providing strong moisturizing and skin hydrating benefits when added to cosmetic applications.

Transepidermal Water Loss (TEWL) Study

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(704) 276-7100 • Fax (704) 276-7101

Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6

Test Request Form #: 1094

Lot #: 39359P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed: Transepidermal Water Loss Study

Introduction

An *in-vivo* study was conducted over a period of three weeks to evaluate the ability of **Leucidal® Liquid** to enhance barrier function through reduction in Transepidermal Water Loss (TEWL). Results indicate that this material is capable of efficiently reducing TEWL, which allows moisture retention.

Materials

A. Equipment: DermaLab Skin Combo

Methods

Ten volunteers M/F between the ages of 23 and 45 and who were known to be free of any skin pathologies participated in this study. A Dermalab Combo was used to measure TEWL on the subject's volar forearms. The instrument consists of a probe that is based upon the vapor gradient with an open chamber. This open chamber design maintains the free natural evaporation from the skin without interfering with the environment over the measurement area. This ensures unbiased and accurate readings. Operation of the water loss module is fully menu drive, allowing for pre-setting and standard deviation or measurement time. Baseline TEWL readings were taken on day one of the study.

Following initial measurements, all subjects were asked to apply 5milligrams of each test material on their volar forearms. Measurements were taken immediately after application of the test materials and then weekly for three weeks. The test material consisted of 2% **Leucidal® Liquid** in a base lotion.

For added perspective, measurements of an untreated test site and a site treated with a base lotion (Cetaphil Moisturizing for All Skin Types) were recorded.

Following initial measurements, all subjects were asked to apply 2 mg of each test material on their volar forearms. Measurements were taken immediately after application of test materials and then weekly for four weeks. The test material consisted of 2% **Leucidal® Liquid** in a base lotion.

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For added perspective, measurements of an untreated test site and a site treated with a base lotion (Cetaphil Moisturizing for All Skin Types) were recorded.

Results

Leucidal® Liquid showed improvements in skin density at a 2.0% concentration. Please note that each value is an average of three consecutive readings per test site.

Averages	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks
Untreated Control	-9.06	-8.06	-7.71	-7.36
Base Lotion Control	-9.31	-9.15	-8.79	-9.12
Experimental (2.0% Leucidal® Liquid) in Base Lotion	-9.99	-10.32	-9.50	-9.70

Chart 1. Average Increase in Skin Density per Individual Test Site

Percent (%) Change	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks
Experimental (2.0% Leucidal® Liquid) vs. Base Lotion	9.0%	22.0%	23.0%	24.0%
Experimental (2.0% Leucidal® Liquid) vs. Untreated Control	3.0%	10.0%	12.0%	15.0%

Chart 2. Comparison of Skin Density Changes between Two Test Sites

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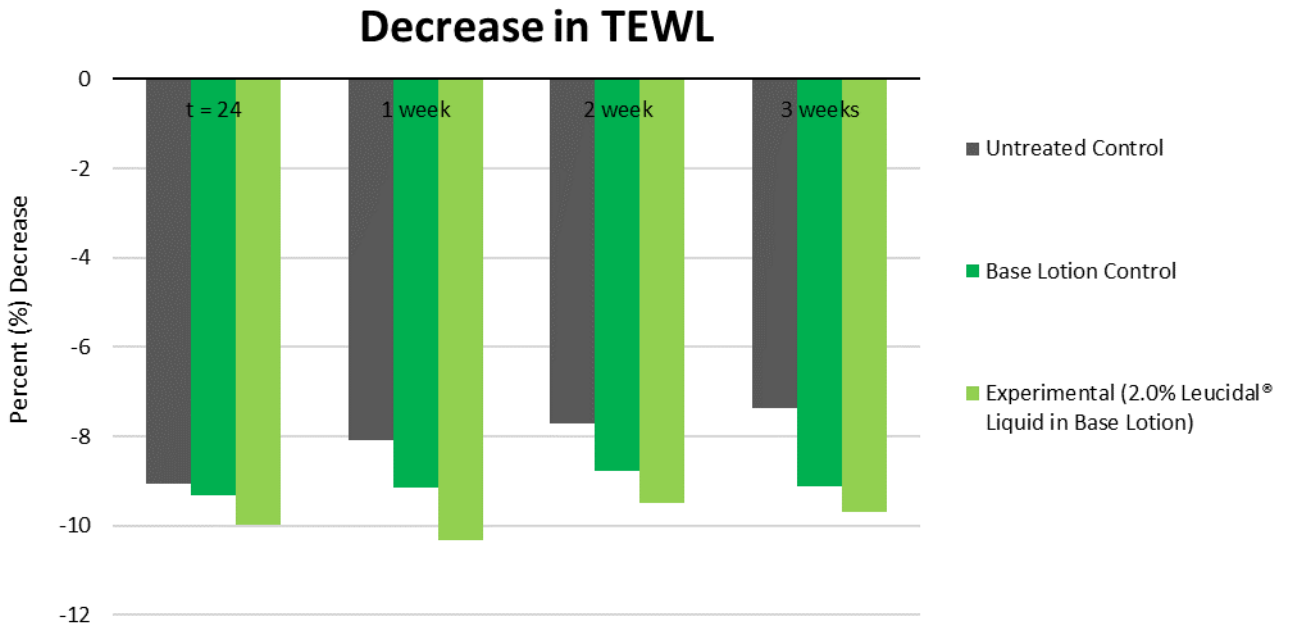
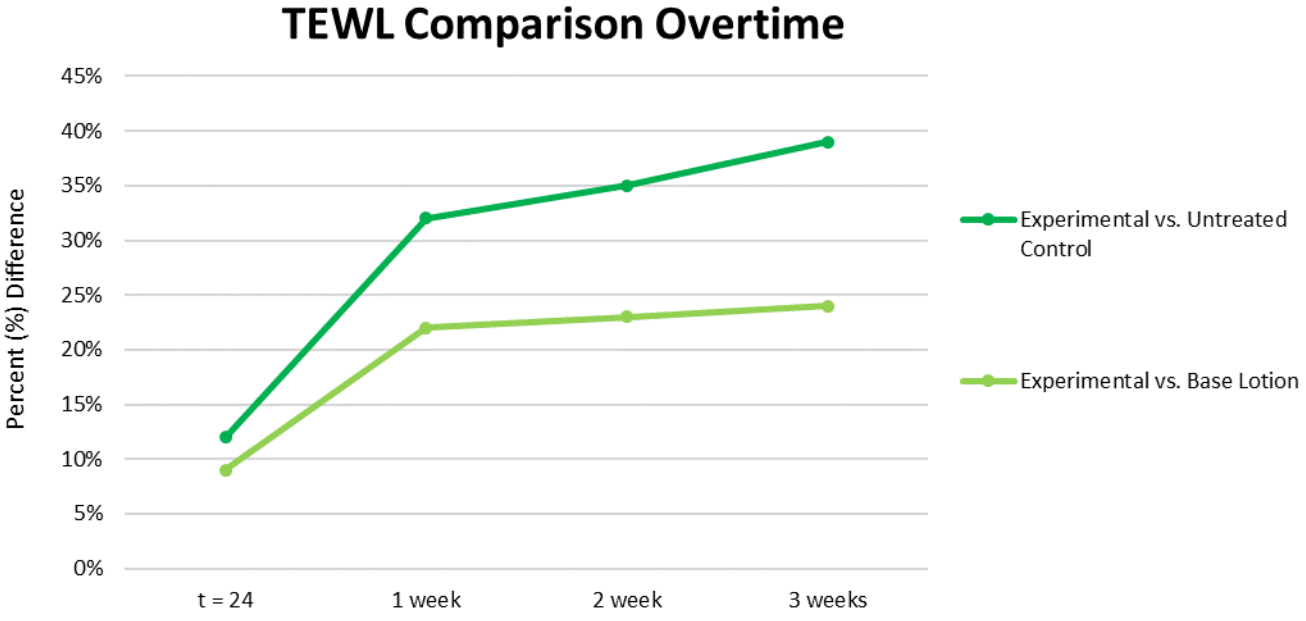


Graph 1. Average Decrease in TEWL per Individual Test Site



Graph 2. Comparison of TEWL Changes between Two Test Sites

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Discussion

As shown, the results indicate continuous improvements in the barrier of the skin throughout the three week test period. After one week, the solution containing 2.0% **Leucidal® Liquid** decreased TEWL 10% more effectively than the base lotion alone. After three weeks, the solution containing 2.0% **Leucidal® Liquid** demonstrated even more effective barrier protection, decreasing TEWL 15% better than the base lotion alone.

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Scratch Assay Analysis

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(704) 276-7100 • Fax (704) 276-7101

Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6

Test Request Form #: 1083

Lot #: 40140P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Scratch Assay

Introduction

Wounded tissue begins a complex and structured series of events in order to repair the damaged region. Some of these events include upregulation of angiogenic factors causing increased vascularization, increased deposition of extracellular matrix, and increased cell proliferation. The wound healing process begins as cells polarize toward the wound, initiate protrusion, migrate, and close the wound area. These processes reflect the behavior of individual cells as well as the entire tissue complex.

The scratch assay was conducted to assess the wound healing properties of **Leucidal® Liquid-**treated *in vitro* cultured human dermal fibroblasts.

Assay Principle

The *in vitro* scratch assay is a well-known and widely used method to study cell migration and proliferation. This assay is based on the observation that when an artificial gap or scratch is made on a confluent cell monolayer, the cells will migrate towards the opening and close the scratch. The basic steps involve creating a scratch in a cell monolayer and capturing images throughout the healing or cell migration process. Through these images we can quantify the rate of cell migration.

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Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% Relative Humidity (RH)
B. Equipment:	Forma Humidified Incubator, ESCO Biosafety Laminar Flow Hood, Inverted Microscope; Camera; Pipettes
C. Cell Line:	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
D. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM); Fetal Bovine Serum (FBS); Penicillin-Streptomycin (50U-50mg/mL); Phosphate Buffered Saline (PBS)
E. Reagents:	Epidermal Growth Factor-1 (100ng/mL); Paraformaldehyde (3.7%); Crystal Violet Stain
F. Culture Plate:	Falcon Flat Bottom 6-Well Tissue Culture Treated Plates
G. Other:	Sterile Disposable Pipette Tips; Wash Bottles; 15mL Conical Tubes

Methods

Human dermal fibroblasts were seeded into 6-well tissue culture plates and allowed to grow to confluency in complete DMEM. A 0.01% concentration of **Leucidal® Liquid** was added to the culture media and incubated with fibroblasts for the extent of the experiment. Epidermal Growth Factor-1 was utilized as the positive control and serum-free media (SFM) was used a negative control. Complete media contains 10% FBS.

When cell growth reached confluency scratches were made across the well in a cross or 'X' pattern. The wells were washed with sterile PBS and fresh media containing **Leucidal® Liquid** and the controls were added. Initial images were captured immediately after the scratch took place and every 24-hours afterwards, up to 72-hours. Cells were fixed with 3.7% paraformaldehyde and stained with crystal violet for enhanced microscopy.

ImageJ software was used to analyze the images and calculate the area of the scratch and the closure rate.

Results

The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated.

Leucidal® Liquid at a 0.01% concentration was able to increase cell migration and wound healing compared to our negative control.

Percent scratch closure and migration rate are expressed by the following formula:

$$\frac{Scratch\ Area_{\ t=x} - Scratch\ Area_{\ t=0}}{Scratch\ Area_{\ t=0}} \times 100 = \%\ Scratch\ Closure$$

$$\frac{Change\ in\ Area\ of\ Scratch\ (nm^2)}{Migration\ Time_{\ t=x}} = Migration\ Rate$$

$$Where\ x = time\ (hours)\ post\ scratch$$

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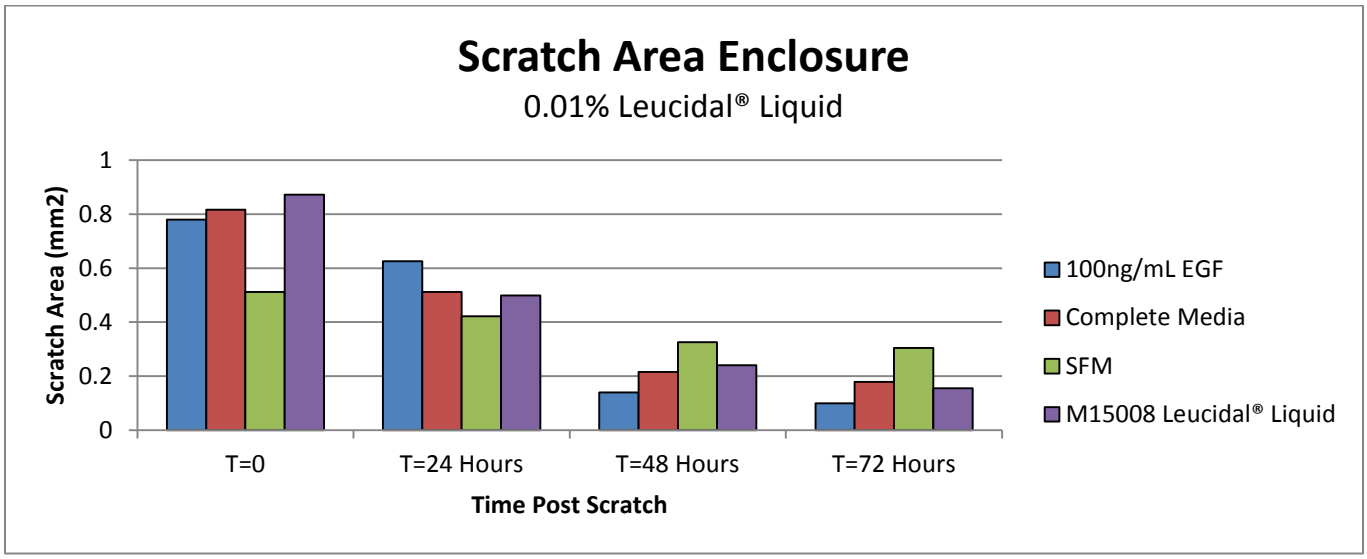

Figure 1: Area of scratch

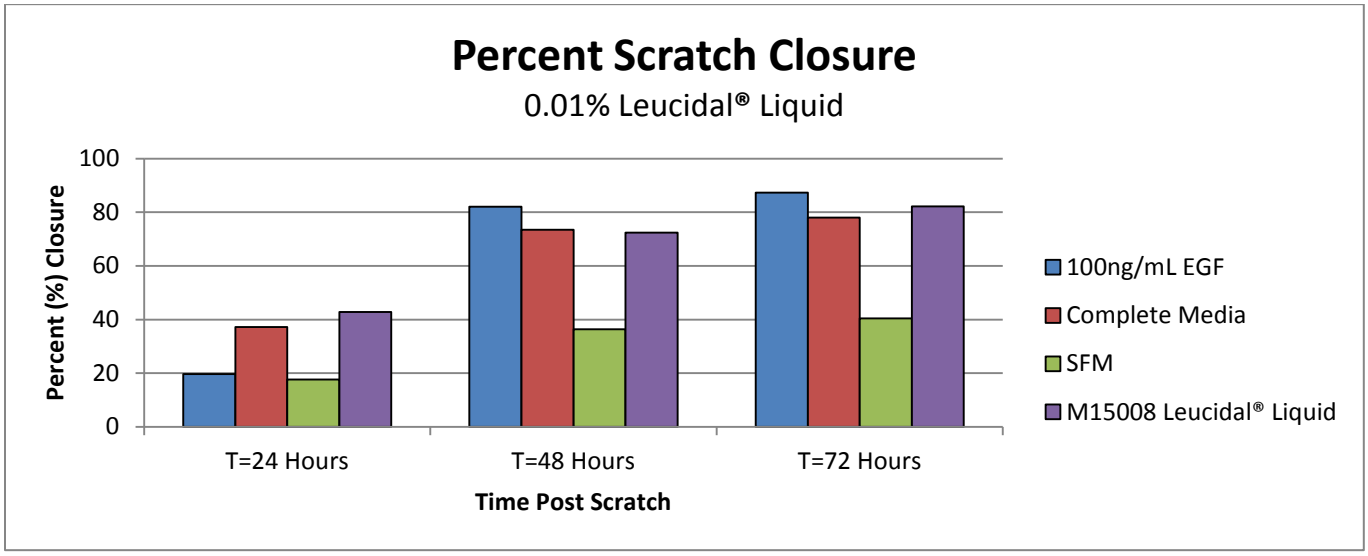

Figure 2: Percent scratch closure

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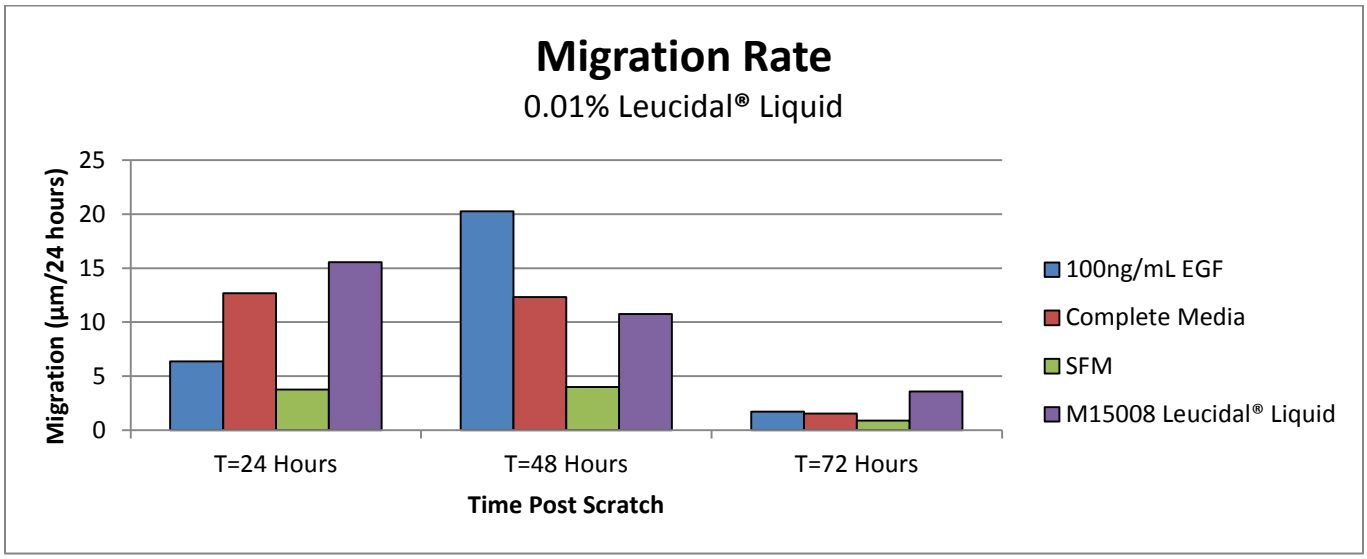

Figure 3: Cell migration rate

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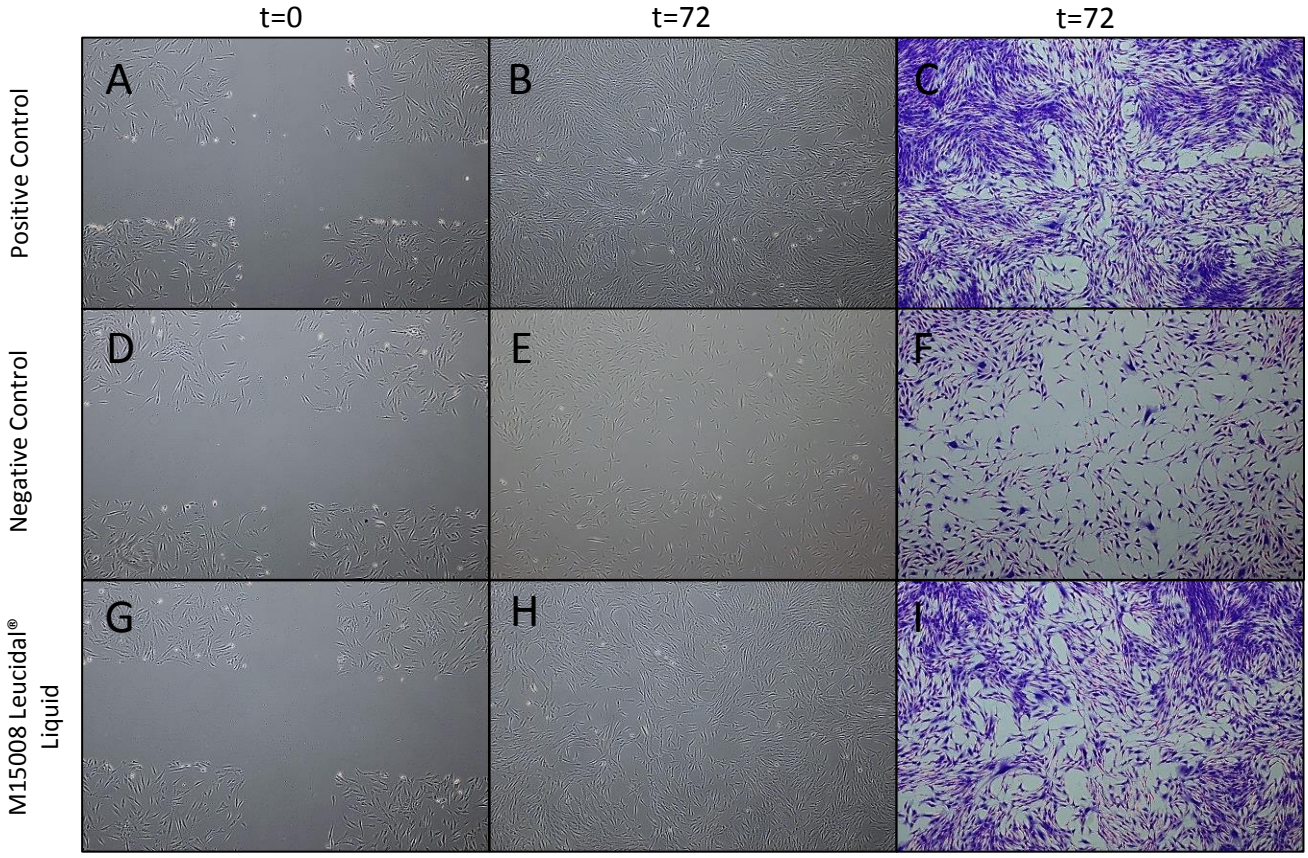

Figure 4: Images at t=0 hours (A, D, G) and t=72 hours (B, E, H) for **Leucidal® Liquid**, positive control (EGF-1), and negative control (SFM). At experiment completion (t=72 hours), cells were fixed in paraformaldehyde and stained with crystal violet (C, F, I).

Discussion

Leucidal® Liquid (code M15008) was able to increase cell migration and close the scratch at a rate comparable to the positive control. The mechanisms of the cells in the *in vitro* scratch assay mimic the mechanisms seen in *in vivo* wound healing therefore we can be assured that our results are translatable outside the laboratory. With the present study we can be confident that this product has healing abilities and cell proliferation properties.

ACTIVE MICRO TECHNOLOGIES

High Resolution Ultrasound Skin Imaging Assay

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Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6

Test Request Form #: 1094

Lot #: 39359P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
High Resolution Ultrasound Skin-Imaging Assay

Introduction

An *in-vivo* study was conducted over a period of four weeks to evaluate the effect on skin density of **Leucidal® Liquid**. 10 M/F subjects between the ages of 23-45 participated in the study. Results indicate that this material is capable of significantly improving skin density compared to the control.

Materials

Equipment: DermaLab Skin Combo (Ultrasound Probe)

Methods

Ultrasound skin imaging is based on measuring the acoustic response after an acoustic pulse is sent into the skin. The energy of the acoustic pulse is low and will not affect the skin in any way. When the acoustic pulse is emitted and hits different areas of the skin, part of the pulse will be reflected and part will be transmitted further into the skin. The reflected signal travels back and is picked up by the ultrasound transducer. After processing the signal, a cross-sectional image appears on the screen. This image represents an intensity, or amplitude, analysis of the signals.

The intensity of the signals that are received refer to a color scale. Dark colors represent areas of the skin with low reflection. This means that there are no changes or very small changes in density between the structures in the skin. Bright colors represent areas with strong reflections, signifying substantial changes in density between structures.

Following initial measurements, all subjects were asked to apply 2 mg of each test material on their volar forearms. Measurements were taken immediately after application of test materials and then weekly for four weeks. The test material consisted of 2% **Leucidal® Liquid** in a base lotion.

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For added perspective, measurements of an untreated test site and a site treated with a base lotion (Cetaphil Moisturizing for All Skin Types) were recorded.

Results

Leucidal® Liquid showed improvements in skin density at a 2.0% concentration. Please note that each value is an average of three consecutive readings per test site.

