ATS LABS

FINAL STUDY REPORT

STUDY TITLE

AOAC Use-Dilution Method

Test Organisms:

Pseudomonas aeruginosa (ATCC 15442)
Staphylococcus aureus (ATCC 6538)
Salmonella enterica (ATCC 10708)

PRODUCT IDENTITY

Ultra-Lyte
UL01, UL02, and UL03

DATA REQUIREMENTS

U.S. EPA 40 CFR Part 158
"Data Requirements for Registration"
Pesticide Assessment Guidelines - Subdivision G, 91-2 (d)

AUTHOR

Joshua Luedtke, M.S.
Study Director

STUDY COMPLETION DATE

March 22, 2010

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

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Belfair, WA 98528

SPONSOR REPRESENTATIVE

Plains ECA Solutions
RR1 Decker Manitoba
R0M 0K0 Canada

PROJECT NUMBER

A09168

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B), or (C).

Company: Clarentis LLC

Company Agent: _____

_____
Title

_____ Date:_____
Signature

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160.

The procedures not performed by or under the direction of ATS Labs are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the compound(s).

Submitter:_____ Date:_____

Sponsor:_____ Date:_____

Study Director: Joshua Luedtke Date: 3·22·10
Joshua Luedtke, M.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: AOAC Use-Dilution Method

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date of Phase Inspection	Date Reported to Study Director	Date Reported to Management
Critical Phase Audit	March 12, 2010	March 12, 2010	March 22, 2010
Final Report	March 19, 2010	March 19, 2010	

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: Joyce Saluda Date: 3/22/10

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STUDY PERSONNEL

STUDY DIRECTOR: Joshua Luedtke, M.S.

Professional personnel involved:

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Adam W. Pitt, B.S.	- Research Assistant II
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Christine Chan, B.S.	- Research Assistant I
Kathryn LaFleur, B.S.	- Research Assistant I

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: AOAC Use-Dilution Method

Project Number: A09168

Protocol Number: CLS01012010.UD.2

Sponsor:
Clarentis LLC
23969 NE SR3, Suite G #143
Belfair, WA 98528

Sponsor Representative:
Plains ECA Solutions
RR1 Decker Manitoba
R0M 0K0 Canada

Test Facility:
ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance Name: Ultra-Lyte

Lot/Batch(s): UL01, UL02 and UL03

Test Substance Characterization
Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor.

STUDY DATES

Date Sample Received: March 12, 2010
Study Initiation Date: March 5, 2010
Experimental Start Date: March 12, 2010
Experimental End Date: March 16, 2010
Study Completion Date: March 22, 2010

OBJECTIVE

The objective of this study was to determine the efficacy of the Sponsor's product following the AOAC Use-Dilution Method in compliance with the U.S. Environmental Protection Agency requirements set forth in the Pesticide Assessment Guidelines and methods approved by Health Canada.

SUMMARY OF RESULTS

Test Substance:	Ultra-Lyte (UL01, UL02, and UL03)
Dilution:	Ready to use (RTU)
Test Organisms:	*Pseudomonas aeruginosa* (ATCC 15442) *Staphylococcus aureus* (ATCC 6538) *Salmonella enterica* (ATCC 10708)
Exposure Time:	10 minutes
Exposure Temperature:	20 ± 1°C (20.0°C)
Organic Soil Load:	5% fetal bovine serum
Efficacy Result:	Ultra-Lyte demonstrated efficacy of 3 lots against *Pseudomonas aeruginosa*, and therefore, meets the requirements set forth by the U.S. EPA and Health Canada for disinfectant label claims following a 10 minute exposure time at 20 ± 1°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load. Ultra-Lyte demonstrated efficacy of 3 lots against *Staphylococcus aureus*, and therefore, meets the requirements set forth by the U.S. EPA and Health Canada for disinfectant label claims following a 10 minute exposure time at 20 ± 1°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load. Ultra-Lyte demonstrated efficacy of 3 lots against *Salmonella enterica*, and therefore, meets the requirements set forth by the U.S. EPA and Health Canada for disinfectant label claims following a 10 minute exposure time at 20 ± 1°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

TEST MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Pseudomonas aeruginosa	15442	Nutrient Broth	35-37°C, aerobic
Staphylococcus aureus	6538	Synthetic Broth	35-37°C, aerobic
Salmonella enterica	10708	Synthetic Broth	35-37°C, aerobic

The microorganisms used in this study were obtained from the American Type Culture Collection (ATCC), Manassas, Virginia.

