FINAL STUDY REPORT

STUDY TITLE

Time Kill Assay For Antimicrobial Agents

Test Organism:

Clostridium difficile – spore form (ATCC 43598)

PRODUCT IDENTITY

AX250
Batch # AX-13196-0210

AUTHOR

Gracia Schroeder, B.S.
Study Director

STUDY COMPLETION DATE

November 6, 2013

PERFORMING LABORATORY

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

SPONSOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PROJECT NUMBER

A15628

EXACT COPY
INITIALS KT DATE 11-6-13

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

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:_____

Sponsor:_____ Date:_____

Study Director: Gracia Schroeder Date: 11/12/13
Gracia Schroeder, B.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: Time Kill Assay For Antimicrobial Agents

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 (21 CFR Part 58) 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 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	October 1, 2013	October 1, 2013	October 1, 2013
Draft Report	October 16, 2013	October 16, 2013	October 17, 2013
Final Report	November 6, 2013	November 6, 2013	November 6, 2013

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

Quality Assurance Auditor: Judy Heidemann Date: 11-6-13

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STUDY PERSONNEL

STUDY DIRECTOR:	Gracia Schroeder, B.S.
Professional personnel involved:	
Scott R. Steinagel, B.S.	- Director, Microbiology Operations
Becky Lien, B.A.	- Manager, Microbiology Operations
Peter Toll, B.S.	- Supervisor, Microbiology Laboratory Operations
Jill Ruhme, B.S.	- Senior Microbiologist
Adam W. Pitt, B.S.	- Senior Microbiologist
Philip Lange, B.S.	- Associate Microbiologist
Kristen Niehaus, B.A.	- Microbiologist
Rebecca Astrup, B.S.	- Associate Microbiologist
Nicole Zroka, B.A.	- Associate Microbiologist

STUDY REPORT

GENERAL STUDY INFORMATION

Protocol Title: Time Kill Assay For Antimicrobial Agents

Project Number: A15628

Protocol Number: INI01091613.TK.2

Sponsor: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

TEST SUBSTANCE IDENTITY

Test Substance Name: AX250

Batch Number: Batch # AX-13196-0210

Test Substance Characterization
Test substance characterization as to content, stability, etc., (21 CFR Part 58, Subpart F [58.105]) is the responsibility of the Sponsor. The Sponsor Test Material Certificate of Analysis Report may be found in Attachment I.

STUDY DATES

Date Sample Received: September 11, 2013
Study Initiation Date: September 24, 2013
Experimental Start Date: October 1, 2013
Experimental End Date: October 4, 2013
Study Completion Date: November 6, 2013

OBJECTIVE

The objective of this testing was to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

SUMMARY OF RESULTS

Test Substance: AX250 (Batch # AX-13196-0210)

Dilution: Ready to use (RTU)

Test Organism: *Clostridium difficile* – spore form (ATCC 43598)

Exposure Times: 15 seconds, 30 seconds, 60 seconds, and 90 seconds

Exposure Temperature: Room Temperature (21°C)

Efficacy Result: AX250 (Batch # AX-13196-0210) demonstrated a 99.5% (2.33 $\log_{10}$) reduction of *Clostridium difficile* – spore form (ATCC 43598) survivors following a 15 second exposure, a >99.999% (>5.35 $\log_{10}$) reduction of *Clostridium difficile* – spore form (ATCC 43598) survivors following a 30 second exposure, a >99.999% (>5.35 $\log_{10}$) reduction of *Clostridium difficile* – spore form (ATCC 43598) survivors following a 60 second exposure and a >99.999% (>5.35 $\log_{10}$) reduction of *Clostridium difficile* – spore form (ATCC 43598) survivors following a 90 second exposure when tested at room temperature (21°C).

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Culture Medium	Incubation Parameters
Clostridium difficile – spore form	43598	CDC Anaerobic Blood Agar	35-37°C, anaerobic

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

Recovery Media

Neutralizer: Letheen Broth + 0.1% Sodium Thiosulfate
Agar Plate Medium: BHI-HT Agar

TEST METHOD

Preparation of Test Organism

From a stock plate prepared on CDC anaerobic Blood agar, two 10 mL tubes of pre-reduced Reinforced Clostridial Medium (RCM) were inoculated using an isolated colony. Each tube was mixed and incubated anaerobically at 35-37°C for 24±2 hours. Following incubation, 18 CDC Anaerobic Blood Agar plates were inoculated with 100 µl of the broth culture. The inoculum was spread evenly with a sterile plate spreader. The plates were inverted and incubated anaerobically for 7 days at 36±1°C under anaerobic conditions

using anaerobe jars to prevent desiccation. The growth was harvested from each plate by adding 5.0 mL of Phosphate Buffered Saline (PBS) + 0.1% Tween 80 to each plate and gently scraping with a cell scraper avoiding the collection of agar fragments where possible. The suspension was pooled into sterile 50 mL conical tubes.

