FINAL STUDY REPORT

STUDY TITLE

AOAC Use-Dilution Method

Test Organism:

Campylobacter jejuni (ATCC 29428)

PRODUCT IDENTITY

Axen 30
Lot # 2006.003.001 and Lot # 2007.008.002

DATA REQUIREMENTS

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

AUTHOR

Jill Ruhme, B.S.
Study Director

STUDY COMPLETION DATE

March 20, 2007

PERFORMING LABORATORY

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

SPONSOR

Pure Bioscience
1725 Gillespie Way
El Cajon, CA 92020

PROJECT NUMBER

A04702

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: Pure Bioscience

Company Agent: DOLANA BLOUNT

DIRECTOR OF REGULATORY AFFAIRS
Title

Signature

Date: 3.30.07

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 studies 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: 3.30.07

Sponsor: _____ Date: 3.30.07

Study Director: _____ Date: 3-2007
Jill Ruhme, B.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. These studies have been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date	Study Director	Management
Critical Phase	February 23, 2007	February 23, 2007	March 20, 2007
Critical Phase	March 9, 2007	March 9, 2007	
Final Report	March 20, 2007	March 20, 2007	

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

Quality Assurance Auditor: Brenda Si Date: 3/20/07

TABLE OF CONTENTS

STUDY PERSONNEL

STUDY DIRECTOR: Jill Ruhme, B.S.

Professional personnel involved:

David Rottjakob, M.T.	- Director, Microbiology Services
Scott R. Steinagel, B.S.	- Microbiology Laboratory Supervisor
Adam W. Pitt, B.S.	- Research Assistant II
Matthew Sathe, B.S.	- Research Assistant I
Lisa Slusser, B.S.	- Research Assistant I
Katherine C. Sager, B.S.	- Research Assistant I
Jessica Rice, B.A.	- Research Assistant I

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: AOAC Use-Dilution Method

Project Number: A04702

Protocol Number: IMS01020807.UD.4

Sponsor: Pure Bioscience
1725 Gillespie Way
El Cajon, CA 92020

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

TEST SUBSTANCE IDENTITY

Test Substance Name: Axen 30

Lot/Batch(s): Lot # 2006.003.001 and Lot # 2007.008.002

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: February 13, 2007
Study Initiation Date: February 15, 2007
Experimental Start Date: February 23, 2007
Experimental End Date: March 12, 2007
Study Completion Date: March 20, 2007

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.

SUMMARY OF RESULTS

Test Substance:	Axen 30 (Lot # 2006.003.001 and Lot # 2007.008.002)
Dilution:	Ready to use (RTU)
Test Organism:	*Campylobacter jejuni* (ATCC 29428)
Exposure Time:	Two and five minutes
Exposure Temperature:	20±1°C
Organic Soil Load:	None required
Efficacy Result:	Axen 30 demonstrated efficacy of two lots against *Campylobacter jejuni*, and therefore, meets the requirements set forth by the U.S. EPA for disinfectant label claims following a two minute and five minute exposure period.

TEST HISTORY

Testing performed February 23, 2007, was invalid due to failing neutralization control results. The dilutions plated were too high and no growth was observed after inoculation of ≤100 colonies. The test was repeated March 9, 2007, and valid results were generated. Valid data is contained within the body of this report. Invalid data is reported in Attachment I.

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium
Campylobacter jejuni	29428	Tryptic Soy Agar with 5% Sheep Blood (BAP)

The microorganism used in this study was obtained from the American Type Culture Collection, Manassas, Virginia.

Recovery Media

Neutralizing Subculture Medium:
Fluid Thioglycollate Medium with 0.07% Lecitin + 0.5% Tween 80 (Primary)
Fluid Thioglycollate Medium (Secondary)

Agar Plate Medium:
Tryptic Soy Agar with 5% Sheep Blood (BAP)

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 at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a 20±1°C water bath and allowed to equilibrate for ≥10 minutes.

