ACTIVE MICRO TECHNOLOGIES

# Technical Dossier

ability natural rowan technology Activity
sustainability benefits Ecocert leuconostoc
moisture Cosmos condition peptide
Improving solar choice antimicrobial

# AMTicide® Coconut

Code Number: M14003

INCI Name: Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract

# Table of Contents

Click on the logo to return to the Table of Contents



# Table of Contents

Click on the logo to return to the Table of Contents



**AMTicide® Coconut** Code Number: M14003
INCI Name: Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract

# AMTicide® Coconut

Patent Pending: Application Number 62/139,908

## Technical Data Sheet

## BACKGROUND

Fueled by the ever-changing regulations and the increasing safety concerns, among consumers, we must steer away from synthetics and focus on natural solutions. **AMTicide® Coconut** is developed by fermenting *Cocos nucifera* (Coconut) fruit with *Lactobacillus* to deliver a non-irritating, effective and multifunctional product. This highly marketable product can provide moisturizing and conditioning benefits in hair and skin care applications. In addition, it is effective at preventing the growth of fungus, specifically yeast and mold, thus providing the perfect addition to any formulation.

As we know, the use of parabens and formaldehyde donor materials are coming to a screeching halt, as controversy continues to surround them. When companies began phasing out parabens and formaldehyde donor materials, they replaced them with ingredients that dissociate into formaldehyde when put in an aqueous solution, such as DMDM Hydantoin. This option is not only cost effective but also protected formulas against bacteria, yeast and mold, which made the life of formulators seemingly effortless. However, as consumers continue to focus on natural solutions, these products are no longer an acceptable option.

**Code Number:** M14003
**INCI Nomenclature:** Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract
**INCI Status:** Approved
**REACH Status:** Fully Compliant
**CAS Number:** 68333-16-4 & 8001-31-8
**EINECS Number:** 232-282-8
**Origin:** Biotechnology/Botanical: *Lactobacillus & Cocos nucifera*
**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:** Water
**Suggested Use Levels:** 2.0 - 4.0%
**Suggested Applications:**
Moisturization, Skin/Scalp Conditioning, Antifungal

This leads the quest for natural antimicrobial products that can suit the needs of formulators and ultimately consumers. The challenge is not only finding one product that will protect against a wide range of microorganisms but also finding another product to supplement it, for protection that will not fail. How do we achieve the protection we need in a formulation without adding any synthetic ingredients?



Active Micro Technologies, LLC - www.activemicrotechnologies.com - info@activemicrotechnologies.com
107 Technology Drive - Lincolnton, NC 28092 - USA - Phone (704) 276-7100 - Fax (704) 276-7101

Version 3 - 05.13.15

# AMTicide® Coconut

Patent Pending: Application Number 62/139,908

## SCIENCE

Active Micro Technologies has prided itself in developing and supplying effective, natural products that provide skin and hair conditioning benefits, along with providing natural antimicrobial activity. As our original Leucidal Liquid product line continues to flourish, we still had the need for an antifungal product to round out our portfolio. This need left us with a long road of trial and error, in efforts to develop a marketable, yet effective antifungal booster. **AMTicide® Coconut** was developed to be used in conjunction with one of our broad-spectrum antimicrobials, however it can be used alongside any preservative package for extra protection against yeast and mold.

We began investigating the antimicrobial effects of medium chain triglycerides (MCT's), which have been studied for years. MCT's, including lauric acid, have natural antifungal activity and work by disrupting the cellular structures of fungus, thus essentially destroying them before they can wreck havoc. This led us to an exotic oil that is rich in MCT's - the well-known coconut oil. Coconut oil has been an important component of the Ayurveda tradition, popular among people of the tropics and currently at the center of a health craze in the U.S. First, it was coconut water for hydration, then coconut oil for health and now coconut is popping up in just about every industry. We cannot seem to get away from this fruit and for good reason.

So what makes coconut oil so unique? As mentioned, coconut oil is rich in MCT's, particularly lauric acid, which comprises ~50% of its total fatty acid content. Coconut oil was traditionally used to treat skin disorders, yeast infections, ringworm and even athletes foot. What many of these skin issues had in common was that they were all a type of yeast infection. Yeast and mold are types of fungus that can be inconspicuous because of their small size and structure and can flourish in our favorite skin care and cosmetic products. This can lead to not only destruction of that lotion you love, but it can also create a health hazard. Natural antimicrobial products are similar to synthetic preservatives systems, in that they are effective against bacteria, however not as effective against fungus, specifically yeast and mold.

One of the first steps in the development of this product was to determine the products potential ability to inhibit the growth of yeast and mold. Using standard serial dilution protocols in growth media, the Minimum Inhibitory Concentrations (MICs) for **AMTicide® Coconut** were determined for both yeast and mold organisms.

| Microorganisms Tested | MIC (%) |
|---|---|
| A. brasiliensis | 0.50 |
| C. albicans | 0.50 |

**Table 1**. MIC Data for **AMTicide® Coconut.**



Active Micro Technologies, LLC - www.activemicrotechnologies.com - info@activemicrotechnologies.com
107 Technology Drive - Lincolnton, NC 28092 - USA - Phone (704) 276-7100 - Fax (704) 276-7101

Version 3 - 05.13.15

# AMTicide® Coconut

Patent Pending: Application Number 62/139,908

The positive MIC screening results warranted further testing to confirm its ability to provide product preservation. A Double Challenge Test was completed using 2% **AMTicide® Coconut** by itself and **AMTicide® Coconut** with **Leucial® Liquid** in a generic cream base formulationat a pH of 7. Samples were inoculated with the microorganisms *E. Coli, P. aeruginosa, S. aureus, A. brasiliensis and C. albicans*. During the first 28-day incubation period, samples were periodically collected and tested for the presence of these fungi. Following this initial 28 days of incubation, the cream samples were then re-inoculated with the cultures and sampled over an additional 28-day period. Tables 2 and 3 shows the positive antifungal results for **AMTicide® Coconut**.

## Challenge Test Results
## AMTicide® Coconut

| Inoculum (initial) CFU/ml | Organisms | | | | |
|---|---|---|---|---|---|
| | E. coli | P. aeruginosa | S. aureus | A. brasiliensis | C. albicans |
| | $8.4 \times 10^6$ | $4.8 \times 10^6$ | $3.2 \times 10^6$ | $4.0 \times 10^5$ | $1.1 \times 10^5$ |
| Day 0* | 99.802 | 99.541 | 99.263 | 99.999% | 99.999% |
| Day 7 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999 |
| Day 14 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 21 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 28 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Inoculum (initial) CFU/ml | $3.5X10^6$ | $3.2X10^6$ | $1.8X10^6$ | $1.2X10^5$ | $2.9X10^5$ |
| Day 7 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 14 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 21 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 28 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |

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



Active Micro Technologies, LLC - www.activemicrotechnologies.com - info@activemicrotechnologies.com
107 Technology Drive - Lincolnton, NC 28092 - USA - Phone (704) 276-7100 - Fax (704) 276-7101

Version 3 - 05.13.15

# AMTicide® Coconut

Patent Pending: Application Number 62/139,908

## Challenge Test Results
## AMTicide® Coconut + Leucidal® Liquid

| Inoculum (initial) CFU/ml | Organisms | | | | |
|---|---|---|---|---|---|
| | E. coli | P. aeruginosa | S. aureus | A. brasiliensis | C. albicans |
| | $4.5 \times 10^6$ | $7.8 \times 10^6$ | $3.1 \times 10^6$ | $4.0 \times 10^5$ | $5.4 \times 10^5$ |
| 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 (initial) CFU/ml | $3.5X10^6$ | $3.2X10^6$ | $1.8X10^6$ | $1.2X10^5$ | $2.9X10^5$ |
| 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 3**. 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.

## BENEFITS

Not only is coconut a fruit with powerful benefits but it also complies with our stringent sustainability standards. We have an array of coconut products, which leaves us with unused portions of this multifunctional fruit. As we continued the development process, we began utilizing the lipid fractions of the unused coconut pericarp, which allowed us to further optimize material usage and coincide with our sustainability commitment. In addition, we incorporated our specialty technique of LAB (lactic acid bacteria) fermentation. Fermentation is an important process that can increase the bioavailability of natural phytocompounds, which can uncover wide array of benefits. A next generation, natural antifungal product was born.



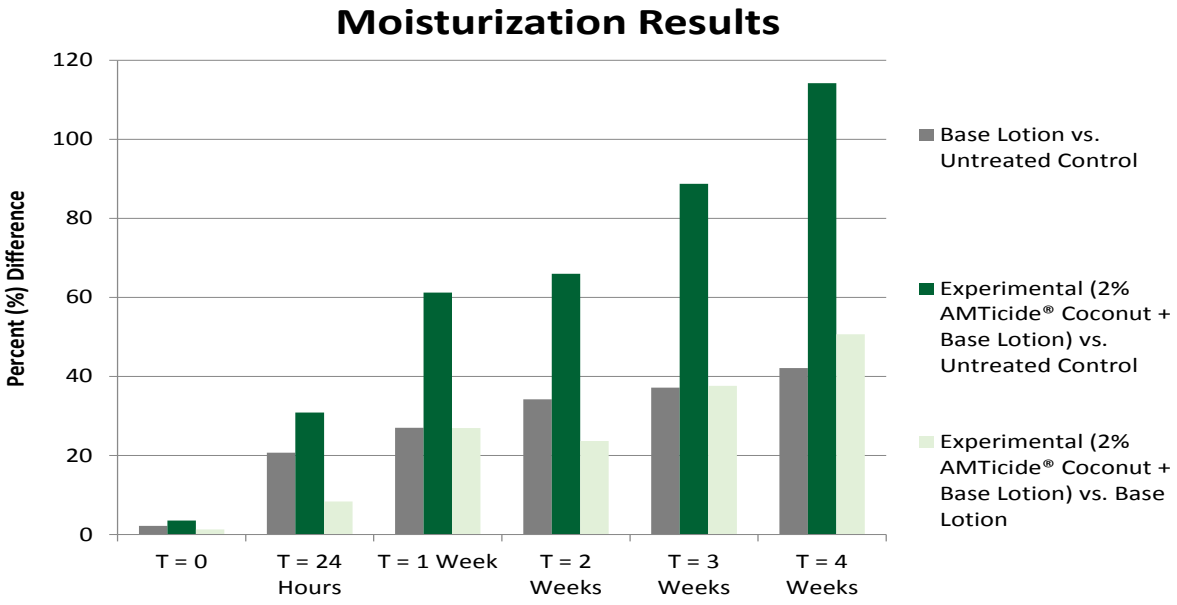
Active Micro Technologies, LLC - www.activemicrotechnologies.com - info@activemicrotechnologies.com
107 Technology Drive - Lincolnton, NC 28092 - USA - Phone (704) 276-7100 - Fax (704) 276-7101

Version 3 - 05.13.15

# AMTicide® Coconut

Patent Pending: Application Number 62/139,908

An *in-vivo* study was also conducted over the course of three weeks to evaluate **AMTicide® Coconut's** ability to increase moisturization. Ten (M/F) subjects between the ages of 23 - 45 participated in the 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. Baseline measurements were taken on day one of the study. Following initial measurements, all subjects were to apply 2 mg of the positive control and test material to the denoted area on their respective forearms, twice a day for three weeks. The test material consisted of 2.0% **AMTicide® Coconut** + Base Lotion and the positive control (base lotion) used was Cetaphil Moisturizing Lotion for All Skin Types.




**Graph 1.** Increase in Moisturization after application of 2% **AMTicide® Coconut**.

**AMTicide® Coconut** was developed to be coupled with one of our broad-spectrum antimicrobials, such as Leucidal® Liquid, or perhaps any preservative package that is lacking protection, against yeast and mold. This added boost of antifungal activity is the natural additive that will protect your product and consumers. Say goodbye to using potassium sorbate or sodium benzoate for added protection. Replace them with an eco-friendly, marketable and consumer acceptable antifungal product for the protection, and brand differentiation your product craves.

## USE RECOMMENDATIONS

As with all biological materials, some attention must paid to the conditions under which **AMTicide® Coconut** is used. Based on bench-scale evaluations, as well as actual product applications, **AMTicide® Coconut** 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).



Active Micro Technologies, LLC - www.activemicrotechnologies.com - info@activemicrotechnologies.com
107 Technology Drive - Lincolnton, NC 28092 - USA - Phone (704) 276-7100 - Fax (704) 276-7101

Version 3 - 05.13.15

ACTIVE MICRO TECHNOLOGIES

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

Patent Pending: Application Number 62/139,908

# Specification

**Product Name:** AMTicide® Coconut
**Code Number:** M14003
**CAS #'s:** 68333-16-4 & 8001-31-8
**EINECS #'s:** N/A & 232-282-8
**INCI Name:** Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract

| Specification | Parameter |
|---|---|
| Appearance | Clear to Slightly Hazy Liquid |
| Color | 5 Gardner Maximum |
| Odor | Characteristic |
| pH | 7.0 – 9.0 |
| Solids (1g-105°C-1hr) | 20.0 – 25.0% |
| Heavy Metals | < 20 ppm |
| Arsenic | < 2 ppm |
| Lipopeptide Content | 0.5% Minimum |
| Minimum Inhibitory Concentration[1] | |
| Organism (ATCC#) | |
| C. albicans (#10231) | 0.25 – 2.00% |
| A. brasiliensis (#16404) | 0.25 – 2.00% |

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

Note:
1) Refer to Inhibition Activity Data

ACTIVE MICRO TECHNOLOGIES

# Compositional Breakdown

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

## AMTicide® Coconut

Code: M14003

Compositional Breakdown:

| Ingredient | % |
|---|---|
| Lactobacillus | 80.00 |
| Cocos Nucifera (Coconut) Fruit Extract | 20.00 |

- **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

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

This is to certify that AMTicide® Coconut 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

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

This is to certify that AMTicide® Coconut 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 |

# Compositional Breakdown

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

| | |
|---|---|
| Parathion | < 0.50 |
| 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 |

ACTIVE MICRO TECHNOLOGIES

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

# Moisturization / Hydration Assay

**Tradename:** AMTicide® Coconut

**Code:** M14003

**CAS #:** 68333-16-4 & 8001-31-8

**Test Request Form #:** 996

**Lot #:** NC14111-E

**Sponsor:** *Active Concepts, 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 **AMTicide® Coconut**. 6 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 **AMTicide® Coconut.**

## 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.

6 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.