Averages	T = 0	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks
Experimental (2.0% Leucidal® Liquid) in Base Lotion	62.3	70	69.2	73.1	77.6
Base Lotion Control	57.9	61.5	60.9	66.2	67.2
Untreated Control	61.6	63.4	61.2	68.4	64.1

Chart 1. Average Increase in Skin Density per Individual Test Site

Percent (%) Change	T = 0	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks
Experimental (2.0% Leucidal® Liquid) vs. Untreated Control	9.25%	10.41%	13.07%	11.55%	17.32%
Experimental (2.0% Leucidal® Liquid) vs. Base Lotion	10.51%	12.18%	13.63%	15.26%	15.87%

Chart 2. Comparison of Skin Density Changes between Two Test Sites

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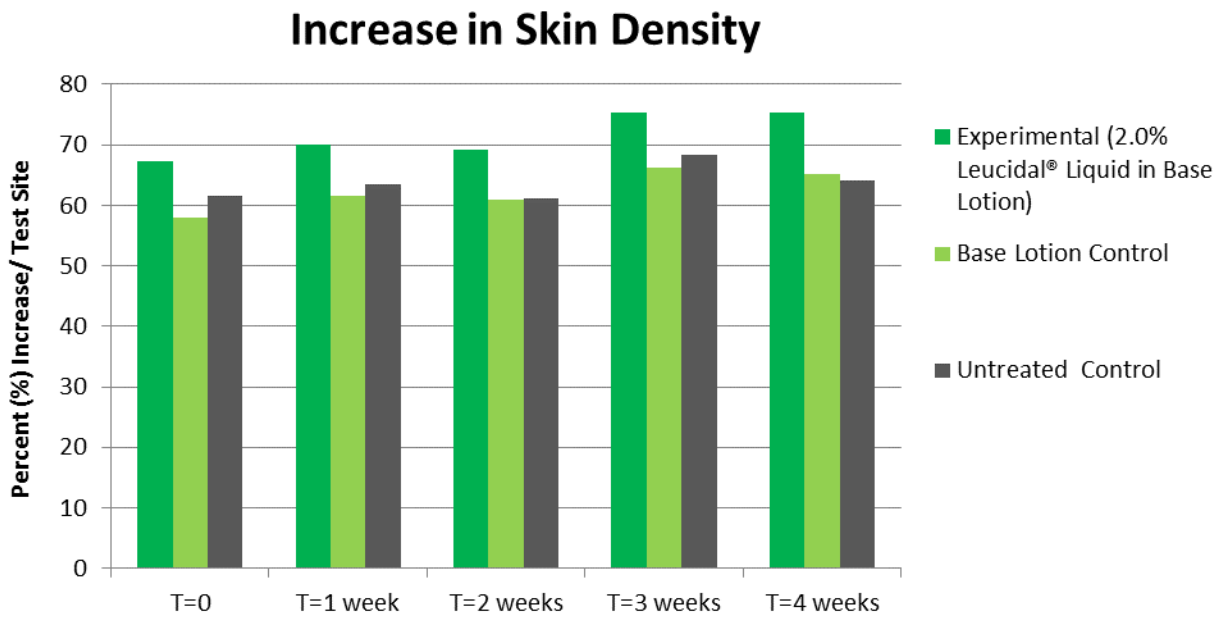
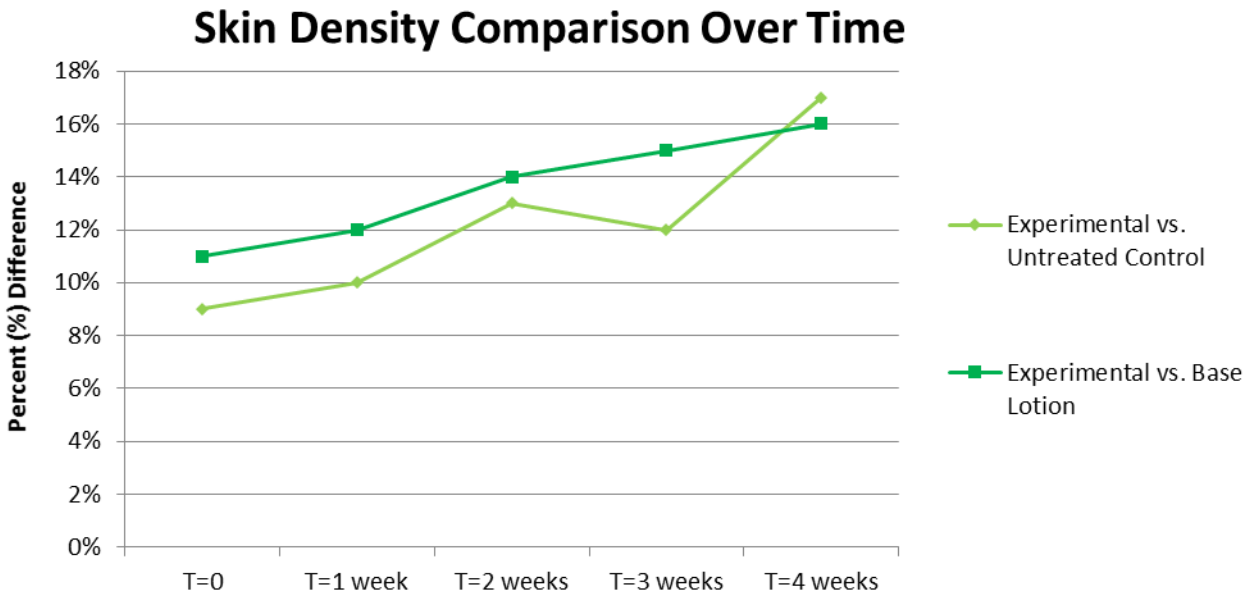


Graph 1. Average Increase in Skin Density per Individual Test Site



Graph2. Comparison of Skin Density Changes between Two Test Sites

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Discussion

As evidenced in a four-week efficacy study of **Leucidal® Liquid** on skin, skin density was improved by 10.41% after one week and by 17.32% after four weeks when compared to the untreated control. When compared to the base cream **Leucidal® Liquid** improved skin density during each week of the trial, working 12.18% better than the base lotion after one week and 15.87% better than the base lotion after four weeks. Results indicate that **Leucidal® Liquid** is capable of improving skin density when compared to both the untreated control as well as the base lotion.

Leucidal® Liquid has a strong positive effect on skin's density when used at recommended use levels.

Cellular Viability Assay Analysis

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Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6

Test Request Form #: 1510

Lot #: 4752P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Cellular Viability Assay

Introduction

The cellular viability assay is useful for quantitatively measuring cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity. Increased metabolism in a cell indicates ample cellular respiration and adenosine triphosphate (ATP) production. ATP is the molecular energy of cells and is required in basic cell function and signal transduction. A decrease in ATP levels indicates cytotoxicity and decreased cell function while an increase in ATP levels indicates healthy cells.

The cellular viability assay was conducted to assess the ability of **Leucidal® Liquid** to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction of growth medium from cell growth and by respiring mitochondria. Healthy cells that are in a proliferative state will be able to easily convert resazurin into resorufin without harming the cells. This method is a more sensitive assay than other commonly used mitochondrial reductase dyes such as MTT. An increase in the signal generated by resazurin-conversion is indicative of a proliferative cellular state.

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Materials

A. Kit:	PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
B. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes
D. Cell Line:	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E. Media/Buffers:	Basal Medium (Fibrolife; LM-0001), 500µg/mL Human Serum Albumins (Fibrolife; LS-1001), 0.6µM Linoleic Acid (Fibrolife; LS-1001), 0.6µg/mL (Fibrolife; LS-1001), 5ng/mL Fibroblast Growth Factor (Fibrolife; LS-1002), 5mg/mL Epidermal Growth Factor (Fibrolife; LS-1003), 30pg/mL Transforming Growth Factor β-1 (Fibrolife; LS-2003), 7.5mM L-Glutamine (Fibrolife; LS-1006), 1µg/mL Hydrocortisone Hemisuccinate (Fibrolife; LS-1007), 50µg/mL Ascorbic Acid (Fibrolife; LS-1005), 5µg/mL Insulin (Fibrolife; LS-1004)
F. Culture Plate:	Falcon flat bottom 96-well tissue culture treated plates
G. Reagents:	PrestoBlue™ reagent (10X)
H. Other:	Sterile disposable pipette tips

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete serum-free media. A 10-fold serial dilution was performed resulting in **Leucidal® Liquid** concentrations of 0.1% and 0.01% in complete serum-free media and incubated with fibroblasts for 24 hours.

Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells and a fluorometric measurement was taken at 560nm for excitation and 590nm for emission.

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Results

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated.

Leucidal® Liquid did not exhibit negative effects on cell metabolism.

Cellular metabolism results are shown as mean fluorescence units (MFU) and expressed as percentage change, calculated by the below equation:

$$Percent\ (\%)Change = \frac{MFU_{Control} - MFU_{Sample}}{MFU_{Control}} \times 100$$

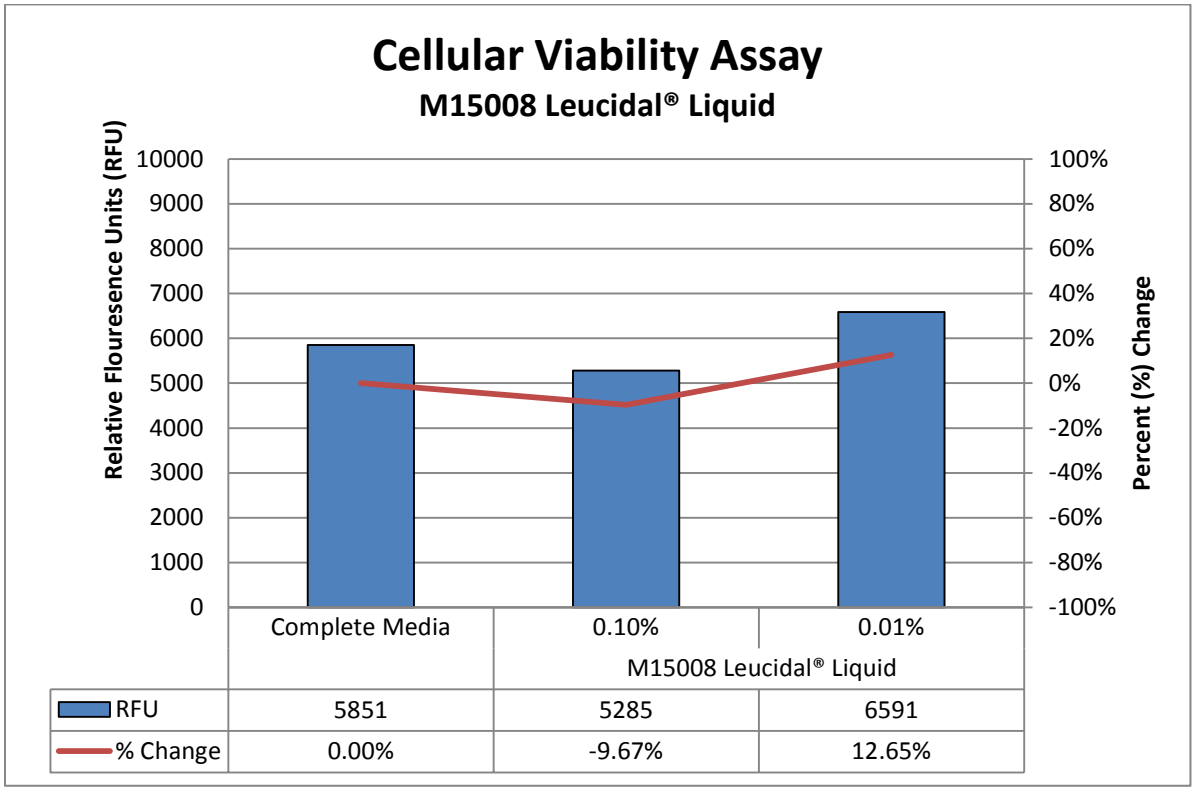

Figure 1: Cellular Metabolism of **Leucidal® Liquid**-treated fibroblasts

Discussion

In this study, **Leucidal® Liquid** (code M15008) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of 0.1% and 0.01%, **Leucidal® Liquid**, nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **Leucidal® Liquid** is not cytotoxic.

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Inhibition Activity Data

Product Name: Leucidal® Liquid
Code Number: M15008
Lot Number: 4869P
Test Request Number: 1492
CAS #'s: 1686112-10-6
EINECS #'s: N/A
INCI Name: *Leuconostoc*/Radish Root Ferment Filtrate

Organism (ATCC #)	Minimum Inhibitory Concentration (%)
E.coli #8739	2.0
S. aureus #6538	1.0
P. aeruginosa #9027	2.0
C. albicans #10231	2.0
A. brasiliensis #16404	2.0

QA Signature Monica Beltran

Date 09-08-2015

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Zone of Inhibition Test

Product Name: Leucidal® Liquid
Code Number: M15008
Lot Number: 39079P
Test Request Number: 1032
CAS #'s: 1686112-10-6
EINECS #'s: N/A
INCI Name: *Leuconostoc*/Radish Root Ferment Filtrate

Organism (ATCC #)	Zone of Inhibition (mm)
E.coli #8379	13.2
S. aureus #6538	12.6
P. aeruginosa #9027	13.5
C. albicans #10231	12.5
A. brasiliensis #16404	14.6

QA Signature Monica Beltran

Date 01-28-2015

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® Liquid
M15008

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on October 23rd, 2013 and was completed on December 26th, 2013.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

Test Method

Fifty grams of Generic Cream Formula pH 3 with 4% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

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The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	1.3×10^7	2.4×10^7	1.4×10^7	3.3×10^5	3.1×10^5
Day 0*	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	3.5×10^6	3.2×10^6	1.8×10^6	1.2×10^5	2.9×10^5
Day 7	>99.999%	99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 3 with 4% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

ACTIVE MICRO TECHNOLOGIES

Challenge Test with 4.0% Leucidal® Liquid

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The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 3 with 4% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast were reduced by greater than 99.9% within 7 days of each challenge. The mold was reduced by greater than 90% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	71.15%
	Carbopol Ultrez 10	Lubrizol	0.10%
II	Dermol 816	Alzo International	5.00%
	Sunflower Oil	Arista Industries	5.00%
	Silderm Emulsifying CS	Active Concepts, LLC	2.00%
	Dermol 2014	Alzo International	5.00%
	Lipomulse 165	Lipo Chemicals	1.60%
	Lipowax D	Lipo Chemicals	1.60%
	Stearic Acid	Acme Hardesty Oleochemicals	0.25%
III	ACB Bamboo Bioferment	Active Concepts, LLC	2.00%
IV	Organic Rice Solution	Arbor Organic Technologies, LLC	5.00%
	ACB Bio-Chelate 5	Active Concepts, LLC	2.00%

Manufacturing Process:

Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Check pH and adjust to 6.0 – 7.0 with Citric Acid (50%) or NaOH (25%).

Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 15 minutes. Begin force cooling.

Phase III:
Add ingredients at 50^{0}C and mix until homogenous.

Phase IV:
Add ingredients and mix until homogenous.

Specifications:

Appearance: White to Off-White Emulsion
pH: 4.0 – 5.5
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

The intent of performing an Antimicrobial Efficacy or Challenge test is to evaluate whether an antimicrobial agent or preservation system in a given cosmetic formulation has the ability to prevent the growth of test microorganisms. The test methodology employed by Active Micro Technologies (AMT) is based on the methods published in the CTFA Microbiology Guidelines. AMT's goal is to assist our customers by providing a screening test of a product formulation that is approaching finalization. It is expected that the formulation(s) submitted for Challenge testing contain AMT antimicrobials and have already passed the customer's internal stability tests. It is also anticipated that formal challenge testing of the final formulation will subsequently be performed by the customer at an outside lab of their choosing.

The information contained in this report is provided by Active Micro Technologies after the exercise of all reasonable care and skill in its compilation, preparation, and issue. It is provided without liability regarding its subsequent application and use. This type of screening test will be conducted only for validation of the efficacy of the antimicrobial agent or preservative system in the specific formulation tested. It does not address the suitability of the overall formula, nor does it address the regulatory status of any component therein. This testing does not account for the possibility of environmental microorganisms and cannot be relied upon as sufficient to justify commercialization of the product tested. By submitting samples for testing, the customer acknowledges that they will not hold Active Micro Technologies responsible for products launched based solely on the support of these studies.

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® Liquid
M15008

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on October 23rd, 2013 and was completed on December 26th, 2013.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

Test Method

Fifty grams of Generic Cream Formula pH 5 with 4% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

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The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	1.3×10^7	2.4×10^7	1.4×10^7	3.3×10^5	3.1×10^5
Day 0*	99.968%	99.999%	99.969%	>99.999%	99.970%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	4.4×10^6	1.4×10^5	1.9×10^6	1.3×10^5	1.0×10^5
Day 7	>99.999%	99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 5 with 4% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

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Challenge Test with 4.0% Leucidal® Liquid

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 5 with 4% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast were reduced by greater than 99.9% within 7 days of each challenge. The mold was reduced by greater than 90% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	71.15%
	Carbopol Ultrez 10	Lubrizol	0.10%
II	Dermol 816	Alzo International	5.00%
	Sunflower Oil	Arista Industries	5.00%
	Silderm Emulsifying CS	Active Concepts, LLC	2.00%
	Dermol 2014	Alzo International	5.00%
	Lipomulse 165	Lipo Chemicals	1.60%
	Lipowax D	Lipo Chemicals	1.60%
	Stearic Acid	Acme Hardesty Oleochemicals	0.25%
III	ACB Bamboo Bioferment	Active Concepts, LLC	2.00%
IV	Organic Rice Solution	Arbor Organic Technologies, LLC	5.00%
	ACB Bio-Chelate 5	Active Concepts, LLC	2.00%

Manufacturing Process:

Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Check pH and adjust to 6.0 – 7.0 with Citric Acid (50%) or NaOH (25%).

Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 15 minutes. Begin force cooling.

Phase III:
Add ingredients at 50^{0}C and mix until homogenous.

Phase IV:
Add ingredients and mix until homogenous.

Specifications:

Appearance: White to Off-White Emulsion
pH: 4.0 – 5.5
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

The intent of performing an Antimicrobial Efficacy or Challenge test is to evaluate whether an antimicrobial agent or preservation system in a given cosmetic formulation has the ability to prevent the growth of test microorganisms. The test methodology employed by Active Micro Technologies (AMT) is based on the methods published in the CTFA Microbiology Guidelines. AMT's goal is to assist our customers by providing a screening test of a product formulation that is approaching finalization. It is expected that the formulation(s) submitted for Challenge testing contain AMT antimicrobials and have already passed the customer's internal stability tests. It is also anticipated that formal challenge testing of the final formulation will subsequently be performed by the customer at an outside lab of their choosing.

The information contained in this report is provided by Active Micro Technologies after the exercise of all reasonable care and skill in its compilation, preparation, and issue. It is provided without liability regarding its subsequent application and use. This type of screening test will be conducted only for validation of the efficacy of the antimicrobial agent or preservative system in the specific formulation tested. It does not address the suitability of the overall formula, nor does it address the regulatory status of any component therein. This testing does not account for the possibility of environmental microorganisms and cannot be relied upon as sufficient to justify commercialization of the product tested. By submitting samples for testing, the customer acknowledges that they will not hold Active Micro Technologies responsible for products launched based solely on the support of these studies.

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® Liquid
M15008

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on October 23rd, 2013 and was completed on December 26th, 2013.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

Test Method

Fifty grams of Generic Cream Formula pH 7 with 4% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

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The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	1.3×10^7	2.4×10^7	1.4×10^7	3.3×10^5	3.1×10^5
Day 0*	99.963%	>99.999%	99.972%	99.984%	99.932%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	3.5×10^6	3.2×10^6	1.8×10^6	1.2×10^5	2.9×10^5
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 7 with 4% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

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Challenge Test with 4.0% Leucidal® Liquid

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 7 with 4% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast were reduced by greater than 99.9% within 7 days of each challenge. The mold was reduced by greater than 90% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	71.15%
	Carbopol Ultrez 10	Lubrizol	0.10%
II	Dermol 816	Alzo International	5.00%
	Sunflower Oil	Arista Industries	5.00%
	Silderm Emulsifying CS	Active Concepts, LLC	2.00%
	Dermol 2014	Alzo International	5.00%
	Lipomulse 165	Lipo Chemicals	1.60%
	Lipowax D	Lipo Chemicals	1.60%
	Stearic Acid	Acme Hardesty Oleochemicals	0.25%
III	ACB Bamboo Bioferment	Active Concepts, LLC	2.00%
IV	Organic Rice Solution	Arbor Organic Technologies, LLC	5.00%
	ACB Bio-Chelate 5	Active Concepts, LLC	2.00%

Manufacturing Process:

Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Check pH and adjust to 6.0 – 7.0 with Citric Acid (50%) or NaOH (25%).

Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 15 minutes. Begin force cooling.

Phase III:
Add ingredients at 50^{0}C and mix until homogenous.

Phase IV:
Add ingredients and mix until homogenous.

Specifications:

Appearance: White to Off-White Emulsion
pH: 4.0 – 5.5
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

The intent of performing an Antimicrobial Efficacy or Challenge test is to evaluate whether an antimicrobial agent or preservation system in a given cosmetic formulation has the ability to prevent the growth of test microorganisms. The test methodology employed by Active Micro Technologies (AMT) is based on the methods published in the CTFA Microbiology Guidelines. AMT's goal is to assist our customers by providing a screening test of a product formulation that is approaching finalization. It is expected that the formulation(s) submitted for Challenge testing contain AMT antimicrobials and have already passed the customer's internal stability tests. It is also anticipated that formal challenge testing of the final formulation will subsequently be performed by the customer at an outside lab of their choosing.

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® Liquid
M15008

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on October 23rd, 2013 and was completed on December 26th, 2013.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

Test Method

Fifty grams of Generic Cream Formula pH 3 with 2% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

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The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	1.3×10^7	2.4×10^7	1.4×10^7	3.3×10^5	3.1×10^5
Day 0*	>99.999%	>99.999%	>99.999%	99.984%	99.932%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	3.5×10^6	3.2×10^6	1.8×10^6	1.2×10^5	2.9×10^5
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 3 with 2% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

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The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 3 with 2% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast were reduced by greater than 99.9% within 7 days of each challenge. The mold was reduced by greater than 90% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	71.15%
	Carbopol Ultrez 10	Lubrizol	0.10%
II	Dermol 816	Alzo International	5.00%
	Sunflower Oil	Arista Industries	5.00%
	Silderm Emulsifying CS	Active Concepts, LLC	2.00%
	Dermol 2014	Alzo International	5.00%
	Lipomulse 165	Lipo Chemicals	1.60%
	Lipowax D	Lipo Chemicals	1.60%
	Stearic Acid	Acme Hardesty Oleochemicals	0.25%
III	ACB Bamboo Bioferment	Active Concepts, LLC	2.00%
IV	Organic Rice Solution	Arbor Organic Technologies, LLC	5.00%
	ACB Bio-Chelate 5	Active Concepts, LLC	2.00%

Manufacturing Process:

Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Check pH and adjust to 6.0 – 7.0 with Citric Acid (50%) or NaOH (25%).

Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 15 minutes. Begin force cooling.

Phase III:
Add ingredients at 50^{0}C and mix until homogenous.

Phase IV:
Add ingredients and mix until homogenous.

Specifications:

Appearance: White to Off-White Emulsion
pH: 4.0 – 5.5
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

The intent of performing an Antimicrobial Efficacy or Challenge test is to evaluate whether an antimicrobial agent or preservation system in a given cosmetic formulation has the ability to prevent the growth of test microorganisms. The test methodology employed by Active Micro Technologies (AMT) is based on the methods published in the CTFA Microbiology Guidelines. AMT's goal is to assist our customers by providing a screening test of a product formulation that is approaching finalization. It is expected that the formulation(s) submitted for Challenge testing contain AMT antimicrobials and have already passed the customer's internal stability tests. It is also anticipated that formal challenge testing of the final formulation will subsequently be performed by the customer at an outside lab of their choosing.

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® Liquid
M15008

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on October 23rd, 2013 and was completed on December 26th, 2013.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

Test Method

Fifty grams of Generic Cream Formula pH 5 with 2% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

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The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	1.3×10^7	2.4×10^7	1.4×10^7	3.3×10^5	3.1×10^5
Day 0*	99.869%	>99.999%	99.982%	>99.999%	99.896%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	3.5×10^6	3.2×10^6	1.8×10^6	1.2×10^5	2.9×10^5
Day 7	>99.999%	99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 5 with 2% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

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Challenge Test with 2.0% Leucidal® Liquid

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 5 with 2% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast were reduced by greater than 99.9% within 7 days of each challenge. The mold was reduced by greater than 90% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	71.15%
	Carbopol Ultrez 10	Lubrizol	0.10%
II	Dermol 816	Alzo International	5.00%
	Sunflower Oil	Arista Industries	5.00%
	Silderm Emulsifying CS	Active Concepts, LLC	2.00%
	Dermol 2014	Alzo International	5.00%
	Lipomulse 165	Lipo Chemicals	1.60%
	Lipowax D	Lipo Chemicals	1.60%
	Stearic Acid	Acme Hardesty Oleochemicals	0.25%
III	ACB Bamboo Bioferment	Active Concepts, LLC	2.00%
IV	Organic Rice Solution	Arbor Organic Technologies, LLC	5.00%
	ACB Bio-Chelate 5	Active Concepts, LLC	2.00%

Manufacturing Process:

Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Check pH and adjust to 6.0 – 7.0 with Citric Acid (50%) or NaOH (25%).

Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 15 minutes. Begin force cooling.

Phase III:
Add ingredients at 50^{0}C and mix until homogenous.

Phase IV:
Add ingredients and mix until homogenous.

Specifications:

Appearance: White to Off-White Emulsion
pH: 4.0 – 5.5
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

Leucidal® Liquid
M15008

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on October 23rd, 2013 and was completed on December 26th, 2013.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

Test Method

Fifty grams of Generic Cream Formula pH 7 with 2% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

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The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	1.3×10^7	2.4×10^7	1.4×10^7	3.3×10^5	3.1×10^5
Day 0*	99.949%	99.995%	99.971%	99.984%	99.958%
Day 7	99.999%	>99.999%	>99.999%	99.993%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	3.5×10^6	3.2×10^6	1.8×10^6	1.2×10^5	2.9×10^5
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 7 with 2% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

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The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 7 with 2% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast were reduced by greater than 99.9% within 7 days of each challenge. The mold was reduced by greater than 90% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	71.15%
	Carbopol Ultrez 10	Lubrizol	0.10%
II	Dermol 816	Alzo International	5.00%
	Sunflower Oil	Arista Industries	5.00%
	Silderm Emulsifying CS	Active Concepts, LLC	2.00%
	Dermol 2014	Alzo International	5.00%
	Lipomulse 165	Lipo Chemicals	1.60%
	Lipowax D	Lipo Chemicals	1.60%
	Stearic Acid	Acme Hardesty Oleochemicals	0.25%
III	ACB Bamboo Bioferment	Active Concepts, LLC	2.00%
IV	Organic Rice Solution	Arbor Organic Technologies, LLC	5.00%
	ACB Bio-Chelate 5	Active Concepts, LLC	2.00%

Manufacturing Process:

Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Check pH and adjust to 6.0 – 7.0 with Citric Acid (50%) or NaOH (25%).

Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 15 minutes. Begin force cooling.

Phase III:
Add ingredients at 50^{0}C and mix until homogenous.

Phase IV:
Add ingredients and mix until homogenous.

Specifications:

Appearance: White to Off-White Emulsion
pH: 4.0 – 5.5
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

The intent of performing an Antimicrobial Efficacy or Challenge test is to evaluate whether an antimicrobial agent or preservation system in a given cosmetic formulation has the ability to prevent the growth of test microorganisms. The test methodology employed by Active Micro Technologies (AMT) is based on the methods published in the CTFA Microbiology Guidelines. AMT's goal is to assist our customers by providing a screening test of a product formulation that is approaching finalization. It is expected that the formulation(s) submitted for Challenge testing contain AMT antimicrobials and have already passed the customer's internal stability tests. It is also anticipated that formal challenge testing of the final formulation will subsequently be performed by the customer at an outside lab of their choosing.

The information contained in this report is provided by Active Micro Technologies after the exercise of all reasonable care and skill in its compilation, preparation, and issue. It is provided without liability regarding its subsequent application and use. This type of screening test will be conducted only for validation of the efficacy of the antimicrobial agent or preservative system in the specific formulation tested. It does not address the suitability of the overall formula, nor does it address the regulatory status of any component therein. This testing does not account for the possibility of environmental microorganisms and cannot be relied upon as sufficient to justify commercialization of the product tested. By submitting samples for testing, the customer acknowledges that they will not hold Active Micro Technologies responsible for products launched based solely on the support of these studies.

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

AMTicide® Coconut
Leucidal® Liquid

Test Request #:

1277

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on February 25th, 2015 and was completed on April 27th, 2015.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

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Test Method

Fifty grams of Generic Cream Formula pH 3 with 4% AMTicide® Coconut and 2% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	4.8×10^6	7.8×10^6	9.7×10^6	1.3×10^5	5.4×10^5
Day 0*	99.999%	99.999%	99.999%	99.999%	99.999%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	7.3×10^6	6.7×10^6	6.4×10^6	2.1×10^5	6.8×10^5
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 3 with 4% AMTicide® Coconut and 2% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 3 with 4% AMTicide® Coconut and 2% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.9% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	85.2
	Carbopol Ultrez 10	Lubrizol	0.1
	Glycerin	PT. Musim Mas	3.0
	Tealan	RITA	0.9
II	Cetyl Alcohol	RITA	2.0
	Stearic Acid	Acme Hardesty Oleochemicals	0.8
	Glyceryl Stearate	Protameen Chem.	1.5
	Isopropyl Myristate	Alzo	1.5
	Mineral Oil	RITA	5.0

Manufacturing Process:

1. Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Add the rest of ingredients.

2. Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 30 minutes. Begin force cooling to 25^{0}C.