The culture was centrifuge concentrated at approximately 3500 RPM (approximately 1650 x g) for approximately 38 minutes. (This provided near equivalent centrifugation of approximately 4500 x g for 15 minutes.) The supernatant was discarded, the pellet was disaggregated as necessary and the pellet was resuspended in approximately 20 mL of PBS + 0.1% Tween 80. This step was the first wash. The washing step was repeated two more times for a total of three washes. After the third wash, the supernatant was discarded and the total culture was resuspended in approximately 4 mL of Phosphate Buffered Saline + 0.1% Tween 80. The culture was combined into one tube after the pellets had been disaggregated as applicable and was vortex mixed.

The spore suspension was heated in a water-bath for 10±1 minutes at 65±2°C (65.0°C). To ensure the spore suspension had reached 65±2°C prior to starting the timer, the temperature of an identical side-by-side tube containing the same volume of deionized water was monitored. Following heat treatment, the suspension was allowed to cool to room temperature.

A 50% (w/v) solution of HistoDenz was prepared in deionized water and was filter sterilized. A 5.0 mL aliquot of 50% HistoDenz was pipetted into a sufficient number of 15 mL conical tubes. One (1.00) mL of spore suspension was layered on top of the HistoDenz in each tube. The tubes were centrifuged at approximately 3500 RPM (approximately 1650 x g) in a swinging bucket rotor for approximately 27 minutes. (This provided near equivalent centrifugation of approximately 4500 x g for 10 minutes.) Four layers were formed in the HistoDenz solution, with spores aggregated in the bottom layer. The top three layers (an upper clear layer, a dense second layer, and a clear third layer) were carefully removed, leaving the pellet and approximately 3-4 mm (visually estimated) of an undisturbed cloudy layer above the pellet. The pellet was resuspended by vortex mixing and approximately 1.00 mL aliquots were transferred to individual microcentrifuge tubes. The culture was centrifuged at approximately 16000 x g for approximately 5 minutes. The supernatant was discarded and the pellet was resuspended in 1.00 mL PBS + 0.1% Tween 80. The culture was vortex mixed to disaggregate the pellet. Each microcentrifuge tube was centrifuged at approximately 16000 x g for approximately 2 minutes. The supernatant was discarded and the pellet was resuspended in approximately 1.00 mL of PBS + 0.1% Tween 80. The culture was vortex mixed to disaggregate the pellet. This two minute wash was the first wash step. The wash step was repeated two additional times for a total of three washes. The supernatant was discarded and the pellet in each microcentrifuge tube was resuspended in 0.50 mL of PBS + 0.1% Tween 80. The purity of the spore suspension was examined to ensure the spore concentration ≥95% using a Malachite green stain. The spore purity was determined to be 98%. The spore titer was determined by standard serial dilution and plating onto BHI-HT recovery agar. The plates were incubated for 48±4 hours at 35-37°C under anaerobic conditions. The plates were refrigerated for 2 days prior to evaluation. The suspension was stored at approximately -20°C for up to 3 months prior to use. The culture was adjusted, to target ≥5 x 10^8 spores/mL in PBS + 0.1% Tween 80 by combining 2.00 mL of spore suspension with 18.0 mL of PBS + 0.1% Tween 80.

Preparation of Test Substance
The test substance was ready to use (RTU), as received from the Sponsor. A 1.90 mL aliquot of the test substance was transferred to a sterile vessel for use in testing. The test substance was homogenous as determined by visual observation.

One replicate sample was set up and evaluated.

Exposure Conditions
A 0.100 aliquot of the standardized inoculum was added to 1.90 mL test substance representing the start of the test exposure. The inoculated test substance was immediately mixed thoroughly using a vortex mixer. The inoculated and mixed test substance was exposed for the exposure times of 15 seconds, 30 seconds, 60 seconds, and 90 seconds at room temperature (21°C).

Test System Recovery
At each Sponsor specified exposure time, each sample was mixed and a 0.100 mL aliquot of the inoculated test substance was transferred to 9.9 mL of neutralizer representing a 10^0 dilution. Additional ten-fold serial dilutions were prepared from the 10^0 neutralized material in Butterfield's Buffer.

Using standard microbiological spread plate procedures, 1.00 mL aliquots of the 10^0 dilution and 0.100 mL aliquots of the 10^0-10^{-3} dilutions were plated in duplicate on appropriate recovery medium for the test organism.

Incubation and Observation
All subculture plates were incubated for 3 days at 35-37°C under anaerobic conditions. Following incubation, the agar plates were visually examined for the presence of growth and enumerated. Log_{10} and percent reductions were determined for each exposure time.

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.

Neutralizer Sterility Control
A 1.00 mL aliquot of the neutralizer was plated as in the test and incubated. The acceptance criterion for this study control is a lack of growth.