Recovery Media

Neutralizing Subculture Medium: Letheen Broth + 0.2% Sodium Thiosulfate
Agar Plate Medium: Tryptic Soy + 5% Sheep Blood Agar (BAP)

Reagents

Organic Soil Load Description: 5% fetal bovine serum (FBS)

Carriers

Stainless steel penicylinders were pre-soaked overnight in 1.0 N NaOH, washed in water until rinse water was neutral to phenolphthalein, and autoclaved in 0.1% asparagine.

TEST METHOD

Preparation of Test Substance

The test substance was ready to use (RTU), as received from the Sponsor. The test substance was homogenous as determined by visual observation.

Ten (10.0) mL aliquots of the test substance were transferred to sterile 25 x 150 mm tubes, placed in a 20 ± 1°C (20.0°C) water bath and allowed to equilibrate for ≥10 minutes.

Preparation of Test Organism

From a stock slant, an initial tube of culture broth was inoculated. From this initial broth suspension a minimum of three daily transfers were performed on consecutive days prior to use in testing procedure. For each test organism, the appropriate growth medium was subcultured using a daily transfer (more than 3, but less than 30 transfers) of the test organism.

A 48-54 hour broth culture incubated at 35-37°C was prepared. On the day of use, the pellicle was aspirated from the *Pseudomonas aeruginosa* culture. The test cultures were thoroughly mixed and allowed to stand for ≥10 minutes prior to use.

Addition of Organic Soil Load

A 9.3 mL aliquot of FBS was added to 176.7 mL of each broth culture to yield a 5% fetal bovine serum organic soil load.

Contamination of Carriers

Sterile penicylinders were immersed for 15 minutes in a 48-54 hour old broth culture of the test organism, at a ratio of 1 carrier per 1.0 mL broth. The penicylinders were then dried on filter paper in a sterile Petri dish at 35-37°C for 40 minutes at a 41% relative humidity.

Exposure Conditions

For each test substance, 60 contaminated and dried carriers were individually transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance and exposed for 10 minutes at 20 ± 1°C (20.0°C).

Test System Recovery

Following the Sponsor specified exposure time, each medicated carrier was then transferred by hook needle at identical staggered intervals to 10 mL of Letheen Broth + 0.2% Sodium Thiosulfate.

Incubation and Observation

The neutralized subculture tubes and plates were incubated for 46 hours at 35-37°C. Subcultures were stored at 2-8°C for 1 day prior to examination. Following incubation and storage, the subcultures were visually examined for the presence or absence of visible growth.

Representative neutralized subcultures showing growth were subcultured onto appropriate agar, stained and biochemically assayed to confirm or rule out the presence of the test organism.

TEST CONTROLS

Purity Control
A "streak plate for isolation" was performed on each organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control
The serum used for soil load was cultured, incubated, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control
A representative uninoculated carrier was added to the subculture medium. The subculture medium containing the carrier was incubated and examined for growth. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control
A representative sample of uninoculated neutralizing subculture medium was incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control
A representative inoculated carrier was added to the subculture medium. The subculture medium containing the carrier was incubated and visually examined for growth. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control
The neutralization of the test substance was confirmed by exposing sterile carriers (representing not less than 10% of the total number of test carriers) to the test substance and transferring them to subculture tubes containing 10 mL of neutralizing subculture medium. The subculture tubes containing the exposed carriers were inoculated with ≤100 colony forming units (CFU) of each test organism, incubated under test conditions and visually examined for the presence of growth. This control was performed with multiple replicates using different dilutions of the test organism. A standardized spread plate procedure was run concurrently in order to enumerate the number of CFU actually added. The control result was reported using data from the most appropriate dilution.