3. Check the pH.

Specifications:

Appearance: White to Off-White Emulsion
pH: 6.5 – 8.0
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

The intent of performing an Antimicrobial Efficacy or Challenge test is to evaluate whether an antimicrobial agent or preservation system in a given cosmetic formulation has the ability to prevent the growth of test microorganisms. The test methodology employed by Active Micro Technologies (AMT) is based on the methods published in the CTFA Microbiology Guidelines. AMT's goal is to assist our customers by providing a screening test of a product formulation that is approaching finalization. It is expected that the formulation(s) submitted for Challenge testing contain AMT antimicrobials and have already passed the customer's internal stability tests. It is also anticipated that formal challenge testing of the final formulation will subsequently be performed by the customer at an outside lab of their choosing.

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

AMTicide® Coconut
Leucidal® Liquid

Test Request #:

1278

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on February 25th, 2015 and was completed on April 27th, 2015.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

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Test Method

Fifty grams of Generic Cream Formula pH 5 with 4% AMTicide® Coconut and 2% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	4.8×10^6	7.8×10^6	9.7×10^6	1.3×10^5	5.4×10^5
Day 0*	99.931%	99.998%	99.918%	99.969%	99.995%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	7.3×10^6	6.7×10^6	6.4×10^6	2.1×10^5	6.8×10^5
Day 7	>99.999%	>99.999%	99.965%	99.995%	99.997%
Day 14	>99.999%	>99.999%	99.985%	>99.999%	99.998%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 5 with 4% AMTicide® Coconut and 2% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 5 with 4% AMTicide® Coconut and 2% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.9% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	85.2
	Carbopol Ultrez 10	Lubrizol	0.1
	Glycerin	PT. Musim Mas	3.0
	Tealan	RITA	0.9
II	Cetyl Alcohol	RITA	2.0
	Stearic Acid	Acme Hardesty Oleochemicals	0.8
	Glyceryl Stearate	Protameen Chem.	1.5
	Isopropyl Myristate	Alzo	1.5
	Mineral Oil	RITA	5.0

Manufacturing Process:

1. Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Add the rest of ingredients.

2. Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 30 minutes. Begin force cooling to 25^{0}C.

3. Check the pH.

Specifications:

Appearance: White to Off-White Emulsion
pH: 6.5 – 8.0
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

The intent of performing an Antimicrobial Efficacy or Challenge test is to evaluate whether an antimicrobial agent or preservation system in a given cosmetic formulation has the ability to prevent the growth of test microorganisms. The test methodology employed by Active Micro Technologies (AMT) is based on the methods published in the CTFA Microbiology Guidelines. AMT's goal is to assist our customers by providing a screening test of a product formulation that is approaching finalization. It is expected that the formulation(s) submitted for Challenge testing contain AMT antimicrobials and have already passed the customer's internal stability tests. It is also anticipated that formal challenge testing of the final formulation will subsequently be performed by the customer at an outside lab of their choosing.

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

AMTicide® Coconut
Leucidal® Liquid

Test Request #:

1101

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on January 12th, 2015 and was completed on March 9th, 2015.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

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Test Method

Fifty grams of Generic Cream Formula pH 7 with 4% AMTicide® Coconut and 2% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	4.5×10^6	7.8×10^6	3.1×10^6	4.0×10^5	5.4×10^5
Day 0*	99.931%	99.998%	99.744%	99.990%	99.951%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	3.5×10^6	3.2×10^6	1.8×10^6	1.2×10^5	2.9×10^5
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 7 with 4% AMTicide® Coconut and 2% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 7 with 4% AMTicide® Coconut and 2% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

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Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.9% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	85.2
	Carbopol Ultrez 10	Lubrizol	0.1
	Glycerin	PT. Musim Mas	3.0
	Tealan	RITA	0.9
II	Cetyl Alcohol	RITA	2.0
	Stearic Acid	Acme Hardesty Oleochemicals	0.8
	Glyceryl Stearate	Protameen Chem.	1.5
	Isopropyl Myristate	Alzo	1.5
	Mineral Oil	RITA	5.0

Manufacturing Process:

1. Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Add the rest of ingredients.

2. Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 30 minutes. Begin force cooling to 25^{0}C.

3. Check the pH.

Specifications:

Appearance: White to Off-White Emulsion
pH: 6.5 – 8.0
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

The intent of performing an Antimicrobial Efficacy or Challenge test is to evaluate whether an antimicrobial agent or preservation system in a given cosmetic formulation has the ability to prevent the growth of test microorganisms. The test methodology employed by Active Micro Technologies (AMT) is based on the methods published in the CTFA Microbiology Guidelines. AMT's goal is to assist our customers by providing a screening test of a product formulation that is approaching finalization. It is expected that the formulation(s) submitted for Challenge testing contain AMT antimicrobials and have already passed the customer's internal stability tests. It is also anticipated that formal challenge testing of the final formulation will subsequently be performed by the customer at an outside lab of their choosing.

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

AMTicide® Coconut
Leucidal® Liquid

Test Request #:

1175

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on February 25th, 2015 and was completed on April 27th, 2015.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

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Test Method

Fifty grams of Generic Cream Formula pH 3 with 2% AMTicide® Coconut and 2% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	4.8×10^6	7.8×10^6	9.7×10^6	1.3×10^5	5.4×10^5
Day 0*	99.999%	99.999%	99.999%	99.999%	99.981%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	7.3×10^6	6.7×10^6	6.4×10^6	2.1×10^5	6.8×10^5
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 3 with 2% AMTicide® Coconut and 2% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 3 with 2% AMTicide® Coconut and 2% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.9% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	85.2
	Carbopol Ultrez 10	Lubrizol	0.1
	Glycerin	PT. Musim Mas	3.0
	Tealan	RITA	0.9
II	Cetyl Alcohol	RITA	2.0
	Stearic Acid	Acme Hardesty Oleochemicals	0.8
	Glyceryl Stearate	Protameen Chem.	1.5
	Isopropyl Myristate	Alzo	1.5
	Mineral Oil	RITA	5.0

Manufacturing Process:

1. Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Add the rest of ingredients.

2. Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 30 minutes. Begin force cooling to 25^{0}C.

3. Check the pH.

Specifications:

Appearance: White to Off-White Emulsion
pH: 6.5 – 8.0
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

AMTicide® Coconut
Leucidal® Liquid

Test Request #:

1176

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on February 25th, 2015 and was completed on April 27th, 2015.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

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Test Method

Fifty grams of Generic Cream Formula pH 5 with 2% AMTicide® Coconut and 2% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	4.8×10^6	7.8×10^6	9.7×10^6	1.3×10^5	5.4×10^5
Day 0*	99.939%	99.993%	99.954%	99.969%	99.951%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	7.3×10^6	6.7×10^6	6.4×10^6	2.1×10^5	6.8×10^5
Day 7	>99.999%	>99.999%	99.950%	99.992%	99.996%
Day 14	>99.999%	>99.999%	99.997%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 5 with 2% AMTicide® Coconut and 2% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 5 with 2% AMTicide® Coconut and 2% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.9% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	85.2
	Carbopol Ultrez 10	Lubrizol	0.1
	Glycerin	PT. Musim Mas	3.0
	Tealan	RITA	0.9
II	Cetyl Alcohol	RITA	2.0
	Stearic Acid	Acme Hardesty Oleochemicals	0.8
	Glyceryl Stearate	Protameen Chem.	1.5
	Isopropyl Myristate	Alzo	1.5
	Mineral Oil	RITA	5.0

Manufacturing Process:

1. Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Add the rest of ingredients.

2. Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 30 minutes. Begin force cooling to 25^{0}C.

3. Check the pH.

Specifications:

Appearance: White to Off-White Emulsion
pH: 6.5 – 8.0
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Product

AMTicide® Coconut
Leucidal® Liquid

Test Request #:

1100

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on January 12th, 2015 and was completed on March 9th, 2015.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

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Test Method

Fifty grams of Generic Cream Formula pH 7 with 2% AMTicide® Coconut and 2% Leucidal® Liquid was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	4.5×10^6	7.8×10^6	3.1×10^6	4.0×10^5	5.4×10^5
Day 0*	99.939%	99.993%	99.858%	99.995%	99.981%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	3.5×10^6	3.2×10^6	1.8×10^6	1.2×10^5	2.9×10^5
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream Formula pH 7 with 2% AMTicide® Coconut and 2% Leucidal® Liquid inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream Formula pH 7 with 2% AMTicide® Coconut and 2% Leucidal® Liquid. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

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Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.9% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.999% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	85.2
	Carbopol Ultrez 10	Lubrizol	0.1
	Glycerin	PT. Musim Mas	3.0
	Tealan	RITA	0.9
II	Cetyl Alcohol	RITA	2.0
	Stearic Acid	Acme Hardesty Oleochemicals	0.8
	Glyceryl Stearate	Protameen Chem.	1.5
	Isopropyl Myristate	Alzo	1.5
	Mineral Oil	RITA	5.0

Manufacturing Process:

1. Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75^{0}C. Slowly sift in Carbopol while mixing. Add the rest of ingredients.

2. Phase II:
In a separate beaker, combine ingredients and heat to 75^{0}C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 75^{0}C and mix for 30 minutes. Begin force cooling to 25^{0}C.

3. Check the pH.

Specifications:

Appearance: White to Off-White Emulsion
pH: 6.5 – 8.0
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

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Time Kill Test

E2315

Assessment of Antimicrobial Activity Using a Time Kill Procedure

Product

Leucidal® Liquid

Test Request #:

1808

Purpose

This study was initiated to measure the change in population of aerobic microorganisms within a specified sampling time when tested against a cosmetic ingredient.

Study Dates

The study was started on March 8th, 2016 and was completed on March 15th, 2016.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Bacillus subtilis* ATCC #6051
5. *Aspergillus brasiliensis:* ATCC #16404
6. *Candida albicans:* ATCC #10231

Neutralization:

Inactivation of the antimicrobial activity of the test material is achieved through the dilution of the test material during the sampling time at specified sampling intervals.

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Test Method

Ten grams of 4% Leucidal® Liquid solution was weighed into six individual containers. Each container was inoculated with one of the six test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition. Serial dilutions from each container were performed to enumerate the surviving microorganisms using the Plate Count Technique.

The activity of the test material inoculated was evaluated at determine time intervals of 30 seconds, 1, 5, 10 and 30 minutes after the inoculation to determine quantitatively the number of viable microorganisms remaining after the incubation time.

Organisms	Inoculum Concentration CFU/ml	Percentage of Reduction				
		30 seconds	1 minute	5 minute	10 minute	30 minutes
E.coli * ATCC# 8739	6.1×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
S.aureus ATCC# 6538	6.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
P.aeruginosa ATCC# 9027	4.6×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
B.subtilis ATCC# 6051	5.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
A.brasiliensis ATCC# 16404	4.6×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
C.albicans ATCC# 10231	2.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%

Table 1. Time Kill Test results for 4% Leucidal® Liquid inoculated with 10^6 microorganisms' population.
Results show % reduction in viable organisms after inoculation and sampling time intervals.

*Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results of this Time Kill Test determine the changes in population of aerobic microorganisms within a specified sampling time when tested against 4% Leucidal® Liquid solution.

The Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.9% within 30 seconds interval of the test after the inoculation.

Leucidal® Liquid Efficacy vs. *Propionibacterium acnes*

ACTIVE MICRO TECHNOLOGIES

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Abstract

Propionibacterium acnes is a gram positive, non-spore-forming, microaerophilic, rod-shaped bacterium that is a common inhabitant of human skin. This microorganism metabolizes fatty acids created by sebaceous glands. The combination of fatty acid metabolites and antigens produced by the bacteria can create intense localized areas of inflammation that can fracture hair follicles. As a consequence, lesions develop on the surface of the skin in the form of pustules. This condition is commonly known as acne.

The purpose of this study was to determine the bactericidal efficacy of **Leucidal® Liquid** against *P. acnes* by establishing the minimum inhibitory concentration (MIC) required to inhibit its growth and proliferation. For comparative purposes, an over-the-counter acne treatment product was used as a benchmark. According to the MIC results, **Leucidal® Liquid** is capable of effectively inhibiting the growth of *P. acnes* at a significantly lower concentration than that of the benchmark product.

Materials and Methods

The products tested were **Leucidal® Liquid** and an over-the-counter, deep cleaning astringent that contains 2% salicylic acid (Benchmark). Each product was tested by preparing a serial dilution in a growth medium, beginning with an initial product concentration of 100%.

To determine the Minimum Inhibitory Concentration (MIC) of each product against *P. acnes,* a standard 9% saline solution was added to a test tube using a sterile pipette. Enough bacteria were added to the saline solution using a sterile loop to match the turbidity of a 0.5 McFarland standard. Two milliliters of this bacterial suspension were then transferred to one additional milliliter of 9% saline solution. Afterwards, 300 μL of the diluted mixture were added to 30 ml of sterile water yielding a final bacterial concentration of approximately 10^6 colony forming units (cfu)/ml. Using an 8-tip pipettor, 150 μL of double strength Tryptic Soy Broth (TSB) were added to the first row of wells in a sterile microwell plate. Then, 150 μL of single strength TSB were pipetted into the remaining rows of the plate.

150 μL of **Leucidal® Liquid** was pipetted into the first row of wells containing the double-strength TSB and mixed 5 times. 150 μL of this mixed material from the first row were then transferred via pipettor into the second row of wells and mixed 5 times. This procedure was repeated for each subsequent row, creating a serial dilution of the **Leucidal® Liquid** ranging from 50% to 0.05% concentration through the first 11 rows of the plate. The last row did not receive any of the serially diluted **Leucidal® Liquid**. This twelfth row, containing only single strength TSB, served as a positive control to demonstrate the viability of the diluted bacterial culture used to inoculate the plate. This same procedure was then repeated using the over-the-counter benchmark product.

Each plate was inoculated using an inoculating plate that had been dipped in the *P. acnes* inoculum suspension, prepared as previously described. The plates were incubated for 48 hours at 35 +/- 2°C. After the 48-hour incubation period the plates were examined for microbial growth, indicated by turbidity in the wells. The row of wells with the lowest concentration of tested product that remained clear (i.e., inhibited growth) was used to establish the MIC value.

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The following formula was used to calculate the MIC values:

$$\% \text{ MIC} = \frac{\text{Initial product concentration (\%) in Row 1}}{2^{\text{(last no growth row)}}}$$

Results

MIC Results		
Product Tested	**Last Clear Row**	**% MIC**
Leucidal® Liquid	6	1.563
Benchmark product	3	12.500

Table 1. MIC Results

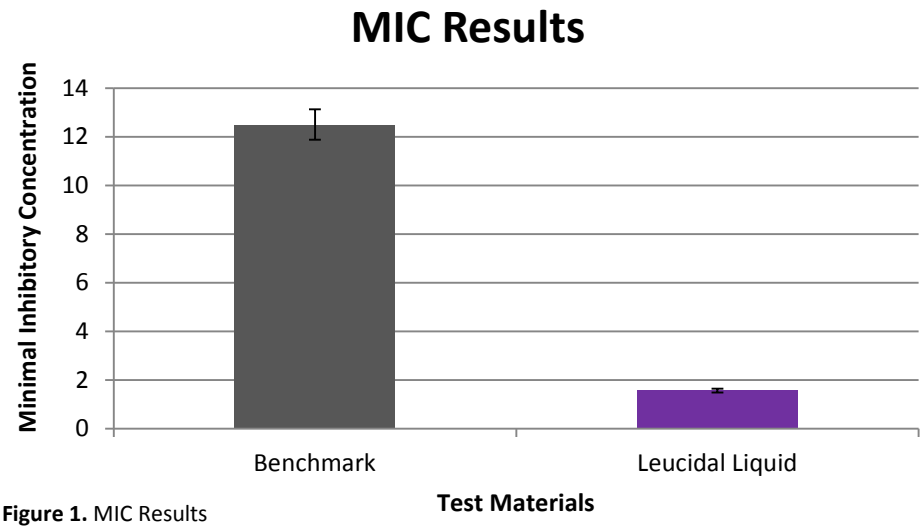

Figure 1. MIC Results

Discussion

Based on these results, we can confirm that **Leucidal® Liquid** is capable of inhibiting the growth of *Propionibacterium acnes* when used at a concentration of approximately 1.5%. This concentration is significantly lower than the 12.5% concentration that is required to equally inhibit growth when using the benchmark product containing 2% salicylic acid.

P. acnes has been identified as the primary factor that causes acne. By inhibiting the proliferation of this bacterium, one may significantly minimize acne formation. **Leucidal® Liquid** is a broad-spectrum antimicrobial that has been shown to be effective against the acne-causing bacterium *Propionibacterium acnes.* These properties make **Leucidal® Liquid** an effective ingredient for formulations developed to address problem skin.

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The following report evaluates a topical sample containing

Leucidal® Liquid (M15008) – AMA Lab No. O-0053

Provided by Active Concepts, LLC to AMA Laboratories, Inc.

An Investigation into the Efficacy of an Acne Treatment Product

June 19, 2015

Study Guidelines:

- The study consisted of 5 M/F subjects between the ages of 19-26 with mild to moderate facial acne.
- The subjects applied the topical sample twice a day (morning and evening) with a cotton swab to the acne affected facial areas for a total of 42 days.
- Subjects were evaluated at baseline and days 3, 7, 14, 30 and 42.

Topical Sample Composition	
Ingredient	**%**
Water	84.00
Leucidal® Liquid	10.00
Liposorb L-20	5.00
Keltrol	1.00

Figure 1. Topical Sample Composition with a pH of 5.8.

AMA LABORATORIES, INC.
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AN INVESTIGATION INTO THE EFFICACY OF AN ACNE TREATMENT PRODUCT

AMA Ref. Nos.: MS15.PHGX.ACNE.REP.O0053.AMT

Date: June 19, 2015

Sponsor: Active Micro Technologies, LLC
107 Technology Drive
Lincolnton, North Carolina 28092

1.0 Objective:

This panel has been convened to evaluate efficacy and tolerance of a topically applied test product in treatment of mild to moderate facial acne over a 30 day period. Counts of visible inflammatory and non-inflammatory acne lesions were conducted by Expert Clinical Evaluator. Each stage in the progression of treatment was photographically documented using highly developed High Resolution Matched Scientific Photography and measured via PhotoGrammetrix™ Image Analysis.

2.0 Test Material:

2.1 Test Sample Description:

On April 6, 2015 test samples labeled Liquid Topical Preparation, Lot # NC150401-E were received from Active Concepts, LLC and assigned AMA Lab No. O-0053.

2.2 Handling:

Upon arrival at AMA Laboratories, Inc., the test material was assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and test requested.

Samples are retained for a period of three months beyond submission of final report unless otherwise specified by the sponsor or if sample is known to be in support of governmental applications, in which case retained samples are kept two years beyond final report submission.

Sample disposition is conducted in compliance with appropriate federal, state and local ordinances.

2.3 Test Material Evaluation Prerequisite:

Prior to induction of a human test panel, animal toxicology, microbiology and other in-vitro performance spectra may be required to assess the feasibility of commencement as dictated by an Institutional Review Board (IRB) described in Section 3.0.

Sponsor purports that prior to sample submission to AMA the following tests were conducted with no adverse results and that the test data are on file at their premises and have not been made available to AMA personnel:

- CTFA Preservative Efficacy Test or equivalent
- 90 Day Accelerated Stability and Container Compatibility Study

3.0 Institutional Review Board:

Reference: CFR Title 21 Part 56, Subparts A, B, C, and D. The IRB of AMA Laboratories, Inc., consists of five or more individuals, chosen from within the company for technical expertise and also from the local community for lay interaction. The list of IRB members is kept on file at AMA Laboratories, Inc., and is available for inspection during the hours of operation.

4.0 Panel Selection:

4.1 Standards for Inclusion in a Study:

a. Male and/or female subjects 18 years of age or older with mild to moderate facial acne as confirmed by the Study Director.
b. Individuals who will complete a preliminary medical history and screening document as mandated by AMA Laboratories, Inc.
c. Individuals who will read, understand and sign an informed consent document as required by Reference 21 CFR Ch. 1 Part 50, Subpart B. Consent forms will be kept on file and will be available for examination on the premises of AMA Laboratories, Inc., only.
d. Individuals in general good health and free of any health problems, including neurological, dermatological, or systemic disorder that would interfere with the results, at the discretion of the Study Director.

e. Individuals able to cooperate with the Investigator and research staff, willing to have the test material(s) applied according to the protocol, and complete the full course of study.
f. Individuals who have abstained from using any anti-acne products for a period of 72 hours prior to study commencement and who will use only the assigned test material during the test period.

4.2 Standards for Exclusion from a Study:

a. Individuals who are under the care of a physician.
b. Individuals currently taking medication that may mask or interfere with the test results.
c. Individuals diagnosed with chronic skin allergies.
d. Females who are pregnant, lactating, have been pregnant, or given birth within the six month period immediately preceding study commencement.
e. Subjects with a history of any form of skin cancer, melanoma, lupus, psoriasis, connective tissue disease, diabetes, or any disease that would increase the risk associated with study participation.
f. Individuals with irritation or sensitivity to any cosmetic products in general and acne treatment products in particular.

4.3 Recruitment:

Panel selection is accomplished by advertisements in local periodicals, community bulletin boards, phone solicitation, electronic media or any combination thereof.

4.4 Informed Consent Document:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form and screening form. These forms, along with the signed consent forms, are available for inspection on the premises of AMA Laboratories, Inc., only. Reference 21 CFR Ch.1 Part 50, Subpart B.

5.0 Population Demographics:

Number of subjects enrolled		5
Number of subjects completing study		5
Age Range		19 - 26
Sex	Male	1
	Female	4
Race	Caucasian	5

6.0 Study Design:

Five panelists exhibiting mild to moderate (Grade 2-3) facial acne were inducted into this study. All participants were advised of the general nature and purpose of the study, and were required to complete medical history forms and informed consent document. Subjects were mandated to adhere to all the restrictions mentioned in the inclusion/exclusion criteria (sections 4.1 and 4.2).

On the initial day of the study, Study Director graded acne condition of each panelist using the Investigator's Global Assessment Scale for Acne Vulgaris recommended by FDA 2005 Guidance.

IGA Scale for Acne Vulgaris [ref. 1]**:**

0 – Clear almost with no inflammatory or non-inflammatory lesions
1 – Almost clear; rare non-inflammatory lesions with no more than one small inflammatory lesion
2 – Mild severity; greater than Grade 1; some non-inflammatory lesions with no more than a few inflammatory lesions (papules/pustules only, no nodular lesions)
3 – Moderate severity; greater than Grade 2; up to many non-inflammatory lesions and may have some inflammatory lesions, but no more than one small nodular lesion
4 – Severe; greater than Grade 3; up to many non-inflammatory and inflammatory lesions, but no more than a few nodular lesions

The study was conducted according to sponsor requested design wherein panelists were instructed to use the test product as follows:

- *Apply with a cotton swab twice a day, in morning and evening*

All subjects were instructed to apply the test product to acne affected facial areas for a period of 42 days.

Study participants were provided with a brief diary to record time of test material application along with any comments related to product usage.

On each evaluation day (at baseline, days 3, 7, 14, 30 and 42) counts of visible inflammatory and non-inflammatory acne lesions were conducted.

Subjects were instructed to report any adverse reactions which might occur during the course of the study. Clients are notified immediately in the case of an adverse reaction and a determination is made as to treatment regimen, if necessary.

Reverse Photo Engineering:

Exclusively detailed, high resolution matched digital photographs were taken, at baseline and again after 3, 7, 14, 30 and 42 days of use. Photographs were taken with fixed camera background, distances, angles, settings, lighting, panelist positioning, color bars, white balance, standardized and digitally certified unretouched. Each stage in the progression of the treatment regimen was photographically documented and the test area of involvement isolated. Photographs were evaluated using PhotoGrammetrix™ Image Analysis which allows areas associated with acne to be captured and quantified, thus providing a visual record of the efficacy of the product.

7.0 Results:

Please refer to attached Tables and Charts.

8.0 Observations:

No adverse effects or unexpected reactions of any kind were observed on any of the subjects during the course of the study.

9.0 Archiving:

All original samples, raw data sheets, technician's notebooks, correspondence files, copies of final reports and remaining specimens are maintained on premises of AMA Laboratories in limited access, marked storage files. A duplicate DVD copy of final reports is separately archived in a bank safe deposit vault.

10.0 References:

1. Guidance for Industry Acne Vulgaris: Developing Drugs for Treatment. U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER). September 2005.
http://www.fda.gov/cder/guidance/6499dft.htm

2. Draize J.H. Dermal and eye toxicity tests. In: Principles and procedures for evaluating the toxicity of household substances. Washington, DC: National Academy of Sciences, 1997:31-2.

11.0 Security Label Disclosure:

To prevent loss of and protect intellectual property, original, certified documents issued by AMA Laboratories Inc. can be identified by a proprietary, tamper evident security hologram affixed to all Conclusion/Signature pages on final reports. Any attempt to remove the hologram will irreversibly damage the label and leave an immediate trace, thus invalidating the document.

Only reports containing the AMA LABS, INC. hologram intact will be recognized by AMA Laboratories Inc. as a certified original.

12.0 Conclusions:

Within the limits imposed by the conduct and population size of the study described herein, the following conclusions are drawn:

The test material (AMA Lab No.: O-0053; Client No.: Liquid Topical Preparation, Lot # NC150401-E) was found to be effective in improving facial acne condition by reducing the mean number of total acne lesions (41.82% reduction) as well as improving overall appearance of the skin.

Total Acne Lesion (Comedones+Papules+Pustules) Counts – SUMMARY					
Time Point:	Day 3	Day 7	Day 14	Day 30	Day 42
% Difference:	*-3.64%*	*-15.45%*	*-22.73%*	*-23.64%*	*-41.82%*

Moreover, the data obtained via PhotoGrammetrix™ Image Analysis demonstrated that the test product reduced facial acne condition by an average of 68.65% with maximum improvement of 93.00% over a 42 day period.