Test Population Control
In a similar manner as the culture inoculum was added to the test substance, an equivalent volume of inoculum (0.100 mL) was added to 1.90 mL Butterfield's buffer). This suspension was neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. The suspension was serially diluted and appropriate dilutions were plated using standard microbiological techniques and 0.100 mL aliquots. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of the test substance was neutralized as in the test procedure. A 1.00 mL aliquot of the neutralized sample was then removed and discarded. To the neutralized sample, 1.00 mL of the organism suspension containing approximately 1000-10,000 CFU/mL was added and the suspension was vortex mixed. A 0.100 mL aliquot of the neutralized mixture was plated in duplicate on appropriate recovery agar and incubated. A numbers control was performed by adding 1.00 mL of the same organism suspension to 9.0 mL of untreated neutralizer. A 0.100 mL aliquot was plated in duplicate and incubated.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log. The most appropriate dilution was reported.

HCl Resistance
Prior to testing, a 990 µL aliquot of 2.5 N HCl was placed into each of three sterile microcentrifuge tubes. As a control, 990 µL of sterile deionized water was placed into one 2.0 mL microcentrifuge tube. Using a positive displacement pipettor, 10 µL of the spore suspension used in testing was transferred to each microcentrifuge tube and vortex mixed. One test tube was held for 5 minutes, one test tube was held for 10 minutes, the third test tube was held for 20 minutes and the control tube was held for 20 minutes at room temperature. Following the holding period, 0.100 mL of suspension was transferred to 900 µL of Phosphate Buffer Dilution Water to neutralize the suspension (10^0). Each neutralized tube was serially diluted to 10^{-4} and duplicate 0.100 mL aliquots of 10^{-2} through 10^{-4} dilutions were spread plated for each test and control tube onto BHI-HT agar. The plates were incubated as in the test. The CFU/mL for each tube and the $\log_{10}$ for each test tube was determined and compared to the control tube. The acceptance criterion for this study control is ≤ 2 $\log_{10}$ reduction following 10 minutes of exposure as compared to the control. (See Protocol Deviation).

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results are expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

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

PROTOCOL CHANGES

Protocol Amendment:
Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58)

Protocol Deviation:
An HCl control was performed prior to testing (during the test organism preparation) and was included in the test organism preparation worksheet. The protocol does not indicate to perform the HCl control, thus a deviation has occurred. There is no impact on the study as this control adds assurance of the resistance of the spore form of the test organism to HCl, indicating the endospores used in testing were in a resistant state.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{\text{(average CFU) x (dilution factor) x (volume neutralized solution in mL)}}{\text{(volume plated in mL)}}$$

A value of <1 was used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros were added together to increase the sensitivity of the test. (A value of 2 mL plated was used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

The geometric mean value for the population control was determined and used to calculate percent reduction as multiple time points were evaluated in the control.

The geometric mean value of the test results were determined and used to calculate percent reduction as more than one replicate is performed.

$$\text{Geometric mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

The average $\log_{10}$ value for the population control was determined and used to calculate $\log_{10}$ reduction as multiple time points are evaluated in the control.

The average $\log_{10}$ value of the test results was determined and used to calculate $\log_{10}$ reduction as more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

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 discarded following study completion. It is the responsibility of the Sponsor to retain a sample of the test substance.

REFERENCES

1. .American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

3. American Society for Testing and Materials (ASTM). Standard Test Method for Production of *Clostridium difficile* Spores for Use in Efficacy Evaluation of Antimicrobial Agents, E 2839-11.

RESULTS

For Control and Neutralization Results, see Tables 1-4.
All data measurements/controls including culture purity, neutralizer sterility, test population control, neutralization confirmation, and HCl controls performed within acceptance criteria.

For Test Results, see Tables 5-6.

ANALYSIS AND STUDY CONCLUSION

AX250 (Batch # AX-13196-0210) demonstrated a 99.5% (2.33 log_{10}) reduction of *Clostridium difficile* – spore form (ATCC 43598) survivors following a 15 second exposure, a >99.999% (>5.35 log_{10}) reduction of *Clostridium difficile* – spore form (ATCC 43598) survivors following a 30 second exposure, a >99.999% (>5.35 log_{10}) reduction of *Clostridium difficile* – spore form (ATCC 43598) survivors following a 60 second exposure and a >99.999% (>5.35 log_{10}) reduction of *Clostridium difficile* – spore form (ATCC 43598) survivors following a 90 second exposure when tested at room temperature (21°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
Purity Control	*Clostridium difficile* – spore form (ATCC 43598)	Pure
Neutralizer Sterility Control		No Growth

TABLE 2: TEST POPULATION CONTROL RESULTS

Test Organism	Results	
	CFU/mL	Log_{10}
Clostridium difficile – spore form (ATCC 43598)	1.13×10^6	6.05