The acceptance criterion for this study control is growth after inoculation with ≤100 CFU.

Carrier Population Control
Inoculated carriers were added at a ratio of 1 carrier to 10 mL neutralizing broth and vortex mixed. Appropriate serial ten-fold dilutions were prepared and aliquots were spread plated on agar plate medium, and incubated. Following incubation, the resulting colonies were enumerated and the CFU/carrier calculated. The acceptance criterion for this study control is a minimum of 1.0×10^4 CFU/carrier.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The EPA efficacy performance requirements for label claims state that the test substance must kill the microorganism on 59 out of the 60 inoculated carriers.

Health Canada performance requirements for label claims state that the test substance must kill the microorganism on 58 out of the 60 inoculated carriers.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section.

PROTOCOL CHANGES

Protocol Amendments:
No protocol amendments were required for this study.

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculation

Carrier Population Control Calculation:

$$\text{CFU/carrier} = \frac{\text{(average number colonies/plate @ dilution) x (dilution factor) x (volume neutralizer)}}{\text{(number of carriers tested) x (volume plated)}}$$

The carrier population was calculated and reported using data from the most appropriate dilution(s).

Statistical Analysis
None used.

STUDY RETENTION

Record Retention

All of the original raw data developed exclusively for this study shall be archived at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. The original data includes, but is not limited to, the following:

1. Certified copy of final study report.
2. Original signed protocol.
3. Any protocol amendments/deviation notifications.
4. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
5. All measured data used in formulating the final report.
6. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
7. Study-specific SOP deviations made during the study.

Test Substance Retention

The test substance will be discarded following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test material.

REFERENCES

1. Association of Official Analytical Chemists (AOAC), 1990. Use-Dilution Tests, p. 135-137. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.

2. Association of Official Analytical Chemists (AOAC), 1990. Germicidal and Detergent Sanitizing Action of Disinfectants, p. 139 [Preparation of Synthetic Hard Water]. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.

3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Efficacy Data Requirements, Disinfectants for Use on Hard Surfaces, DIS/TSS-1.

4. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1979. Efficacy Data Requirements, Supplemental Recommendations, DIS/TSS-2.

5. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Subseries 91A: Public Health Uses. *In* Pesticide Assessment Guidelines – Subdivision G (Product Performance).

6. Assessment of Efficacy of Antimicrobial Agents for Use on Environmental Surfaces and Medical Devices, CAN/CGSB-2. 161-97, August 1997.

7. Guidance Document: Disinfectant Drugs, Health Products and Food Branch, Health Canada, 10/29/2007.

RESULTS

For Control and Neutralization Results, see Tables 1-3.
All data measurements/controls including the culture purity, viability, organic soil sterility, neutralizing subculture medium sterility, carrier sterility, neutralization confirmation, and carrier population were within acceptance criteria.

For Test Results, see Table 4.

ANALYSIS

Ultra-Lyte (UL01), ready to use, demonstrated no growth of *Pseudomonas aeruginosa* (ATCC 15442) in any of the 60 subculture tubes following a 10 minute exposure time at 20 ± 1°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

Ultra-Lyte (UL02 and UL03), ready to use, both demonstrated growth of *Pseudomonas aeruginosa* (ATCC 15442) in 1 of the 60 subculture tubes following a 10 minute exposure time at 20 ± 1°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

Ultra-Lyte (UL01, UL02, and UL03), ready to use, demonstrated no growth of *Staphylococcus aureus* (ATCC 6538) in any of the 60 subculture tubes following a 10 minute exposure time at 20 ± 1°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

Ultra-Lyte (UL01, UL02, and UL03), ready to use, demonstrated no growth of *Salmonella enterica* (ATCC 10708) in any of the 60 subculture tubes following a 10 minute exposure time at 20 ± 1°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum organic soil load, Ultra-Lyte (UL01, UL02 and UL03), ready to use, demonstrated efficacy against *Pseudomonas aeruginosa* as required by the U.S. EPA and Health Canada for disinfectant label claims following a 10 minute exposure time at 20 ± 1°C (20.0°C).