Reverse Photo Engineering - Acne Reduction Analysis - SUMMARY					
Time Point:	Day 3	Day 7	Day 14	Day 30	Day 42
% Difference:	*-60.32%*	*-54.47%*	*-55.48%*	*-32.88%*	*-68.65%*

Mayya Tatsene

Mayya Tatsene, M.D.
Study Director

James Van Zetta, B.A. Candidate
Photography Department Study Director

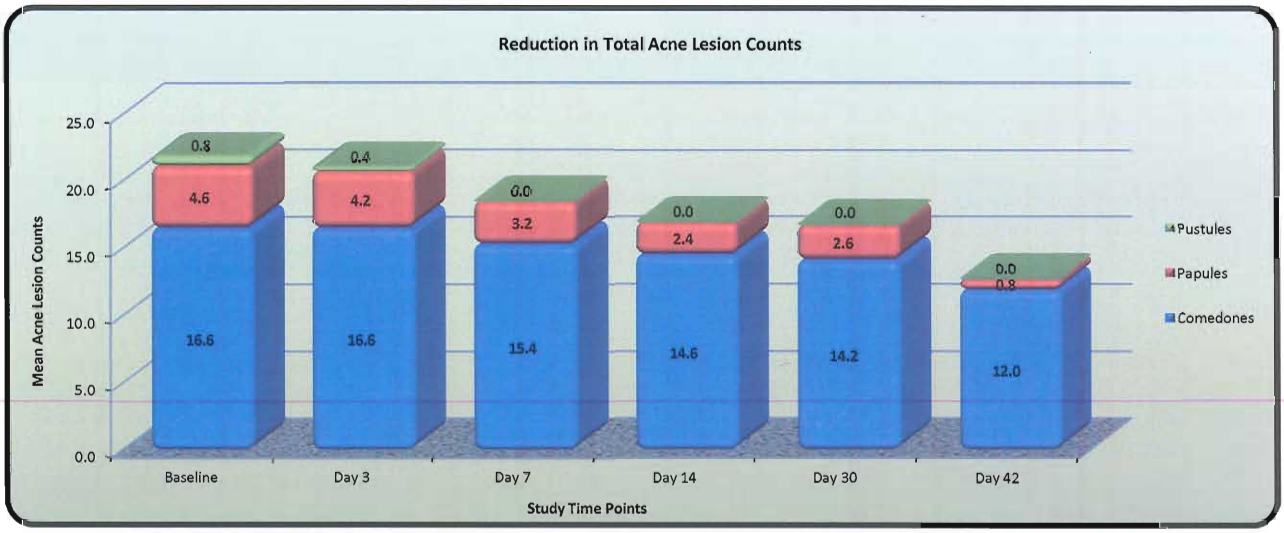
Claudia Cohen, A.A.
Photography Department Coordinator

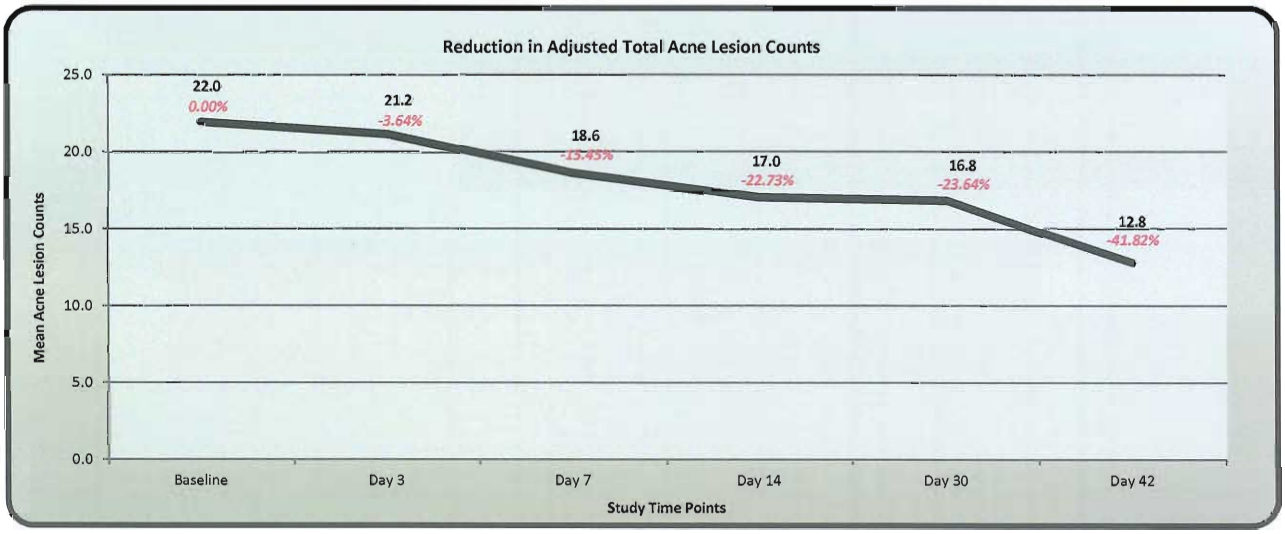
David R. Winne, B.S.
Technical Director

6/19/15

Date

AN INVESTIGATION INTO THE EFFICACY OF AN ACNE TREATMENT REGIMEN - SUMMARY													
AMA Lab Nos.:	Client Nos.:												
O-0053	Liquid Topical Preparation, Lot # NC150401-E												
Panelist ID Number:	Baseline Acne Lesion Counts:				Day 3 Acne Lesion Counts:				Day 7 Acne Lesion Counts:				
	Non-Inflammatory	Inflammatory		Total	Non-Inflammatory	Inflammatory		Total	Non-Inflammatory	Inflammatory		Total
	Comedones	Papules	Pustules		Comedones	Papules	Pustules		Comedones	Papules	Pustules	
80 2591	12	1	1	14	12	2	0	14	11	1	0	12
84 1537	11	6	3	20	11	6	2	19	10	6	0	16
73 3502	24	5	0	29	24	3	0	27	22	2	0	24
84 5853	9	3	0	12	9	3	0	12	8	2	0	10
94 5905	27	8	0	35	27	7	0	34	26	5	0	31
Mean:	16.6	4.6	0.8	22.0	16.6	4.2	0.4	21.2	15.4	3.2	0.0	18.6
		2.7				2.3				1.6		
% Difference:	0.00%	0.00%	0.00%	0.00%	0.00%	-8.70%	-50.00%	-3.64%	-7.23%	-30.43%	-100.00%	-15.45%
		0.00%				-14.81%				-40.74%		
Panelist ID Number:	Day 14 Acne Lesion Counts:				Day 30 Acne Lesion Counts:				Day 42 Acne Lesion Counts:			
	Non-Inflammatory	Inflammatory		Total	Non-Inflammatory	Inflammatory		Total	Non-Inflammatory	Inflammatory		Total
	Comedones	Papules	Pustules		Comedones	Papules	Pustules		Comedones	Papules	Pustules	
80 2591	10	1	0	11	9	2	0	11	8	0	0	8
84 1537	10	4	0	14	10	3	0	13	7	1	0	8
73 3502	21	1	0	22	21	2	0	23	18	0	0	18
84 5853	8	2	0	10	8	2	0	10	6	1	0	7
94 5905	24	4	0	28	23	4	0	27	21	2	0	23
Mean:	14.6	2.4	0.0	17.0	14.2	2.6	0.0	16.8	12.0	0.8	0.0	12.8
		1.2				1.3				0.4		
% Difference:	-12.05%	-47.83%	-100.00%	-22.73%	-14.46%	-43.48%	-100.00%	-23.64%	-27.71%	-82.61%	-100.00%	-41.82%
		-55.56%				-51.85%				-85.19%		





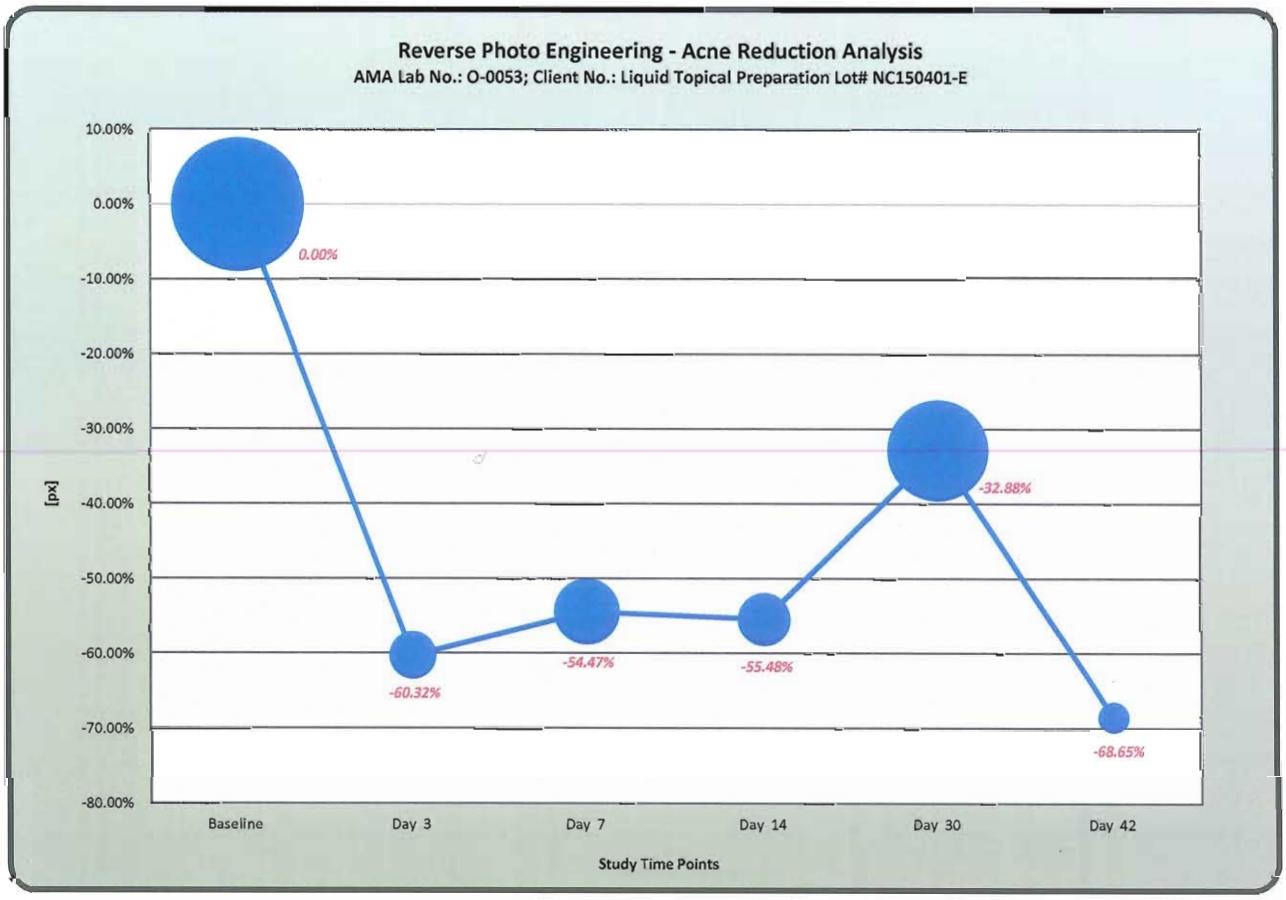
Reverse Photo Engineering - Acne Reduction Analysis											
AMA Lab No.:	Client No.:										
O-0053	Liquid Topical Preparation Lot# NC150401-E										
Panelist ID Number:	Baseline [px]	Day 3 [px]	Individual % Difference	Day 7 [px]	Individual % Difference	Day 14 [px]	Individual % Difference	Day 30 [px]	Individual % Difference	Day 42 [px]	Individual % Difference
73 3502	3341	11	-99.67%	2007	-39.93%	117	-96.50%	489	-82.36%	234	-93.00%
80 2591	2268	452	-80.07%	271	-88.05%	186	-91.80%	46	-97.97%	225	-90.08%
84 1537	15738	10178	-35.33%	5785	-63.24%	7388	-53.06%	7665	-51.30%	8583	-45.46%
84 5853	30883	9778	-68.34%	17403	-43.65%	17705	-42.67%	30851	-0.10%	8890	-71.21%
94 5905	7063	3109	-55.98%	1530	-78.34%	1001	-85.83%	748	-89.41%	657	-90.70%
Average:	11858.60	4705.60		5399.20		5279.40		7959.80		3717.80	
Average % Difference		-60.32%		-54.47%		-55.48%		-32.88%		-68.65%	
Maximum % Reduction		-99.67%		-88.05%		-96.50%		-97.97%		-93.00%	
p		0.113		0.050		0.029*		0.055		0.086	
t		2.021		2.773		3.322*		2.687		2.265	

* Statistically significant

Reverse Photo Engineering Exclusively detailed, high resolution before and after digital photography was taken, with fixed camera background, distances, angles, settings, lighting, panelist positioning, color bars, white balance, standardized and digitally certified unretouched. Each stage in the progression of the treatment regimen was photographically documented and the test area of involvement isolated. Photographs were evaluated using image analysis software which allows the Acne to be captured and quantified. The size of the area of involvement differed for each test panelist, therefore percent difference was calculated individually and then averaged.

Student's t-test was used in this investigation. This is the test of the null hypothesis that the difference between two responses measured on the same statistical unit has a mean value of zero. In this investigation the changes in acne (area affected by acne measured in px2) before and after the treatment were measured. If the treatment is effective, we expect the area affected by acne for many of the patients to be smaller following the treatment. This is often referred to as the "paired" or "repeated measures" t-test. Dependent samples (or "paired") t-tests typically consist of a sample of matched pairs of similar units, or one group of units that has been tested twice (a "repeated measures" t-test). Once a t value is determined, a p-value can be found using a table of values from Student's t-distribution. If the calculated p-value is below the threshold chosen for statistical significance (0.05 (5%)), then the null hypothesis (Null Hypothesis p>0.05) is rejected in favor of the alternative hypothesis.

Statistical analysis was computed using appropriate Excel statistical software functions, where one function returns the probability associated with a Student's t-Test and the other returns the t-value of the Student's t-distribution as a function of the probability and the degrees of freedom.



13.0 Quality Assurance Statement:

This study was inspected in accordance with the Standard Operating Procedures of AMA Laboratories, Inc. To assure compliance with the study protocol, the Quality Assurance Unit completed an audit of the study records and report.

Report reviewed by:

Christian Gorgione

Christian Gorgione, B.S.
Quality Assurance Supervisor

6/19/15
Date

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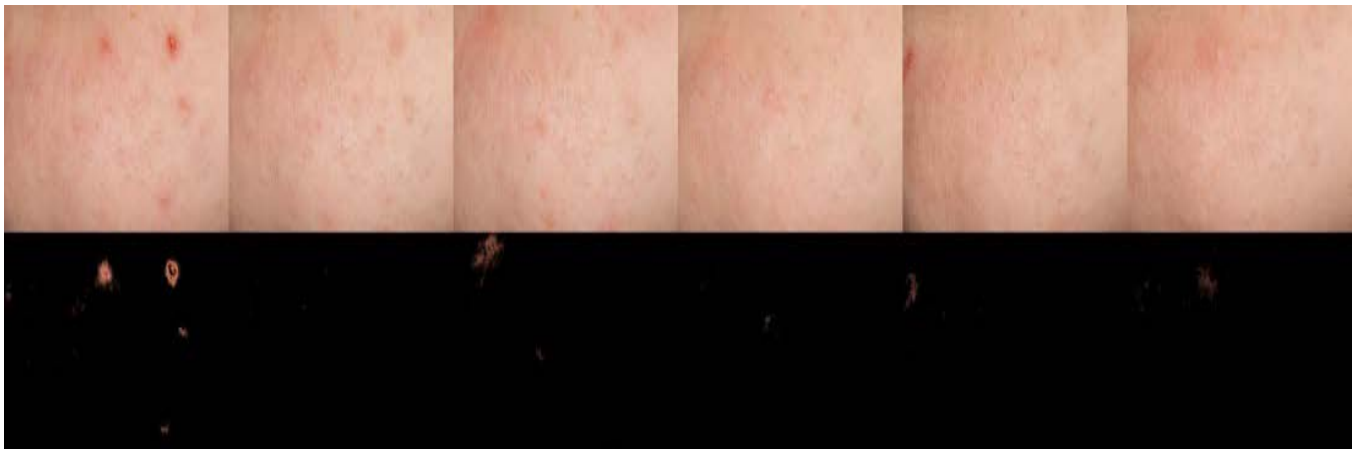
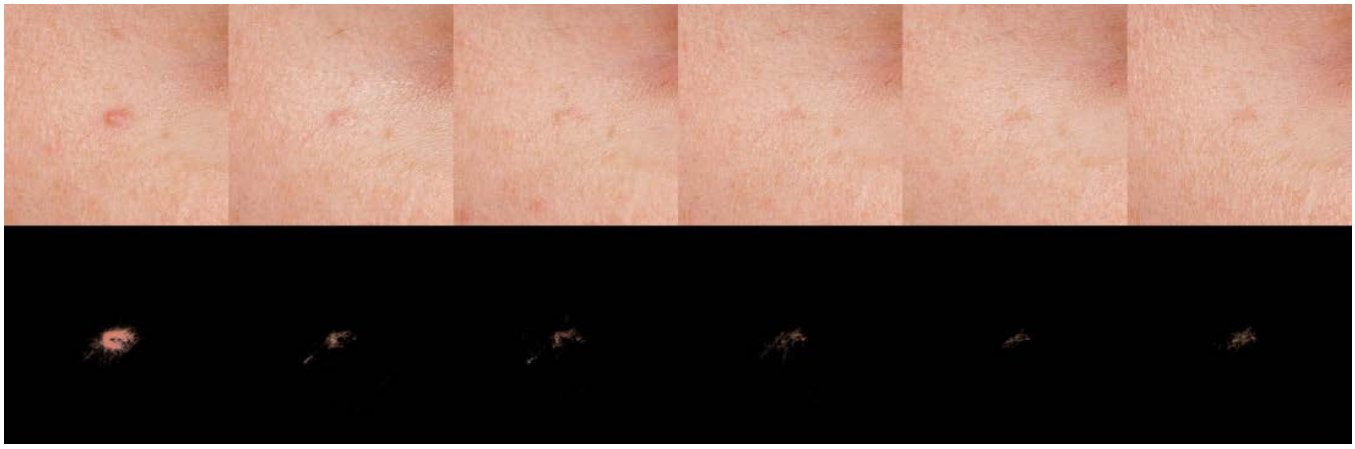
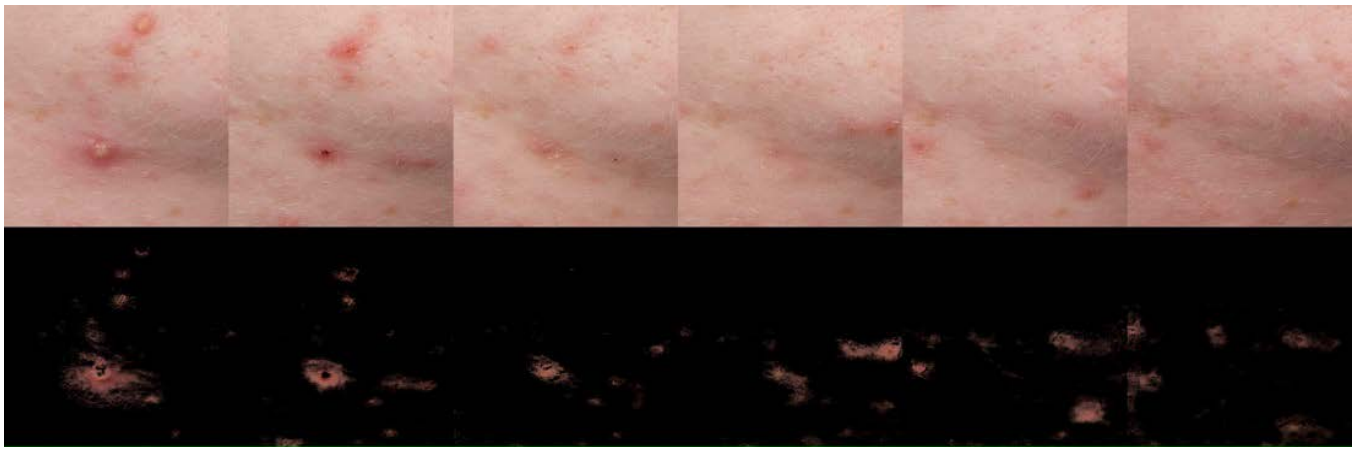
High Resolution Digital Photographs



Baseline	Day 3	Day 7	Day 14	Day 30	Day 42
3,341PX	11PX 99.67% Acne Reduction	2,007PX 39.93% Acne Reduction	117PX 96.5% Acne Reduction	117PX 85.36% Acne Reduction	234PX 93.00% Acne Reduction

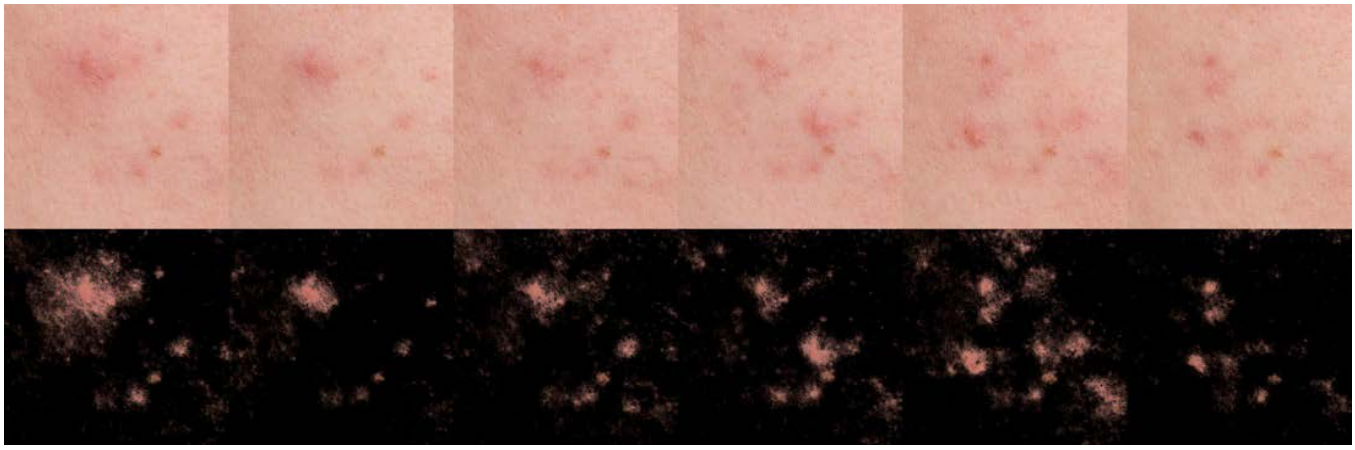


Baseline	Day 3	Day 7	Day 14	Day 30	Day 42
2,268PX	452PX 80.07% Acne Reduction	271PX 88.05% Acne Reduction	186PX 91.80% Acne Reduction	46PX 97.97% Acne Reduction	225PX 90.08% Acne Reduction

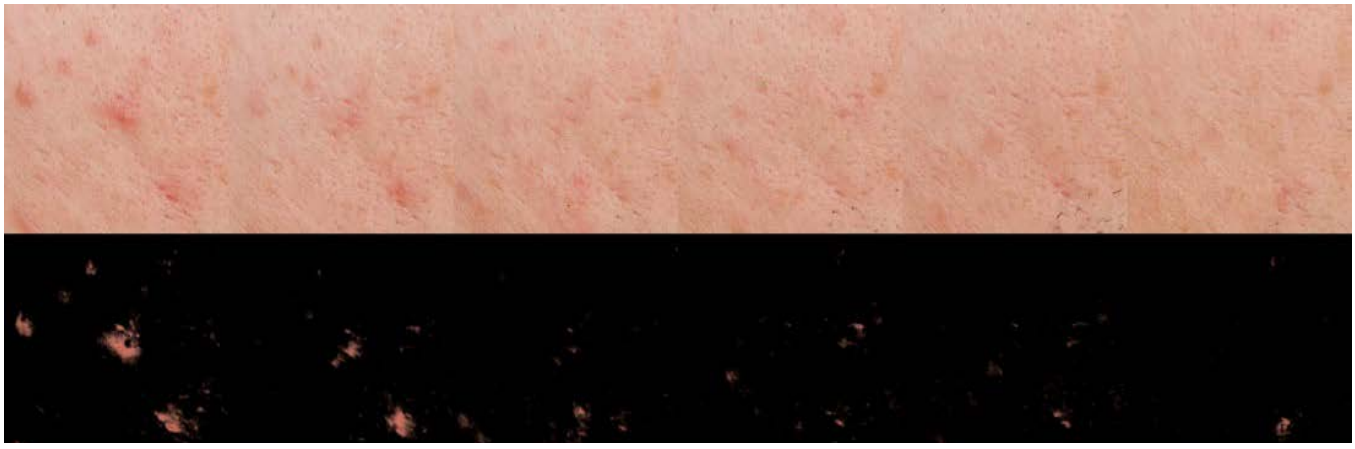


Baseline	Day 3	Day 7	Day 14	Day 30	Day 42
15,738PX	10,178PX 35.33% Acne Reduction	5,785PX 63.24% Acne Reduction	7,388PX 53.06% Acne Reduction	7,665PX 51.30% Acne Reduction	8,583PX 45.46% Acne Reduction

High Resolution Digital Photographs



Baseline - 30,883PX | **Day 3** - 9,778PX 68.34% Acne Reduction | **Day 7** - 17,403PX 43.65% Acne Reduction | **Day 14** - 17,705PX 42.67% Acne Reduction | **Day 30** - 30,851PX 00.10% Acne Reduction | **Day 42** - 8,890PX 71.21% Acne Reduction



Baseline - 7,063PX | **Day 3** - 3,109PX 55.98% Acne Reduction | **Day 7** - 1,530PX 78.34% Acne Reduction | **Day 14** - 1,001PX 85.83% Acne Reduction | **Day 30** - 748PX 89.41% Acne Reduction | **Day 42** - 657PX 90.70% Acne Reduction

DISCUSSION

The test material was found to be effective in improving facial acne by the mean number of total acne lesions (41.82% reduction) and improved the appearance of the skin. PhotoGrammetrix™ Image Analysis demonstrated that the test product also reduced facial acne by an average of 68.65% with a maxium improvement of 93.00% over a 42 week period. **Leucidal® Liquid** can be incorporated into applications to improve the appearance of the skin.

Active Micro Technologies, LLC - www.activemicrotechnologies.com - info@activemicrotechnologies.com
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Version 1- 08.04.15

Safety Statement

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Product Name: Leucidal® Liquid

Product Code: M15008

INCI Name: Leuconostoc/Radish Root Ferment Filtrate

INCI Status: Approved

Leucidal® Liquid is created by the fermentation of radish root in the presence of *Leuconostoc kimchii*. This process creates antimicrobial peptides that are capable of providing broad-spectrum antimicrobial activity and hydrating benefits.

To comply with global animal testing regulations (Directive 76/768/ECC), Active Micro Technologies, LLC does not test its products on animals. The component materials that are used to make our products have not been subject to animal testing or re-testing for cosmetic purposes by us or on our behalf.

Leuconostoc is a genus of microorganisms used to produce a variety of fermented food products, most commonly sauerkraut. *Leuconostoc* is a type of Lactic Acid Bacteria (LAB) and converts various sugars into lactic acid. Any existing LAB in Leucidal® Liquid is removed by filtration.

The FDA (Food and Drug Administration) states in sections 201 and 409 of the Federal Food, Drug and Cosmetic Act that "any substance that is intentionally added to food is a food additive, that is subject to review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under conditions of its use or unless the use of the substance is otherwise excluded for the definition of a food additive."[1] Therefore, *Leuconostoc* and its fermentation products are considered GRAS (generally recognized as safe) by the FDA.

In vitro dermal and ocular irritation studies were conducted to evaluate whether Leucidal® Liquid would induce dermal or ocular irriation in the EpiDerm™ and EpiOcular™ model assays. Test substances were applied to the tissue inserts and incubated. Cell viability was measured by dehydrogenase conversion of MTT, present in cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical was dictated by the reduction in tissue viability of exposed tissues compared to the negative control. Under conditions of this assay, the test article was considered to be non-irritating in both models. The substances used in these assays were undiluted. Please find attached a copy of these results.

In vitro phototoxicity irritation studies were conducted to evaluate whether Leucidal® Liquid would induce photoxic irritation in the EpiDerm™ model assay. Test solution was applied to tissue inserts at concentrations of 0.4%, 1.23%, and 3.7%. After the required incubation, tissue inserts were irritated for 60 minutes with 1.7 mW/cm^2 (=6 J/cm^2). Cell viability was measured by dehydrogenase conversion of MTT, present in cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The photoirritation potential of the test chemical was dictated by the reduction in tissue viability of UVA exposed tissues compared to non-UVA exposed tissues. Under conditions of this assay the test article was considered to be non-phototoxic at tested concentrations. The negative and positive controls performed as anticipated.