CFU = Colony Forming Units

Note: *The highest challenge level was achieved for this control based on the use of standard propagation methods.*

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Neutralization Confirmation (CFU)		Pass/Fail ± 1 log_{10} (Log_{10} Difference)
		Numbers Control	Test Substance Results	
AX250 Batch # AX-13196-0210	*Clostridium difficile* – spore form (ATCC 43598)	40, 33	31, 25	Pass (0.12)

CFU = Colony Forming Units

TABLE 4: HCl RESISTANCE CONTROL RESULTS*

Test Organism: *Clostridium difficile* – spore form (ATCC 43598)						
Exposure Time	**10^{-2}**	**10^{-3}**	**10^{-4}**	**CFU/mL (Log_{10})**	**Log_{10} Reduction from Control**	**Pass/Fail (≤ 2 log_{10} difference)**
5 minutes (test)	T, T	62, 82	11, 6	7.2×10^5 (5.86)	0.53	Not Applicable
10 minutes (test)	T, T	65, 64	4, 2	6.5×10^5 (5.81)	0.58	Pass
20 minutes (test)	189, 162	37, 20	2, 0	2.9×10^5 (5.46)	0.93	Not Applicable
20 minutes (control)	T, T	244, 242	25, 31	2.43×10^6 (6.39)	Not Applicable	Not Applicable

CFU = Colony Forming Units
T = Too Numerous To Count (>300 colonies)
*See Protocol Deviation.

Note: *The acceptance criterion for this study control is ≤ 2 log_{10} reduction following the 10 minutes of exposure as compared to the control. Performance of the 5 minute and 20 minute tests are for additional information only and do not have an acceptance criterion.*

TABLE 5: TEST RESULTS FOR AX250 Batch # AX-13196-0210

DILUTION (VOLUME PLATED)	Test Organism: *Clostridium difficile* – spore form (ATCC 43598)			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)	T, T	0, 0*	0, 0*	0, 0*
10^0 (0.100 mL)	55, 49*	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0

T= Too Numerous to Count
* Indicates dilution used for calculation purposes.

TABLE 6: CALCULATED DATA FOR AX250 Batch # AX-13196-0210

Test Organism	Exposure Time	CFU/mL in Test Population Control (Log_{10})	CFU/mL of Survivors	Log_{10} Survivors	Percent Reduction	Log_{10} Reduction
Clostridium difficile – spore form (ATCC 43598)	15 seconds	1.13×10^6 (6.05)	5.2×10^3	3.72	99.5%	2.33
	30 seconds		<5	<0.70	>99.999%	>5.35
	60 seconds		<5	<0.70	>99.999%	>5.35
	90 seconds		<5	<0.70	>99.999%	>5.35

CFU = Colony Forming Units

Note: *For samples with a "<" value sign, a value of <1 was used in place of zero for calculation purposes. For these samples with a "<" value sign, no growth was observed on the duplicate test plates at the lowest dilution plated. The zeros were added together to increase the sensitivity of the test and a value of 2 mL plated was used in the calculation. The limit of detection of this test is a value of <5 CFU/mL.*

Attachment I: Sponsor Test Material Certificate of Analysis - Batch AX-13196-0210

Issued: July 16, 2013
Last Revised: September 10, 2013

FORM-COA-02

AQUAOX INDUSTRIES INC
16155, Sierra Lakes Parkway,
Suite 160-714,
Fontana. CA 92336. USA.

Certificate of Analysis

Date of Manufacture:	07 / 15 / 2013
Product Name:	AX250
Batch / Lot #:	AX-13196-0210
Production Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Testing Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377

TEST	ANALYSIS	UNITS
FAC	207	ppm
pH	5.91	n/a
Conductivity	1230	µS/cm
ORP	966	mV
Osmolality	22	mOsm/kg

This certification states that the intermediate product AX250, bearing the above description and lot number, has been found to conform to the internal specifications established for this product. The above lot was made in accordance with our internal specifications and current good manufacturing practices under controlled procedures.

This lot has been appropriately inspected and tested, and, to the best of our knowledge, conforms to all applicable test methods, standards and internal specifications.

This certification does not constitute any written or expressed warranty or guarantee of any kind.

Rebecca Lei
QA Regulatory Specialist

Date: 9/10/13

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AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	10/10/13
Sponsor:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Time Kill Assay For Antimicrobial Agents
ATS Labs Protocol Number:	INI01091613.TK.2
ATS Labs Project Number:	A15628

Modifications to Protocol:

Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

Changes to the protocol are acceptable as noted.