Preparation of Test Organism

From frozen stock, a stock culture plate of *Campylobacter jejuni* was created on BAP and incubated for 2-4 days at 35-37°C under microaerophilic conditions (CampyPak™ Plus). From the stock culture BAP plate, multiple BAP were inoculated and incubated for 2-4 days at 35-37°C under microaerophilic conditions (CampyPak™ Plus). Following incubation, a bacterial subculture suspension was prepared by swabbing bacterial growth and placing the swab in Fluid Thioglycollate Medium to yield approximately 1.0 x 10^8 CFU/mL as compared to a 0.5 McFarland Turbidity Standard. The test cultures were thoroughly mixed and allowed to stand for ≥10 minutes prior to use.

Contamination of Carriers

Sterile penicylinders were immersed for 15 minutes in the test organism suspension at a ratio of 1 carrier per 1 mL broth. The penicylinders were then dried on filter paper in a sterile petri dish at 25-30°C for 20 minutes at a 68% relative humidity.

Exposure Conditions

For each lot of test substance, 10 contaminated and dried carriers were individually transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the requested dilution and exposed for two minute and five minutes at 20±1°C.

Test System Recovery

Following exposure, each exposed carrier was then transferred by hook needle at identical staggered intervals to 10 mL of Fluid Thioglycollate Medium with 0.07% Lecitin + 0.5% Tween 80. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Fluid Thioglycollate Medium ≥30 minutes after subculture of the first carrier.

Incubation and Observation

The neutralized subculture tubes were incubated for 3 days at 35-37°C in 5.0% CO_2. The subculture plates were incubated for 3 days at 35-37°C under microaerophilic conditions (CampyPak™ Plus). Following incubation, the subcultures were visually examined for the presence or absence of visible growth.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" was 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
None used.

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 for two minutes and transferring them to primary subculture tubes containing 10 mL of neutralizing subculture medium for two minutes. Carriers were then transferred from primary subculture tubes into individual secondary subculture tubes ≥30 minutes following the primary transfer. The subculture tubes containing the exposed carriers were inoculated with ≤100 CFU of the 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 disinfectant must kill the microorganism on 10 out of the 10 inoculated carriers.

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

PROTOCOL CHANGES

Protocol Amendment:
This protocol is amended to correct an entry error on page 7 of the protocol. The expiration date is being changed from "Axen 30 2006.003.001" to "NA".

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

Carrier Population Control Calculation:

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

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. 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.

Test Substance Retention
The test substance will be returned 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).

RESULTS

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

For Test Results, see Table 4.

ANALYSIS

Axen 30 (Lot # 2006.003.001 and Lot # 2007.008.002), ready to use, demonstrated no growth of *Campylobacter jejuni* (ATCC 29428) in any of the 10 primary subculture tubes and no growth in any of the 10 secondary subculture tubes following two and five minute exposure periods.

STUDY CONCLUSION

Under the conditions of this investigation, Axen 30 (Lot # 2006.003.001 and Lot # 2007.008.002), ready to use, demonstrated efficacy against *Campylobacter jejuni* as required by the U.S. EPA for disinfectant label claims following two and five minute exposure periods.

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
		***Campylobacter jejuni* (ATCC 29428)**
Purity Control		Pure
Viability Control		Growth
Neutralizing Subculture Medium Sterility Control	Primary	No Growth
	Secondary Lot FTM012907-11	No Growth
	Secondary Lot FTM021607-11	No Growth
Carrier Sterility Control		No Growth

TABLE 2: CARRIER POPULATION CONTROL RESULTS

Test Organism	Date Performed	Result
Campylobacter jejuni (ATCC 29428)	3/9/07	6.5×10^6 CFU/carrier

CFU = Colony Forming Unit

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Date Performed	Average Inoculum (CFU/mL)	Number of Subculture Tubes	
				Tested	Positive
Axen 30, Lot # 2006.003.001	*Campylobacter jejuni* (ATCC 29428)	3/9/07	27	1	1
Axen 30, Lot # 2007.008.002				1	1

CFU = Colony Forming Unit

TABLE 4: TEST RESULTS

Test Substance	Test Organism	Date Performed	Sample Dilution *	Exposure Time	Number of Carriers	
					Exposed	Showing Growth**
Axen 30 Lot # 2006.003.001	*Campylobacter jejuni* (ATCC 29428)	3/9/07	RTU	2 minutes	1°=10 2°=10	1°=0 2°=0
				5 minutes	1°=10 2°=10	1°=0 2°=0
Axen 30 Lot # 2007.008.002				2 minutes	1°=10 2°=10	1°=0 2°=0
				5 minutes	1°=10 2°=10	1°=0 2°=0

* RTU = Ready to use.
** Number of carriers showing growth of the test organism.
1° Primary Subculture
2° Secondary Subculture

ATTACHMENT I: Invalid Data

NOTE: Due to invalid neutralization control results, this assay was repeated.