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum organic soil load, Ultra-Lyte (UL01, UL02, UL03), ready to use, demonstrated efficacy against *Staphylococcus aureus* as required by the U.S. EPA and Health Canada for disinfectant label claims following a 10 minute exposure time at 20 ± 1°C (20.0°C).

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum organic soil load, Ultra-Lyte (UL01, UL02, UL03), ready to use, demonstrated efficacy against *Salmonella enterica* as required by the U.S. EPA and Health Canada for disinfectant label claims following a 10 minute exposure time at 20 ± 1°C (20.0°C).

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.

TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Type of Control		Results		
		Pseudomonas aeruginosa **(ATCC 15442)**	***Staphylococcus aureus*** **(ATCC 6538)**	***Salmonella enterica*** **(ATCC 10708)**
Purity Control		Pure	Pure	Pure
Viability Control		Growth	Growth	Growth
Organic Soil Sterility Control	Vial #16	No Growth		
	Vial #18	No Growth		
	Vial #19	No Growth		
Neutralizing Subculture Medium Sterility Control		No Growth		
Carrier Sterility Control		No Growth		

TABLE 2: CARRIER POPULATION CONTROL RESULTS

Test Organism	Date Performed	Result
Pseudomonas aeruginosa (ATCC 15442)	3/12/10	1.58×10^6 CFU/carrier
Staphylococcus aureus (ATCC 6538)		1.47×10^7 CFU/carrier
Salmonella enterica (ATCC 10708)		1.27×10^6 CFU/carrier

CFU = Colony Forming Unit

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Organism	Test Substance	Date Performed	Average Inoculum (CFU/mL)	Number of Subculture Tubes	
				Tested	Positive
Pseudomonas aeruginosa (ATCC 15442)	Ultra-Lyte UL01	3/12/10	13	6	6
	Ultra-Lyte UL02			6	6
	Ultra-Lyte UL03			6	6
Staphylococcus aureus (ATCC 6538)	Ultra-Lyte UL01		9	6	6
	Ultra-Lyte UL02			6	6
	Ultra-Lyte UL03			6	6
Salmonella enterica (ATCC 10708)	Ultra-Lyte UL01		8	6	6
	Ultra-Lyte UL02			6	6
	Ultra-Lyte UL03			6	6

CFU = Colony Forming Unit

TABLE 4: TEST RESULTS

Test Substance	Test Organism	Date Performed	Sample Dilution*	Number of Carriers	
				Exposed	Showing Growth**
Ultra-Lyte UL01	*Pseudomonas aeruginosa* (ATCC 15442)	3/12/10	RTU	60	0
	Staphylococcus aureus (ATCC 6538)			60	0
	Salmonella enterica (ATCC 10708)			60	0
Ultra-Lyte UL02	*Pseudomonas aeruginosa* (ATCC 15442)			60	1
	Staphylococcus aureus (ATCC 6538)			60	0
	Salmonella enterica (ATCC 10708)			60	0
Ultra-Lyte UL03	*Pseudomonas aeruginosa* (ATCC 15442)			60	1
	Staphylococcus aureus (ATCC 6538)			60	0
	Salmonella enterica (ATCC 10708)			60	0

* RTU = Ready to use.
** Number of carriers showing growth of the test organism.