Study Director

10/10/13
Date

EXACT COPY
INITIALS SC DATE 11/6/13

(For Laboratory Use Only)
ATS Labs Project # A15628
9-25-13

ATS LABS

PROTOCOL

Time Kill Assay For Antimicrobial Agents

Test Organism:

Clostridium difficile - spore form (ATCC 43598)

PROTOCOL NUMBER

INI01091613.TK.2

PREPARED FOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PERFORMING LABORATORY

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

PREPARED BY

Anne Stemper, B.S.
Senior Microbiologist

DATE

September 16, 2013

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INITIALS CJM DATE 11/6/13

Time Kill Assay For Antimicrobial Agents

SPONSOR: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

PURPOSE
The objective of this testing is to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

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. 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 September 24, 2013. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of October 21, 2013. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

A "case-by-case" approach is generally taken by the regulatory authorities and cannot be over-emphasized when considering a testing regimen. While this protocol is based upon our experience in the field of germicidal testing, and the current regulatory guidelines, each product presents a different set of issues to the regulatory authorities. We recommend that you consult with the appropriate agency before finalizing your testing regimen, as ATS Labs cannot guarantee acceptance of this protocol by the regulating authorities.

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 nor 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 regulating 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
Analyzing the efficacy of antimicrobial agents may be performed by various suspension and susceptibility methods. This study is designed to examine the rate-of-kill of a test substance against a pure test culture. This is accomplished by exposing the test culture to the test substance and assaying for survivors following a variety of exposure times.

TEST PRINCIPLE

A suspension of the test organism is exposed to the test substance for specified exposure times. After exposure, an aliquot of the suspension is transferred to a neutralizer and assayed for survivors. Appropriate culture purity, sterility, population and neutralization confirmation controls are performed. The current version of Standard Operating Procedure CGT-4130 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Culture Medium	Incubation Parameters
Clostridium difficile – spore form	43598	CDC Anaerobic Blood Agar (or equivalent)	35-37°C, anaerobic

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

The Neutralizer will be appropriate for the test substance.
Agar medium used will be appropriate for *C. difficile* spore recovery (e.g. BHI-HT agar)

Preparation of Test Organism

From a stock plate prepared on CDC anaerobic Blood agar, inoculate a sufficient number of 10 mL tubes of pre-reduced Reinforced Clostridial Medium (RCM) using an isolated colony, mix, and incubate anaerobically at 35-37°C for 24±2 hours. Following incubation, inoculate each of a minimum of 10 CDC Anaerobic Blood Agar plates with 100 µl of the broth culture. Spread the inoculum evenly with a sterile plate spreader (or equivalent). Invert plates and incubate anaerobically for 7-10 days at 36±1°C under anaerobic conditions. This should provide sporulation at ≥90%. Anaerobic jars are recommended for use to prevent desiccation. Harvest growth from each plate by adding 5 mL of Phosphate Buffered Saline (PBS) + 0.1% Tween 80 to each plate and gently scraping with a cell scraper or other appropriate device avoiding the collection of agar fragments where possible. Pool the suspension into sterile 50 mL conical tubes.

Centrifuge the culture at approximately 3500 RPM (approximately 1650 x g) for approximately 38 minutes. (This provides near equivalent centrifugation of approximately 4500 x g for 15 minutes.) Discard the supernatant, disaggregate the pellet as necessary and resuspend the pellet in approximately 20 mL of PBS + 0.1% Tween 80. This step is the first wash. Repeat the washing step two more times for a total of three washes. After the third wash, discard the supernatant and resuspend the total culture in approximately 4 mL of Phosphate Buffered Saline + 0.1% Tween 80. The culture may be combined into one tube after the pellets have been disaggregated. Vortex mix the culture. Heat the spore suspension in a water-bath for 10±1 minutes at 65±2°C. To ensure the spore suspension has reached 65±2°C prior to starting the timer, monitor the temperature of an identical side-by-side tube containing the same volume of deionized water. Following heat treatment, allow the suspension to cool to room temperature.

Prepare a 50% (w/v) solution of HistoDenz in deionized water and filter sterilize. Pipet 5 mL of 50% HistoDenz into a sufficient number of (i.e. 4) 15 mL conical tubes. Layer 1 mL of spore suspension on top of the HistoDenz in each tube. Centrifuge the tubes at approximately 3500 RPM (approximately 1650 x g) in a swinging bucket rotor for approximately 27 minutes. (This provides near equivalent centrifugation of approximately 4500 x g for 10 minutes.) Four layers will be formed in the HistoDenz solution, with spores aggregated in the bottom layer. Carefully remove the top three layers (an upper clear layer, a dense second layer, and a clear third layer), leaving the pellet and approximately 3-4 mm of the cloudy layer (visually estimated) above the pellet undisturbed. Resuspend the pellet by vortex mixing and transfer approximately 1 mL aliquots to individual microcentrifuge tubes. Centrifuge the culture at approximately 16000 x g for approximately 5 minutes. Discard the supernatant and resuspend the pellet in approximately 1 mL PBS + 0.1% Tween 80. Vortex mix to disaggregate the pellet. Centrifuge each microcentrifuge tubes at approximately 16000 x g for approximately 2 minutes. Discard the supernatant and resuspend the pellet in approximately 1 mL of PBS + 0.1% Tween 80. Vortex mix to disaggregate the pellet. This two minute wash is the first wash step. Repeat the wash step two additional times for a total of three washes. Discard the supernatant and resuspend the pellet in each microcentrifuge tube in approximately 0.5 mL of PBS + 0.1% Tween 80. Examine the spore purity to ensure the spore concentration is ≥95% by phase contrast microscopy or using a Malachite green spore stain. Determine the spore titer by standard serial dilution and plating onto appropriate recovery agar (e.g. BHI-HT). Incubate the plates for 48±4 hours at 35-37°C under anaerobic conditions. Plates may be refrigerated for up to 3 days prior to evaluation. Adjust the culture, as necessary, to target ≥5 x 10^8 spores/mL. Applicable culture dilutions will be prepared in PBS + 0.1% Tween 80. The culture may be frozen at approximately -20°C for up to 3 months prior to use in testing.