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

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% **AMTicide® Coconut** 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.

## Results

**AMTicide® Coconut** 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 | 102 | 133 | 247 | 262 | 280 | 285 |
| | Base Lotion | 83 | 109 | 100 | 192 | 184 | 191 |
| | Untreated | 109 | 123 | 139 | 151 | 139 | 130 |
| Panelist 2 | Experimental | 119 | 200 | 220 | 261 | 280 | 300 |
| | Base Lotion | 176 | 185 | 200 | 250 | 250 | 300 |
| | Untreated | 119 | 110 | 106 | 92 | 100 | 105 |
| Panelist 3 | Experimental | 113 | 145 | 288 | 262 | 300 | 355 |
| | Base Lotion | 90 | 105 | 200 | 216 | 210 | 225 |
| | Untreated | 139 | 120 | 209 | 215 | 200 | 230 |
| Panelist 4 | Experimental | 80 | 150 | 211 | 230 | 225 | 300 |
| | Base Lotion | 84 | 160 | 193 | 190 | 179 | 180 |
| | Untreated | 70 | 72 | 117 | 110 | 73 | 90 |
| Panelist 5 | Experimental | 102 | 119 | 212 | 289 | 295 | 325 |
| | Base Lotion | 75 | 100 | 173 | 168 | 165 | 150 |
| | Untreated | 100 | 120 | 118 | 164 | 170 | 122 |
| Panelist 6 | Experimental | 100 | 54 | 141 | 160 | 200 | 260 |
| | Base Lotion | 100 | 80 | 173 | 168 | 160 | 165 |
| | Untreated | 58 | 67 | 129 | 150 | 155 | 175 |

**Chart 1.** Panelist Moisturization Measurements

| Averages | T = 0 | T = 24 Hours | T = 1 Week | T = 2 Weeks | T = 3 Weeks | T = 4 Weeks | T = -24 Hours | T = -1 Week | T = -2 Weeks |
|---|---|---|---|---|---|---|---|---|---|
| Experimental (2.0% AMTicide® Coconut in Base Lotion) | 102.6 | 133.5 | 219.8 | 244 | 263.3 | 304.2 | 244.3 | 175.8 | 135.8 |
| Base Lotion Control | 101.3 | 123.2 | 173.2 | 197.3 | 191.3 | 201.8 | 141.6 | 101.6 | 63.16 |
| Untreated Control | 99.2 | 102 | 136.3 | 147.0 | 140.0 | 142.0 | 115.8 | 104.3 | 93.33 |

**Chart 2.** Average Moisture Increase and Regression Scores of Individual Test Sites

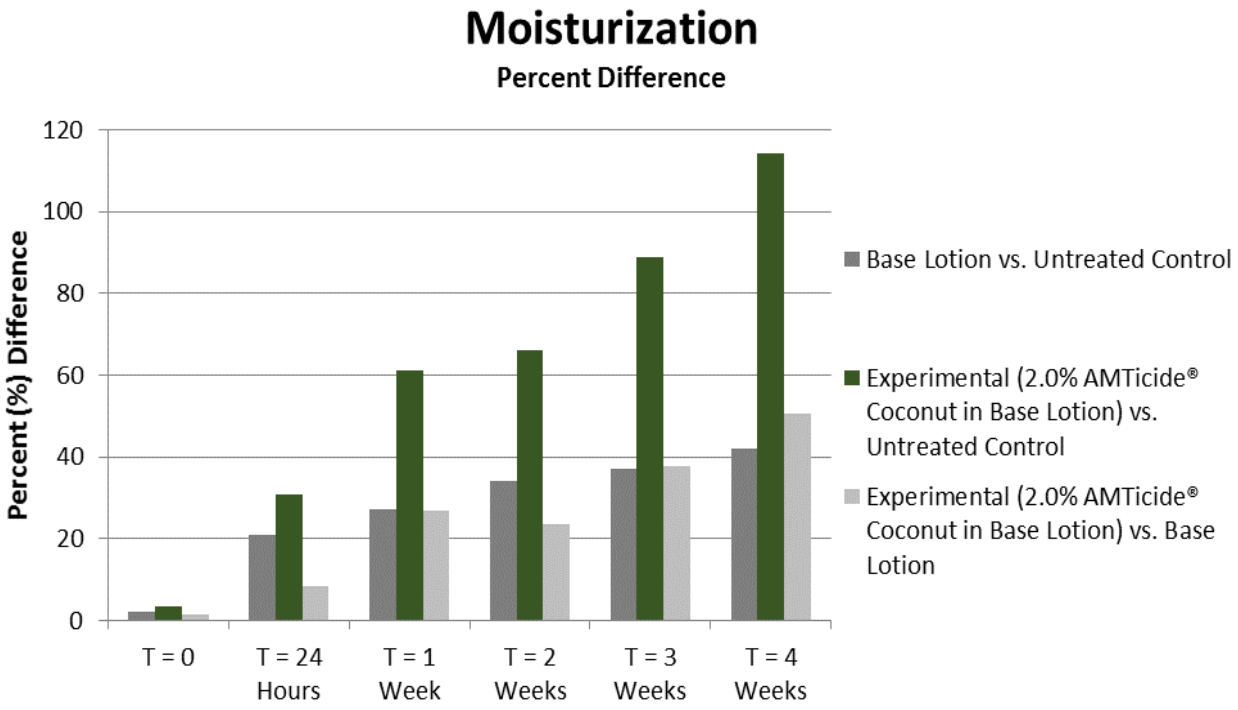
ACTIVE MICRO TECHNOLOGIES

# Moisturization / Hydration Assay

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

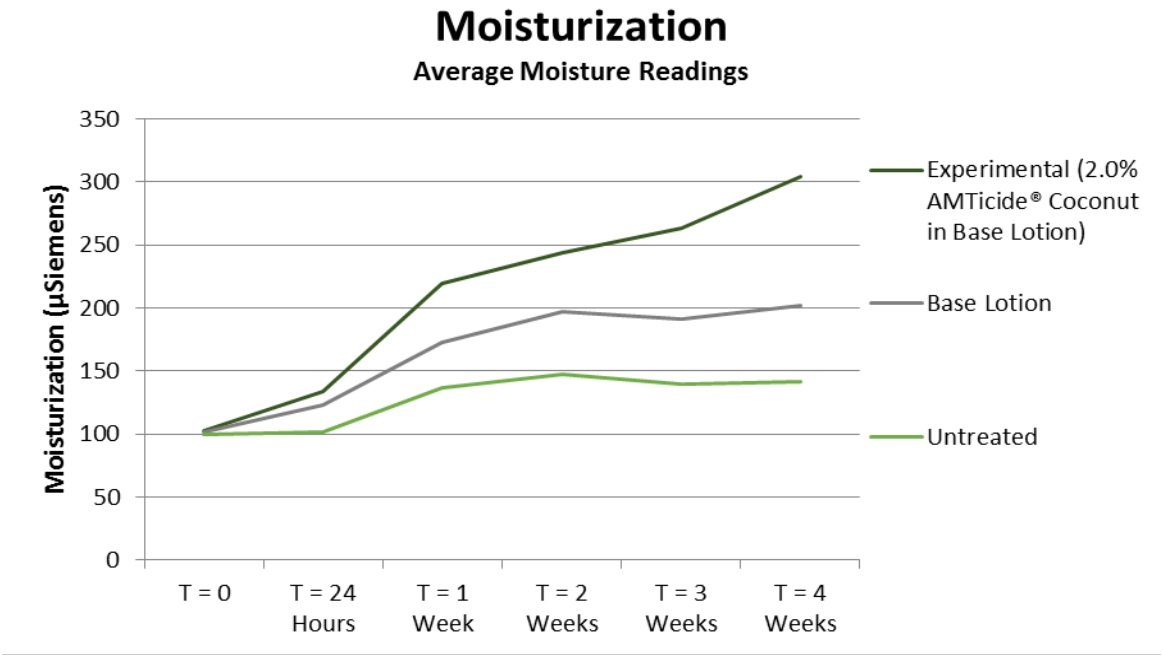
| Percent (%) Change | T = 0 | T = 24 Hours | T = 1 Week | T = 2 Weeks | T = 3 Weeks | T = 4 Weeks | T = -24 Hours | T = -1 Week | T = -2 Weeks |
|---|---|---|---|---|---|---|---|---|---|
| **Base Lotion vs. Untreated Control** | 2.19 | 20.75 | 27.02 | 34.2 | 37.12 | 42.14 | 22.30 | -2.55 | -32.32 |
| **Experimental (2.0% AMTcide® Coconut in Base Lotion) vs. Untreated Control** | 3.53 | 30.88 | 61.24 | 65.99 | 88.77 | 114.2 | 110.9 | 68.53 | 45.53 |
| **Experimental (2.0% AMTicide® Coconut in Base Lotion) vs. Base Lotion** | 1.32 | 8.95 | 26.95 | 23.64 | 37.63 | 50.70 | 72.47 | 72.95 | 115.0 |

**Chart 3.** Comparative Moisture Increase and Regression Scores Between Individual Test Sites

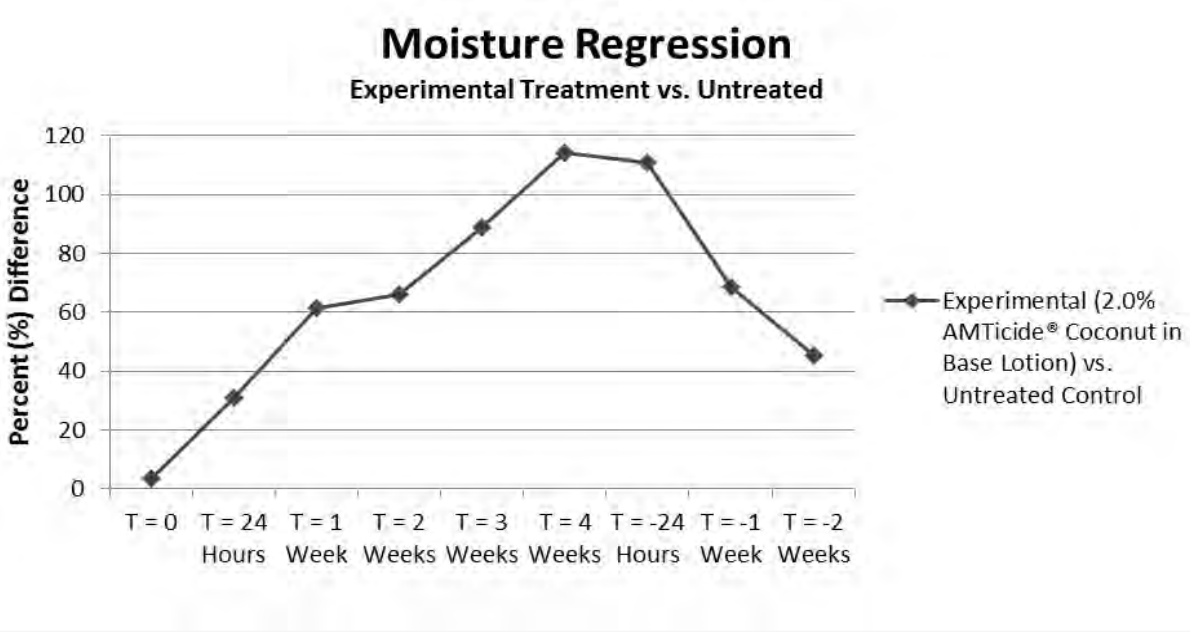



**Graph 1.** Percent Difference between test sites

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




**Graph 2.** Average increase in moisturization per test site




**Graph 3.** Experimental vs. Untreated Control Moisture Regression

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

## Discussion

As evidenced in a four-week efficacy study of **AMTicide® Coconut**, moisture levels were improved by 30.88% after 24 hours and by 114.20% after four weeks when compared to the untreated control. When compared to the base cream **AMTicide® Coconut** improved moisturization by 8.95% and after 24 hours and by 50.70% after four weeks. Results indicate that **AMTicide® Coconut** is capable of increasing moisturization when compared to both the untreated control as well as the base lotion.

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

# Transepidermal Water Loss (TEWL) Study

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

**Tradename:** AMTicide® Coconut

**Code:** M14003

**CAS #:** 68333-16-4 & 8001-31-8

**Test Request Form #:** 996

**Lot #:** NC14111-E

**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 **AMTicide® Coconut** 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% **AMTicide® Coconut** 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% **AMTicide® Coconut** in a base lotion.