Additionally, a Human Subject Repeat Insult Patch Test Skin Irritation/Sensitization evaluation was completed to determine if Leucidal Liquid was classified as a sensitizing agent. Under the reported testing conditions, results indicated that Leucidal Liquid was not a primary sensitizer and a non-irritating material. Please find attached a copy of these results as well.

Safety Statement

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A *Salmonella typhimurium* reverse mutation standard plate incorporation study was conducted to evaluate whether Leucidal® Liquid would cause mutagenic changes in the average number of revertants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and WP2*uvr*A in the presence and absence of S9 metabolic activation. This study was conducted to satisfy, in part, the Genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity. Under the conditions of this assay, the test article solution was considered to be nonmutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and WP2*uvr*A. The product was tested undiluted and the negative and positive controls performed as anticipated.

The active antimicrobial components of Leucidal® Liquid are peptides. Peptides are similar to proteins, distinguished from them only on the basis of size. The approximate molecular weight of Leucidal® Liquid is 3,950 Da. Studies have been completed for proteins and protein-like biomolecules when inhaled. The Journal of Aerosol Medicine has determined that if protein or protein-like biomolecules are inhaled the threat for adverse respiratory effects is minimal.[2] Furthermore, Leucidal® Liquid is presented in an aqueous carrier. Water is not a volatile material and thereby presents a negligible risk of inhalation.

In summary, several data sets exist to support the safety of Leucidal® Liquid. The molecular weight of this product is larger than what is required to penetrate skin. Therefore, hazards that may otherwise occur via this route are not an issue. It is presented in an aqueous carrier, all but eliminating its risk for inhalation. Toxicological, irritation, and sensitization assays have all been performed with favorable results for each. Therefore, it is logically concluded that Leucidal® Liquid is safe for use at the recommended use level of 2.0 - 4.0% and no further testing is required.

[1] Federal Food, Drug & Cosmetic Act. US Food & Drug Administration.
http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/default.htm
[2] R.K. WOLFF. Journal of Aerosol Medicine. *Safety of Inhaled Proteins for Therapeutic Use.*1998, 11(4): 197-219

ACTIVE MICRO TECHNOLOGIES

Dermal and Ocular Irritation Tests

107 Technology Drive • Lincolnton, NC 28092
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Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6

Test Request Form #: 23

Lot #: 26051

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
In Vitro EpiDerm™ Dermal Irritation Test (EPI-200-SIT)
EpiOcular™ Eye Irritation Test (OCL-200-EIT)

SUMMARY

In vitro dermal and ocular irritation studies were conducted to evaluate whether **Leucidal® Liquid** would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays.

The product was tested according to the manufacture's protocol. The test article solution was found to be a **non-irritant**. Reconstructed human epidermis and cornea epithelial model were incubated in growth media overnight to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substances were applied to the tissue inserts and incubated for 60 minutes for liquid and solid substances in the EpiDerm™ assay and 30 minutes for liquid substances and 90 minutes for solid substances in the EpiOcular™ assay at 37°C, 5% CO_2, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

Under the conditions of this assay, the test article was considered to be **non-irritating**. The negative and positive controls performed as anticipated.

I. Introduction

A. Purpose

In vitro dermal and ocular irritation studies were conducted to evaluate whether a test article would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays. MatTek Corporation's reconstructed human epidermal and human ocular models are becoming a standard in determining the irritancy potential of test substances. They are able to discriminate between irritants and non-irritants. The EpiDerm™ assay has accuracy for the prediction of UN GHS R38 skin irritating and no-label (non-skin irritating) test substances. The EpiOcular™ assay can differentiate chemicals that have been classified as R36 or R41 from the EU classifications based on Dangerous Substances Directive (DSD) or between the UN GHS Cat 1 and Cat 2 classifications.

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II. Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity
B. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes
C. Media/Buffers:	DMEM based medium; DPBS; sterile deionized H_2O
D. Preparation:	Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
E. Tissue Culture Plates:	Falcon flat bottom 96-well, 24-well, 12-well, and 6-well tissue culture plates
F. Reagents:	MTT (1.0mg/mL); Extraction Solution (Isopropanol); SDS (5%); Methyl Acetate
G. Other:	Nylon Mesh Circles (EPI-MESH); Cotton tip swabs; 1mL tuberculin syringes; Ted Pella micro-spatula; 220mL specimen containers; sterile disposable pipette tips; Parafilm

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™, and cornea epithelial model, EpiOcular™, consist of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis and cornea epithelium. These models consist of organized basal, spinous, and granular layers, and the EpiDerm™ systems also contains a multilayer stratum corneum containing intercellular lamellar lipid layers that the EpiOcular™ system is lacking. Both the EpiDerm™ and EpiOcular™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile DPBS and sterile deionized water are used as negative controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

C. Positive Control

Known dermal and eye irritants, 5% SDS solution and Methyl Acetate, were used as positive controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

D. Data Interpretation Procedure

a. EpiDerm™

An irritant is predicted if the mean relative tissue viability of the 3 tissues exposed to the test substance is reduced by 50% of the mean viability of the negative controls and a non-irritant's viability is > 50%.

b. EpiOcular™

An irritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance is reduced by 60% of the mean viability of the negative controls and a non-irritant's viability is > 40%.

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Micro Technologies, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for an additional 18 to 21 hours.

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B. Test Substance Exposure

a. EpiDerm™

30µL (liquid) or 25mg (solid) of the undiluted test substance is applied to 3 tissue inserts and allowed to incubate for 60 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

b. EpiOcular™

Each tissue is dosed with 20µL DPBS prior to test substance dosing. 50µL (liquid) or 50mg (solid) of the undiluted test substance is applied to 2 tissue inserts and allowed to incubate for 90 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

C. Tissue Washing and Post Incubation

a. EpiDerm™

All tissue inserts are washed with DPBS, dried with cotton tipped swab, and transferred to fresh media and culture plates. After 24 hours the inserts are again transferred into fresh media and culture plates for an additional 18 to 20 hours.

b. EpiOcular™

Tissue inserts are washed with DPBS and immediately transferred into 5mL of assay medium for 12 to 14 minutes. After this soak the inserts are transferred into fresh media and tissue culture plates for 120 minutes for liquid substances and 18 hours for solid substances.

D. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO_2, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

B. Positive Control

a. EpiDerm™

The assay meets the acceptance criterion if the mean viability of positive control tissues expressed as a % of the negative control is ≤ 20%.

b. EpiOcular™

The assay meets the acceptance criterion if the mean viability of positive control tissues is < 60% of control viability.

C. Standard Deviation

Since each irritancy potential is predicted from the mean viability of 3 tissues for EpiDerm™ and 2 tissues for EpiOcular™, the variability of the replicates should be < 18% for EpiDerm™ and < 20% EpiOcular™.

VI. Results

A. Tissue Characteristics

The tissue inserts included in the MatTek EpiDerm™ and EpiOcular™ assay kits were in good condition, intact, and viable.

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B. Tissue Viability Assay
The results are summarized in Figures 1 and 2. In no case was the tissue viability ≤ 50% for EpiDerm™ or ≤ 60% for EpiOcular™ in the presence of the test substance. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited substantial loss of tissue viability and cell death.

C. Test Validity
The data obtained from this study met criteria for a valid assay.

VII. Conclusion
Under the conditions of this assay, the test article substance was considered to be **non-irritating**. The negative and positive controls performed as anticipated.

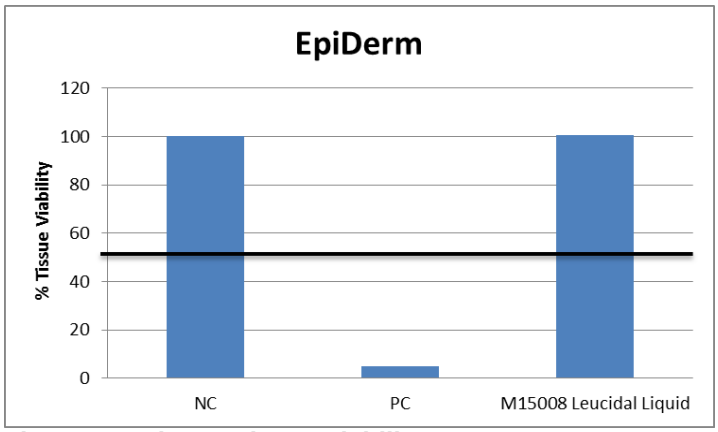

Figure 1: EpiDerm tissue viability

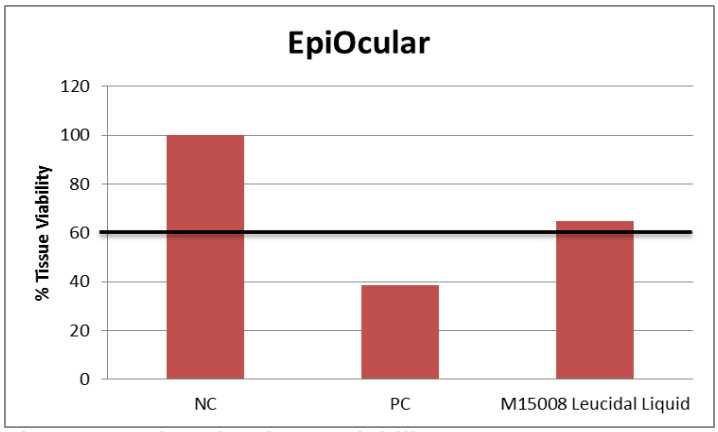

Figure 2: EpiOcular tissue viability

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The following report evaluates a sample of

Leucidal® Liquid (M15008) – AMA Lab No. L-2090

Provided by Active Concepts, LLC to AMA Laboratories, Inc.

Utilizing the Repeat Insult Patch Test
Skin Irritation / Sensitization Evaluation (Occlusive Patch)

February 18, 2008

Version#1/08-04-15

AMA LABORATORIES, INC.
216 Congers Road, Bldg. 1
New City, NY 10956 USA
(845) 634-4330
FAX: (845) 634-5565
www.amalabs.com

50 HUMAN SUBJECT REPEAT INSULT PATCH TEST SKIN IRRITATION/SENSITIZATION EVALUATION (Occlusive Patch)

AMA Ref. No.: MS08.RIPT.L2090O.50.ACTC

Date: February 18, 2008

Sponsor: Active Concepts, LLC
121 Ethel Road West, Suite 3
Piscataway, New Jersey 08854

1.0 Objective:

Consumer products or raw materials designed for consistent reapplication to areas of the skin may, under proper conditions, prove to be contact sensitizers or irritants in certain individuals. It is the intention of a Repeat Insult Patch Test (RIPT) to provide a basis for evaluation of this irritation/sensitization potential if such exists.

2.0 Test Material:

2.1 Test Material Description:

On January 11, 2008 one test sample labeled EN080110-E was received from Active Concepts, LLC and assigned AMA Lab No. L-2090.

2.2 Handling:

Upon arrival at AMA Laboratories, Inc., the test material is assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and tests requested.

Samples are retained for a period of three months beyond submission of final report unless otherwise specified by the sponsor or, if sample is known to be in support of governmental applications, representative retained samples are kept two years beyond final report submission.

Sample disposition is conducted in compliance with appropriate federal, state and local ordinances.

2.3 Test Material Evaluation Prerequisite:

Prior to induction of a human test panel, toxicology, microbiology or in-vitro performance spectra may be required to assess the feasibility of commencement as dictated by an Institutional Review Board (IRB) described in Section 3.0.

Sponsor purports that prior to sample submission the following tests were conducted with no adverse results and that the test data are on file on their premises and have not been made available to AMA personnel:

- USP or CTFA Preservative Efficacy Test or equivalent
- 90 Day Accelerated Stability and Container Compatibility Study

3.0 Institutional Review Board:

Reference: CFR Title 21 Part 56, Subparts A, B, C, and D. The IRB of AMA Laboratories, Inc., consists of five or more individuals, chosen from within the company for technical expertise and from the local community for lay interaction. The list of IRB members is kept on file at AMA Laboratories, Inc. and is available for inspection during the hours of operation.

4.0 Panel Selection:

4.1 Standards for Inclusion in a Study:

- Individuals who are not currently under a doctor's care.
- Individuals free of any dermatological or systemic disorder which would interfere with the results, at the discretion of the Investigator.
- Individuals free of any acute or chronic disease that might interfere with or increase the risk of study participation.
- Individuals who will complete a preliminary medical history form mandated by AMA Laboratories, Inc. and are in general good health.
- Individuals, who will read, understand and sign an informed consent document relating to the specific type of study they are subscribing. Consent forms are kept on file and are available for examination on the premises of AMA Laboratories, Inc. only.
- Individuals able to cooperate with the Investigator and research staff, willing to have test materials applied according to the protocol, and complete the full course of the study.

4.2 Standards for Exclusion from a Study:

- Individuals under 18 years of age.
- Individuals who are currently under a doctor's care.
- Individuals who are currently taking any medication (topical or systemic) that may mask or interfere with the test results.
- Subjects with a history of any acute or chronic disease that might interfere with or increase the risk associated with study participation.
- Individuals diagnosed with chronic skin allergies.
- Female volunteers who indicate that they are pregnant or lactating.

4.3 Recruitment:

Panel selection is accomplished by advertisements in local periodicals, community bulletin boards, phone solicitation, electronic media or any combination thereof.

4.4 Informed Consent and Medical History Forms:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form. These forms along with the signed consent forms, are available for inspection on the premises of AMA Laboratories, Inc. only. Reference 21 CFR Ch. 1 Part 50, Subpart B.

The parties agree to comply with applicable state and federal privacy laws for the use and disclosure of a subject's personal health information by taking reasonable steps to protect the confidentiality of this information. This obligation shall survive the termination or expiration of this Agreement.

5.0 Population Demographics:

Number of subjects enrolled		52
Number of subjects completing study		50
Age Range		26-64
Sex	Male	7
	Female	45
Race	Caucasian	42
	Hispanic	9
	Asian	1

6.0 Equipment:

- Patch Description: Parke-Davis Hypoallergenic Readi Bandages or the equivalent.
- 1ml volumetric syringe without a needle.

7.0 Procedure:

- Subjects are requested to bathe or wash as usual before arrival at the facility.
- 0.2 ml or 0.2g of the test material is dispensed onto the occlusive, hypoallergenic patch.
- The patch is then applied directly to the skin of the infrascapular regions of the back, to the right or left of the midline and the subject is dismissed with instructions not to wet or expose the test area to direct sunlight.
- After 24 hours the patch is removed by the panelist at home.
- This procedure is repeated until a series of nine consecutive 24 hour exposures have been made for every Monday, Wednesday, and Friday for three consecutive weeks.
- In the event of an adverse reaction, the area of erythema and edema is measured. The edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin. Reactions are scored just before applications two through nine and the next test date following application nine. In most instances this is approximately 24 hours after patch removal. Clients are notified immediately in the case of adverse reaction and determination is made as to treatment program if necessary.
- Subjects are then given a 10 - 14 day rest period after which a challenge or retest dose is applied once to a previously unexposed test site. The retest dose is equivalent to any one of the original nine exposures. Reactions are scored 24 and 48 hours after application.
- Comparison is made between the nine inductive responses and the retest dose.

8.0 Results:

Please refer to attached Table.

9.0 Observations:

No adverse reactions of any kind were noted during the course of this study.

10.0 Archiving:

All original samples, raw data sheets, technician's notebooks, correspondence files and copies of final reports and remaining specimens are maintained on premises of AMA Laboratories, Inc. in limited access storage files marked "Archive". A duplicate disk copy of final reports is separately archived in a bank safe deposit vault.

11.0 Reference:

Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics, published by The Association of Food and Drug Officials of The United States, 1965 (modified).

12.0 Conclusions:

The test material (AMA Lab. No.: L-2090; Client No.: EN080110-E) when tested under occlusion as described herein, may be considered:
a **NON-PRIMARY IRRITANT** and **NON-PRIMARY SENSITIZER** to the skin according to the reference.

Mayya Tatsene, M.D.
Study Director

Patrycja Bienias, M.S.
Technician

David R. Winne, B.S.
Technical Director

2/18/08
Date

TABLE
SUMMARY OF RESULTS
(Occlusive Patch)

AMA Lab No.: L-2090
Client No.: EN080110-E

No.	Subject ID	RACE	SEX	Response 1	2	3	4	5	6	7	8	9	Chall. 24 HR	48 HR	Score
1	25 0215	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
2	28 0971	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
3	34 4672	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
4	36 2168	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
5	36 7304	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
6	36 7970	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
7	36 8248	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
8	40 6489	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
9	42 1835	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
10	42 1837	C	F	0	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	N/A
11	44 9258	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
12	46 4172	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
13	48 4004	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
14	50 1699	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
15	50 1729	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
16	50 3800	A	M	0	0	0	0	0	0	0	0	0	0	0	0.0
17	50 5772	C	M	0	0	0	0	0	0	0	0	0	0	?	0.0
18	50 8253	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
19	52 4898	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
20	52 5000	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
21	54 0763	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
22	54 1935	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
23	54 2951	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
24	54 4408	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
25	54 6357	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
26	56 0719	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
27	56 3659	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
28	56 4962	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
29	56 5529	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0

TABLE (CONT'D)
SUMMARY OF RESULTS
(Occlusive Patch)

AMA Lab No.: L-2090
Client No.: EN080110-E

No.	Subject ID	RACE	SEX	Response 1	2	3	4	5	6	7	8	9	Chall. 24 HR	48 HR	Score
30	58 3087	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
31	58 3965	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
32	58 7412	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
33	58 9750	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
34	60 0082	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
35	60 1825	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
36	60 2888	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
37	60 3135	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
38	60 6328	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
39	60 9336	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
40	62 3596	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
41	62 5624	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
42	62 8070	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
43	64 2464	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
44	64 4340	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
45	64 6653	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
46	64 8003	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
47	66 1927	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
48	70 5391	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
49	72 2318	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
50	76 2719	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
51	82 4417	H	M	0	0	0	0	0	0	0	0	0	0	0	0.0
52	90 3845	H	F	0	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	N/A

Evaluation Period:

This study was conducted from January 14, 2008 through February 15, 2008.

Scoring Scale and Definition of Symbols Shown in Table:

0 - No evidence of any effect

? - (Barely perceptible) minimal faint (light pink) uniform or spotty erythema

1 - (Mild) pink uniform erythema covering most of contact site

2 - (Moderate) pink\red erythema visibly uniform in entire contact area

3 - (Marked) bright red erythema with accompanying edema, petechiae or papules

4 - (Severe) deep red erythema with vesiculation or weeping with or without edema

D - Patch eliminated due to reaction

Dc - Discontinued due to absence of subject on application date

M - Patch applied to an adjacent site after strong test reaction

N/A - Score is not calculated for subjects discontinued before challenge

S - Skin stained from pigment in product

T - Tan

NOTE: All technical employees of AMA LABORATORIES, INC. are required to take and pass a visual discrimination examination conducted by a Board Certified Ophthalmologist using the Farnsworth-Munsell 100 Hue Test as published; which determines a person's ability to discern color against a black background. This test was additionally modified to include a flesh tone background more nearly approaching actual use conditions, wherein erythematous skin is graded according to intensity.

13.0 Quality Assurance Statement:

This study was inspected in accordance with the Standard Operating Procedures of AMA Laboratories, Inc. To assure compliance with the study protocol, the Quality Assurance Unit completed an audit of the study records and report.

Report reviewed by:

Kamil Wojtowicz

Kamil Wojtowicz, M.S.
Quality Assurance Supervisor

2/18/08
Date

ACTIVE MICRO TECHNOLOGIES

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OECD TG 442C: *In Chemico* Skin Sensitization

Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6

Test Request Form #: 1237

Lot #: 4786P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
OECD TG 442C: *In Chemico* Skin Sensitization
Direct Peptide Reactivity Assay (DPRA)

Introduction

A skin sensitizer is a substance that will lead to an allergic response following skin contact[1]. Haptenation is the covalent binding of a hapten, or low-molecular weight substance or chemical, to proteins in the skin. This is considered the prominent mechanism which defines a chemical as a sensitizer. Haptenation is described as a "molecular initiating event" in the OECD Adverse Outcome Pathway (AOP) for skin sensitization which summarizes the key events known to be involved in chemically-induced allergic contact dermatitis[2]. The direct peptide reactivity assay (DPRA) is designed to mimic the covalent binding of electrophilic chemicals to nucleophilic centers in skin proteins by quantifying the reactivity of chemicals towards the model synthetic peptides containing cysteine and lysine. The DPRA is able to distinguish sensitizers from non-sensitizer with 82% accuracy (sensitivity of 76%; specificity of 92%)[3].

This assay was conducted to determine skin sensitization hazard of **Leucidal® Liquid** in accordance with European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) and OECD Test Guideline 442C.

Assay Principle

The DPRA is an *in chemico* method which addresses peptide reactivity by measuring depletion of synthetic heptapeptides containing either cysteine or lysine following 24 hours incubation with the test substance. The peptide is a custom material containing phenylalanine to aid in detection. Depletion of the peptide in the reaction mixture is measured by HPLC with gradient elution and UV detection at 220 nm. Cysteine and lysine peptide percent depletion values are then calculated and used in a prediction model which allows assigning the test chemical to one of four reactivity classes used to support the discrimination between sensitizers and non-sensitizers.

1. United Nations Economic Commission (UNECE) (2013) Global Harmonized System of Classification and Labelling of Chemicals (GHS) 5th Revised Edition
2. OECD (2012). The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence. Series on Testing and Assessment No. 168
3. EC EURL ECVAM (2012) Direct peptide reactivity assay (DPRA) validation study report; pp 1 -74.

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Materials

A.	**Equipment:**	HPLC-UV (Waters Alliance 2695 - Waters 996 Photodiode Array); Pipettes; Analytical balance
B.	**HPLC/Guard Columns:**	Agilent Zorbax SB-C18 2.1mm x 100mm x 3.5µm; Phenomenex Security Guard C18 4mm x 2mm
C.	**Chemicals:**	Trifluoroacetic acid; Ammonium acetate; Ammonium hydroxide; Acetonitrile; Cysteine peptide (Ac-RFAA**C**AA-COOH); Lysine peptide (Ac-RFAA**K**AA-COOH); Cinnamic aldehyde
D.	**Reagents/Buffers:**	Sodium phosphate buffer (100mM); Ammonium acetate buffer (100mM)
E.	**Other:**	Sterile disposable pipette tips

Methods

Solution Preparation:

- 0.667mM Cysteine Peptide in 100mM Phosphate Buffer (pH 7.5)
- 0.667mM Lysine Peptide in 100mM Ammonium Acetate Buffer (pH 10.2)
- 100mM Cinnamic Aldehyde in Acetonitrile
- 100mM **Leucidal® Liquid** in Acetonitrile

Reference Controls:

- Reference Control A: For calibration curve accuracy
- Reference Control B: For peptide stability over analysis time of experiment
- Reference Control C: For verification that the solvent does not impact percent peptide depletion

Sample, Reference Control, and Co-Elution Control Preparation:

- Once these solutions have been made they should be incubated at room temperature, protected from light, for 24±2 hours before running HPLC analysis.
- Each chemical should be analyzed in triplicate.

1:10 Ratio, Cysteine Peptide 0.5mM Peptide, 5mM Test Chemical	1:50 Ratio, Lysine Peptide 0.5mM Peptide, 25mM Test Chemical
• 750µL Cysteine Peptide Solution (or 100mM Phosphate Buffer, pH 7.5, for Co-Elution Controls) • 200µL Acetonitrile • 50µL Test Chemical Solution (or Acetonitrile for Reference Controls)	• 750µL Lysine Peptide Solution (or 100mM Ammonium Acetate Buffer, pH 10.2, for Co-Elution Controls) • 250µL Test Chemical Solution (or Acetonitrile for Reference Controls)

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Calibration Curve:

- Standards are prepared in a solution of 20% Acetonitrile:Buffer
  - For the Cysteine peptide using the phosphate buffer, pH 7.5
  - For the Lysine peptide using the ammonium acetate buffer, pH 10.2

	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
mM Peptide	0.534	0.267	0.1335	0.0667	0.0334	0.0167	0.000

HPLC Analysis:

- HPLC-UV system should be equilibrated at 30°C with 50% Mobile Phase A (0.1% (v/v) trifluoroacetic acid in water) and 50% Mobile Phase B (0.085% (v/v) trifluoroacetic acid in acetonitrile) for 2 hours
- Absorbance is measured at 220nm
- Flow Conditions:

Time	Flow	%A	%B
0 minutes	0.35 mL/min	90	10
10 minutes	0.35 mL/min	75	25
11 minutes	0.35 mL/min	10	90
13 minutes	0.35 mL/min	10	90
13.5 minutes	0.35 mL/min	90	10
20 minutes	End Run		

Data and Reporting

Acceptance Criteria:

1. The following criteria must be met for a run to be considered valid:
   a. Standard calibration curve should have an $r^2 > 0.99$.
   b. Mean percent peptide depletion values of three replicates for the positive control cinnamic aldehyde should be between 60.8% and 100% for the cysteine peptide and between 40.2% and 69% for the lysine peptide and the maximum standard deviation should be <14.9 for the percent cysteine depletion and <11.6 for the percent lysine depletion.
   c. Mean peptide concentration of reference controls A should be 0.50±0.05mM and the coefficient of variable of the peptide peak areas for reference B and C in acetonitrile should be <15.0%.

2. The following criteria must be met for a test chemical's results to be considered valid:
   a. Maximum standard deviation should be <14.9 for percent cysteine depletion and <11.6 for percent lysine depletion.
   b. Mean peptide concentration of the three reference control C should be 0.50±0.05mM.

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Prediction Model:

Cysteine 1:10/Lysine 1:50 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
0% < Mean % Depletion < 6.38%	Minimal Reactivity	Non-sensitizer
6.38% < Mean % Depletion < 22.62%	Low Reactivity	Sensitizer
22.62% < Mean % Depletion < 42.47%	Moderate Reactivity	Sensitizer
42.47% < Mean % Depletion < 100%	High Reactivity	Sensitizer

If co-elution occurs with the lysine peptide, than the cysteine 1:10 prediction model can be used:

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
0% < Cys % Depletion < 13.89%	Minimal Reactivity	Non-sensitizer
13.89% < Cys % Depletion < 23.09%	Low Reactivity	Sensitizer
23.09% < Cys % Depletion < 98.24%	Moderate Reactivity	Sensitizer
98.24% < Cys % Depletion < 100%	High Reactivity	Sensitizer

Results and Discussion

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated.

Percent peptide depletion is determined by the following equation:

$$Percent\ Peptide\ Depletion = \left[1 - \left(\frac{Peptide\ Peak\ Area\ in\ Replicate\ Injection}{Mean\ Peptide\ Peak\ Area\ in\ Reference\ Controls\ C}\right)\right] \times 100$$

Based on HPLC-UV analysis of **Leucidal® Liquid (code M15008)** we can determine that this product is not a sensitizer and will not cause allergic contact dermatitis. The Mean Percent Depletion of Cysteine and Lysine was 2.89% causing minimal reactivity in the assay giving us the prediction of a non-sensitizer.

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OECD TG 442D: *In Vitro* Skin Sensitization

Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6

Test Request Form #: 1192

Lot #: 4752P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
OECD TG 442D: In Vitro Skin Sensitization
ARE-Nrf2 Luciferase Test Method

Introduction

Skin sensitization refers to an allergic response following skin contact with the tested chemical, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals[1]. Substances are classified as skin sensitizers if there is evidence in humans that the substance can lead to sensitization by skin contact or positive results from appropriate tests, both *in vivo* and *in vitro*. Utilization of the KeratinoSens™ cell line allows for valid *in vitro* testing for skin sensitization.