A standard organic soil load may be added to the test organism suspension per Sponsor's request. Alternate soils may be used at the request of the Sponsor.

Preparation of Test Substance
The test substance to be tested is prepared according to the directions supplied 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. A 1.9 mL aliquot of the prepared test substance will be transferred to a sterile vessel (15 mL conical tube, sterile snap-cap tube etc.) for testing procedures. If necessary, 1.9 g of test substance may be used. Multiple replicate vessels may be set up if requested.

Exposure Conditions
A 100 µL aliquot of the standardized inoculum will be added to the test substance representing the start of the test exposure. The inoculated test substance will be immediately mixed thoroughly using a vortex mixer, stirring with a pipette or by any other applicable method. The inoculated and mixed test substance will be held at the Sponsor specified temperature. If the requested exposure temperature lies outside of achievable ambient conditions, the test substance may be placed in a water bath (or other appropriate device) to equilibrate to the desired exposure temperature prior to testing. For very short exposure times or exposure times which are close together, individual test substance vessels may be utilized where necessary.

Test System Recovery
At each Sponsor specified exposure time, the sample will be mixed and a 0.1 mL aliquot of the inoculated test substance will be transferred to 9.9 mL of neutralizer broth (10^0 dilution). Additional ten-fold serial dilutions will be prepared in Butterfield's buffer. Using a standard microbiological spread plate count procedure, 1.0 mL aliquots of the 10^0 dilution and 0.1 mL aliquots of the (10^0-10^{-3}) will be plated in duplicate to the appropriate recovery media.

Incubation and Observation
All subculture plates are incubated for 3-5 days at 35-37°C under anaerobic conditions.

Following incubation, the subcultures will be visually examined for growth and enumerated. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination. Log_{10} and percent reductions will be determined for each time point. Representative subcultures demonstrating growth may 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
If applicable, 1.0 mL of the serum used for soil load will be added to a tube of Fluid Thioglycollate, incubated, and observed for lack of growth. The acceptance criterion for this study control is lack of growth.

Neutralizer Sterility Control
A 1.0 mL aliquot of the neutralizer will be plated as in the test and incubated. The acceptance criterion for this study control is lack of growth.

Test Population Control
In a similar manner as the culture inoculum is added to the test substance, add an equivalent volume of inoculum to Butterfield's buffer (same volume as the test substance). This suspension will be neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. If requested, the sample may be exposed as in the test and evaluated at an additional time point. (If requested, the final time point is recommended.) The suspension will be serially diluted and appropriate dilutions plated using standard microbiological techniques. *If swarming is a concern, 0.1 mL aliquots will be plated.*

Following incubation, the organism plates will be observed and enumerated. If more than one time point is evaluated, the geometric mean will be determined prior to reduction calculations. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of test substance will be neutralized as in the test procedure. Only the most concentrated test substance needs to be evaluated in this control. Remove and discard 1.0 mL of the neutralized sample. To the neutralized sample, add 1.0 mL of an organism suspension to target approximately 1000-10,000 CFU per mL of neutralizer and vortex mix. Plate, in duplicate, 0.1 mL of neutralized mixture to appropriate recovery agar and incubate. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 0.1 mL aliquots, in duplicate, and incubate. This control may be performed prior to or concurrent with testing.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log.

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 test organism 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 subcultures, etc. during the course of the test. Test subcultures 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
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results will be expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol.

REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism 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.