DATE PERFORMED: February 23, 2007
SAMPLE NAME OR CODE: Axen 30, Lot # 2006.003.001 and Lot # 2007.008.002
ORGANISM: *Campylobacter jejuni* (ATCC 29428)
CULTURE MEDIUM: Tryptic Soy Agar with 5% Sheep Blood (BAP)

NEUTRALIZING SUBCULTURE MEDIUM:
Fluid Thioglycollate Medium with 0.07% Lecitin + 0.5% Tween 80 (Primary)
Fluid Thioglycollate Medium (Secondary)

CARRIER POPULATION: 7.1×10^5 CFU/carrier

STUDY CONTROLS

Type of Control		Results
		***Campylobacter jejuni* (ATCC 29428)**
Purity Control		Pure
Viability Control		Growth
Neutralizing Subculture Medium Sterility Control	Primary	No Growth
	Secondary	No Growth
Carrier Sterility Control		No Growth

EVALUATION OF GROWTH IN CARRIER SUBCULTURES

Test Substance	Test Organism	Sample Dilution*	Exposure Time	Number of Carriers	
				Exposed	Showing Growth**
Axen 30 Lot # 2006.003.001	*Campylobacter jejuni*	RTU	2 minutes	1°=10 2°=10	1°=0 2°=0
			5 minutes	1°=10 2°=10	1°=0 2°=0
Axen 30 Lot # 2007.008.002			2 minutes	1°=10 2°=10	1°=0 2°=0
			5 minutes	1°=10 2°=10	1°=0 2°=0

* RTU = Ready to use.
** Number of carriers showing growth of the test organism.
1° Primary Subculture
2° Secondary Subculture

NEUTRALIZATION CONFIRMATION RESULTS

Test Substance	Test Organism	Neutralization Confirmation			
		Date Performed	Average Inoculum CFU/mL	Number Subculture Tubes	
				Tested	Tubes Positive
Axen 30 Lot # 2006.003.001	*Campylobacter jejuni*	2/26/07	<1	1	0
Axen 30 Lot # 2007.008.002				1	0

ATS LABS

ATS LABS

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	March 19, 2007
Sponsor:	Pure Bioscience 1725 Gillespie Way El Cajon, CA 92020
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	AOAC Use-Dilution Method
ATS Labs Protocol Number:	IMS01020807.UD.4
ATS Labs Project Number:	A04702

Modifications to Protocol:

This protocol is amended to correct an entry error on page 7 of the protocol. The expiration date is being changed from "Axen 30 2006.003 001" to "NA"

Changes to the protocol are acceptable as noted.

Study Director

3-19-07
Date

EXACT COPY
INITIALS JM DATE 3-20-07

(For Laboratory Use Only)
ATS Labs Project # A 0 4 7 0 2

ATS LABS

PROTOCOL

AOAC Use-Dilution Method

Test Organism:

Campylobacter jejuni
(ATCC 29428)

PROTOCOL NUMBER

IMS01020807.UD.4

PREPARED FOR

Pure Bioscience
1725 Gillespie Way
El Cajon, CA 92020

PERFORMING LABORATORY

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

PREPARED BY

David Rottjakob, M.T.
Director, Microbiology Services

DATE

February 8, 2007

EXACT COPY
INITIALS MR DATE 3-20-07

1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

AOAC Use-Dilution Method

SPONSOR: Pure Bioscience
1725 Gillespie Way
El Cajon, CA 92020

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.

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, 2007. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of March 19, 2007. 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 United States FDA or EPA 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 requires 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.