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

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

**AMTicide® Coconut** 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.

| TEWL | | T = 0 | T = 1 Week | T = 2 Weeks | T = 3 Weeks | T = 4 Weeks |
|---|---|---|---|---|---|---|
| Panelist 1 | Experimental | 2.1 | 2.3 | 2 | 2 | 2 |
| | Base Lotion | 4 | 2.5 | 1.5 | 2.7 | 1.9 |
| | Untreated | 3.3 | 3.1 | 2.4 | 3 | 3.2 |
| Panelist 2 | Experimental | 5.2 | 3 | 3.5 | 3 | 2.7 |
| | Base Lotion | 5 | 2.6 | 2.7 | 2.5 | 2.6 |
| | Untreated | 3.6 | 1.7 | 3.5 | 3 | 3.1 |
| Panelist 3 | Experimental | 4 | 4.3 | 2.5 | 2 | 2.1 |
| | Base Lotion | 3.3 | 3 | 2.1 | 2.5 | 2.8 |
| | Untreated | 3.2 | 4.2 | 2.7 | 3 | 2.8 |
| Panelist 4 | Experimental | 4.5 | 4 | 3.5 | 3 | 2.4 |
| | Base Lotion | 5 | 4 | 3.8 | 4.8 | 4 |
| | Untreated | 4.7 | 4.1 | 4 | 4.9 | 3.2 |
| Panelist 5 | Experimental | 1.4 | 2.4 | 2.7 | 2.5 | 2 |
| | Base Lotion | 3.7 | 2.8 | 3.9 | 4 | 3.8 |
| | Untreated | 3 | 5.7 | 4.5 | 4.5 | 6.2 |
| Panelist 6 | Experimental | 3.5 | 2.4 | 2.3 | 2.1 | 1.8 |
| | Base Lotion | 0.4 | 1.6 | 1.6 | 2.5 | 3.7 |
| | Untreated | 2 | 2.3 | 2.2 | 2.5 | 3.8 |

**Chart 1.** Panelist TEWL Measurements

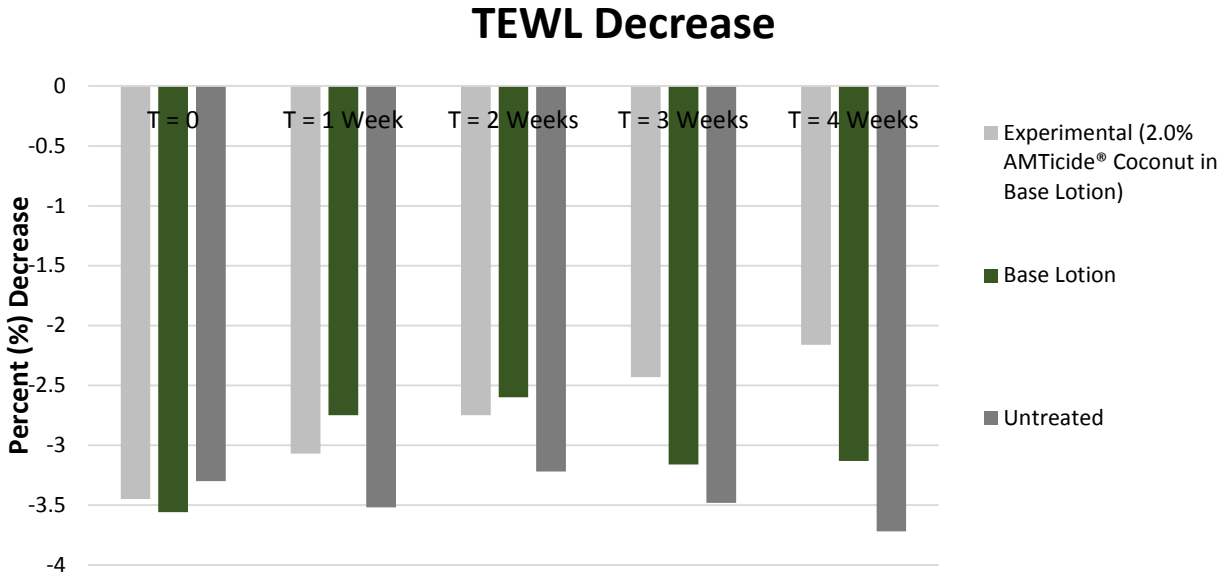
| Averages | T = 0 | T = 1 Week | T = 2 Weeks | T = 3 Weeks | T = 4 Weeks |
|---|---|---|---|---|---|
| Untreated Control | 3.45 | 3.07 | 2.75 | 2.43 | 2.16 |
| Base Lotion Control | 3.56 | 2.75 | 2.60 | 3.16 | 3.13 |
| Experimental (2.0% AMTicide® Coconut ) in Base Lotion | 3.30 | 3.52 | 3.22 | 3.48 | 3.72 |

**Chart 2.** Average Increase in Skin Density per Individual Test Site

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

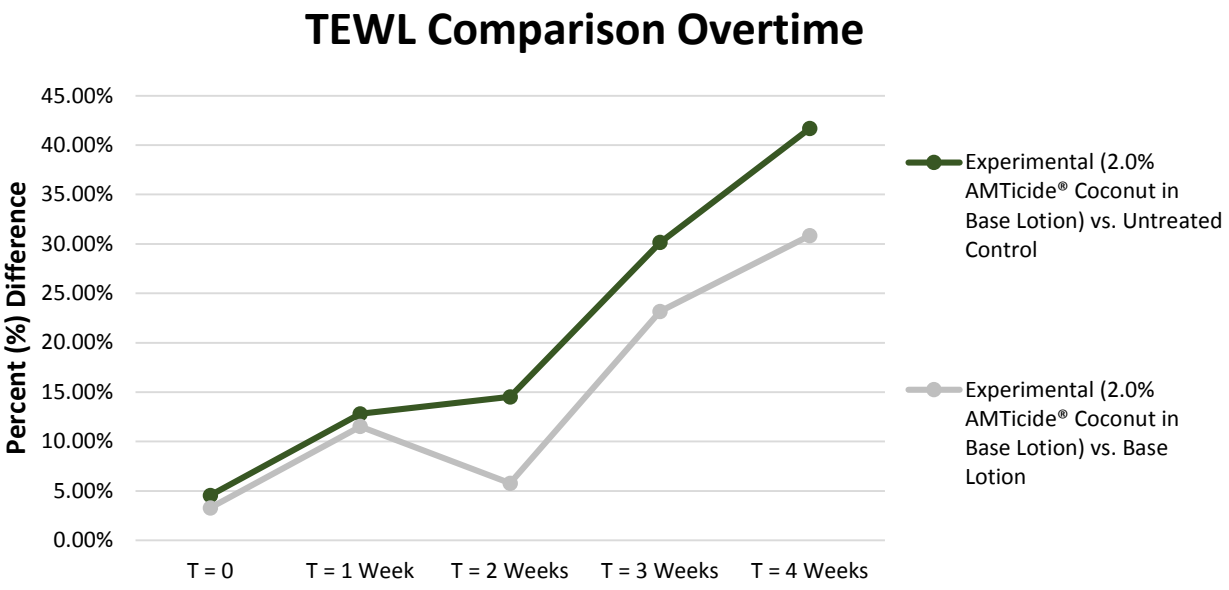
| Percent (%) Change | T = 0 | T = 1 Week | T = 2 Weeks | T = 3 Weeks | T = 4 Weeks |
|---|---|---|---|---|---|
| **Experimental (2.0% AMTicide® Coconut ) vs. Base Lotion** | 8.08 | -21.80 | -19.17 | -9.09 | -15.70 |
| **Experimental (2.0% AMTicide® Coconut ) vs. Untreated Control** | 4.55 | -12.80 | -14.51 | -30.14 | -41.70 |
| **Base Lotion vs. Untreated Control** | 3.27 | 11.52 | 5.77 | -23.16 | -30.85 |

**Chart 3.** Comparison of Skin Density Changes between Two Test Sites




**Graph 1.** Average Decrease in TEWL per Individual Test Site

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




**Graph 2.** Comparison of TEWL Changes between Two Test Sites

## Discussion

While there is directional improvements between the individual test sites, the results as a whole are not statistically significant. However, this lack of difference clearly indicates that **AMTicide® Coconut** does not disrupt or otherwise adversely affect barrier function.

ACTIVE MICRO TECHNOLOGIES

# High Resolution Ultrasound Skin Imaging Assay

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

**Tradename:** AMTicide® Coconut

**Code:** M14003

**CAS #:** 68333-16-4 & 8001-31-8

**Test Request Form #:** 996

**Lot #:** NC14111-E

**Sponsor:** *Active Micro Technologies, 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 **AMTicide® Coconut**. 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)

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% **AMTicide® Coconut** 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.

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

## Results

**AMTicide® Coconut** 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.

| Ultrasound | | T = 0 | T = 1 Week | T = 2 Weeks | T = 3 Weeks | T = 4 Weeks |
|---|---|---|---|---|---|---|
| Panelist 1 | Experimental | 68 | 75 | 67 | 72 | 75 |
| | Base Lotion | 60 | 61 | 55 | 59 | 60 |
| | Untreated | 74 | 71 | 68 | 70 | 72 |
| Panelist 2 | Experimental | 76 | 78 | 75 | 82 | 86 |
| | Base Lotion | 76 | 80 | 78 | 77 | 75 |
| | Untreated | 60 | 60 | 53 | 55 | 61 |
| Panelist 3 | Experimental | 80 | 78 | 85 | 83 | 85 |
| | Base Lotion | 60 | 68 | 70 | 73 | 77 |
| | Untreated | 65 | 77 | 57 | 60 | 62 |
| Panelist 4 | Experimental | 58 | 61 | 65 | 67 | 72 |
| | Base Lotion | 64 | 66 | 64 | 68 | 70 |
| | Untreated | 50 | 66 | 65 | 59 | 60 |
| Panelist 5 | Experimental | 66 | 86 | 86 | 87 | 86 |
| | Base Lotion | 61 | 62 | 63 | 65 | 67 |
| | Untreated | 71 | 72 | 75 | 76 | 79 |
| Panelist 6 | Experimental | 30 | 45 | 36 | 45 | 57 |
| | Base Lotion | 50 | 58 | 57 | 59 | 65 |
| | Untreated | 27 | 28 | 36 | 35 | 34 |

**Chart 1.** Panelist Ultrasound Measurements

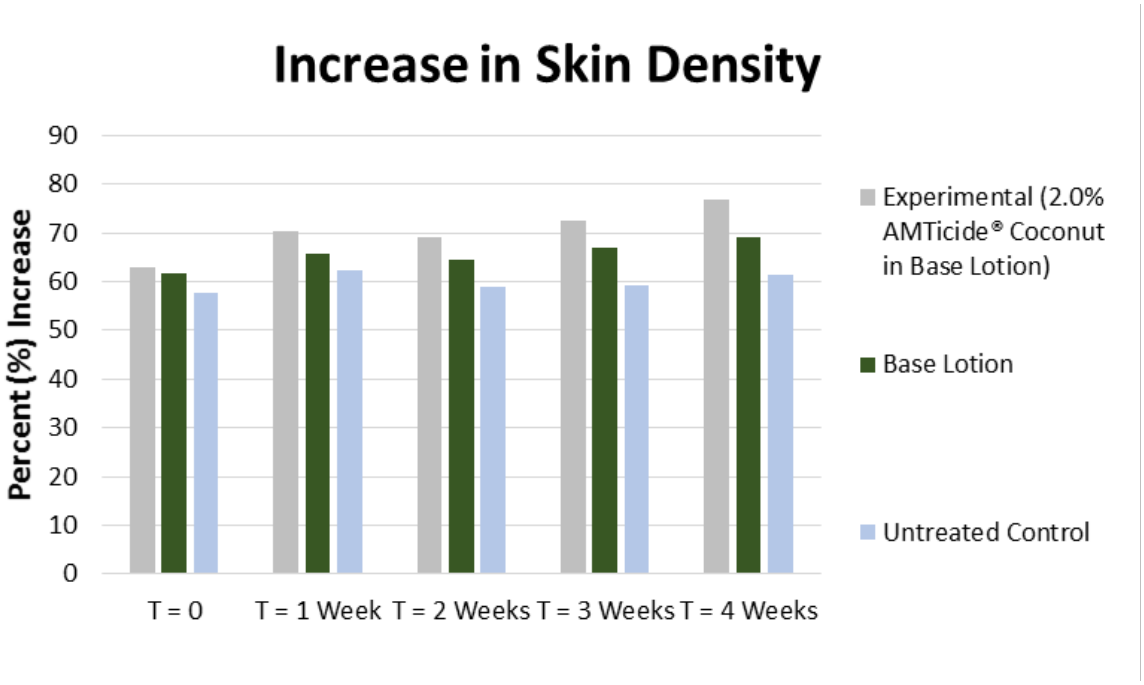
| Averages | T = 0 | T = 1 Week | T = 2 Weeks | T = 3 Weeks | T = 4 Weeks |
|---|---|---|---|---|---|
| Experimental (2.0% AMTicide® Coconut ) in Base Lotion | 63.0 | 70.5 | 69.0 | 72.6 | 76.8 |
| Base Lotion Control | 61.8 | 65.8 | 64.5 | 66.8 | 69.0 |
| Untreated Control | 57.8 | 62.3 | 59.0 | 59.1 | 61.3 |