TEST SUBSTANCE RETENTION

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. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

3. American Society for Testing and Materials (ASTM). Standard Test Method for Production of *Clostridium difficile* Spores for Use in Efficacy Evaluation of Antimicrobial Agents, E 2839-11.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{(\text{average CFU}) \times (\text{dilution factor}) \times (\text{volume neutralized solution in mL})}{(\text{volume plated in mL})}$$

A value of <1 may be used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros may be added together to increase the sensitivity of the test. (A value of 2 mL plated is used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

If applicable, the geometric mean value for the population control will be determined and used to calculate percent reduction if multiple time points are evaluated in the control. The geometric mean value of the test results will be determined and used to calculate percent reduction if more than one replicate is performed.

$$\text{Geometric mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

If applicable, the average $\log_{10}$ value for the population control will be determined and used to calculate $\log_{10}$ reduction if multiple time points are evaluated in the control. The average $\log_{10}$ value of the test results will be determined and used to $\log_{10}$ reduction if more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

Statistical Analysis
None used.

Study Information

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number - exactly as it should appear on final report):
AX250 Batch # AX-13196-0210

Expiration Date: 07/2015

Test Substance Active Concentration (upon submission to ATS Labs): 0.024% HOCl

Product Description:

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

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: _____ defined as _____ + _____
  (example: 1 oz/gallon) (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.

Exposure Times: 15 seconds, 30 seconds, 60 seconds, and 90 seconds

Number of Test Replicate(s) per sample: 1

Exposure Temperature:

- ☑ Ambient
- ☐ Other _____

Organic Soil Load:

- ☐ Yeast Extract, bovine serum albumin, and bovine mucin soil load (as described in the protocol)
- ☐ Minimum 5% Organic Soil Load (fetal bovine serum)
- ☑ No Organic Soil Load Required
- ☐ Other _____

Test Organism: ☑ *Clostridium difficile* - spore form (ATCC 43598)

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: 7/11/13 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

_____

_____

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Dr. Fred Ma TITLE: M.D., Ph.D. Chief Medical Officer

SIGNATURE: Dr. Fred Ma DATE: 09/17/13

PHONE: (909) 822 - 6000 FAX: _____ EMAIL: fma@Innovacyn.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

Hannah Carroll (hannahc@innovacyn.com)

ATS Labs:

NAME: Gracia Schroeder
Study Director

SIGNATURE: _____
Study Director

DATE: 9/24/13

ATS LABS

FINAL STUDY REPORT

STUDY TITLE

Time Kill Assay For Antimicrobial Agents

Test Organism:

Mycobacterium bovis - BCG (Organon Teknika)

PRODUCT IDENTITY

AX250
Batch # AX-13196-0210

AUTHOR

Jill Ruhme, B.S.
Study Director

STUDY COMPLETION DATE

November 6, 2013

PERFORMING LABORATORY

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

SPONSOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PROJECT NUMBER

A15655

EXACT COPY
INITIALS KT DATE 11-20-13

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

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:_____

Sponsor:_____ Date:_____

Study Director:_____ Date: 11-6-13
Jill Ruhmle, B.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: Time Kill Assay For Antimicrobial Agents

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 (21 CFR Part 58) 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 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	October 2, 2013	October 2, 2013	October 3, 2013
Final Report	November 4, 2013	November 4, 2013	November 6, 2013

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

Quality Assurance Auditor: _____ Date: 11/6/13

TABLE OF CONTENTS

STUDY PERSONNEL

STUDY DIRECTOR:	Jill Ruhme, B.S.

Professional personnel involved:

Scott R. Steinagel, B.S.	- Director, Microbiology Operations
Becky Lien, B.A.	- Manager, Microbiology Operations
Peter Toll, B.S.	- Supervisor, Microbiology Laboratory Operations
Gracia Schroeder, B.S.	- Microbiologist
Kristen Niehaus, B.A.	- Microbiologist
Elizabeth Schwandt, B.S.	- Associate Microbiologist
Nicole Zroka, B.A.	- Associate Microbiologist

STUDY REPORT

GENERAL STUDY INFORMATION

Protocol Title: Time Kill Assay For Antimicrobial Agents

Project Number: A15655

Protocol Number: INI01091613.TK.5

Sponsor: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

TEST SUBSTANCE IDENTITY

Test Substance Name: AX250

Batch Number: Batch # AX-13196-0210

Test Substance Characterization
Test substance characterization as to content, stability, etc., (21 CFR, Part 58, Subpart F [58.105]) is the responsibility of the Sponsor. The Sponsor Test Material Certificate of Analysis Report may be found in Attachment I.