This assay was conducted to determine skin sensitization potential of **Leucidal® Liquid** in accordance with the UN GHS.

Assay Principle

The ARE-Nrf2 luciferase test method addresses the induction of genes that are regulated by antioxidant response elements (ARE) by skin sensitizers. The Keap1-Nrf2-ARE pathways have been shown to be major regulator of cytoprotective responses to oxidative stress or electrophilic compounds. These pathways are also known to be involved in the cellular processes in skin sensitization. Small electrophilic substances such as skin sensitizers can act on the sensor protein Keap1 (Kelch-like ECH-associated protein 1), by covalent modification of its cysteine residue, resulting in its dissociation from the transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2). The dissociated Nrf2 can then activate ARE-dependent genes such as those coding for phase II detoxifying enzymes.

The skin sensitization assay utilizes the KeratinoSens™ method which uses an immortalized adherent human keratinocyte cell line (HaCaT cell line) that has been transfected with a selectable plasmid to quantify luciferase gene induction as a measure of activation of Keap1-Nrf2-antioxidant/electrophile response element (ARE). This test method has been validated by independent peer review by the EURL-ECVAM. The addition of a luciferin containing reagent to the cells will react with the luciferase produced in the cell resulting in luminescence which can be quantified with a luminometer.

1. United Nations (UN) (2013). Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Fifth revised edition, UN New York and Geneva, 2013

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Materials

A.	**Incubation Conditions:**	37°C at 5% CO_2 and 95% relative humidity (RH)
B.	**Equipment:**	Humidified incubator; Biosafety laminar flow hood; Microplate Reader; Pipettes
C.	**Cell Line:**	KeratinoSens™ by Givaudan Schweiz AG
D.	**Media/Buffers:**	Dulbecco's Modified Eagle Medium (DMEM); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Geneticin
E.	**Culture Plate:**	Flat bottom 96-well tissue culture treated plates
F.	**Reagents:**	Dimethyl Sulfoxide (DMSO); Cinnamic Aldehyde; ONE-Glo Reagent; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); sodium lauryl sulfate (SLS)
G.	**Other:**	Sterile disposable pipette tips; wash bottles

Methods

KeratinoSens™ were into seeded four 96-well tissue culture plates and allowed to grow to 80 – 90% confluency in DMEM containing 10% FBS and 500µg/mL G418 geneticin. Twelve test concentrations of **Leucidal® Liquid** were prepared in DMSO with a concentration range from 0.98 – 2000 µM. These 12 concentrations were assayed in triplicate in 2 independently performed experiments. The positive control was cinnamic aldehyde for which a series of 5 concentrations prepared in DMSO had final test concentrations of 4 – 64 µM. The negative control was a 1% test concentration of DMSO.

24 hour post KeratinoSens™ seeding, the culture media was removed and replaced with fresh media containing 10% FBS without G418 geneticin. 50 µL of the above described test concentrations was added to the appropriate wells. The treated plates were then incubated for 48 hours at 37°C in the presence of 5% CO_2 and 95% relative humidity. After treatment incubation was complete the media was removed and the wells were washed with PBS 3 times.

One of the four plates was used for a cytotoxicity endpoint, where MTT was added to the wells and incubated for 4 hours at 37°C in the presence of 5% CO_2. SLS was then added to the wells and incubated overnight at room temperature. A spectrometer measured the absorbance at 570 nm. The absorbance values (optical density) were then used to determine the viability of each well by comparing the optical density of each test material treated well to that of the solvent control wells to determine the IC_{50} and IC_{30} values.

The remaining 3 plates were used in the luciferase induction endpoint of the assay. 100 µL of Promega's ONE-Glo Reagent was added to 100 µL of fresh media containing 10% FBS without geneticin. Cells were incubated for 5 minutes to induce cell lysis and release luciferin into the media. Plates were read with a luminometer and $EC_{1.5}$ and maximum response (I_{max}) values were obtained.

Data and Reporting

Acceptance Criteria:

1. Gene induction obtained with the positive control, cinnamic aldehyde, should be statistically significant above the threshold of 1.5 in at least one of the tested concentrations (from 4 to 64 µM).
2. The EC1.5 value should be within two standard deviations of the historical mean and the average induction in the three replicates for cinnamic aldehyde at 64 µM should be between 2 and 8.
3. The average coefficient of variability of the luminescence reading for the negative (solvent) control DMSO should be below 20% in each experiment.

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A KeratinoSens™ prediction is considered positive if the following conditions are met:

1. The Imax is higher than 1.5-fold and statistically significantly higher as compared to the solvent (negative) control
2. The cellular viability is higher than 70% at the lowest concentration with a gene induction above 1.5 fold (i.e., at the EC1.5 determining concentration)
3. The $EC_{1.5}$ value is less than 1000 µM (or < 200 µg/ml for test chemicals with no defined MW)
4. There is an apparent overall dose-response for luciferase induction

Results

Compound	Classification	$EC_{1.5}$ (µM)	IC_{50}	I_{max}
Cinnamic aldehyde	Sensitizer	19	289.19 µM	31.6
DMSO	Non-Sensitizer	No Induction	243.24 µM	1.2
Leucidal® Liquid	Non-Sensitizer	No Induction	> 1000 µM	0.5

Table 1: Overview of KeratinoSens™ Assay Results

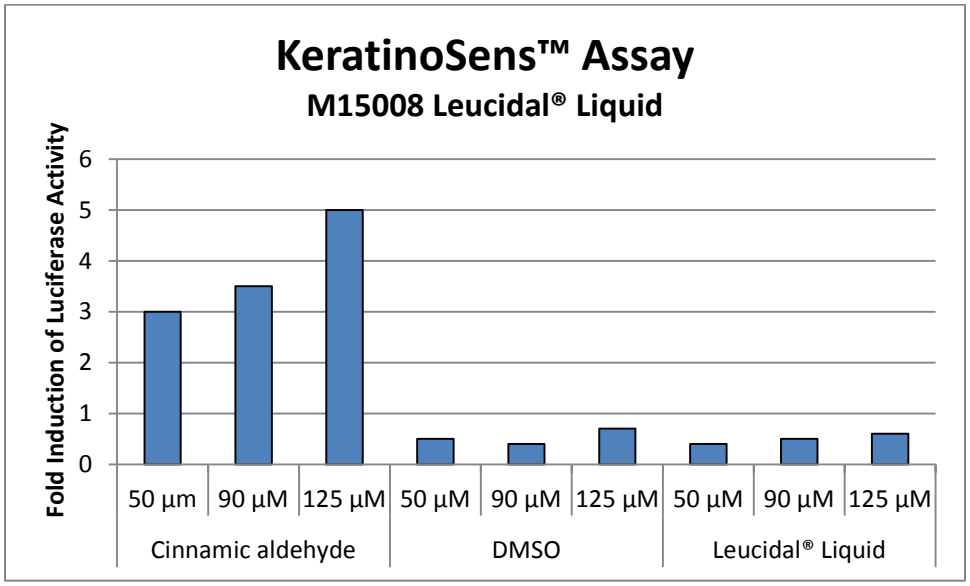

Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **Leucidal® Liquid (code M15008)** was not predicted to be a skin sensitizer based on the KeratinoSens™ ARE-Nrf2 Luciferase Test Method as there was not a significant increase in luciferase expression. It can be concluded that **Leucidal® Liquid** can be safely used in cosmetics and personal care products at typical use levels.

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Bacterial Reverse Mutation Test

Test Article: Leucidal® Liquid
Code Number: M15008
CAS #: 1686112-10-6

Sponsor:
Active Micro Technologies, LLC
107 Technology Drive
Lincolnton, NC 28092

Study Director: *Erica Segura*
Principle Investigator: *Monica Beltran*

Test Performed:
Genotoxicity: Bacterial Reverse Mutation Test

Reference:
OECD471/ISO10993.Part 3

Test Request Number: 1004

SUMMARY

A *Salmonella typhimurium/Escherichia coli* reverse mutation standard plate incorporation study described by Ames *et al.* (1975) was conducted to evaluate whether a test article solution **Leucidal® Liquid** would cause mutagenic changes in the average number of reveratants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2*uvr*A in the presence and absence of Aroclor-induced rat liver S9. This study was conducted to satisfy, in part, the Genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

The stock test article was tested at eight doses levels along with appropriate vehicle control and positive controls with overnight cultures of tester strains. The test article solution was found to be noninhibitory to growth of tester strain TA98, TA100, TA1537, TA1535 and WP2*uvr*A after Sport Inhibition Screen.

Separate tubes containing 2 ml of molten top agar at 45^0C supplemented with histidine-biotin solution for the *Salmonella typhimurium* strains and supplemented with tryptophan for *Escherichia coli* strain were inoculated with 100 µl of tester strains, 100 µl of vehicle or test article dilution were added and 500 µl aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. After vortexing, the mixture was poured across the Minimal Glucose Agar (GMA) plates. Parallel testing was also conducted with positive control correspond to each strain, replacing the test article aliquot with 50µl aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for 48 hours at 37^0C. The mean numbers of revertants of the test plates were compared to the mean number of revertants of the negative control plates for each of the strains tested. The means obtained for the positive controls were used as points of reference.

Under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* tester strain WP2*uvr*A. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

I. Introduction

A. **Purpose**
A *Salmonella typhimurium/Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a test article solution would cause mutagenic changes in the average number of revertants for *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2*uvr*A in the presence and absences of the S9 metabolic activation. Bacterial reverse mutation tests have been widely used as rapid screening procedures for the determination of mutagenic and potential carcinogenic hazards.

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II. Materials

A. **Storage Conditions:** Room temperature (23-25C).
B. **Vehicle:** Sterile DI Water.
C. **Preparation:** Eight different doses level were prepared immediately before use with sterile DI water.
D. **Solubility/Stability:** 100% Soluble and Stable.
E. **Toxicity:** No significant inhibition was observed.

III. Test System

A. Test System

Each *Salmonella typhimurium* and *Escherichia coli* tester strain contains a specific deep rough mutation (*rfa)*, the deletion of *uvr*B gene and the deletion in the *uvr*A gene that increase their ability to detect mutagens, respectively. These genetically altered *Salmonella typhimurium* strains (TA98, TA100, TA1537 and TA1535) and *Escherichia coli* strain (WP2*uvr*A) cannot grow in the absence of histidine and tryptophan, respectively. When placed in a histidine-tryptophan free medium, only those cells which mutate spontaneously back to their wild type states are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

Tester strain	Mutations/Genotypic Relevance
TA98	hisD3052, Dgal chlD bio *uvr*B *rfa* pKM101
TA100	hisG46, Dgal chlD BIO *uvr*B *rfa* pKM101
TA1537	hisC3076, *rfa*, Dgal chlD bio *uvrB*
TA 1535	hisG46, Dgal chlD bio *uvr*B *rfa*
WP2*uvr*A	trpE, *uvr*A

rfa	=	causes partial loss of the lip polysaccharide wall which increases permeability of the cell to large molecules.
uvrB	=	deficient DNA excision-repair system (i.e., ultraviolet sensitivity)
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens.
*uvr*A	=	All possible transitions and transversions, small deletions.

B. Metabolic Activation

Aroclor induced rat liver (S9) homogenate was used as metabolic activation. The S9 homogenate is prepared from male Sprague Dawley rats. Material is supplied by MOLTOX, Molecular Toxicology, Inc.

C. Preparation of Tester strains

Cultures of *Salmonella typhimurium* TA98, TA100,TA1537, TA1535 and *Escherichia coli* WP2uvrA were inoculated to individual flasks containing Oxoid broth No.2. The inoculated broth cultures were incubated at 37°C in an incubator shaker operating at 140-150 rpm for 12-16 hours.

D. Negative Control

Sterile DI water (vehicle without test material) was tested with each tester strain to determine the spontaneous reversion rate. Each strain was tested with and without S9 activation. These data represented a base rate to which the number of reveratants colonies that developed in each test plate were compared to determine whether the test material had significant mutagenic properties.

E. Positive Control

A known mutagen for each strain was used as a positive control to demonstrate that tester strains were sensitive to mutation to the wild type state. The positive controls are tested with and without the presence of S9 homogenate.

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F. Titer of the Strain Cultures:
Fresh cultures of bacteria were grown up to the late exponential or early stationary phase of growth; to confirm this, serial dilutions from each strain were conducted, indicating that the initial population was in the range of 1 to $2x10^9$/ml.

IV. Method

A. Standard Plate Incorporation Assay:
Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *Salmonella typhimurium* and tryptophan for *Escherichia coli* were inoculated with 100 µl of culture for each strain and 100 µl of testing solution or vehicle without test material. A 500 µl aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across Minimal Glucose Agar plates labeled with strain number and S9 activation (+/-). When plating the positive controls, the test article aliquot was replaced by 50µl aliquot of appropriate positive control. The test was conducted per duplicate. The plates were incubated for 37°C for 2 days. Following the incubation period, the revertant colonies on each plate were recorded. The mean number of reveratnts was determined. The mean numbers of revertants of the test plates were compared to the mean number of reverants of the negative control of each strain used.

V. Evaluation

For the test solution to be evaluated as a test failure or "potential mutagen" there must have been a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control for any or all strains. Each positive control mean must have exhibited at least a 3-fold increase over the respective negative control mean of the *Salmonella* tester strain used.

VI. Results and Discussion

A. Solubility:
Water was used as a solvent. Solutions from the test article were made from 0.015 to 50mg/ml.

B. Dose levels tested:
The maximum dose tested was 5000 µg per plate. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate.

C. Titer (Organisms/ml):
5×10^8 UFC/ml plate count indicates that the initial population was in the range of 1 to 2×10^9 UFC/ml.

C. Standard Plate Incorporation Assay
In no case was there a 2-fold or greater increase in the mean number of revertant testing strains TA98, TA100, TA1537, TA1535 and WP2*uvr*A in the presence of the test solution compared with the mean of vehicle control value. The positive controls mean exhibited at least a 3-fold increase over the respective mean of the *Salmonella typhimurium* and *Escherichia coli* tester strains used. The results are summarized in Appendix 2.

VII. Conclusion

All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2*uvr*A.The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

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Appendix 2:

Bacterial Mutation Assay
Plate Incorporation Assay Results

	Concentration µg per Plate	***TA98***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	32	34	33
	1500	15	17	16
	500	28	32	30
	150	26	36	31
	50	28	18	23
	15	14	20	17
	5.0	24	21	23
	1.5	26	26	26
Test Solution w/o S9	5000	18	16	17
	1500	33	45	39
	500	15	19	17
	150	21	35	28
	50	18	23	21
	15	25	27	26
	5.0	21	21	21
	1.5	25	15	20
DI Water w/S9		36	36	36
DI Water w/o S9		28	32	30
2-aminoanthracen w/ S9		410	398	404
2-nitrofluorene w/o S9		257	225	241
Historical Count Positive w/S9		**43-1893**		
Historical Count Positive w/o S9		**39-1871**		
Historical Count Negative w/S9		**4-69**		
Historical Count Negative w/o S9		**3-59**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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	Concentration µg per Plate	***TA100***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	112	110	111
	1500	108	144	126
	500	115	117	116
	150	114	132	123
	50	128	156	142
	15	144	162	153
	5.0	132	146	139
	1.5	168	134	151
Test Solution w/o S9	5000	132	148	140
	1500	112	124	118
	500	152	126	139
	150	112	68	90
	50	102	44	73
	15	116	125	121
	5.0	136	112	124
	1.5	126	124	125
DI Water w/S9		154	185	170
DI Water w/o S9		194	210	202
2-aminoanthracen w/ S9		425	368	397
Sodium azide w/o S9		398	410	404
Historical Count Positive w/S9		**224-3206**		
Historical Count Positive w/o S9		**226-1837**		
Historical Count Negative w/S9		**55-268**		
Historical Count Negative w/o S9		**47-250**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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	Concentration µg per Plate	***TA1537***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	10	8	9
	1500	16	22	19
	500	14	12	13
	150	24	16	20
	50	22	24	23
	15	14	14	14
	5.0	12	32	22
	1.5	19	25	22
Test Solution w/o S9	5000	42	22	32
	1500	12	12	12
	500	10	8	9
	150	10	12	11
	50	14	18	16
	15	22	14	18
	5.0	16	22	19
	1.5	16	11	14
DI Water w/S9		10	5	8
DI Water w/o S9		15	16	16
2-aminoanthracen w/ S9		355	347	351
2-aminoacridine w/o S9		348	306	327
Historical Count Positive w/S9		**13-1934**		
Historical Count Positive w/o S9		**17-4814**		
Historical Count Negative w/S9		**0-41**		
Historical Count Negative w/o S9		**0-29**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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	Concentration µg per Plate	***TA1535***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	24	35	25
	1500	25	28	27
	500	42	31	37
	150	22	16	19
	50	21	24	23
	15	18	15	17
	5.0	17	17	17
	1.5	14	22	18
Test Solution w/o S9	5000	45	61	53
	1500	48	33	41
	500	82	81	82
	150	65	42	54
	50	15	28	22
	15	12	25	19
	5.0	44	36	40
	1.5	22	24	23
DI Water w/S9		15	18	17
DI Water w/o S9		25	33	29
2-aminoanthracen w/ S9		224	256	240
Sodium azide w/o S9		416	475	446
Historical Count Positive w/S9		**22-1216**		
Historical Count Positive w/o S9		**47-1409**		
Historical Count Negative w/S9		**1-50**		
Historical Count Negative w/o S9		**1-45**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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	Concentration µg per Plate	***WP2uvrA***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	20	32	26
	1500	21	11	16
	500	26	24	25
	150	25	42	34
	50	29	36	33
	15	20	12	16
	5.0	45	47	46
	1.5	51	55	53
Test Solution w/o S9	5000	62	36	49
	1500	44	62	53
	500	26	38	32
	150	16	16	16
	50	35	52	44
	15	61	47	54
	5.0	52	37	45
	1.5	40	60	50
DI Water w/S9		44	42	43
DI Water w/o S9		62	51	56
2-aminoanthracen w/ S9		482	502	492
Methylmethanesulfonate w/o S9		385	363	374
Historical Count Positive w/S9		**44-1118**		
Historical Count Positive w/o S9		**42-1796**		
Historical Count Negative w/S9		**8-80**		
Historical Count Negative w/o S9		**8-84**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

Phototoxicity Assay Analysis

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Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6

Test Request Form #: 23

Lot #: 24723

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
In Vitro EpiDerm™ Model (EPI-200-SIT) Phototoxicity

SUMMARY

In vitro phototoxicity irritation studies were conducted to evaluate whether **Leucidal® Liquid** would induce phototoxic irritation in the EpiDerm™ model assay.

The product was tested according to the manufacturer's protocol. The test article solution was found to be a **non-photoirritant** at concentrations of 0.4%, 1.2%, and 3.7%. Reconstructed human epidermis was incubated in growth media for one hour to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substance was applied to the tissue inserts in five varying concentrations and incubated overnight at 37°C, 5% CO_2, and 95% relative humidity (RH). The following day, the appropriate tissue inserts were irradiated (UVA) for 60 minutes with 1.7 mW/cm^2 (=6 J/cm^2). After substance incubation, irradiation, and washing was completed, the cell viability test was conducted. Cell viability was measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that was measured after extraction from the tissue. The photoirritation potential of the test chemical was dictated by the reduction in tissue viability of UVA exposed tissues compared to non-UVA exposed tissues.

Under the conditions of this assay, the test article was considered to be **non-phototoxic** at concentrations of 0.4%, 1.2%, and 3.7%. The negative and positive controls performed as anticipated.

I. Introduction

A. Purpose

In vitro dermal phototoxicity study was conducted to evaluate whether a test article would induce photoirritation in the EpiDerm™ model assay. MatTek Corporation's reconstructed human epidermal model is becoming a standard in determining the phototoxicity potential of a test substance. This assay is able to discriminate between photoirritants and non-photoirritants at varying concentrations.

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II. Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity
B. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; UVA-vis Irradiation Equipment; UVA meter; Pipettes
C. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM) based medium; Dulbecco's Phosphate-Buffered Saline (DPBS); sterile deionized H_2O
D. Preparation:	Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
E. Tissue Culture Plates:	Falcon flat bottom 96-well, 24-well, and 6-well tissue culture plates
F. Reagents:	MTT (*3-4,5-dimethyl thiazole 2-yl*) (1.0mg/mL); Extraction Solution (Isopropanol); Chlorpromazine; Triton X-100 (1%)
G. Other:	Wash bottle; sterile disposable pipette tips; Parafilm; forceps

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™ consists of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis. This model consists of organized basal, spinous, and granular layers, and contains a multilayer stratum corneum containing intercellular lamellar lipid layers. The EpiDerm™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile deionized water is used as the negative controls for the EpiDerm™ Phototoxicity assay.

C. Positive Control

Concentrations of chloropromazine, ranging from 0.001% to 0.1%, were used as positive controls for the EpiDerm™ Phototoxicity assay.

D. Data Interpretation Procedure

A photoirritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance and 60 minutes of 6 J/cm^2 is reduced by 20% compared to the non-irradiated control tissues.

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Micro Technologies, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and tissue insert dosing begins.

B. Test Substance Exposure

50µL of the diluted test substance in their respective concentrations are applied to 2 tissue inserts and allowed to incubate for overnight in a humidified incubator (37°C, 5% CO_2, 95% RH).

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C. Tissue Irradiation

Tissue inserts in their 6-well plates are UVA-irradiated for 60 minutes with 6 J/cm^2 at room temperature. The non-irradiated tissue inserts are incubated at room temperature in the dark.

D. Tissue Washing and Post Incubation

After UVA-irradiation and dark incubation is complete the tissue inserts are washed using sterile DPBS and transferred to fresh 6-well plates and media for overnight incubation at 37°C, 5% CO_2, 95% RH.

E. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO_2, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD_{570}) is ≥ 0.8.

B. Positive Control

The assay meets the acceptance criterion if a dose dependent reduction in cell viability in the UVA-irradiated tissues is between 0.00316% and 0.0316%.

C. Standard Deviation

Since the phototoxicity potential is predicted from the mean viability of 2 tissues for the EpiDerm™ Phototoxicity Protocol, the variability of the replicates should not exceed 30%.

VI. Results

A. Tissue Characteristics

The tissue inserts included in the MatTek EpiDerm™ assay kit were in good condition, intact, and viable.

B. Tissue Viability Assay

The results are summarized in Figure 1. Cell viability is calculated for each tissue as a percentage of the corresponding vehicle control either irradiated or non-irradiated. Tissue viability was not reduced by 20% in the presence of the test substance and UVA-irradiation at concentrations of 0.4%, 1.23%, and 3.7%. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited dose dependent loss of tissue viability and cell death.

C. Test Validity

The data obtained from this study met criteria for a valid assay. The negative and positive controls performed as anticipated.

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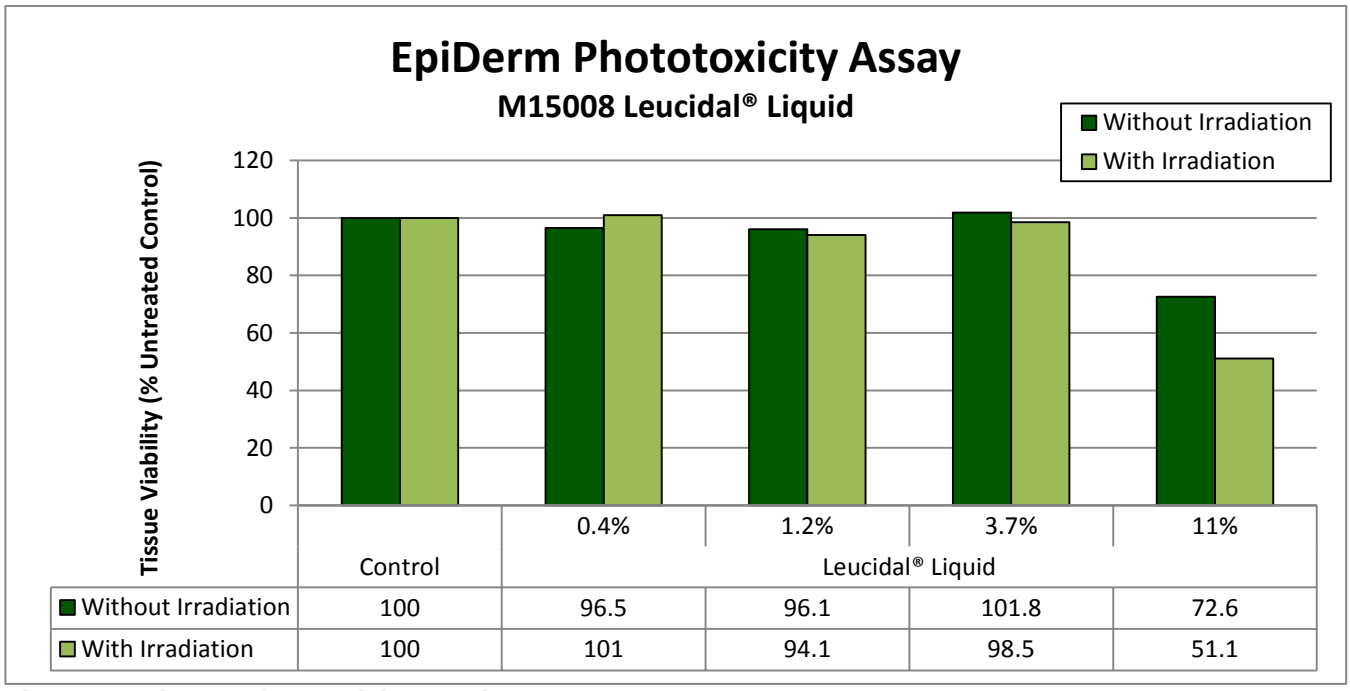
VII. Conclusion

Phototoxicity (photoirritation) is defined as an acute toxic response that is elicited after exposure of the skin to certain chemicals and subsequent exposure to light. Under the conditions of this assay, the test article substance was considered to be **non-phototoxic** at concentrations of 0.4%, 1.2%, and 3.7%. There is a decrease in viability at the 11% test concentration with and without irradiation but this concentration is significantly higher than the suggested use levels. We can safely say that **Leucidal® Liquid** is not a photoirritant when used at the suggested use levels of 2 – 4%.



	Control	0.4%	1.2%	3.7%	11%
Without Irradiation	100	96.5	96.1	101.8	72.6
With Irradiation	100	101	94.1	98.5	51.1

Figure 1: EpiDerm Phototoxicity Graph

OECD 202 Acute *Daphnia* Assay

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Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6

Test Request Form #: 580

Lot #: 32011

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
OECD 202
Daphnia spp. Acute Immobilization Test

Introduction

The purpose of the present study is to determine the toxicity of **Leucidal® Liquid** by exposing Daphnia spp. to the test substance for 48 hours and measuring the immobilization rate against the control. The present study defines an organism as being immobilized when it does not move for 15 seconds after the test vessel is gently shaken.

OECD Guideline 202 on "*Daphnia* spp., Acute Immobilization Test and Reproduction Test", adopted in 1984, included two parts: Part I – the 24 hour EC_{50} acute immobilization test and Part II – the reproduction test (at least 14 days). Revision of the reproduction test resulted in the adoption and publication of Test Guideline 211 on "*Daphnia magna* Reproduction Test" in September 1998. Consequently, the new version of Guideline 202 is restricted to the acute immobilization test.