**Chart 2.** Average Increase in Skin Density per Individual Test Site

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

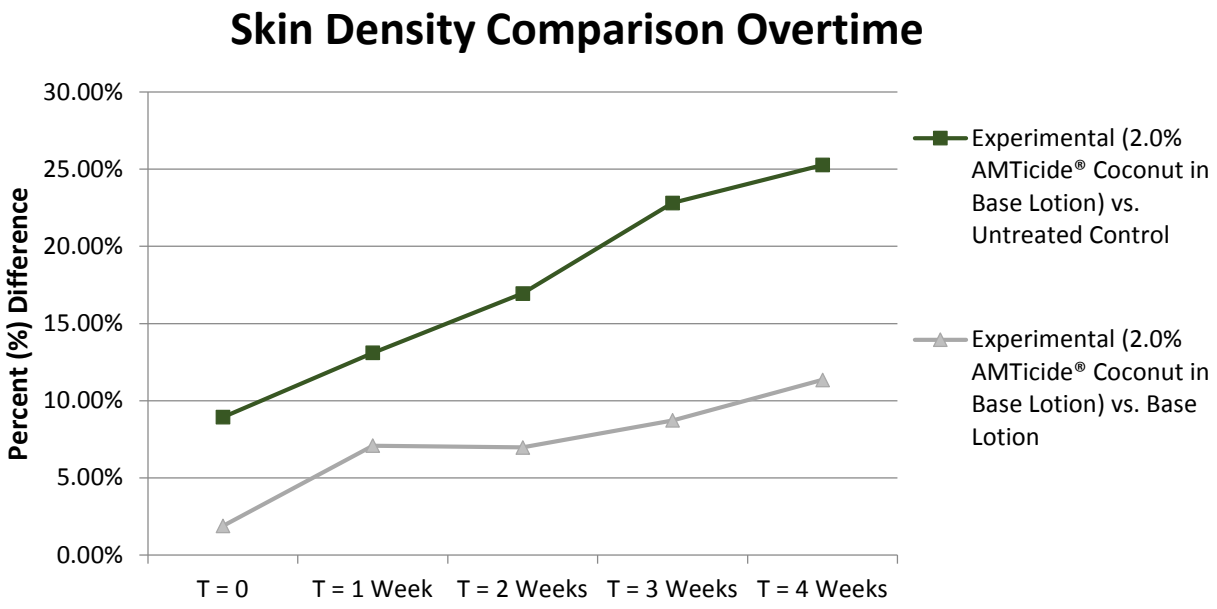
| Percent (%) Change | T = 0 | T = 1 Week | T = 2 Weeks | T = 3 Weeks | T = 4 Weeks |
|---|---|---|---|---|---|
| **Experimental (2.0% AMTicide® Coconut ) vs. Untreated Control** | 8.93 | 13.10 | 16.95 | 22.82 | 25.27 |
| **Experimental (2.0% AMTicide® Coconut) vs. Base Lotion** | 1.89 | 7.09 | 6.98 | 8.73 | 11.35 |

**Chart 3.** Comparison of Skin Density Changes between Two Test Sites




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

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




**Graph2.** Comparison of Skin Density Changes between Two Test Sites

## Discussion

As evidenced in a four-week efficacy study of **AMTicide® Coconut**, skin density was improved by 13.10% after one week and by 25.27% after four weeks when compared to the untreated control. When compared to the base cream **AMTicide® Coconut** improved skin density during each week of the trial, working 7.09% better than the base lotion after one week and 11.35% better than the base lotion after four weeks. Results indicate that **AMTicide® Coconut** is capable of improving skin density when compared to both the untreated control as well as the base lotion.

**AMTicide® Coconut** has a strong positive effect on skin's density when used at recommended use levels.

ACTIVE MICRO TECHNOLOGIES

# Cellular Viability Assay Analysis

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

**Tradename:** AMTicide® Coconut

**Code:** M14003

**CAS #:** 68333-16-4 & 8001-31-8

**Test Request Form #:** 1514

**Lot #:** 41286

**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 **AMTicide® Coconut** 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.

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

## 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 **AMTicide® Coconut** 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.

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

## Results

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

**AMTicide® Coconut** exhibited positive effects on cell metabolism.

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

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

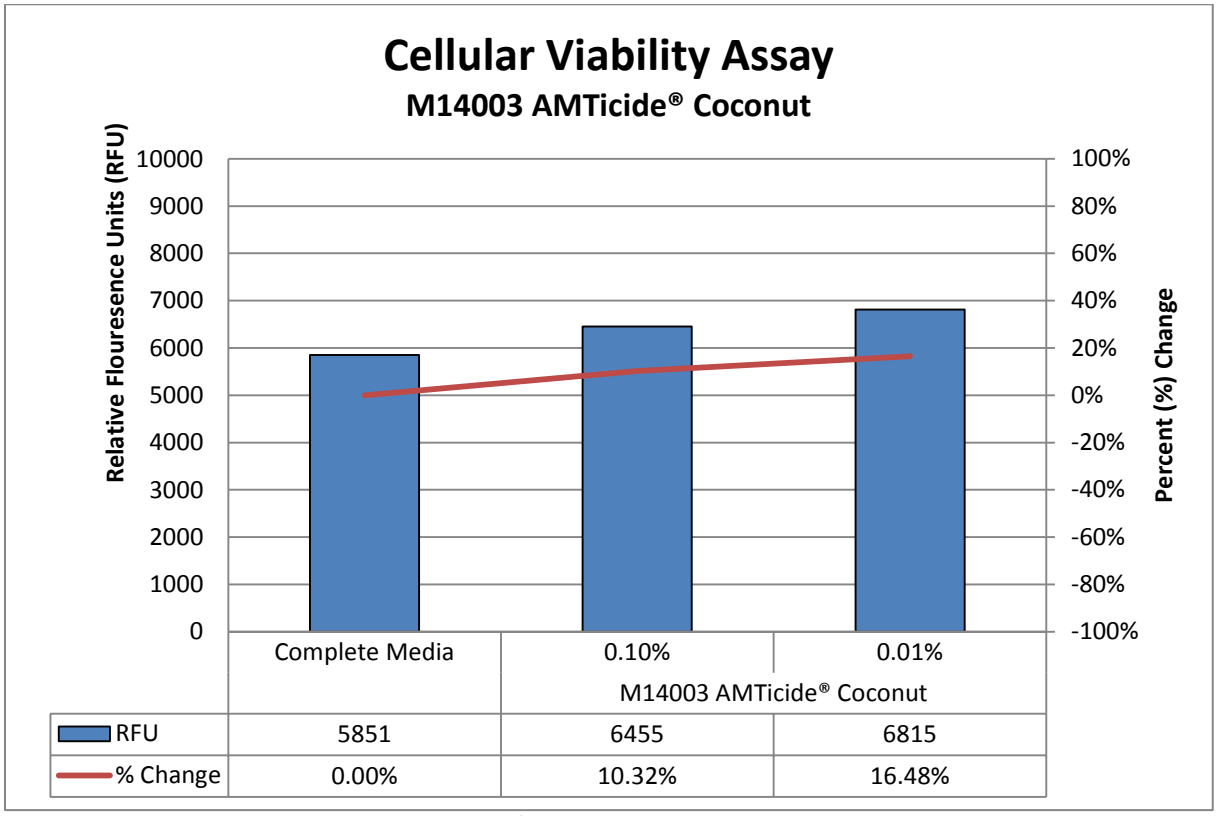



**Figure 1:** Cellular Metabolism of **AMTicide® Coconut-**treated fibroblasts

## Discussion

In this study, **AMTicide® Coconut** (code M14003) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of both 0.1% and 0.01% **AMTicide® Coconut** increases cellular viability by 10.3% and 16.5%, respectively. It can therefore be concluded that at normal use concentrations **AMTicide® Coconut** enhances cellular viability.

ACTIVE MICRO TECHNOLOGIES

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

# Minimum Inhibition Concentration

**Product Name:** AMTicide® Coconut
**Code Number:** M14003
**Lot Number:** NC141216-C
**Test Request Number:** 1010
**CAS #'s:** 68333-16-4 & 8001-31-8
**EINECS #'s:** N/A & 232-282-8
**INCI Name:** *Lactobacillus* & *Cocos nucifera* (Coconut) Fruit Extract

| Organism (ATCC #) | Minimum Inhibitory Concentration (%) |
|---|---|
| *E.coli* #8739 | 8.0 |
| *S. aureus* #6538 | 8.0 |
| *P. aeruginosa* #9027 | 8.0 |
| *C. albicans* #10231 | 0.5 |
| *A. brasiliensis* #16404 | 0.5 |

QA Signature Monica Beltran

Date 01-12-2015

**This is an Electronically Generated Document**

ACTIVE MICRO TECHNOLOGIES

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

# Zone of Inhibition Test

**Product Name:** AMTicide® Coconut
**Code Number:** M14003
**Lot Number:** NC150204-A
**Test Request Number:** 1162
**CAS #'s:** 68333-16-4 & 8001-31-8
**EINECS #'s:** N/A & 232-282-8
**INCI Name**: *Lactobacillus* & Cocos Nucifera (Coconut) Fruit Extract

| Organism (ATCC #) | Zone of Inhibition (mm) |
|---|---|
| *C. albicans* #10231 | 15.0 |
| *A. brasiliensis* #16404 | 14.0 |

QA Signature Monica Beltran

Date 03-16-2015

**This is an Electronically Generated Document**

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut

## Test Request #:

1273

## 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.

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

## Test Method

Fifty grams of Generic Cream Formula pH 3 with 4% AMTicide® Coconut 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* |
| | $4.8 \times 10^6$ | $7.8 \times 10^6$ | $9.7 \times 10^6$ | $1.3 \times 10^5$ | $5.4 \times 10^5$ |
| Day 0* | <99.000% | <99.000% | 99.615% | >99.999% | >99.999% |
| Day 7 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 14 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 21 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 28 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Inoculum (re-inoculated) CFU/ml | $7.3 \times 10^6$ | $6.7 \times 10^6$ | $6.4 \times 10^6$ | $2.1 \times 10^5$ | $6.8 \times 10^5$ |
| Day 7 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 14 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 21 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 28 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |

Table 1. Challenge Test results for Generic Cream Formula pH 3 with 4% AMTicide® Coconut 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.

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

## 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. 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 were not reduced by greater than 99.9% within 7 days of each challenge. The mold and the yeast was reduced by 99.999% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria were not reduced by greater than 99.9. The yeast and mold were reduced by 99.999% or greater.

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

| 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.

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

# 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.

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut

## Test Request #:

1274

## 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.

ACTIVE MICRO TECHNOLOGIES

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

# Challenge Test with 4.0% AMTicide® Coconut

## Test Method

Fifty grams of Generic Cream Formula pH 5 with 4% AMTicide® Coconut 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* |
| | $4.8 \times 10^6$ | $7.8 \times 10^6$ | $9.7 \times 10^6$ | $1.3 \times 10^5$ | $5.4 \times 10^5$ |
| Day 0* | <99.000% | <99.000% | 99.615% | >99.999% | >99.999% |
| Day 7 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 14 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 21 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 28 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Inoculum (re-inoculated) CFU/ml | $7.3 \times 10^6$ | $6.7 \times 10^6$ | $6.4 \times 10^6$ | $2.1 \times 10^5$ | $6.8 \times 10^5$ |
| Day 7 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 14 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 21 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 28 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |

Table 1. Challenge Test results for Generic Cream Formula pH 5 with 4% AMTicide® Coconut 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.

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

## 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. 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 were not reduced by greater than 99.9% within 7 days of each challenge. The mold and the yeast was reduced by 99.999% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria were not reduced by greater than 99.9. The yeast and mold were reduced by 99.999% or greater.

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

| 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.

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

# 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.

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut

## Test Request #:

1153

## 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.

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

## Test Method

Fifty grams of Generic Cream Formula pH 7 with 4% AMTicide® Coconut 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* |
| | $8.4 \times 10^6$ | $4.8 \times 10^6$ | $3.2 \times 10^6$ | $4.0 \times 10^5$ | $1.1 \times 10^5$ |
| Day 0* | 99.823% | 99.714% | 99.615% | >99.999% | >99.999% |
| Day 7 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 14 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 21 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 28 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Inoculum (re-inoculated) CFU/ml | $3.5 \times 10^6$ | $3.2 \times 10^6$ | $1.8 \times 10^6$ | $1.2 \times 10^5$ | $2.9 \times 10^5$ |
| Day 7 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 14 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 21 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |
| Day 28 | <99.000% | <99.000% | <99.000% | >99.999% | >99.999% |

Table 1. Challenge Test results for Generic Cream Formula pH 7 with 4% AMTicide® Coconut 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.

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

## 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. 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 were not reduced by greater than 99.9% within 7 days of each challenge. The mold and the yeast was reduced by 99.999% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria were not reduced by greater than 99.9. The yeast and mold were reduced by 99.999% or greater.

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

| 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.

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

# 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.

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

# 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.

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

## 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* |
| | $4.8 \times 10^6$ | $7.8 \times 10^6$ | $9.7 \times 10^6$ | $1.3 \times 10^5$ | $5.4 \times 10^5$ |
| 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 | $7.3 \times 10^6$ | $6.7 \times 10^6$ | $6.4 \times 10^6$ | $2.1 \times 10^5$ | $6.8 \times 10^5$ |
| 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.