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, dried organism 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 Organism	ATCC #	Growth Medium	Incubation Parameters
Campylobacter jejuni	29428	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, microaerophilic (CampyPak™ Plus)

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

Carriers

Carriers will be screened according to AOAC Official Method of Analysis and any carrier 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 Organism

From frozen stock, a stock culture plate of *Campylobacter jejuni* will be created on BAP and incubated for 2-4 days at 35-37°C under microaerophilic conditions (CampyPak™ Plus). From the stock culture BAP plate, multiple BAP will be inoculated and incubated for 2-4 days at 35-37°C under microaerophilic conditions (CampyPak™ Plus). Following incubation, a bacterial culture suspension will be prepared by swabbing bacterial growth and placing the swab in Fluid Thioglycollate Medium to yield approximately 1.0 x 10^8 CFU/mL as compared to a 0.5 McFarland Turbidity Standard. The test culture 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 the test culture 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 25-30°C, at a relative humidity of ≥60%, for 20-40 minutes. 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 allowed 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.

Test System Recovery

Following the Sponsor specified exposure period each medicated carrier will be transferred by hook needle at staggered intervals to 10 mL of neutralizing broth. If necessary, carriers may 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 controls will be incubated at 35-37°C in CO_2 for 2-5 days. All plates will be incubated at 35-37°C for 2-5 days under microaerophilic conditions (CampyPak™ Plus).

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 showing growth will be subcultured, stained and/or biochemically assayed to confirm or rule out the presence 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. In order to enumerate the number of CFU actually added, a standardized spread plate procedure will be run concurrently. The enumeration will be accomplished by plating 0.1 mL of each dilution in duplicate. 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 enumeration will be accomplished by plating 0.1 mL of each dilution in duplicate. 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.

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 0.1 mL aliquots of the appropriate dilutions will be spread plated on agar 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 disinfectant must kill the microorganism on 10 out of the 10 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.

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)

DATA ANALYSIS

Calculations

Carrier Population Control Calculation:

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

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

Statistical Analysis

None used.

02/12/2007 14:45 6195968790 PURE BIOSCIENCE PAGE 08

STUDY INFORMATION

(All sections must be completed prior to submitting protocol)

Sponsor (Date/Initial): 02.12.07 [initials]

Test Substance (Name & Batch Numbers - exactly as it should appear on final report):
AXEN30 LOT# 2006-003.001 ; AXEN30 LOT# 2007.008-002

Expiration Date: AXEN30 2006-003-001

Product Description:

- ☐ Quaternary ammonia
- ☐ Iodophor
- ☐ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☑ Other Silver ion / citric acid

Test Substance Active Concentration (upon submission to ATS Labs): 30ppm silver ion / 4.64% citric acid

Neutralization/Subculture Broth:

- ☐ _____
- ☑ ATS Labs' Discretion. The Sponsor authorizes additional fees for special neutralization media (if necessary).

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)
- ☐ *Dilutions/Concentrations to be tested _____
  - ☐ Deionized Water (Filter Sterilized)
  - ☐ Tap Water (Filter Sterilized)
  - ☐ AOAC Synthetic Hard Water: _____PPM
  - ☐ Other _____

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

Test Organism: *Campylobacter jejuni* (ATCC 29428)

Carrier Number: 10 per batch

Exposure Time: 2 minutes & 5 minutes

Exposure Temperature: 20 ± 1 °C

Organic Soil Load:

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

02/12/2007 14:45 6195968790 PURE BIOSCIENCE PAGE 09

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: 02.12.2007 Sent via *overnight* delivery? ☑ Yes ☐ No
- ☐ Will be shipped to ATS Labs.
  Date of expected receipt at ATS Labs: _____
- ☐ 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: Dolana Blount TITLE: Assistant to President

SIGNATURE: [signature] DATE: 02.12.07

PHONE: 619-596-8800 x105 FAX: 619-596-8790 EMAIL: dblount@purebio.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

MICHAEL KRAU, PURE BIOSCIENCE

ATS Labs:

NAME: Jill Ruhme
Study Director

SIGNATURE: [signature]
Study Director DATE: 2-15-07