Assay Principle

Young daphnids, aged less than 24 hours at the start of the test, are exposed to the test substance at a range of concentrations for a period of 48 hours. Immobilization is recorded at 24 hours and 48 hours and compared with control values. The results are analyzed in order to calculate the EC_{50} at 48 hours. EC_{50} is the concentration estimated to immobilize 50% of the daphnids within a stated exposure period. Immobilization refers to those animals that are not able to swim within 15 seconds after gentle agitation of the test vessel, even if they can still move their antennae.

The water solubility and vapor pressure of the test substance should be known. A reliable analytical method for the quantification of the substance in the test solutions with reported recovery efficiency and limit of determination should also be available.

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A reference substance may be tested for EC_{50} as a means of assuring that the test conditions are reliable.

For this assay to be valid, the following performance criteria apply:

- In the control, not more than 10% of the daphnids should have been immobilized.
- The dissolved oxygen concentration at the end of the test should be at least 3 mg/L in control and test vessels.

Materials

- Glass Test Tubes and/or Beakers
- Dissolved Oxygen Meter
- pH Meter
- Temperature Control Apparatus
- Total Organic Carbon (TOC) Analyzer
- Chemical Oxygen Demand (COD) Analyzer
- *Daphnia magna* Straus
    - Use organisms less than 24 hours old. Do not use first offspring of parents.
- Water
    - Use water suitable for culturing and testing Daphnia spp. It can be natural water (surface water or groundwater), dechlorinated tap water, or artificially prepared water (Table 1), but must satisfy the conditions listed in Table 2. Do not use Elendt M4 or M7 media or water containing chelating agents for testing metal-containing substances.

Substance	Concentration
Particulate Matter	<20 mg/L
Total Organic Carbon	<2 mg/L
Unionized Ammonia	<1 ug/L
Residual Chlorine	<10 ug/L
Total Organophosphorus Pesticides	<50 ng/L
Total Organochlorine Pesticides plus Polychlorinated Biphenyls	<50 ng/L
Total Organic Chlorine	<25 ng/L

Table 1: Chemical Characteristics of Suitable Water

Substance	Amount Added to 1 Liter Water	To prepare the reconstituted water, add the following volumes of stock solutions to 1 liter water
Calcium Chloride	11.76 grams	25 mL
Magnesium Sulfate	4.93 grams	25 mL
Sodium Bicarbonate	2.59 grams	25 mL
Potassium Chloride	0.23 grams	25 mL

Table 2: Examples of Suitable Reconstituted Test Water

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Methods

Test Conditions

- Test Method
  - Test is performed under a static, semi-static, or flow-through condition. If test substance is unstable, a semi-static or flow-through test is recommended.
- Exposure Period
  - 48 hours
- Test Volume
  - At least 2 milliliters
- Number of Test Organisms
  - At least 20 organisms for each test concentration and the control.
- Test Concentration
  - Adopt a concentration range of at least 5 concentrations, with the highest concentration inducing 100% immobilization and no effect at the lowest concentration.
- Culture Method
  - Illumination: The photoperiod is set to 16 hours light and 8 hours dark
  - Temperature: The temperature is between 18°C to 22°C
  - Dissolved Oxygen Concentration: Must be kept at 3mg/L or higher
  - Feeding: Do not feed test organisms

Observation

- Observe mobility of the organisms at least twice (i.e., at 24 and 48 hours after exposure).
- The organisms are considered immobilized when they do not move for 15 seconds after test vessel is gently shaken.

Measurement of Test Substance Concentrations

- At the beginning and end of exposure, measure test substance concentrations at the lowest and highest test concentration groups.
  - For volatile or adsorptive substances, additional measurements are recommended at 24 hours intervals during exposure period.

Test Condition Measurements

- Measure dissolved oxygen in the control and at the highest test concentration at the beginning and end of the exposure period.
- Measure pH in the control and at the highest test concentration at the beginning and end of the exposure period.
- Water temperature should be measured at the beginning and end of the exposure period.

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Data and Reporting

I. Data
   a. Data should be summarized in tabular form, showing for each treatment group and control, the number of daphnids used, and immobilization at each observation. The percentages immobilized at 24 and 48 hours are plotted against test concentrations. Data are analyzed by appropriate statistical methods (e.g. probit analysis, etc.) to calculate the slopes of the curves and the EC_{50} with 95% confidence limits (p = 0.95).
   b. Where the standard methods of calculating the EC_{50} are not applicable to the data obtained, the highest concentration causing no immobility and the lowest concentration producing 100% immobility should be used as an approximation for the EC_{50} (this being considered the geometric mean of these two concentrations).

II. Test Report
   a. The test report must include the following:
      i. Test substance:
         1. Physical nature and relevant physical-chemical properties
         2. Chemical identification data, including purity
      ii. Test species:
         1. Source and species of *Daphnia*, supplier of source (if known), and the culture conditions (including source, kind and amount of food, feeding frequency)
      iii. Test conditions:
         1. Description of test vessels: type and volume of vessels, volume of solution, number of daphnids per test vessel, number of test vessels (replicates) per concentration
         2. Methods of preparation of stock and test solutions including the use of any solvent or dispersants, concentrations used
         3. Details of dilution water: source and water quality characteristics (pH, hardness, Ca/Mg ratio, Na/K ratio, alkalinity, conductivity, etc); composition of reconstituted water if used
         4. Incubation conditions: temperature, light intensity and periodicity, dissolved oxygen, pH, etc.
      iv. Results:
         1. The nominal test concentrations and the result of all analyses to determine the concentration of the test substance in the test vessels; the recovery efficiency of the method and the limit of determination should also be reported
         2. All physical-chemical measurements of temperature, pH and dissolved oxygen made during the test
         3. The EC_{50} at 48 hours for immobilization with confidence intervals and graphs of the fitted model used for calculation, the slopes of the dose-response curves and their standard error; statistical procedures used for determination of EC_{50}.

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Results

General Information:

Name of new chemical substance	Leucidal® Liquid		
INCI Nomenclature	*Leuconostoc*/Radish Root Ferment Filtrate		
CAS number	1686112-10-6		
Structural or rational formula (if neither is available, summarize its formulation method)	Biotechnology/Botanical: Leuconostoc kimchii & Raphanus Sativus		
Molecular weight	3960 Daltons		
Purity of the new chemical substance used for the test (%)	100%		
Lot number of the new chemical substance used for the test	32011		
Names and contents of impurities	n/a		
Solubility in water	100%		
Properties at room temperature	Clear to Slightly Hazy Liquid		
Stability	Heat Stable up to 70°C		
Solubility in solvents, etc.	**Solvent**	**Solubility**	**Stability in solvent**
	n/a	n/a	n/a

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Test Materials and Methods:

Items			Contents
Test Organisms	Species		*Daphnia magna*
	Source		Carolina Biological Supply Company
	Reference substance (EC_{50})		Potassium dichromate (0.94 mg/L)
Culture	Kind of Medium		Elendt Medium M4
	Conditions (Temperature/Photoperiod)		20°C/16 Hour Light-8 Hour Dark
Test Conditions	Test Vessel		Glass
	Material Water	Kind	Elendt Medium M4
		Hardness	250 mg/L
		pH	7.4
	Date of Exposure		09/25/2013
	Test Concentrations		200, 90.9, 41.3, 18.8, 8.5 mg/L
	Number of organisms		120
	Number of Replicates	Exposure Group	4
		Control Group	4
	Test Solution Volume		2 mL
	Vehicle	Use or Not	N/A
		Kind	N/A
		Concentration	N/A
		Number of Replicates	N/A
	Culture Method (Static, Semi-Static, Flow-Through)		Static
	Water Temperature		20°C ± 2°C
	Dissolved Oxygen Concentration (DO)		3 mg/L
	Photoperiod		16 Hour Light-8 Hour Dark
	Statistical Method		Probit Analysis

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Test Results:

Items		**Contents**
Toxicity Value	48hr EC50	131 mg/L
Exposure Concentrations Used for Calculation	Nominal Values	200, 90.9, 41.3, 18.8, 8.5 mg/L
Remarks		Not harmful to aquatic organisms

Discussion

After 48 hours, the EC50 value for **Leucidal® Liquid** was determined to be 131 mg/L. The conditions of OECD guideline 202 for the validity of the test were adhered to: The immobility of controls in purified drinking water (dilution water) did not exceed 10%. According to the EU Directive 93/67/EEC, this product is not classified and therefore not harmful to aquatic organisms.

OECD 301B Ready Biodegradability Assay

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Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6

Test Request Form #: 579

Lot #: 32011

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
OECD 301 B
Ready Biodegradability: CO_2 Evolution (Modified Sturm Test)

Introduction

A study was conducted to assess the readily biodegradability of **Leucidal® Liquid** in an aerobic aqueous medium. In the OECD guideline 301 for ready biodegradability, six methods are provided as options. This report uses method B, CO_2 Evolution, also known as a Modified Sturm Test. This method was chosen based on the solubility, volatility, and adsorbing capabilities of the test sample.

Assay Principle

A solution or suspension of the test substance in a mineral medium is inoculated and incubated under aerobic conditions in the dark or in diffuse light. The amount of DOC (Dissolved Organic Carbon) in the test solution due to the inoculum should be kept as low as possible compared to the amount of organic carbon due to the test substance. Allowance is made for the endogenous activity of the inoculum by running parallel blanks with inoculum but without test substance. A reference compound is run in parallel to check the procedures' operation.

In general, degradation is followed by the determination of parameters such as DOC, carbon dioxide production, and oxygen uptake. Measurements are taken at sufficiently frequent intervals to allow the identification of the beginning and end of biodegradation.

Normally this test lasts for 28 days, but it may be ended before that time if the biodegradation curve reaches a plateau for at least three determinations. Tests may also be prolonged beyond 28 days when the curve shows that biodegradation has started but the plateau has not yet been reached. In such cases the test substance would not be classified as readily biodegradable.

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The pass levels for ready biodegradability are 70% removal of DOC and 60% of ThOD (Theoretical Oxygen Demand) or $ThCO_2$ (Theoretical Carbon Dioxide) production for respirometric methods. They are lower in the respirometric methods since, as some of the carbon from the test chemical is incorporated into new cells, the percentage of CO_2 produced is lower than the percentage of carbon being used. These pass values have to be reached in a 10-day window within the 28-day period of the test. The 10-day window begins when the degree of biodegradation has reached 10% DOC, ThOD, or $ThCO_2$ and must end before day 28 of the test. Test substances which reach the pass levels after the 28-day period are not deemed to be readily biodegradable.

In order to check the procedure, reference compounds which meet the criteria for ready biodegradability are tested by setting up an appropriate vessel in parallel as part of normal test runs. Suitable compounds are freshly distilled aniline, sodium acetate, and sodium benzoate. These compounds all degrade in this method even when no inoculum is deliberately added.

Because of the nature of biodegradation and of the mixed bacterial populations used as inocula, determinations should be carried out at least in duplicate. It is usually found that the larger the concentration of microorganisms initially added to the test medium, the smaller the variation between replicates.

Materials

- Water
  - Deionized or distilled, free from inhibitory concentrations of toxic substances
  - Must contain no more than 10% of the organic carbon content introduced by the test material
  - Use only one batch of water for each series of tests
- Mineral media
  - To prepare the mineral medium, mix 10 mL of solution A with 800 mL water. Then add 1 mL each of solutions B, C, and D and make up to 1 liter with water.
  - Solution A (Dissolve in water and make up to 1 liter; pH 7.4)
    - Potassium dihydrogen orthophosphate, KH_2PO.....8.5g
    - Dipotassium hydrogen orthophosphate, K_2HPO_4.....21.8g
    - Disodium hydrogen orthophosphate dehydrate, $Na_2HPO_4.2H_2O$.....33.4g
    - Ammonium chloride, NH_4Cl.....0.5g
  - Solution B (Dissolve in water and make up to 1 liter)
    - Calcium chloride, anhydrous, $CaCl_2$.....27.50g
      Or
    - Calcium chloride dehydrate, $CaCl_2.2H_2O$.....36.40g
  - Solution C (Dissolve in water and make up to 1 liter)
    - Magnesium sulphate heptahydrate, $MgSO_4.7H_2O$..... 22.50g
  - Solution D (Dissolve in water and make up to 1 liter.)
    - Iron (III) chloride hexahydrate, $FeCl_3.6H_2O$.....0.25g
  - Flasks, 2-5 liters each, fitted with aeration tubes reaching nearly to the bottoms of the vessels and an outlet
  - Magnetic stirrers
  - Gas absorption bottles
  - Device for controlling and measuring air flow
  - Apparatus for carbon dioxide scrubbing, for preparation of air which is free from carbon dioxide; alternatively, a mixture of CO_2-free oxygen and CO_2-free nitrogen from gas cylinders in the correct proportions (20% O_2 : 80% N_2)
  - Device for determination of carbon dioxide, either titrimetrically or by some form of inorganic carbon analyzer

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- Stock solutions of test substances
  - When solubility of the substance exceeds 1 g/L, dissolve 1-10 g, as appropriate, of test or reference substance in water and make up to 1 liter. Otherwise, prepare stock solutions in mineral medium or add the chemical directly to the mineral medium, making sure it
- Inoculum
  - The inoculum may be derived from the following sources
    - Activated sludge
    - Sewage effluents
    - Surface waters
    - Soils
    - Or from a mixture of these.
  - Inoculum may be pre-conditioned to the experimental conditions, but not pre-adapted to the test substance. Pre-conditioning consists of aerating activated sludge in mineral medium or secondary effluent for 5-7 days at the test temperature. Pre-conditioning sometimes improves the precision of the test method by reducing blank values.

Methods

I. Preparation of flasks: As an example, the following volumes and weights indicate the values for 5-liter flasks containing 3 liters of suspension. If smaller volumes are used, modify the values accordingly.
   a. To each 5-liter flask, add 2,400 mL mineral medium.
   b. Add an appropriate volume of the prepared activated sludge to give a concentration of suspended solids of not more than 30 mg/L in the final 3 liters of inoculated mixture. Alternatively, first dilute the prepared sludge to give a suspension of 500-1000 mg/L in the mineral medium before adding an aliquot to the contents of the 5-liter flask to attain a concentration of 30 mg/L.
   c. Aerate these inoculated mixtures with CO_2-free air overnight to purge the system of carbon dioxide.
   d. Add the test material and reference compound, separately, as known volumes of stock solutions, to replicate flasks to yield concentrations, contributed by the added chemicals, of 10 – 20 mg DOC or TOC per liter. Leave some flasks without addition of chemicals as inoculum controls. Add poorly soluble test substances directly to the flasks on a weight or volume basis. Make up the volumes of suspensions in all flasks to 3 liters by the addition of mineral medium previously aerated with CO_2-free air.
   e. If required, use one flask to check the possible inhibitory effect of the test substance by adding both the test and reference substances at the same concentrations as present in the other flasks.
   f. If required, check whether the test substance is degraded abiotically by using a sterilized uninoculated solution of the chemical. Sterilize by the addition of a toxic substance at an appropriate concentration.
   g. If barium hydroxide is used, connect three absorption bottles, each containing 100 mL of 0.0125M barium hydroxide solution, in series to each 5-liter flask. The solution must be free of precipitated sulfate and carbonate and its strength must be determined immediately before use.
   h. If sodium hydroxide is used, connect two traps, the second acting as a control to demonstrate that all the carbon dioxide was absorbed in the first. Absorption bottles fitted with serum bottle closures are suitable. Add 200 mL 0.05M sodium hydroxide to each bottle. This is sufficient to absorb the total quantity of carbon dioxide evolved when the test substance is completely degraded.
   i. In a typical run, the following flasks are used:
      i. Flasks 1 & 2: containing test substance and inoculum (test suspension)
      ii. Flasks 3 & 4: containing only inoculum (inoculum blank)
      iii. Flask 5: containing reference compound and inoculum (procedure control)
      iv. Flask 6: containing test substance and sterilizing agent (abiotic sterile control)
      v. Flask 7: containing test substance, reference compound and inoculum (toxicity control)

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II. Start the test by bubbling CO_2-free air through the suspensions at a rate of 30-100 mL/minute.

III. CO_2 Determination
   a. It is mandatory to follow the CO_2 evolution from the test suspensions and inoculum blanks in parallel and it is advisable to do the same for the other test vessels.
   b. During the first ten days it is recommended that analyses of CO_2 should be made every second or third day and then at least every fifth day until the 28th day so that the 10-day window period can be identified. On the days of CO_2 measurement, disconnect the barium hydroxide absorber closest to the test vessel and titrate the hydroxide solution with 0.05M HCl using phenolphthalein as the indicator. Move the remaining absorbers one place closer to the test vessel and place a new absorber containing 100 mL fresh 0.0125M barium hydroxide at the far end of the series. Make titrations are needed (for example, when substantial precipitation is seen in the first trap and before any is evident in the second, or at least weekly). Alternatively, with NaOH as absorbent, withdraw a sample of the sodium hydroxide solution from the absorber nearest to the test vessel using a syringe. The sample volume needed will depend on the carbon analyzer used, but sampling should not significantly change the absorbent volume over the test period. Inject the sample into the IC part of the carbon analyzer for analysis of evolved carbon dioxide directly. Analyze the contents of the second trap only at the end of the test in order to correct for any carry-over of carbon dioxide.
   c. On the 28th day withdraw samples, optionally, for DOC and/or specific chemical analysis. Add 1 mL of concentrated hydrochloric acid to each test vessel and aerate them overnight to drive off the carbon dioxide present in the test suspensions. On day 29 make the last analysis of evolved carbon dioxide.

Data and Reporting

I. Treatment of Results
   a. Data from the test should be entered onto the data sheet below.
   b. The amount of CO_2 produced is calculated from the amount of base remaining in the absorption bottle. When 0.0125M $Ba(OH)_2$ is used as the absorbent, the amount remaining is assessed by titrating with 0.05M HCl.
   c. Since 1 mmol of CO_2 is produced for every mol of $Ba(OH)_2$ reacted to $BaCl_2$ and 2 mmol of HCl are needed for the titration of the remaining $Ba(OH)_2$ and given that the molecular weight of CO_2 is 44 g, the weight of CO_2 produced (in mg) is calculated by:

$$\frac{0.05 \times (50 - mL\ HCl\ Titrated) \times 44}{2} = 1.1 \times (50 - mL\ HCl\ Titrated)$$

   Therefore, the factor to convert volume of HCl titrated to mg CO_2 produced is 1.1 in this case. Calculate the weights of CO_2 produced from the inoculum alone and from the inoculum plus test substance using the respective titration values. The difference is the weight of CO_2 produced from the test substance alone.

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d. The percentage biodegradation is calculated from:

$$\%\ Degradation = \frac{mg\ CO_2\ Produced}{ThCO_2 \times mg\ Test\ Substance\ Added} \times 100$$

Or

$$\%\ Degradation = \frac{mg\ CO_2\ Produced}{mg\ TOC\ Added\ in\ Test\ \times 3.67} \times 100$$

$$Where\ 3.67\ is\ the\ conversion\ factor\ \left(\frac{44}{12}\right)\ for\ carbon\ to\ carbon\ dioxide$$

e. When NaOH is used as the absorbent, calculate the amount of CO_2 produced after any time interval from the concentration of inorganic carbon and the volume of absorbent used. Calculate the percentage degradation from:

$$\%\ ThCO_2 = \frac{mg\ IC\ from\ Test\ Flask - mg\ IC\ from\ Blank}{mg\ TOC\ Added\ as\ Test\ Substances} \times 100$$

f. Display the course of degradation graphically and indicate the 10-day window. Calculate and report the percentage removal achieved at the plateau, at the end of the test, and/or at the end of the 10-day window, whichever is appropriate.
g. When appropriate, calculate DOC removals using the equation given in 301 A paragraph 27.
h. When an abiotic control is used, calculate the percentage abiotic degradation by:

$$\%\ Abiotic\ Degradation = \frac{CO_2\ Produced\ by\ Sterile\ Flask\ After\ 28\ Days\ (mg)}{ThCO_2\ (mg)} \times 100$$

Validity of Tests

I. The IC content of the test substance suspension in the mineral medium at the beginning of the test must be less than 5% of the TC, and the total CO_2 evolution in the inoculum blank at the end of the test should not normally exceed 40 mg/L medium. If values greater than 70 mg CO_2/L are obtained, the data and experimental technique should be examined critically.

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OECD 301B Ready Biodegradability Assay

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Data Sheet

Laboratory	Active Concepts Tissue Culture Laboratory		
Test Start Date	9/25/2013		
Test Substance	**Name**	Leucidal® Liquid	
	Stock Solution Concentration	2 g/L	
	Initial Concentration in Medium	20 mg/L	
Inoculum	**Source**	Activated Sludge	
	Treatment Given	Centrifugation	
	Pre-conditioning	N/A	
	Suspended Solids Concentration in Reaction Mixture	4 mg/L	
Reference Material	Sodium Benzoate	**Concentration**	20 mg/L
CO_2 Production and Degradability	**Method**	**$Ba(OH)_2$**	0.0125M
		NaOH	N/A
		Other	N/A
Total Contact Time	28 Days		
Total CO_2 Evolved Measurements	**Days**	2, 4, 11, 17, 23, 28	
Degradation Over Time	95% and 89% after 28 days		
Remarks	Test material was readily biodegradable		
Conclusion	This test met the criteria for a valid assay		

Discussion

Based on the testing conducted in accordance with the specified test method, **Leucidal® Liquid** achieved 92% biodegradation after 28 days of testing. The product met method requirements for the Readily Biodegradable classification.

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Date Issued: January 23, 2015

ALLERGEN DECLARATION

RE: *Leucidal® Liquid (M15008)*

Please be advised that this form is to certify that the above referenced product, manufactured at Active Micro Technologies, LLC, does not contain any of the allergens listed below:

Eggs – or egg products

Milk – or milk products (includes whey, lactose, casein, milk, cream)

Peanuts – or peanut products

Fish – (includes fish: surimi, cod, pollack, whitefish)

Shellfish – (shrimp, lobster, crab, clams, etc.)

Soybeans – or soybean products (includes soya powder, protein, oil, lecithin, tofu)

Wheat – or wheat products (includes Gluten)

Tree nuts – (almond, brazil nut, cashew, chestnut, hazelnut, filbert, pine nuts (pinyon, pinon), pistachio, pecan, macadamia, walnut).

Palm Oil – or palm kernel oil

Corn – or corn products

If you have any further questions or concerns, please contact us at: 1-704-276-7100

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Certificate of Origin

Leucidal® Liquid
Code: M15008

Active Micro Technologies, LLC certifies that all raw material(s) used to manufacture the above listed ingredient originate in the United States of America.

Active Micro Technologies, LLC certifies that all raw material(s) used to manufacture the above listed ingredient are prepared from non-GMO organisms and are BSE/TSE Free.

Active Micro Technologies, LLC certifies the below sources for each item listed in our INCI Name:

INCI Name	Source
Water	Water
Leuconostoc/Radish Root Ferment Filtrate	Bacteria/Plant (*Leuconostoc/ Raphanus sativus*)

Active Micro Technologies, LLC certifies that the above listed ingredient can be classified as Vegan Compliant.

Active Micro Technologies, LLC certifies that the above listed ingredient has never been tested on animals.

SECTION 1. IDENTIFICATION

Product Name/Identifier	Leucidal® Liquid
Product Code	M15008
Recommended Use	Topical Cosmetic Use; Antimicrobial
Restrictions on Use	Refer to the detailed list of labeling/restrictions (Section 15 Regulatory Information)
Supplier/Manufacturing Site	Active Micro Technologies, LLC
Address	107 Technology Drive Lincolnton, NC 28092, USA
Telephone No. (24hrs)	1-704-276-7100
Fax No.	1-704-276-7101
Emergency Telephone #	1-704-276-7100 (Mon-Fri: 8:00AM – 5:00PM EST)

SECTION 2. HAZARD(S) IDENTIFICATION

Classification:

GHS / CLP
Basis for Classification: Based on present data no classification and labeling is required according to GHS, taking into account the national implementation (United Nations version 2011)

USA
OSHA Regulatory Status: This material is non-hazardous as defined by the American OSHA Hazard Communication Standard (29 CFR 1910.1200).

Europe
Basis for Classification:
-According to present data no classification and labeling is required according to Directives 67/548/EEC or 1999/45/EC.
-This product is not classified as hazardous to health or environment according to the CLP regulation.

Labeling Elements:

Pictograph: No hazard symbol expected

Hazard statements/Signal Word: Not applicable

Precautionary statements:
P233: Keep container tightly closed
P281: Use personal protective equipment as required
P402: Store in a dry place
P404: Store in a closed container
P410: Protect from sunlight
P411: Store at temperatures not exceeding 25°C

Other hazards which do not result in classification:

No particular fire or explosion hazard.
By mechanical effect: No particular hazards.
By hydroscopic effect: No particular hazards.

US NFPA 704 (National Fire Protection Association) Hazard Rating System:

Health hazard: Rating 0; Normal Material
Flammability: Rating 0, Will Not Burn
Reactivity: Rating 0, Stable
Other Hazard Information: None

Results of PBT and vPvB assessment:

-PBT: Not applicable
-vPvB: Not applicable

SECTION 3. COMPOSITION / INFORMATION ON INGREDIENTS

Common Chemical Name: Leuconostoc/Radish Root Ferment Filtrate

Generic name:

Chemical Family: Ferment

Description: Mixture: consisting of the following components. This section describes all components of the mixture

Substance	CAS Numbers	EC Numbers	Percentage
Water	7732-18-5	231-791-2	48.00 – 52.00%
Leuconostoc/Radish Root Ferment Filtrate	1686112-10-6	N/A	48.00 – 52.00%

Formula: Not applicable

SECTION 4. FIRST-AID MEASURES

General: In all cases of doubt, or when symptoms persist, seek medical attention.

Inhalation: Move to fresh air from exposure area. Get medical attention for any breathing difficulty.

Skin contact: Rinse with soap and water. Get medical advice if irritation develops.

Eye contact: Immediately rinse with water for at least 15 minutes, while keeping the eyes wide open. Consult with a physician.

Ingestion:	Consult with a physician.
Protection of first-aiders:	No special protection required.

SECTION 5. FIRE-FIGHTING MEASURES

Fire and explosion hazards:	Not considered to be a fire and explosion hazard
Extinguishing media:	
Suitable:	Water, dry chemicals, foam & carbon dioxide.
Not suitable:	None known
Fire fighting:	Move container from fire area if it can be done without risk. Avoid inhalation of material or combustion by-products. Stay upwind and keep out of low area
Protection for fire-fighters:	Boots, gloves, goggles.

SECTION 6. ACCIDENTAL RELEASE MEASURES

Personal precautions:	Avoid contact with eyes. Personal Protective Equipment: -Protective goggles
Environmental precautions:	Prevent entry into sewers and waterways. Do not allow material to contaminate ground water system
Methods for cleaning up:	
Recovery:	Pick up free liquid for recycling or disposal. Residual liquid can be absorbed on an inert material.
Cleaning/Decontamination:	Wash non-recoverable remainder with water.
Disposal:	For disposal of residues refer to sections 8 & 13.

SECTION 7. HANDLING AND STORAGE

Handling

Technical measures:	Labeling: Keep out of the reach of children.
Measures:	For industrial use, only as directed.
Safe handling advice:	Wash hands after use. Avoid storage near feed or food stuff.