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

## 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.

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

| 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.

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

# 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.

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

# 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.

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

## 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* |
| | $4.8 \times 10^6$ | $7.8 \times 10^6$ | $9.7 \times 10^6$ | $1.3 \times 10^5$ | $5.4 \times 10^5$ |
| 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 | $7.3 \times 10^6$ | $6.7 \times 10^6$ | $6.4 \times 10^6$ | $2.1 \times 10^5$ | $6.8 \times 10^5$ |
| 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.

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

## 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.

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

| 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.

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

# 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.

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

# 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.

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

## 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* |
| | $4.5 \times 10^6$ | $7.8 \times 10^6$ | $3.1 \times 10^6$ | $4.0 \times 10^5$ | $5.4 \times 10^5$ |
| 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 | $3.5 \times 10^6$ | $3.2 \times 10^6$ | $1.8 \times 10^6$ | $1.2 \times 10^5$ | $2.9 \times 10^5$ |
| 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.

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

## 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:

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.

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

| 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.

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

# 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.

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut
Leucidal® Liquid SF

## Test Request #:

1281

## 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.

ACTIVE MICRO TECHNOLOGIES

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

# Challenge Test with 4.0% AMTicide® Coconut + 2.0% Leucidal® Liquid SF

## Test Method

Fifty grams of Generic Cream Formula pH 3 with 4% AMTicide® Coconut and 2% Leucidal® Liquid SF 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* |
| | $4.8 \times 10^6$ | $7.8 \times 10^6$ | $9.7 \times 10^6$ | $1.3 \times 10^5$ | $5.4 \times 10^5$ |
| 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 | $7.3 \times 10^6$ | $6.7 \times 10^6$ | $6.4 \times 10^6$ | $2.1 \times 10^5$ | $6.8 \times 10^5$ |
| 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 SF 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.

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

## 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 SF. 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.

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

| 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.

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

# 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.

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut
Leucidal® Liquid SF

## Test Request #:

1282

## 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.

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

## Test Method

Fifty grams of Generic Cream Formula pH 5 with 4% AMTicide® Coconut and 2% Leucidal® Liquid SF 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* |
| | $4.8 \times 10^6$ | $7.8 \times 10^6$ | $9.7 \times 10^6$ | $1.3 \times 10^5$ | $5.4 \times 10^5$ |
| Day 0* | 99.901% | 99.992% | 99.955% | 99.961% | 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 | $7.3 \times 10^6$ | $6.7 \times 10^6$ | $6.4 \times 10^6$ | $2.1 \times 10^5$ | $6.8 \times 10^5$ |
| 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% AMTicide® Coconut and 2% Leucidal® Liquid SF 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.

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

## 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 SF. 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.

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

| 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.

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

# 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.

ACTIVE MICRO TECHNOLOGIES

**Challenge Test with 4.0% AMTicide® Coconut + 2.0% Leucidal® Liquid SF**

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut
Leucidal® Liquid SF

## Test Request #:

984

## 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.

ACTIVE MICRO TECHNOLOGIES

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

# Challenge Test with 4.0% AMTicide® Coconut + 2.0% Leucidal® Liquid SF

## Test Method

Fifty grams of Generic Cream Formula pH 7 with 4% AMTicide® Coconut and 2% Leucidal® Liquid SF 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* |
| | $4.5 \times 10^6$ | $7.8 \times 10^6$ | $3.1 \times 10^6$ | $4.0 \times 10^5$ | $5.4 \times 10^5$ |
| Day 0* | 99.901% | 99.992% | 99.860% | 99.987% | 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 | $3.5 \times 10^6$ | $3.2 \times 10^6$ | $1.8 \times 10^6$ | $1.2 \times 10^5$ | $2.9 \times 10^5$ |
| 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 SF 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.

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

## 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 SF. 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.

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

| 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.

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

# 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.

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

# 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.

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

## 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* |
| | $4.8 \times 10^6$ | $7.8 \times 10^6$ | $9.7 \times 10^6$ | $1.3 \times 10^5$ | $5.4 \times 10^5$ |
| 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 | $7.3 \times 10^6$ | $6.7 \times 10^6$ | $6.4 \times 10^6$ | $2.1 \times 10^5$ | $6.8 \times 10^5$ |
| 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.

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

## 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.

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

| 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.

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

# 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.

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

# 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.

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

## 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* |
| | $4.8 \times 10^6$ | $7.8 \times 10^6$ | $9.7 \times 10^6$ | $1.3 \times 10^5$ | $5.4 \times 10^5$ |
| 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 | $7.3 \times 10^6$ | $6.7 \times 10^6$ | $6.4 \times 10^6$ | $2.1 \times 10^5$ | $6.8 \times 10^5$ |
| 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.

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

## 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.

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

| 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.

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

# 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.

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

# 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.

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

## 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* |
| | $4.5 \times 10^6$ | $7.8 \times 10^6$ | $3.1 \times 10^6$ | $4.0 \times 10^5$ | $5.4 \times 10^5$ |
| 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 | $3.5 \times 10^6$ | $3.2 \times 10^6$ | $1.8 \times 10^6$ | $1.2 \times 10^5$ | $2.9 \times 10^5$ |
| 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.

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

## 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:

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.

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

| 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.

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

# 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.

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut
Leucidal® Liquid SF

## Test Request #:

1279

## 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.

ACTIVE MICRO TECHNOLOGIES

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

# Challenge Test with 2.0% AMTicide® Coconut + 2.0% Leucidal® Liquid SF

## Test Method

Fifty grams of Generic Cream Formula pH 7 with 2% AMTicide® Coconut and 2% Leucidal® Liquid SF 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* |
| | $4.5 \times 10^6$ | $7.8 \times 10^6$ | $3.1 \times 10^6$ | $4.0 \times 10^5$ | $5.4 \times 10^5$ |
| Day 0* | 99.960% | 99.983% | 99.894% | 99.995% | 99.985% |
| 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 | $3.5 \times 10^6$ | $3.2 \times 10^6$ | $1.8 \times 10^6$ | $1.2 \times 10^5$ | $2.9 \times 10^5$ |
| 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 SF 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.

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

## 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 SF. 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.

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

| 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.

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

# 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.

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut
Leucidal® Liquid SF

## Test Request #:

1280

## 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.

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

## Test Method

Fifty grams of Generic Cream Formula pH 5 with 2% AMTicide® Coconut and 2% Leucidal® Liquid SF 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* |
| | $4.8 \times 10^6$ | $7.8 \times 10^6$ | $9.7 \times 10^6$ | $1.3 \times 10^5$ | $5.4 \times 10^5$ |
| Day 0* | 99.960% | 99.983% | 99.966% | 99.990% | 99.988% |
| 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 | $7.3 \times 10^6$ | $6.7 \times 10^6$ | $6.4 \times 10^6$ | $2.1 \times 10^5$ | $6.8 \times 10^5$ |
| 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% AMTicide® Coconut and 2% Leucidal® Liquid SF 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.

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

## 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 SF. 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.

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

| 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.

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

# 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.

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut
Leucidal® Liquid SF

## Test Request #:

1099

## 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.

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

## Test Method

Fifty grams of Generic Cream Formula pH 7 with 2% AMTicide® Coconut and 2% Leucidal® Liquid SF 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* |
| | $4.5 \times 10^6$ | $7.8 \times 10^6$ | $3.1 \times 10^6$ | $4.0 \times 10^5$ | $5.4 \times 10^5$ |
| Day 0* | 99.960% | 99.983% | 99.894% | 99.995% | 99.985% |
| 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 | $3.5 \times 10^6$ | $3.2 \times 10^6$ | $1.8 \times 10^6$ | $1.2 \times 10^5$ | $2.9 \times 10^5$ |
| 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 SF 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.

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

## 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 SF. 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.

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

| 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.

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

# 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.

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut
1, 2 Hexanediol

## Test Request #:

1388

## 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 August 12th, 2015 and was completed on October 13th, 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.

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

## Test Method

Fifty grams of Generic Cream Formula with 4% AMTicide® Coconut and 3% 1, 2 Hexanediol 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* |
| | $7.7 \times 10^6$ | $4.0 \times 10^6$ | $2.2 \times 10^6$ | $3.0 \times 10^5$ | $3.9 \times 10^5$ |
| Day 0* | 99.999% | >99.999% | 99.807% | 99.973% | 99.956% |
| 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 | $7.3 \times 10^6$ | $6.5 \times 10^6$ | $3.9 \times 10^6$ | $5.3 \times 10^5$ | $5.2 \times 10^5$ |
| 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 with 4% AMTicide® Coconut and 3% 1, 2 Hexanediol 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.

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

## 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 with 4% AMTicide® Coconut and 2% 1, 2 Hexanediol. 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 reduce by 99.999% or greater.

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

## 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.

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut
1, 2 Hexanediol

## Test Request #:

1388

## 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 August 12th, 2015 and was completed on October 13th, 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.

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

## Test Method

Fifty grams of Generic Cream Formula with 4% AMTicide® Coconut and 2% 1, 2 Hexanediol 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* |
| | $7.7 \times 10^6$ | $4.0 \times 10^6$ | $2.2 \times 10^6$ | $3.0 \times 10^5$ | $3.9 \times 10^5$ |
| Day 0* | 99.955% | >99.999% | 99.720% | 99.993% | 99.956% |
| 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 | $7.3 \times 10^6$ | $6.5 \times 10^6$ | $3.9 \times 10^6$ | $5.3 \times 10^5$ | $5.2 \times 10^5$ |
| 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 with 4% AMTicide® Coconut and 2% 1, 2 Hexanediol 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.

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

## 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 with 4% AMTicide® Coconut and 2% 1, 2 Hexanediol. 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 reduce by 99.999% or greater.

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

## 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.

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut
1, 2 Hexanediol

## Test Request #:

1388

## 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 August 12th, 2015 and was completed on October 13th, 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.

ACTIVE MICRO TECHNOLOGIES

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

# Challenge Test with 2.0% AMTicide® Coconut and 3.0% 1,2 Hexanediol

## Test Method

Fifty grams of Generic Cream Formula with 2% AMTicide® Coconut and 3% 1, 2 Hexanediol 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* |
| | $7.7 \times 10^6$ | $4.0 \times 10^6$ | $2.2 \times 10^6$ | $3.0 \times 10^5$ | $3.9 \times 10^5$ |
| Day 0* | 99.999% | >99.999% | 99.792% | 99.976% | 99.948% |
| 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 | $7.3 \times 10^6$ | $6.5 \times 10^6$ | $3.9 \times 10^6$ | $5.3 \times 10^5$ | $5.2 \times 10^5$ |
| 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 with 2% AMTicide® Coconut and 3% 1, 2 Hexanediol 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.

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

## 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 with 4% AMTicide® Coconut and 2% 1, 2 Hexanediol. 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 reduce by 99.999% or greater.

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

## 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.

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

# Antimicrobial Efficacy Test
# PCPC Section 20
# Method 3

**Determination of Preservation Adequacy of Water- Miscible Personal Care Products**

## Product

AMTicide® Coconut
1, 2 Hexanediol

## Test Request #:

1388

## 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 August 12th, 2015 and was completed on October 13th, 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.

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

## Test Method

Fifty grams of Generic Cream Formula with 2% AMTicide® Coconut and 2% 1, 2 Hexanediol 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* |
| | $7.7 \times 10^6$ | $4.0 \times 10^6$ | $2.2 \times 10^6$ | $3.0 \times 10^5$ | $3.9 \times 10^5$ |
| Day 0* | 99.964% | >99.999% | 99.792% | 99.990% | 99.907% |
| 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 | $7.3 \times 10^6$ | $6.5 \times 10^6$ | $3.9 \times 10^6$ | $5.3 \times 10^5$ | $5.2 \times 10^5$ |
| 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 with 2% AMTicide® Coconut and 2% 1, 2 Hexanediol 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.

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

## 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 with 2% AMTicide® Coconut and 2% 1, 2 Hexanediol. 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 reduce by 99.999% or greater.

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

## 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.

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

# Time Kill Test
## E2315
### Assessment of Antimicrobial Activity Using a Time Kill Procedure

## Product

AMTicide® Coconut

## Test Request #:

1814

## 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 18th, 2016 and was completed on March 24th, 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.

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

## Test Method

Ten grams of 4% AMTicide® Coconut 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 | $6.3 \times 10^6$ | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% |
| *S.aureus* ATCC# 6538 | $4.8 \times 10^6$ | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% |
| *P.aeruginosa* ATCC# 9027 | $1.1 \times 10^6$ | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% |
| *B.subtilis* ATCC# 6051 | $2.5 \times 10^5$ | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% |
| *A.brasiliensis* ATCC# 16404 | $1.2 \times 10^6$ | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% |
| *C.albicans* ATCC# 10231 | $1.6 \times 10^6$ | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% |

Table 1. Time Kill Test results for 4% AMTicide® Coconut 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.

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

## 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% AMTicide® Coconut 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.

# Safety Statement

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

Product Name: AMTicide® Coconut

Product Code: M14003

INCI Name: Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract

INCI Status: Approved

AMTicide® Coconut is created by fermentation of coconut using *Lactobacillus* in a defined media under controlled conditions of pH, temperature, and time.