(For Laboratory Use Only)
ATS Labs Project # A 0 9 1 6 8
3/8/10

PROTOCOL

AOAC Use-Dilution Method

EXACT COPY
INITIALS JL DATE 3-22-10

Test Organisms:

Pseudomonas aeruginosa (ATCC 15442)
Staphylococcus aureus (ATCC 6538)
Salmonella enterica (ATCC 10708)

PROTOCOL NUMBER

CLS01012010.UD.2

PREPARED FOR

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PERFORMING LABORATORY

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PREPARED BY

Amy S. Jeske, B.S.
Manager, Microbiology Operations

DATE

January 20, 2010

Revised Date: February 18, 2010

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AOAC Use-Dilution Method

SPONSOR:
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SPONSOR REPRESENTATIVE:
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TEST FACILITY:
ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE
The purpose of this study is to determine the efficacy of the sponsor's product following the AOAC Use Dilution Method in compliance with the U.S. Environmental Protection Agency requirements set forth in the Pesticide Assessment Guidelines and methods approved by Health Canada.

SUBSTANCE CHARACTERIZATION
Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor. The test substance shall be characterized by the Sponsor prior to the experimental start date of this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to ATS Labs.

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is February 19, 2010. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of March 9, 2010. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

If a test must be repeated, or a portion of it, due to failure by ATS Labs to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of ATS Labs or any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

The Sponsor is responsible for any rejection of the final report by the regulatory agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the ATS Labs final report and notify ATS Labs of any perceived deficiencies in these areas before submission of the report to the regulatory agency. ATS Labs will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM
The U.S. Environmental Protection Agency and Health Canada require that a specific bacterial claim for a test substance intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed bacteria. This is accomplished in the laboratory by treating the target bacteria with the disinfectant (test substance) under conditions which simulate as closely as possible the actual conditions under which the test substance is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting data. The experimental design in this protocol meets these requirements.

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TEST PRINCIPLE

A film of bacterial cells dried on a surface of stainless steel carriers is exposed to the test substance for a specified contact time. After exposure, the carriers are transferred to vessels containing neutralizing subculture media and assayed for survivors. Appropriate viability, carrier population and neutralization controls are performed. The current version of Standard Operating Procedure CGT-4400 reflects the methods which shall be used in this study.

TEST METHOD

Test Organisms	ATCC #	Growth Medium	Incubation Parameters
Pseudomonas aeruginosa	15442	Nutrient Broth	35-37°C, aerobic
Staphylococcus aureus	6538	Synthetic Broth	35-37°C, aerobic
Salmonella enterica	10708	Synthetic Broth	35-37°C, aerobic

The test organisms to be used in this study were obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Carriers

Carriers will be screened according to AOAC Official Method of Analysis and any carriers positive for growth will be discarded. Only penicylinders showing no growth may be used. Stainless steel penicylinders will be pre-soaked overnight in 1.0N NaOH, washed in water until neutral and autoclaved in 0.1% asparagine.

Preparation of Test Organisms

From a stock slant, an initial tube of culture broth will be inoculated. This culture is termed the "initial broth suspension." From this initial broth suspension, a minimum of three daily transfers will be performed on consecutive days prior to use in testing procedure. For each test organism, the appropriate growth medium will be subcultured using a daily transfer (more than 3, but less than 30 transfers) of the test organism.

A 48-54 hour broth culture incubated at the parameters listed above will be prepared.

On the day of use, the pellicle will be aspirated from the *Pseudomonas aeruginosa* culture. The test cultures will be thoroughly mixed and allowed to stand for ≥10 minutes prior to use.

An organic soil load may be added to the test culture per Sponsor's request.

Contamination of Carriers

The penicylinders will be transferred to the culture and immersed for 15 minutes in a prepared suspension at a ratio of 1 carrier per 1.0 mL culture. The inoculated carriers will be dried on filter paper in a sterile petri dish at 35-37°C for 40 minutes. The drying conditions (temperature and humidity) will be appropriate for the test organism. The actual drying conditions will be clearly documented.