Storage

Technical measures:	Keep container closed.
Recommended Storage Conditions:	Store in a cool, dry place. This product should be stored at room temperature (23 - 25°C). It should not be exposed to excessive heat or cold. Do not freeze.
Incompatible products:	Avoid contact with strong oxidizers. Refer to the detailed list of incompatible materials (Section 10 Stability/Reactivity)
Packaging:	Product may be packaged in normal commercial packaging.
Packaging materials:	Recommended - Polypropylene & High Density Polyethylene

SECTION 8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Precautionary statements: Ensure adequate ventilation

Control parameters

Occupational exposure Limits:

France:	Not Determined
ACGIH:	Not Determined
Korea:	Not Determined
UK:	Not Determined
Surveillance procedures:	Not Determined
Engineering measures:	Not Determined

Personal Protective Equipment:

Respiratory protection:	Local exhaust
Hand protection:	Protective gloves made of rubber or neoprene.
Eye protection:	Safety glasses.
Collective emergency equipment:	Eye fountain.
Skin and Body Protection:	Suitable protective clothing
Hygiene measures:	Handle in accordance with good industrial hygiene and safety practice.
Measures related to the Environment:	No particular measures.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	Clear to slightly hazy liquid
Color:	Yellow to light amber
Odor:	Characteristic
Solids (1g-105°C-1hr):	48.0 – 52.0%

pH:	4.0 – 6.0
Specific Gravity (25°C):	1.140 – 1.180
Ninhydrin:	Positive
Phenolics (tested as Salicylic Acid):	18.0 – 22.0%
Heavy Metals:	< 20 ppm
Arsenic:	< 2 ppm
Minimum Inhibitory Concentration	
Organism (ATCC#):	
E. coli (#8739):	0.50 – 4.00%
S. aureus (#6538):	0.25 – 2.00%
P. aeruginosa (#9027):	1.00 – 4.00%
C. albicans (#10231):	0.25 – 2.00%
A. brasiliensis (#16404):	0.25 – 2.00%
Vapor pressure (@ 20°C):	~20 mm Hg
Vapor density:	Not applicable
Boiling Point:	100°C
Freezing Point:	0°C
Melting point:	Not applicable
Flash point:	> 200°F
Oxidizing properties:	Non oxidizing material according to EC criteria.
Solubility:	
In water:	Soluble
In organic solvents:	Not determined
Log P:	Not determined

SECTION 10. STABILITY AND REACTIVITY

Stability: Stable under ordinary conditions of use and storage up to one year then re-test to full product specifications to extend shelf life

Hazardous reactions: None known

Conditions to avoid: No dangerous reactions known under use of normal conditions. Avoid extreme heat.

Materials to avoid: No dangerous reaction known with common products.

Hazardous decomposition products: None known

SECTION 11. TOXICOLOGICAL INFORMATION

Ingestion: Not Determined
Dermal: Non-Irritant (Dermal Irritection Model)
Ocular: Non-Irritant (Ocular Irritection Model)
Inhalation: Not Determined

Acute toxicity data: EC_{50} (Acute Daphnia): 131 mg/L - Not harmful to aquatic organisms

Sensitization: Non-Primary Irritant & Non-Primary Sensitizer (RIPT, In-Vitro Skin Sensitization Report & Direct Peptide Reactivity Assay)

Repeated dose toxicity: No known effects
Subacute to chronic toxicity: Not Determined

Mutagenicity/genotoxicity: Non-mutagenic

Additional Toxicological Information: This product is not subject to classification according to the calculation method of the General EU Classification Guidelines for Preparations as issued in the latest version.

Specific effects:

Carcinogenicity: No known effects
Mutagenicity: No known effects
Reproductive toxicity: No known effects
Neuro-toxicity: No known effects

For more information: Does not present any particular risk on handling under normal conditions of good occupational hygiene practice.

This product has not been tested for the following:
-Primary cutaneous and corrosive irritation
-Acute oral toxicity

SECTION 12. ECOLOGICAL INFORMATION

Ecotoxicity
Effects on the aquatic environment: Not Determined

Biodegradability:
Persistence: Readily Biodegradable

Bioaccumulation:
Octanol / water partition coefficient: Not Determined

Mobility:
Precipitation:
Expected behavior of the product: Ultimate destination of the product: Soil & sediment.

Other Adverse Effects: None known

SECTION 13. DISPOSAL CONSIDERATIONS

Residues from product

Prohibition: Do not allow the product to be released into the Environment.
Destruction/Disposal: Dispose of in accordance with relevant local regulations

Contaminated packaging

Decontamination/cleaning: Cleaning is not required prior to disposal.
Destruction/Disposal:

Note: Take all necessary precautions when disposing of this product according to local regulations.

SECTION 14. TRANSPORT INFORMATION

UN Number: None
UN Shipping Name: None

Transport Hazard Class: Not classified as dangerous for transport

Land (rail/road): Material is not restrictive for land transport and is not regulated by ADR/RID
Sea: Material is not restrictive for sea transport and is not regulated by IMO/IMDG
Air: Material is not restrictive for land transport and is not regulated by ICA/IATA

Marine Pollutant: No

Transport/Additional Information: Not regulated for US DOT Transport in non-bulk containers
This material is not dangerous or hazardous

Special Precautions for User: None known

The above regulatory prescriptions are those valid on the date of publication of this sheet. However, given the possible evolution of transport regulations for hazardous materials and in the event of the MSDS in your possession dating back more than 12 months, it is advisable to check their validity with your sales office.

SECTION 15. REGULATORY INFORMATION

Labeling/Restrictions:

EC regulations:	Not to be used for children under three years of age
Chinese regulations:	Not to be used for children under three years of age
Brazilian regulations:	Not to be used for children under three years of age
ASEAN regulations:	Not to be used for children under three years of age
Mexico regulations:	Not to be used for children under three years of age

Further regulations

United Kingdom:	Handle in accordance with relevant British regulation: control of substance Hazardous to Health Regulations Environmental Hygiene Guidance: EH40 Workplace Exposure Limits (revised annually)	
Korea regulations:	Industrial safety and hygiene regulation:	No
	Hazardous material control regulation:	No
	Fire prevention regulation:	No

Other regulations:

EINECS inventory status:	Aqua:	231-791-2
	Leuconostoc/Radish Root Ferment Filtrate:	N/A
TSCA inventory status:	Exempt	
AICS inventory status:	Not Listed: 1686112-10-6 Listed: 7732-18-5	
Canadian (CEPA DSL) inventory status:	Not Listed: Leuconostoc/Radish Root Ferment Filtrate (1686112-10-6) Listed as Water (DSL)	
Japan (MITI list):	Water & Leuconostoc/Radish Root Ferment Filtrate	
Korea:	Water & Leuconostoc/Radish Root Ferment Filtrate**	
China inventory status:	Water & Leuconostoc/Radish Root Ferment Filtrate	
Philippines inventory status:	Not Listed: Leuconostoc/Radish Root Ferment Filtrate (1686112-10-6) Listed as Water	

*Listed on 2010 INCI Standard Chinese Name Directory
**Not listed in 2004 CTFA Dictionary – Registered with Personal Care Products Council

Note: The regulatory information given above only indicates the principal regulations specifically applicable to the products described in this sheet. The user's attention is drawn to the possible existence of additional provision which complete these regulations. Please refer to all applicable international, national and local regulations and provisions

SECTION 16. OTHER INFORMATION

Prohibited uses:	For specific uses, food industry, ask the manufacturer for more information.
Last Revision Date:	05/14/2015
Preparation Date:	08/13/2015
MSDS summary of changes	- Removed freezing range under Section 7 (Handling and Storage) - New Logo - Added Precautionary Statements - Section 2 (Hazards Identification) - Added Minimum Inhibitory Concentration – Section 9 (Physical & Chemical Properties) - Updated Transport Information – Section 14 (Transport Information) - Added Sensitization Data – Section 11 (Toxicological Information) - Updated CAS/EINECS#'s – Section 3 (Composition / Information on Ingredients) & Section 15 (Regulatory Information) - Added Sensitization Data – Section 11 (Toxicological Information)

The information given is based on our knowledge of this product, at the time of publication in good faith. The attention of the user is drawn to the possible risks incurred by using the product for any other purpose other than which it was intended. This is not in any way excuse the user from knowing and applying all the regulations governing their activity. It is sole responsibility of the user to take all precautions required in handling the product. The purpose of mandatory regulation mentioned is to help the user to fulfill his obligations regarding the use of products. This information is not exhaustive, this is not exonerate the user from ensuring that legal obligations other than those mentioned, relating to the use and storage.

M15008-Leucidal® Liquid Manufacturing Flow Chart

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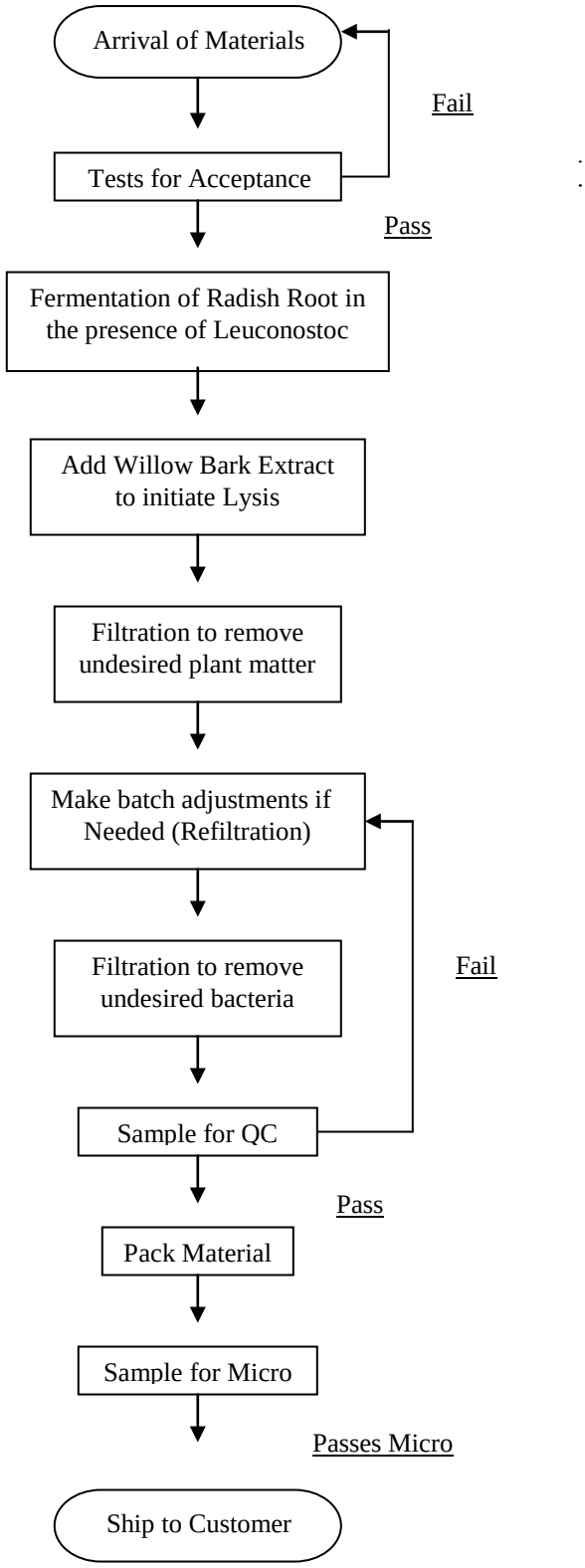

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Leucidal® Liquid
Certificate of Compliance

Code: M15008
INCI Name: Leuconostoc/Radish Root Ferment Filtrate
INCI Status: Approved
CAS #: 1686112-10-6
EINECS #: N/A

The following information on regulatory clearances is believed to be accurate and is given in good faith as a guide to a global use of our ingredients in cosmetic applications. No representation or warranty as to its competences or accuracy is made. Information is offered for use in general cosmetic applications and may vary in particular applications. Users are responsible for determining the suitability of these products for their own particular use. All regulatory decisions should be made on the advice of your regulatory group or legal counsel.

Country / Regulatory Body	Status of Product
EU (REACH)	Compliant at Suggested Use Levels Labeling requirements: Not to be used for children under three years of age Restrictions: Not to be used in preparations for children under 3 years of age, except for shampoos
USA (TSCA)	Exempt
Australia (AICS)	Contact Us
Japan (METI)	Compliant at Suggested Use Levels
Canada (DSL)	Contact Us
China (IECSC)	Compliant at Suggested Use Levels Labeling requirements: Not to be used for children under three years of age Restrictions: Not to be used in preparations for children under 3 years of age, except for shampoos
Brazil (ANVISA)	Compliant at Suggested Use Levels Labeling requirements: Not to be used for children under three years of age Restrictions: Not to be used in preparations for children under 3 years of age, except for shampoos
Korea (KECI)	Compliant
Philippines (PICCS)	Contact Us Labeling requirements: Not to be used for children under three years of age Restrictions: Not to be used in preparations for children under 3 years of age, except for shampoos

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Leucidal® Liquid
Code: M15008

Attention must be paid to the use of Leucidal® Liquid in the equivalent of OTC formulations (eg. quasi-drugs in Japan, or therapeutic goods in Australia). Some countries maintain restricted inventories of raw materials that can be used in those applications so more detailed guidance may be required.

Leucidal® Liquid and any components or impurities are in compliance with the rules governing cosmetic products in the European Union (Directive 76/768/ECC & Regulation No. 1223/2009). However, Leucidal® Liquid contains natural phenolics which will test positive for salicylic acid (see also Specification). This should be borne in mind when formulating products containing Leucidal® Liquid. The recommended use levels for Leucidal® Liquid is 2.00 – 4.00%.

Leucidal® Liquid is in compliance with the standardized set of rules developed and approved by the NPA (Natural Products Association). Leucidal® Liquid is manufactured by the fermentation of radish root in the presence of Leuconostoc. The fermentation media consists of Ammonium Sulfate, Magnesium Sulfate, Disodium Phosphate, Yeast Autolysate & Raphanus Sativus Roots. After fermentation, Willow Bark Extract is added to initiate lysis, resulting material is then filtered to remove undesired plant matter & bacteria.

Leucidal® Liquid is considered a non-hazardous material. All significant toxicological routes of absorption have been considered as well as the systemic effects and margin of safety (MoS) based on a no observed adverse effects level (NOAEL). Due to the restriction placed on animal testing of cosmetic raw materials, and Active Micro Technologies, LLC's internal non-animal testing policy, this product was not tested for NOAEL.

Leucidal® Liquid was tested using *in vitro* dermal and ocular irritation models. This product was found to be non-irritating in both models.

As of December 13, 2013, Leucidal® Liquid does not contain any substances present on the so called "candidate list" provided by the European Chemicals Agency (ECHA). We further certify that to the best of our knowledge our product does not contain any chemicals known or suspected by the State of California to cause cancer or reproductive toxicity as listed under the California Safe Cosmetics Act.

To our knowledge the above material is free of CMR (*) substances, as defined according to Regulation (EC) No 1272/2008 and Cosmetic Regulation (EC) No 1223/2009 as amended.

(*) Carcinogenic, Mutagenic, toxic for Reproduction

Leucidal® Liquid is REACH Compliant. Water is an Annex IV Exemption and Leuconostoc/Radish Root Ferment Filtrate has been pre-registered, reference #17-2119384831-33-0000. Tonnage band for Leuconostoc/Radish Root Ferment Filtrate is between 1 to 10 tonnes/year with a Registration deadline of May 31, 2018.

Active Micro Technologies, LLC certifies that Leucidal® Liquid does not contain any materials prohibited by Halal laws.

Leucidal® Liquid is free of the following:

- Additives
- Alcohol
- BHA & BHT
- Diethylene glycol (DEG)
- Dimethylfuramate
- Dioxin/Dioxane
- Ether
- Formaldehyde/formaldehyde donors
- Formol
- Gluten
- Glycols
- Hydrolyzed Wheat Protein
- Irradiation
- Lactose
- Nanoparticles
- Nitrosamines
- Oil
- Palm oil/palm kernel oil (or derivatives)
- Parabens
- Pesticide residues
- Petrochemicals
- Phthalates
- Polyacrylamides
- Polyethylene Glycol (PEG)
- Quaternary Ammoniums
- Residual Solvents
- Sulfates
- Synthetic preservatives
- Terpene

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Raw Component Regulations

Please note that the below are global regulations for the raw materials used to manufacture Leucidal® Liquid and are not for the product itself.

Leucidal® Liquid contains 18.00 – 22.00% Phenolics, which is the salts and esters of salicylic acid. See below for a list of regulations:

Salicylic Acid and salts:

- **Europe: Maximum Authorized Concentration up to 3.00% (0.50% as acid) when used other than a preservative, depending on the application:**
  a) Rinse-off products: Up to 3.00%
  b) Other products: Up to 2.00%
  *Limitations and requirements: Not to be used in preparations for children under 3, except for shampoos
  *Conditions of use and warnings which must be printed on the label: Not to be used for children under three years of age (1)
  *Note (1): Solely for products which might be used for children under three years of age and which remain in prolonged contact with the skin
  *Intended Purpose must be apparent from the presentation of the final product (e.g. facial toner, anti-acne lotion, peeling gel, etc.)

- **USA: Salicylic Acid is safe when formulated to avoid irritation and to avoid increasing sun sensitivity, or when increased sun sensitivity would be expected, directions for use include the daily use of sun protection.** (*Journal Citation: IJT 22(3):1-108)

- **Japan: Maximum Authorized Concentration:**
  *Salicylic Acid: 0.20 (per 100 grams)
  *Salicylic Acid Salts: 1.00 as total (per 100 grams)

- **Canada: Salicylic Acid permitted in concentrations of 2.00% or less**

- **China: Maximum Authorized Concentration of 0.50% (as acid)**
  *Limitations and requirements: Not to be used in products for children under age 3, except for shampoo
  *Warnings: Do not use for children under 3

- **Brazil: Maximum authorized concentration 0.50% (as acid)**:
  *Limitations: Not to be used in children's products under 3 years, except for shampoos
  *Warnings: Not to be used for children under 3 years of age (1)
  *Note (1): Solely for products which might be used for children under three years of age and which remain in prolonged contact with the skin

- **Mexico: Maximum authorized concentration 0.50% (as acid)**:
  *Limitations: Not to be used in preparations for children under 3 years of age

- **ASEAN: Maximum authorized concentration 0.50% (as acid)**:
  *Limitations: Not to be used in preparations for children under 3 years of age, except for shampoos
  *Warnings: Not to be used for children under 3 years of age

- **Mercosur: Maximum authorized concentration 0.50% (as acid)**:
  *Limitations: Not to be used in preparations for children under 3 years of age, except for shampoos
  *Warnings: Not to be used for children under 3 years of age

ACTIVE MICRO TECHNOLOGIES

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Nanoparticles Statement

Leucidal® Liquid

Code: M15008

Active Micro Technologies, LLC certifies that we are dedicated to providing technologies to support the rapidly developing marketing environment of the Cosmetic Industry. Our products are designed to meet the needs of the Personal Care Industry so nanoparticles are avoided. We can confirm that Leucidal® Liquid (M15008) does not contain nanoparticles nor does its manufacture employ nanotechnologies.

ACTIVE MICRO TECHNOLOGIES

107 Technology Drive • Lincolnton, NC 28092
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Peptide Statement

Leucidal® Liquid

Code: M15008

Leucidal® Liquid contains peptides. Exposure to time, light, and heat can cause browning of peptide solutions. Although this visible phenomenon can occur over time, it does not alter the antimicrobial efficacy of the product.

ACTIVE MICRO TECHNOLOGIES

Rare Earth Elements

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Leucidal® Liquid
Code: M15008

Active Micro Technologies, LLC certifies that we have not analyzed the above listed ingredient for rare earth elements listed in the Chinese FDA PRC regulatory documents. However, based on the origin of the raw materials and the manufacturing processes utilized in this production, we do not expect any of the below listed rare earth elements to be present in Leucidal® Liquid. These elements include:

Element	Symbol
Cerium	Ce
Dysprosium	Dy
Erbium	Er
Europium	Eu
Gadolinium	Gd
Holmium	Ho
Lanthanum	La
Lutetium	Lu
Neodymium	Nd
Praseodymium	Pr
Samarium	Sm
Terbium	Tb
Thulium	Tm
Yttrium	Y
Ytterbium	Yb

ACTIVE MICRO TECHNOLOGIES

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CEPA Statement

Leucidal® Liquid
Code: M15008

According to the **Canadian Environmental Protection Act, 1999,** any products listed under the **Domestic Substance List** are considered acceptable in Canada for Cosmetic use.

According to Part I 6(a), if a product is not listed on the **Domestic Substance List** and the import amount exceeds 20kg but does not exceed 1000kg per calendar year, **Schedule 1** states that the trade name and the material safety data sheet is acceptable documentation for determining the product's safety and toxicity for use in Canada.

These consist of the following materials:
- Water (CAS 7732-18-5): *listed on DSL*
- *Leuconostoc*/Radish Root Ferment Filtrate (CAS 84775-94-0): *listed on MSDS*

REACH Compliance Statement

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Trade Name: Leucidal® Liquid (M15008)

INCI Name: Leuconostoc/Radish Root Ferment Filtrate

This is to certify that Leucidal® Liquid is REACH compliant. Water is an Annex IV Exemption and Leuconostoc/Radish Root Ferment Filtrate has been pre-registered.

If you have further questions, please feel free to contact Heather Ferguson at hferguson@activeconceptsllc.com.

GROUPE ECOCERT

VERIFICATION OF THE RAW MATERIALS CONFORMITY TO THE ECOCERT AND COSMOS COSMETIC STANDARDS

THIS DOCUMENT IS NOT AN ORGANIC CERTIFICATE

Company: **ACTIVE MICRO TECHNOLOGIES LLC** Attestation n° : 5468

The conformity (conf.) is established according to the requirements related to the raw materials contained in the applicable standard(s).

The present document is only valid for ECOCERT until official COSMOS publication of the raw materials on the website: http://www.cosmos-standard-rm.org/

*reference related to the appendices II and/or V of the Cosmos standard.

AMTicide Coconut (M14003) Function: **Skin conditioning, Hair conditioning**

INCI: Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract

Conf. ECOCERT: YES **100** % of natural origin (**0** % of physically processed vegetal ingredients)
0 % synthetic

Conf. COSMOS: YES PPAI : **0** % CPAI : **100** % Petrochemical moiety : **0** %
Non natural ingredient : **0** %

Comments:

Leucidal Advanced - Aloe (M15015) Function: **Moisturizing, Skin conditioning, Antimicrobial**

INCI: Water (and) Leuconostoc/Aloe Barbadensis Leaf/Sorbus Aucuparia Fruit Ferment Filtrate

Conf. ECOCERT: YES **100** % of natural origin (**0** % of physically processed vegetal ingredients)
0 % synthetic

Conf. COSMOS: YES PPAI : **0** % CPAI : **18** % Petrochemical moiety : **0** %
Non natural ingredient : **0** %

Comments:

Drawn up in l'Isle Jourdain, valid from 01/01/2016
until 31/12/2016

Matthieu Bouffartigue

Raw Materials Service Manager

Leucidal Advanced - Rowan (M15018) Function: **Emollient, Skin conditioning, Antimicrobial**

INCI: Water (and) Leuconostoc/Sorbus Aucuparia Fruit Ferment Filtrate

Conf. ECOCERT: YES **100** % of natural origin (**0** % of physically processed vegetal ingredients)
0 % synthetic

Conf. COSMOS: YES PPAI : **0** % CPAI : **16** % Petrochemical moiety : **0** %
Non natural ingredient : **0** %

Comments:

Leucidal Liquid (M15008) Function: **Moisturizing, Skin conditioning, Antimicrobial**

INCI: Leuconostoc/Radish Root Ferment Filtrate

Conf. ECOCERT: YES **100** % of natural origin (**0** % of physically processed vegetal ingredients)
0 % synthetic

Conf. COSMOS: YES PPAI : **0** % CPAI : **52** % Petrochemical moiety : **0** %
Non natural ingredient : **0** %

Comments:

Leucidal Liquid PT (M15021) Function: **Skin conditioning, Antimicrobial**

INCI: Lactobacillus Ferment

Conf. ECOCERT: YES **100** % of natural origin (**0** % of physically processed vegetal ingredients)
0 % synthetic

Conf. COSMOS: YES PPAI : **0** % CPAI : **18** % Petrochemical moiety : **0** %
Non natural ingredient : **0** %

Comments:

Drawn up in l'Isle Jourdain, valid from 01/01/2016
until 31/12/2016

Matthieu Bouffartigue

Raw Materials Service Manager

GROUPE ECOCERT

The conformity (conf.) is established according to the requirements related to the raw materials contained in the applicable standard(s).

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*reference related to the appendices II and/or V of the Cosmos standard.

Leucidal Liquid SF (M15019) Function: **Moisturizing, Skin conditioning, Antimicrobial**

INCI: Lactobacillus Ferment

Conf. ECOCERT: YES **100** % of natural origin (**0** % of physically processed vegetal ingredients)
0 % synthetic

Conf. COSMOS: YES PPAI : **0** % CPAI : **10** % Petrochemical moiety : **0** %
Non natural ingredient : **0** %

Comments:

Leucidal Liquid SF (M15019CHI) Function: **Skin conditioning, Antimicrobial**

INCI: Leuconostoc/Radish Root Ferment Filtrate

Conf. ECOCERT: YES **100** % of natural origin (**0** % of physically processed vegetal ingredients)
0 % synthetic

Conf. COSMOS: YES PPAI : **0** % CPAI : **10** % Petrochemical moiety : **0** %
Non natural ingredient : **0** %

Comments:

PhytoCide Aspen Bark Extract Powder (M16002) Function: **Skin conditioning, Antimicrobial**

INCI: Populus Tremuloides Bark Extract

Conf. ECOCERT: YES **100** % of natural origin (**100** % of physically processed vegetal ingredients)
0 % synthetic

Conf. COSMOS: YES PPAI : **100** % CPAI : **0** % Petrochemical moiety : **0** %
Non natural ingredient : **0** %

Comments:

Drawn up in l'Isle Jourdain, valid from 01/01/2016
until 31/12/2016

Matthieu Bouffartigue

Raw Materials Service Manager

GROUPE ECOCERT

The conformity (conf.) is established according to the requirements related to the raw materials contained in the applicable standard(s).

The present document is only valid for ECOCERT until official COSMOS publication of the raw materials on the website: http://www.cosmos-standard-rm.org/

*reference related to the appendices II and/or V of the Cosmos standard.

PhytoCide Black Currant Powder (M16001) Function: **Soothing, Skin conditioning, Antimicrobial**

INCI: Ribes Nigrum (Black Currant) Fruit Extract

Conf. ECOCERT: YES **100** % of natural origin (**100** % of physically processed vegetal ingredients)
0 % synthetic

Conf. COSMOS: YES PPAI : **100** % CPAI : **0** % Petrochemical moiety : **0** %
Non natural ingredient : **0** %

Comments:

PhytoCide Elderberry OS (M16003) Function: **Skin conditioning, Antimicrobial**

INCI: Sambucus Nigra Fruit Extract

Conf. ECOCERT: YES **100** % of natural origin (**100** % of physically processed vegetal ingredients)
0 % synthetic

Conf. COSMOS: YES PPAI : **100** % CPAI : **0** % Petrochemical moiety : **0** %
Non natural ingredient : **0** %

Comments:

Drawn up in l'Isle Jourdain, valid from 01/01/2016
until 31/12/2016

Matthieu Bouffartigue

Raw Materials Service Manager