*Lactobacillus* is a genus of microorganisms used to produce a variety of food products. It is a type of Lactic Acid Bacteria (LAB) and converts various sugars into lactic acid. Any existing LAB in AMTicide® Coconut 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]

Due to its status as a product of LAB and a fruit that is used in food preparations globally, the Federal Food, Drug and Cosmetic Act classifies materials such as AMTicide® Coconut as Generally Recognized as Safe (GRAS). This knowledge combined with dermal and ocular irritation assays allows us to support the safety of AMTicide® Coconut in cosmetic applications at the recommended use level.

Due to the restriction placed on the animal testing of cosmetic raw materials, and our internal non-animal testing policy Active Micro Technologies, LLC does not test for NOAEL.

1. Federal Food, Drug and Cosmetic Act. U.S Food and Drug Administration. www.fda.gov.

ACTIVE MICRO TECHNOLOGIES

# Dermal and Ocular Irritation Tests

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

**Tradename:** AMTicide® Coconut

**Code:** M14003

**CAS #:** 68333-16-4 & 8001-31-8

**Test Request Form #:**999

**Lot #:** NC141202-D

**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 **AMTicide® Coconut** 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.

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

## 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 Concepts, 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.

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

**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.

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

**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**

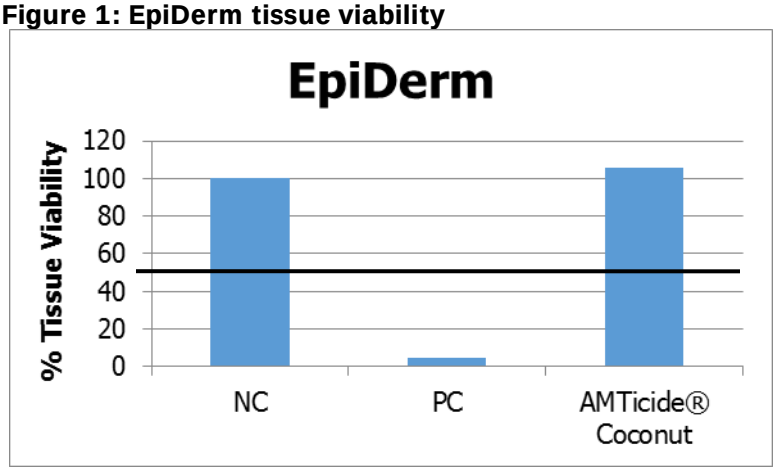
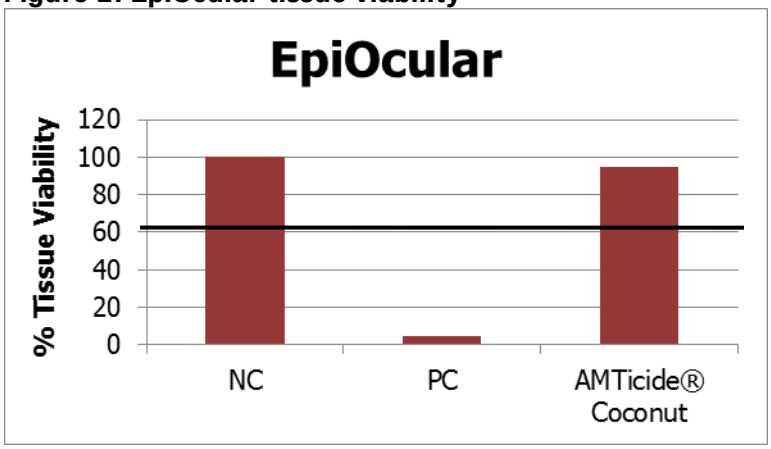



**Figure 2: EpiOcular tissue viability**



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

# 48 HOUR PATCH TEST
# SKIN IRRITATION EVALUATION
# (Occlusive Patch)

AMA Ref. No.: MS14.48HR.N8016O.50.ACTC

Date: December 15, 2014

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

## 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 irritants in certain individuals. It is the intention of a 48 Hour Patch Test to provide a basis for evaluation of this irritation potential if such exists.

## 2.0 Test Material:

### 2.1 Test Material Description:

On November 18, 2014 one test sample labeled Test Sample 1, Lot # NC141111-E was received from Active Concepts, LLC and assigned AMA Lab No. N-8016.

### 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, be 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 | | 50 |
| Number of subjects completing study | | 50 |
| Age Range | | 19-68 |
| Sex | Male | 5 |
| | Female | 45 |
| Race | Caucasian | 40 |
| | Hispanic | 7 |
| | Asian | 3 |

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.
- As per client request, the test material N-8016 was diluted to 5% in distilled water.
- 0.2 ml of the test material is dispensed onto the occlusive, hypoallergenic patch.
- The patch is then affixed 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 48 hours the patch is removed at the facility, and test sites evaluated by trained laboratory personnel.
- 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 again 48 hours following the initial evaluation. Subjects are instructed to report any delayed reactions which might occur after the final reading.
- Clients are notified immediately in the case of an adverse reaction and a determination is made as to treatment program if necessary.

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 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:

The test material (AMA Lab. No.: N-8016; Client No.: Test Sample 1, Lot # NC141111-E) when tested under 48 hour occlusive patching conditions at a 5% dilution in distilled water as described herein, may be considered:
a **NON-PRIMARY IRRITANT** to the skin.

Mayya Tatsene, M.D.
Study Director

Breanna Wanamaker, A.A. (Candidate)
Technician

David R. Winne, B.S.
Technical Director

12/11/14
Date

## TABLE
## SUMMARY OF RESULTS
## (Occlusive Patch)

AMA Lab No.: N-8016
Client No.: Test Sample 1, Lot # NC141111-E
Dilution: 5% in distilled water

| No. | SUBJECT ID | RACE | SEX | RESPONSE 0 HR | RESPONSE 48 HR |
|---|---|---|---|---|---|
| 1 | 25 0215 | C | M | 0 | 0 |
| 2 | 32 4178 | C | F | 0 | 0 |
| 3 | 36 1000 | C | M | 0 | 0 |
| 4 | 36 2041 | C | F | 0 | 0 |
| 5 | 36 8214 | C | F | 0 | 0 |
| 6 | 38 0748 | C | F | 0 | 0 |
| 7 | 40 0533 | C | F | 0 | 0 |
| 8 | 44 7255 | C | F | 0 | 0 |
| 9 | 44 8295 | H | F | 0 | 0 |
| 10 | 44 9258 | C | F | 0 | 0 |
| 11 | 44 9339 | C | F | 0 | 0 |
| 12 | 44 9509 | C | F | 0 | 0 |
| 13 | 46 1691 | C | F | 0 | 0 |
| 14 | 46 4172 | C | F | 0 | 0 |
| 15 | 48 0738 | C | F | 0 | 0 |
| 16 | 48 1427 | C | F | 0 | 0 |
| 17 | 48 1868 | C | F | 0 | 0 |
| 18 | 48 2320 | C | F | 0 | 0 |
| 19 | 48 9460 | C | F | 0 | 0 |
| 20 | 50 7536 | C | F | 0 | 0 |
| 21 | 52 3942 | C | F | 0 | 0 |
| 22 | 56 3122 | C | F | 0 | 0 |
| 23 | 56 3379 | C | F | 0 | 0 |
| 24 | 56 9114 | C | F | 0 | 0 |
| 25 | 60 3496 | C | F | 0 | 0 |
| 26 | 62 0956 | C | F | 0 | 0 |
| 27 | 62 3596 | C | F | 0 | 0 |
| 28 | 62 4776 | C | F | 0 | 0 |
| 29 | 62 5697 | C | F | 0 | 0 |
| 30 | 62 9431 | C | F | 0 | 0 |

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

AMA Lab No.: N-8016
Client No.: Test Sample 1, Lot # NC141111-E
Dilution: 5% in distilled water

| No. | SUBJECT ID | RACE | SEX | RESPONSE 0 HR | RESPONSE 48 HR |
|---|---|---|---|---|---|
| 31 | 64 4521 | A | F | 0 | 0 |
| 32 | 64 7603 | C | F | 0 | 0 |
| 33 | 66 1649 | C | F | 0 | 0 |
| 34 | 66 3958 | C | F | 0 | 0 |
| 35 | 70 5391 | C | F | 0 | 0 |
| 36 | 70 6353 | C | F | 0 | 0 |
| 37 | 70 7182 | A | F | 0 | 0 |
| 38 | 72 3483 | H | M | 0 | 0 |
| 39 | 73 6193 | H | F | 0 | 0 |
| 40 | 76 2719 | C | F | 0 | 0 |
| 41 | 80 7035 | A | M | 0 | 0 |
| 42 | 82 4417 | H | M | 0 | 0 |
| 43 | 82 6379 | H | F | 0 | 0 |
| 44 | 84 4033 | C | F | 0 | 0 |
| 45 | 84 7426 | H | F | 0 | 0 |
| 46 | 84 8405 | C | F | 0 | 0 |
| 47 | 84 9711 | C | F | 0 | 0 |
| 48 | 88 4232 | C | F | 0 | 0 |
| 49 | 90 3845 | H | F | 0 | 0 |
| 50 | 96 6992 | C | F | 0 | 0 |

Evaluation Period:

This study was conducted from December 8, 2014 through December 12, 2014.

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

Dc - Discontinued due to absence of subject on evaluation date

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:

Tasmiya Masud, B.A.
Quality Assurance Supervisor

12/15/14
Date

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.: MS14.RIPT.N8016O.50.ACTC

Date: January 8, 2015

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

## 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 November 18, 2014 one test sample labeled Test Sample 1 Lot # NC141111-E was received from Active Concepts, LLC and assigned AMA Lab No. N-8016.

### 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 | | 19-68 |
| Sex | Male | 5 |
| | Female | 47 |
| Race | Caucasian | 44 |
| | Hispanic | 6 |
| | Asian | 2 |

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.
- As per client request, the test material N-8016 was diluted to 5% in distilled water. Dilutions were freshly prepared on each application day.
- 0.2 ml 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 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.

13.0 Conclusions:

The test material (AMA Lab. No.: N-8016; Client No.: Test Sample 1 Lot # NC141111-E) when tested under occlusion at a 5% dilution in distilled water 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

Breanna Wanamaker, A.A. (Candidate)
Technician

Vera Jelic, B.A. (Candidate)
Technician

David R. Winne, B.S.
Technical Director

11/3/15
Date

## TABLE
## SUMMARY OF RESULTS
## (Occlusive Patch)

AMA Lab No.: N-8016
Client No.: Test Sample 1 Lot # NC141111-E
Dilution: 5% in distilled water

| No. | Subject ID | RACE | SEX | Response 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Chall. 24 HR | 48 HR | Score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 02 4519 | C | F | 0 | 0 | Dc | Dc | Dc | Dc | Dc | Dc | Dc | Dc | Dc | N/A |
| 2 | 25 0215 | C | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 3 | 36 2041 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 4 | 38 0748 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 5 | 44 7255 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 6 | 44 8295 | H | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 7 | 44 9258 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 8 | 44 9509 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 9 | 48 1427 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 10 | 48 2320 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 11 | 48 9460 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 12 | 54 2855 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 13 | 54 3619 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 14 | 54 6257 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 15 | 54 7997 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 16 | 54 9929 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 17 | 56 0875 | H | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 18 | 56 3122 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 19 | 56 3659 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 20 | 56 5529 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 21 | 56 8787 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 22 | 58 5003 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 23 | 60 3225 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 24 | 60 3496 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 25 | 60 4534 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 26 | 60 7979 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 27 | 60 9372 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 28 | 62 0602 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 29 | 62 0956 | 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.: N-8016
Client No.: Test Sample 1 Lot # NC141111-E
Dilution: 5% in distilled water

| No. | Subject ID | RACE | SEX | Response | | | | | | | | | Chall. | | Score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 24 HR | 48 HR | |
| 30 | 62 1837 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 31 | 62 7431 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 32 | 64 2319 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 33 | 64 4521 | A | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 34 | 64 5779 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 35 | 64 6126 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 36 | 64 6663 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 37 | 64 7603 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 38 | 68 0458 | C | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 39 | 70 3559 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 40 | 70 5391 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 41 | 70 6353 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 42 | 72 6994 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 43 | 76 0042 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 44 | 78 8767 | H | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 45 | 80 1527 | C | M | 0 | 0 | 0 | 0 | Dc | Dc | Dc | Dc | Dc | Dc | Dc | N/A |
| 46 | 80 7035 | A | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 47 | 82 5542 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 48 | 84 7426 | H | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 49 | 84 9711 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 50 | 86 1121 | C | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 51 | 90 3845 | H | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| 52 | 90 6566 | H | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |

Evaluation Period:

This study was conducted from December 3, 2014 through January 7, 2015.

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.

14.0 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:

Tasmiya Masud, B.A.
Quality Assurance Supervisor

Date

# OECD TG 442C: *In Chemico* Skin Sensitization

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

**Tradename:** AMTicide® Coconut

**Code:** M14003

**CAS #:** 68333-16-4 & 8001-31-8

**Test Request Form #**: 1239

**Lot #:** 41286P

**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 **AMTicide® Coconut** 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.

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

## 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 **AMTicide® Coconut** 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<br>0.5mM Peptide, 5mM Test Chemical | 1:50 Ratio, Lysine Peptide<br>0.5mM Peptide, 25mM Test Chemical |
|---|---|
| • 750µL Cysteine Peptide Solution (or 100mM Phosphate Buffer, pH 7.5, for Co-Elution Controls)<br>• 200µL Acetonitrile<br>• 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)<br>• 250µL Test Chemical Solution (or Acetonitrile for Reference Controls) |

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

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.