Preparation of Test Substance

The test substance(s) to be assayed will be used as directed by the Sponsor. If a dilution of the test substance is requested by the Sponsor, the diluted test substance(s) shall be used within three hours of preparation. Ten (10) mL of the test substance at its use-dilution will be aliquoted into the required number of sterile 25 x 150 mm tubes. The tubes will be placed into a waterbath at the specified exposure temperature, and allow to equilibrate for ≥10 minutes prior to testing.

Exposure Conditions

Each contaminated and dried carrier will be placed into a separate tube containing 10 mL of the test substance at its use-dilution for the desired exposure time and temperature.

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Test System Recovery
Following the Sponsor specified exposure period, each medicated carrier will be transferred by wire hook at staggered intervals to 10 mL of neutralizing broth. If necessary, carriers will be transferred into individual secondary subculture tubes containing 10 mL neutralizing broth ≥30 minutes after subculture of first carrier.

Incubation and Observation
All subculture tubes and plates will be incubated for 48±4 hours at 35-37°C (or other appropriate time/temperatures).

Following incubation, the subculture tubes will be visually examined for growth. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination.

Representative subculture tubes demonstrating growth (positive tubes) will be subcultured onto appropriate agar for confirmation of the test organism.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" will be performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control
The serum used for soil load will be cultured, incubated, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control
A representative uninoculated carrier will be added to the neutralizing subculture medium. The subculture medium containing the carrier will be incubated and examined for growth. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control
A representative sample of uninoculated neutralizing subculture medium will be incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control
A representative inoculated carrier will be added to the subculture medium. The subculture medium containing the carrier will be incubated and visually examined for growth. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control
The neutralization of the test substance will be confirmed by exposing sterile carriers (representing not less than 10% of the total number of test carriers) to the test substance and transferring them to primary subcultures containing 10 mL of neutralizing subculture medium. If performed in the test procedure, carriers will then be transferred from primary subcultures into individual secondary subcultures ≥30 minutes following the primary transfer. The subcultures containing the exposed carriers will be inoculated with ≤100 colony forming units (CFU) of each test organism, incubated under test conditions and visually examined for the presence of growth. This control will be performed with multiple replicates using different dilutions of the test organism. A standardized spread plate procedure will be run concurrently in order to enumerate the number of CFU actually added. The control result will be reported using data from the most appropriate dilution.

The acceptance criterion for this study control is growth following inoculation with ≤100 CFU.

OR:

Ten percent of the subcultures containing carriers showing no growth will be inoculated with ≤100 CFU of each test organism and incubated. This control will be performed with multiple replicates representing different dilutions of the test organism. A standardized spread plate procedure will be run concurrently in order to enumerate the number of CFU actually added. The control result will be reported using data from the most appropriate dilution.

The acceptance criterion for this study control is growth following inoculation with ≤100 CFU.

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Carrier Population Control
Inoculated carriers will be added at a ratio of 1 carrier to 10 mL neutralizing broth and vortex mixed. Appropriate serial ten-fold dilutions will be prepared and the aliquots spread plated on agar plate medium, and incubated. Following incubation, the resulting colonies will be enumerated and the CFU/carrier calculated. The acceptance criterion for this study control is a minimum of 1.0×10^4 CFU/carrier.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

ATS Labs maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including bacterial strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subculture tubes, etc. during the course of the test. Test subculture tubes are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: NA

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The EPA efficacy performance requirements for label claims state that the test substance must kill the microorganism on 59 out of the 60 inoculated carriers.

Health Canada performance requirements for label claims state that the test substance must kill the microorganism on 58 out of the 60 inoculated carriers.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section.

REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the bacterial strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for changes will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the correct effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

PRODUCT DISPOSITION

It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

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RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at ATS Labs. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at ATS Labs. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. Association of Official Analytical Chemists (AOAC), 1990. Use-Dilution Tests, p. 135-137. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.
2. Association of Official Analytical Chemists (AOAC), 1990. Germicidal and Detergent Sanitizing Action of Disinfectants, p. 139 [Preparation of Synthetic Hard Water]. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.
3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Efficacy Data Requirements, Disinfectants for Use on Hard Surfaces, DIS/TSS-1.
4. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1979. Efficacy Data Requirements, Supplemental Recommendations, DIS/TSS-2.
5. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Subseries 91A: Public Health Uses. *In* Pesticide Assessment Guidelines – Subdivision G (Product Performance).
6. Assessment of Efficacy of Antimicrobial Agents for Use on Environmental Surfaces and Medical Devices, CAN/CGSB-2.161-97, August 1997.
7. Guidance Document: Disinfectant Drugs, Health Products and Food Branch, Health Canada, 10/29/2007.

DATA ANALYSIS

Calculations

Carrier Population Control Calculation:

$$\text{Carrier population, CFU/carrier} = \frac{\text{(average number colonies/plate @ dilution) x (dilution factor) x (volume neutralizer)}}{\text{(number of carriers tested) x (volume plated)}}$$

The carrier population is calculated and reported using data from the most appropriate dilution(s).

Statistical Analysis

None used.

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STUDY INFORMATION

(All sections must be completed prior to submitting protocol)

Sponsor (Date/Initial): 2-23-10 JK

Test Substance (Name & Batch Numbers, *including ≥60 day old batch* - exactly as it should appear on final report): Ultra-Lyte, UL01, UL02, UL03

Specify ≥60 day old batch: UL01

Expiration Date: 2-22-11

Product Description:

- ☐ Quaternary ammonia
- ☐ Iodophor
- ☐ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☑ Other HOCL

Test Substance Active Concentration (upon submission to ATS Labs): 500 ppm

Neutralization/Subculture Broth:

- ☐ _____
- ☑ ATS Labs' Discretion. By checking, the Sponsor authorizes ATS Labs, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions:

- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other: _____

Hazards:

- ☑ None known: Use Standard Precautions
- ☐ Material Safety Data Sheet, Attached for each product
- ☐ As Follows: _____

Product Preparation

- ☑ No dilution required, Use as received (RTU)
- ☐ *Dilution(s) to be tested: _____ (amount of test substance) + _____ (amount of diluent)
  - ☐ Deionized Water (Filter or Autoclave Sterilized)
  - ☐ Tap Water (Filter or Autoclave Sterilized)
  - ☐ AOAC Synthetic Hard Water: _____PPM
  - ☐ Other _____

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Organism(s): *Pseudomonas aeruginosa* (ATCC 15442)
Staphylococcus aureus (ATCC 6538)
Salmonella enterica (ATCC 10708)

Carrier Number: 60 per batch

Exposure Time: 10 Minutes

Exposure Temperature: 20 ± 1 °C

Organic Soil Load:

- ☑ Minimum 5% Organic Soil Load (Fetal Bovine Serum)
- ☐ No Organic Soil Load Required
- ☐ Other: _____

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TEST SUBSTANCE SHIPMENT STATUS

- ☐ Has been used in one or more previous studies at ATS Labs .
- ☐ Has been shipped to ATS Labs (but has not been used in a previous study).
  Date shipped to ATS Labs:_____ Sent via *overnight* delivery? ☐ Yes ☐ No
- ☑ Will be shipped to ATS Labs.
  Date of expected receipt at ATS Labs: 3-11-10
- ☐ Sender (if other than Sponsor):_____

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP Study)

PROTOCOL MODIFICATIONS

- ☑ Approved without modification
- ☐ Approved with modification - Supplemental Information Form Attached - ☐ Yes ☐ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Duke Van Kalken (Clarentis LLC) TITLE: President

SIGNATURE: Duke van Kalken DATE: 2-23-10

PHONE: 561-799-9299 FAX: 561-799-9219 EMAIL: dukevankalken@hotmail.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

Nathan Waldner

ATS LABS:

NAME: Joshua Luedtke
Study Director

SIGNATURE: Joshua Luedtke DATE: 3.5.10
Study Director