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

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 **AMTicide® Coconut (code M14003)** 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 2.37% causing minimal reactivity in the assay giving us the prediction of a non-sensitizer.

ACTIVE MICRO TECHNOLOGIES

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

# OECD TG 442D: *In Vitro* Skin Sensitization

**Tradename:** AMTicide® Coconut

**Code:** M14003

**CAS #:** 68333-16-4 & 8001-31-8

**Test Request Form #**: 1194

**Lot #:** NC150212-A

**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 **AMTicide® Coconut** 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

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

## 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 **AMTicide® Coconut** 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 – 64µM. 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.

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

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 |
| AMTicide® Coconut | Non-Sensitizer | No Induction | > 1000 µM | 0.4 |

Table 1: Overview of KeratinoSens™ Assay Results

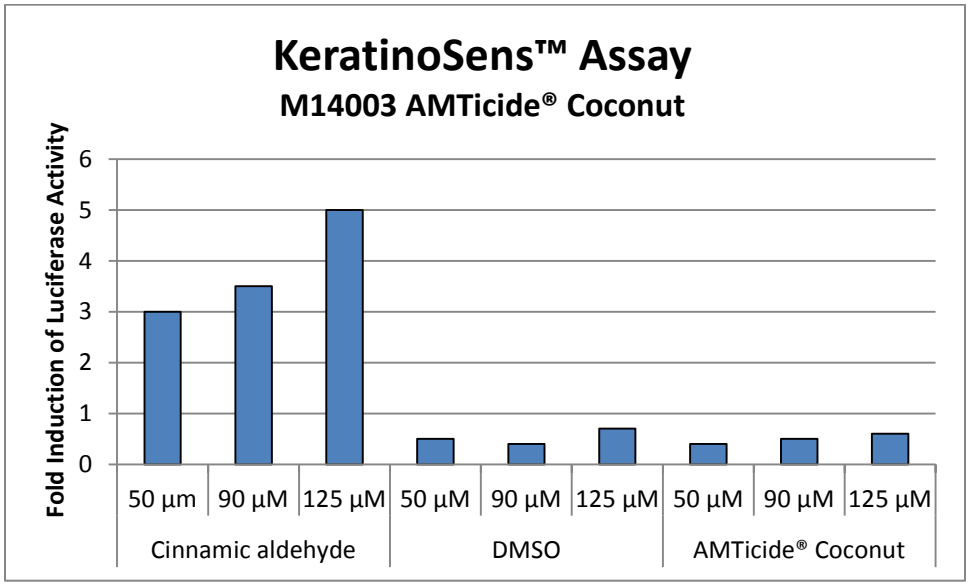



Figure 1: Fold Induction of Luciferase

**Discussion**

As shown in the results, **AMTicide® Coconut (code M14003)** 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 **AMTicide® Coconut** can be safely used in cosmetics and personal care products at typical use levels.

# Bacterial Reverse Mutation Test

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

**Test Article:** AMTicide® Coconut
**Code Number:** M14003
**CAS #:** 68333-16-4 & 8001-31-8

**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:** 1000

## 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 **AMTicide® Coconut** 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 $45^0$C 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 $37^0$C. 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.

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

## 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.

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

**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* and *Escherichia coli* ester 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)**:
$5 \times 10^8$ UFC/ml plate count indicates that the initial population was in the range of 1 to $2 \times 10^9$ 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.

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

**Appendix 2:**

## Bacterial Mutation Assay
## Plate Incorporation Assay Results

| | Concentration µg per Plate | *TA98* | | |
|---|---|---|---|---|
| | | Revertants per plate (CFU) | | Mean |
| Test Solution w/ S9 | 5000 | 30 | 22 | 26 |
| | 1500 | 31 | 41 | 36 |
| | 500 | 70 | 73 | 72 |
| | 150 | 56 | 57 | 57 |
| | 50 | 53 | 68 | 61 |
| | 15 | 26 | 38 | 32 |
| | 5.0 | 79 | 57 | 68 |
| | 1.5 | 42 | 48 | 45 |
| Test Solution w/o S9 | 5000 | 39 | 73 | 56 |
| | 1500 | 56 | 60 | 58 |
| | 500 | 78 | 82 | 80 |
| | 150 | 52 | 79 | 66 |
| | 50 | 91 | 80 | 86 |
| | 15 | 53 | 86 | 70 |
| | 5.0 | 78 | 76 | 77 |
| | 1.5 | 73 | 63 | 68 |
| DI Water w/S9 | | 54 | 58 | 56 |
| DI Water w/o S9 | | 56 | 61 | 59 |
| 2-aminoanthracen w/ S9 | | 301 | 322 | 312 |
| 2-nitrofluorene w/o S9 | | 210 | 351 | 281 |
| 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

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

| | Concentration µg per Plate | ***TA100*** | | |
|---|---|---|---|---|
| | | Revertants per plate (CFU) | | Mean |
| Test Solution w/ S9 | 5000 | 13 | 68 | 41 |
| | 1500 | 124 | 148 | 136 |
| | 500 | 196 | 204 | 200 |
| | 150 | 172 | 112 | 142 |
| | 50 | 172 | 124 | 148 |
| | 15 | 196 | 140 | 168 |
| | 5.0 | 148 | 104 | 126 |
| | 1.5 | 116 | 80 | 98 |
| Test Solution w/o S9 | 5000 | 84 | 24 | 54 |
| | 1500 | 49 | 102 | 76 |
| | 500 | 184 | 124 | 154 |
| | 150 | 180 | 128 | 154 |
| | 50 | 176 | 144 | 160 |
| | 15 | 132 | 152 | 142 |
| | 5.0 | 136 | 196 | 166 |
| | 1.5 | 116 | 136 | 126 |
| DI Water w/S9 | | 120 | 148 | 134 |
| DI Water w/o S9 | | 124 | 68 | 96 |
| 2-aminoanthracen w/ S9 | | 630 | 540 | 585 |
| Sodium azide w/o S9 | | 840 | 1104 | 972 |
| 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

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

| | Concentration µg per Plate | ***TA1537*** | | |
|---|---|---|---|---|
| | | Revertants per plate (CFU) | | Mean |
| Test Solution w/ S9 | 5000 | 4 | 4 | 4 |
| | 1500 | 1 | 2 | 2 |
| | 500 | 5 | 12 | 9 |
| | 150 | 11 | 14 | 13 |
| | 50 | 6 | 9 | 8 |
| | 15 | 6 | 3 | 5 |
| | 5.0 | 11 | 9 | 10 |
| | 1.5 | 10 | 8 | 9 |
| Test Solution w/o S9 | 5000 | 3 | 1 | 2 |
| | 1500 | 6 | 7 | 7 |
| | 500 | 11 | 7 | 9 |
| | 150 | 4 | 11 | 8 |
| | 50 | 13 | 14 | 14 |
| | 15 | 6 | 10 | 8 |
| | 5.0 | 9 | 11 | 10 |
| | 1.5 | 9 | 10 | 10 |
| DI Water w/S9 | | 18 | 33 | 26 |
| DI Water w/o S9 | | 20 | 5 | 13 |
| 2-aminoanthracen w/ S9 | | 150 | 136 | 143 |
| 2-aminoacridine w/o S9 | | 210 | 202 | 206 |
| 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

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

| | Concentration µg per Plate | ***TA1535*** | | |
|---|---|---|---|---|
| | | Revertants per plate (CFU) | | Mean |
| Test Solution w/ S9 | 5000 | 5 | 6 | 6 |
| | 1500 | 11 | 24 | 18 |
| | 500 | 19 | 20 | 20 |
| | 150 | 30 | 21 | 26 |
| | 50 | 27 | 24 | 26 |
| | 15 | 8 | 30 | 19 |
| | 5.0 | 26 | 26 | 26 |
| | 1.5 | 19 | 9 | 14 |
| Test Solution w/o S9 | 5000 | 6 | 3 | 5 |
| | 1500 | 8 | 10 | 9 |
| | 500 | 27 | 17 | 22 |
| | 150 | 15 | 23 | 19 |
| | 50 | 27 | 18 | 23 |
| | 15 | 21 | 24 | 23 |
| | 5.0 | 14 | 36 | 25 |
| | 1.5 | 25 | 19 | 22 |
| DI Water w/S9 | | 18 | 21 | 20 |
| DI Water w/o S9 | | 34 | 20 | 27 |
| 2-aminoanthracen w/ S9 | | 231 | 304 | 268 |
| Sodium azide w/o S9 | | 616 | 632 | 624 |
| 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

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

| | Concentration µg per Plate | ***WP2uvrA*** | | |
|---|---|---|---|---|
| | | Revertants per plate (CFU) | | Mean |
| Test Solution w/ S9 | 5000 | 43 | 35 | 39 |
| | 1500 | 22 | 57 | 40 |
| | 500 | 53 | 50 | 52 |
| | 150 | 42 | 46 | 44 |
| | 50 | 53 | 52 | 53 |
| | 15 | 37 | 52 | 45 |
| | 5.0 | 72 | 72 | 72 |
| | 1.5 | 46 | 58 | 52 |
| Test Solution w/o S9 | 5000 | 52 | 51 | 52 |
| | 1500 | 63 | 50 | 57 |
| | 500 | 62 | 57 | 60 |
| | 150 | 47 | 34 | 41 |
| | 50 | 55 | 45 | 50 |
| | 15 | 49 | 52 | 51 |
| | 5.0 | 56 | 58 | 57 |
| | 1.5 | 48 | 42 | 45 |
| DI Water w/S9 | | 67 | 55 | 61 |
| DI Water w/o S9 | | 49 | 59 | 54 |
| 2-aminoanthracen w/ S9 | | 274 | 263 | 269 |
| Methylmethanesulfonate w/o S9 | | 310 | 324 | 317 |
| 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

ACTIVE MICRO TECHNOLOGIES

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

# Phototoxicity Assay Analysis

**Tradename:** AMTicide® Coconut

**Code:** M14003

**CAS #:** 68333-16-4 & 8001-31-8

**Test Request Form #:** 1141

**Lot #:** 42873P

**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 **AMTicide® Coconut** 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.

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

## 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).

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

**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.

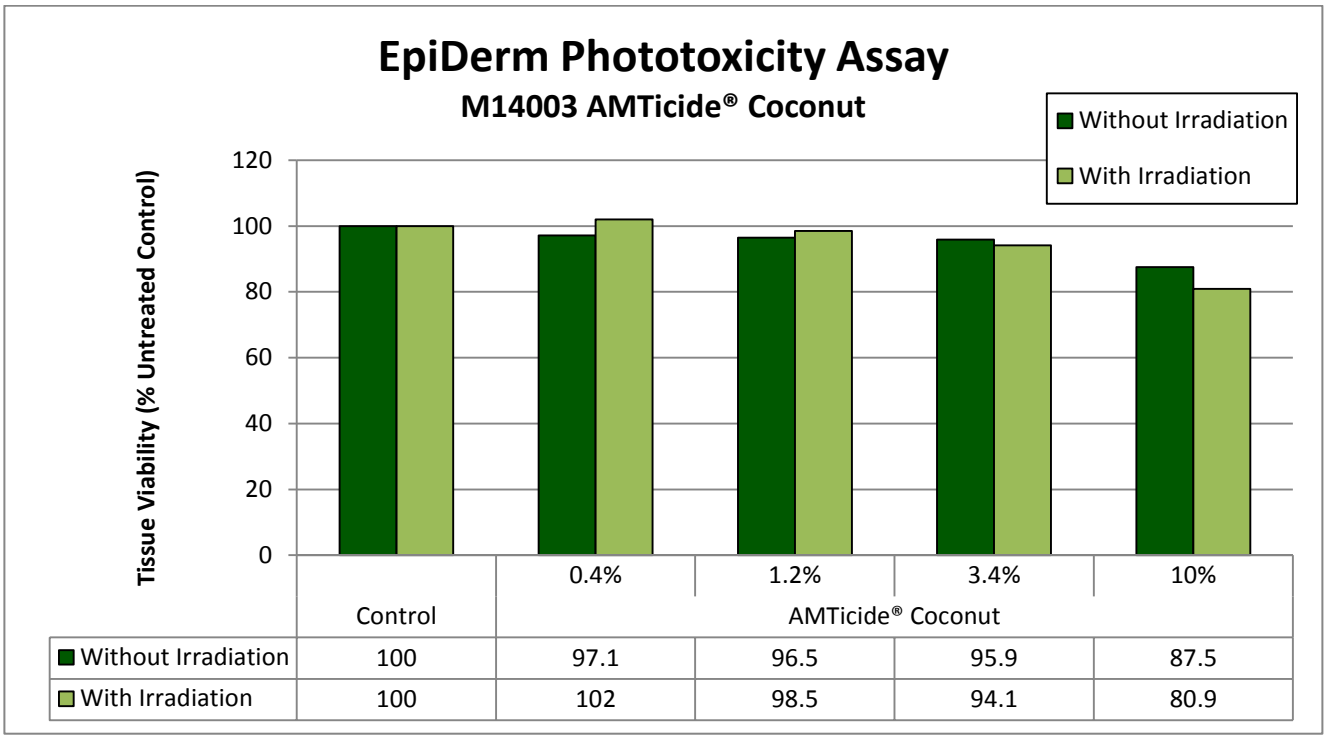
**C. Test Validity**

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

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

## 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. Using any test substance at this high of a concentration will have noticeable effects on cellular viability due to the fact that that substance is replacing the cell's nutrients. We can safely say that **AMTicide® Coconut** is not a photoirritant when used at the suggested use levels of 2 – 4%.




| | Control | 0.4% | 1.2% | 3.4% | 10% |
|---|---|---|---|---|---|
| Without Irradiation | 100 | 97.1 | 96.5 | 95.9 | 87.5 |
| With Irradiation | 100 | 102 | 98.5 | 94.1 | 80.9 |

**Figure 1: EpiDerm Phototoxicity Graph**

# OECD 202 Acute *Daphnia* Assay

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

**Tradename:** AMTicide® Coconut

**Code:** M14003

**CAS #:** 68333-16-4 & 8001-31-8

**Test Request Form #**: 1040

**Lot #:** NC141216-C

**Sponsor:** *Active Micro Technologies, 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 **AMTicide® Coconut** 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.

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

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. The water hardness should be 250 mg/L or smaller in terms of calcium carbonate concentration, and the pH should be 6-9. Aerate the material water before using it for the test.

| 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**

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

## 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.

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

## 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 $EC_{50}$

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

## Results

General Information:

| | | | |
|---|---|---|---|
| **Name of new chemical substance** | AMTicide ® Coconut | | |
| **INCI Nomenclature** | Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract | | |
| **CAS number** | 68333-16-4 & 8001-31-8 | | |
| **Structural or rational formula** (if neither is available, summarize its formulation method) | Biotechnology/Botanical: Leuconostoc kimchii & Cocos Nucifera | | |
| **Molecular weight** | 1500 Daltons | | |
| **Purity of the new chemical substance used for the test (%)** | 100% | | |
| **Lot number of the new chemical substance used for the test** | NC141216-C | | |
| **Names and contents of impurities** | n/a | | |
| **Solubility in water** | 100% | | |
| **Melting point** | n/a | | |
| **Boiling point** | 100°C | | |
| **Properties at room temperature** | Clear to Slightly Hazy Liquid | | |
| **Stability** | Stable at temperatures between 23 - 28°C | | |
| **Solubility in solvents, etc.** | **Solvent** | **Solubility** | **Stability in solvent** |
| | n/a | n/a | n/a |

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

Test Materials and Methods:

| Items | | | Contents |
|---|---|---|---|
| **Test Organisms** | Species | | *Daphnia magna* |
| | Source | | Carolina Biological Supply Company |
| | Susceptibility to 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 | | 1/13/2015 |
| | Test Concentrations | | 200, 89.5, 42.3, 20.6, 7.9 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 |
| **Calculation of Results** | Statistical Method | | Probit Analysis |

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

Test Results:

| Items | | Contents |
|---|---|---|
| **Toxicity Value** | 48hr EC50 | 138.4 mg/L |
| **Exposure Concentrations Used for Calculation** | Nominal Values | 200, 89.5, 42.3, 20.6, 7.9 mg/L |
| **Remarks** | | Not harmful to aquatic organisms |

## Discussion

After 48 hours, the EC50 value for **AMTicide® Coconut** was determined to be 138.4 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

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

**Tradename:** AMTicide® Coconut

**Code:** M14003

**CAS #:** 68333-16-4 & 8001-31-8

**Test Request Form #**: 1041

**Lot #:** NC141216-C

**Sponsor:** *Active Micro Technologies, 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 ready biodegradability of **AMTicide® Coconut** 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.

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

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

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

- 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.
- 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)

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

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 attached data sheet.
   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.

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

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:

f.

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

g. 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.

h. When appropriate, calculate DOC removals using the equation given in 301 A paragraph 27.

i. 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.

ACTIVE MICRO TECHNOLOGIES

# OECD 301B Ready Biodegradability Assay

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

## Data Sheet

| | | | |
|---|---|---|---|
| **Laboratory** | Active Micro Technologies Tissue Culture Laboratory | | |
| **Test Start Date** | 12/29/2014 | | |
| **Test Substance** | **Name** | AMTicide® Coconut | |
| | **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** | 95.8% | | |
| **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 method, test **AMTicide® Coconut** achieved 95.8% biodegradation after 28 days of testing. The product met method requirements for Readily Biodegradability classification.

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

Date Issued: October 8, 2015

## ALLERGEN DECLARATION

**RE:** *AMTicide® Coconut (M14003)*

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**

# Heavy Metals Statement

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

May 10, 2016

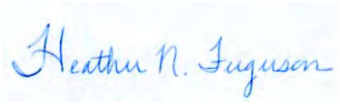
To Whom It May Concern,

This letter is to certify that AMTicide® Coconut (M14003) has the following heavy metals profile:

| | |
|---|---|
| Heavy Metals: | Less than 20 ppm |
| Lead: | Less than 10 ppm |
| Antimony: | Less than 5 ppm |
| Arsenic: | Less than 2 ppm |
| Mercury: | Less than 1 ppm |
| Cadmium: | Less than 1 ppm |

**Please note: The above levels illustrate the Maximum Limits. Values for Lead, Antimony, Mercury and Cadmium do not appear on the Specification for AMTicide® Coconut.

Best Regards,



Tomorrow's Vision... *Today!*®

Heather Ferguson |R&D Coordinator

107 Technology Drive | Lincolnton, NC 28092

Direct: 704.276.7083|Main: 704.276.7100|Fax: 704.276.7101

Email: hferguson@activeconceptsllc.com

www.activeconceptsllc.com

ACTIVE MICRO TECHNOLOGIES

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

# Certificate of Origin

## AMTicide® Coconut
## Code: M14003

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-Free.

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

| INCI Name | Source |
|---|---|
| Lactobacillus | Bacteria (*Lactobacillus*) |
| Cocos Nucifera (Coconut) Fruit Extract | Plant (*Cocos nucifera*) |

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** | AMTicide® Coconut |
| **Product Code** | M14003 |
| **Recommended Use** | Topical Cosmetic Use; Antimicrobial |
| **Restrictions on Use** | None |
| **Supplier/Manufacturing Site** | Active Micro Technologies, LLC |
| Address | 107 Technology Drive<br>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:** Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract

**Generic name:**

**Chemical Family:** Extract

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

| Substance | CAS Numbers | EC Numbers | Percentage |
|---|---|---|---|
| Lactobacillus | 68333-16-4 | N/A | 80.00% |
| Cocos Nucifera (Coconut) Fruit Extract | 8001-31-8 | 232-282-8 | 20.00% |

**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.<br>Avoid inhalation of material or combustion by-products.<br>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.<br><br>Personal Protective Equipment:<br>-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.<br>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:** | 5 Gardner Maximum |
| **Odor:** | Characteristic |
| **pH:** | 7.0 – 9.0 |

| | |
|---|---|
| **Solids (1g-105°C-1hr):** | 20.0 – 25.0% |
| **Heavy Metals:** | < 20 ppm |
| **Arsenic:** | < 2 ppm |
| **Lipopeptide Content:** | 0.5% Minimum |
| **Minimum Inhibitory Concentration Organism (ATCC#):** | |
| **C. albicans (#10231):** | 0.25 – 2.00% |
| **A. brasiliensis (#16404):** | 0.25 – 2.00% |
| **Specific Gravity:** | 1.033 – 1.040 |
| **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 & 48 Hour Patch Test) |
| **Ocular:** | Non-Irritant (Ocular Irritection Model) |
| **Inhalation:** | Not Determined |

**Acute toxicity data:** EC50 (Acute Daphnia): 138.4 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:**
EC regulations: This product does not need to be labeled in accordance with EC Directives or respective national laws

**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: | Lactobacillus: | N/A |
| | Cocos Nucifera Fruit Extract: | 232-282-8 |
| TSCA inventory status: | Exempt | |
| AICS inventory status: | 68333-16-4 & 8001-31-8 | |
| Canadian (CEPA DSL) inventory status: | Listed as Lactobacillus acidophilus (Revised ICL) & Coconut Oil (DSL) | |
| Japan (MITI list): | Lactobacillus & Cocos Nuifera (Coconut) Fruit Extract | |
| Korea: | Lactobacillus^ & Cocos Nuifera (Coconut) Fruit Extract^ | |
| China inventory status: | Lactobacillus & Cocos Nuifera (Coconut) Fruit Extract | |
| Philippines inventory status: | Not Listed: Lactobacillus (68333-16-4) | |
| | Listed as Coconut oil | |

^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

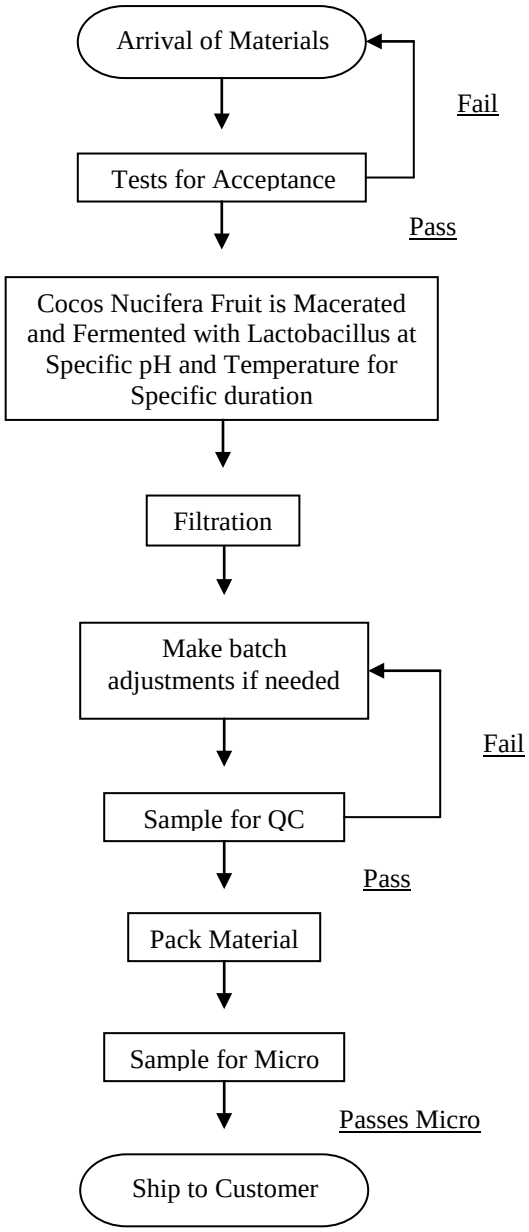
## SECTION 16. OTHER INFORMATION

| | |
|---|---|
| Prohibited uses: | For specific uses, food industry, ask the manufacturer for more information. |
| Last Revision Date: | 08/13/2015 |
| Preparation Date: | 08/20/2015 |
| MSDS summary of changes | - Updated Transport Information – Section 14 (Transport Information)<br>- Added Irritation Data – Section 11 (Toxicological Information)<br>- Added Dermal Irritation Data & Sensitization Data – Section 11 (Toxicological Information)<br>- Added Acute Toxicity & Mutagenicity Data – Section 11 (Toxicological Information) & Added Biodegradability Data – Section 12 (Ecological Information)<br>- Added Sensitization Data – Section 11 (Toxicological Information)<br>- Added Lipopeptide Content – Section 9 (Physical & Chemical Properties)<br>- Added Sensitization Data – Section 11 (Toxicological Information)<br>- Added Specific Gravity – Section 9 (Physical & Chemical Properties) |

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.

# M14003-AMTicide® Coconut Manufacturing Flow Chart

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



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

# AMTicide® Coconut
# Certificate of Compliance

**Code:** M14003
**INCI Name:** Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract
**INCI Status:** Approved
**CAS #:** 68333-16-4 & 8001-31-8
**EINECS #:** N/A & 232-282-8

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 |
| USA (TSCA) | Exempt |
| Australia (AICS) | Compliant |
| Japan (METI) | Compliant |
| Canada (DSL) | Compliant |
| China (IECSC) | Compliant |
| Brazil (ANVISA) | Compliant |
| Korea (KECI) | Compliant |
| Philippines (PICCS) | Contact Us |
| Taiwan (FDA 2.0!) | Compliant |

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

# AMTicide® Coconut
## Code: M14003

Attention must be paid to the use of AMTicide® Coconut 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.

AMTicide® Coconut 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). The recommended use levels for AMTicide® Coconut is 2.00 – 4.00%.

AMTicide® Coconut 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.

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

As of June 18, 2012, AMTicide® Coconut does not contain any substances present on the so called "candidate list" provided by the European Chemicals Agency (ECHA). We further certify that our product is not listed on CITES.

AMTicide® Coconut is in compliance with the standardized set of rules developed and approved by the NPA (Natural Products Association).

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

Active Micro Technologies, LLC certifies 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 & Proposition 65.

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

AMTicide® Coconut is REACH Compliant and free of the following:

- Formaldehyde or formaldehyde donors
- Glycol ethers
- Gluten
- Lactose
- Nanoparticles
- Nitrosamines
- Palm oil/palm kernel oil (or derivatives)
- Parabens
- Paraffin/petroleum products
- Phthalates
- Polyethylene glycol (PEG)
- Residual solvents
- Sulfates
- Volatile organic compounds/solvents

# REACH Compliance Statement

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

Trade Name: AMTicide® Coconut (M14003)

INCI Name: Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract

This is to certify that AMTicide® Coconut is REACH compliant. Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract are exempt from registration under REACH as they're currently manufactured and/or exported at less than 1 tonne per year.

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

# 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).

**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.

**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