STUDY DATES

Date Sample Received: September 11, 2013
Study Initiation Date: September 26, 2013
Experimental Start Date: October 2, 2013
Experimental End Date: October 21, 2013
Study Completion Date: November 6, 2013

OBJECTIVE

The objective of this testing was to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

SUMMARY OF RESULTS

Test Substance:	AX250 (Batch # AX-13196-0210)
Dilution:	Ready to use (RTU)
Test Organism:	*Mycobacterium bovis* - BCG (Organon Teknika)
Exposure Times:	15 seconds, 30 seconds, 60 seconds, and 90 seconds
Exposure Temperature:	Ambient temperature (20.61°C)
Organic Soil Load:	No organic soil load required
Efficacy Result:	AX250 (Batch # AX-13196-0210) demonstrated a 99.8% (2.65 $\log_{10}$) reduction of *Mycobacterium bovis* - BCG (Organon Teknika) survivors following a 15 second exposure, >99.99% (4.43 $\log_{10}$) reduction of *Mycobacterium bovis* - BCG (Organon Teknika) survivors following a 30 second exposure, >99.999% (>5.21 $\log_{10}$) reduction of *Mycobacterium bovis* - BCG (Organon Teknika) survivors following a 60 second exposure and >99.999% (>5.21 $\log_{10}$) reduction of *Mycobacterium bovis* - BCG (Organon Teknika) survivors following a 90 second exposure when tested at ambient temperature (20.61°C).

STUDY MATERIALS

Test System/Growth Media

Test Organism	Growth Medium	Incubation Parameters
Mycobacterium bovis - BCG (Organon Teknika)	Modified Proskauer-Beck Broth	35-37°C, aerobic

The test organism used in this study was obtained from the Organon Teknika, Durham, NC.

Recovery Media

Neutralizer:	Horse Serum + 0.1% Sodium Thiosulfate
Agar Plate Medium:	Middlebrook 7H11 agar

TEST METHOD

Preparation of Test Organism

Mycobacterium bovis - BCG (Organon Teknika) was prepared by inoculating Modified Proskauer-Beck broth (MPB) from a stock culture and incubating at 35-37°C for 21 days. Following incubation, the suspension was homogenized using a sterile tissue grinder to target approximately 1 x 10^8 CFU/mL or greater. A biosafety cabinet was utilized when working with culture.

Preparation of Test Substance

The test substance was ready to use (RTU), as received from the Sponsor. A 9.5 mL aliquot of the test substance was transferred to a sterile vessel for use in testing. The test substance was homogenous as determined by visual observation.

One replicate sample was set up and evaluated.

Exposure Conditions

A 0.50 mL aliquot of the standardized inoculum was added to 9.5 mL test substance representing the start of the test exposure. The inoculated test substance was immediately mixed thoroughly using a vortex mixer. The inoculated and mixed test substance was exposed for the exposure times of 15 seconds, 30 seconds, 60 seconds, and 90 seconds at ambient temperature (20.61°C).

Test System Recovery

At each Sponsor specified exposure time, the sample was mixed and a 0.100 mL aliquot of the inoculated test substance was transferred to 9.9 mL of neutralizer representing a 10^0 dilution. Additional ten-fold serial dilutions were prepared from the 10^0 neutralized material in Butterfield's Buffer.

Using standard microbiological spread plate procedures, 1.00 mL aliquots of the 10^0-10^{-4} dilutions were plated in duplicate onto an appropriate recovery medium.

Incubation and Observation

The subculture plates were incubated at 35-37°C for 19 days in a manner to prevent desiccation. Following incubation, the agar plates were visually examined for the presence of growth and enumerated. Log_{10} and percent reductions were determined for each exposure time.

Representative subcultures demonstrating growth were 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" 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.

Neutralizer Sterility Control

A 1.00 mL aliquot of the neutralizer was plated as in the test and incubated. The acceptance criterion for this study control is a lack of growth.

Test Population Control

In a similar manner as the culture inoculum was added to the test substance, an equivalent volume of inoculum (0.50 mL) was added to 9.5 mL Butterfield's buffer. This suspension was neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. The suspension was serially diluted and appropriate dilutions were plated using standard microbiological techniques and 1.00 mL aliquots. Following incubation, the organism plates were observed and enumerated. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control

An aliquot of the test substance was neutralized as in the test procedure. A 1.00 mL aliquot of the neutralized sample was then removed and discarded. To the neutralized sample, 1.00 mL of the organism suspension containing approximately 100 CFU/mL was added and the suspension was vortex mixed. A 1.00 mL aliquot of the neutralized mixture was plated in duplicate on appropriate recovery agar and incubated. A numbers control was performed by adding 1.00 mL of the same organism suspension to 9.0 mL of untreated neutralizer. A 1.00 mL aliquot was plated in duplicate and incubated.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log. The most appropriate dilution was reported.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results are expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

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

PROTOCOL CHANGES

Protocol Amendment:
Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{(\text{average CFU}) \times (\text{dilution factor}) \times (\text{volume neutralized solution in mL})}{(\text{volume plated in mL})}$$

A value of <1 was used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros were added together to increase the sensitivity of the test. (A value of 2 mL plated was used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

The geometric mean value for the population control was determined and used to calculate percent reduction as multiple time points were evaluated in the control.

The geometric mean value of the test results were determined and used to calculate percent reduction as more than one replicate is performed.

$$\text{Geometric mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

The average $\log_{10}$ value for the population control was determined and used to calculate $\log_{10}$ reduction as multiple time points are evaluated in the control.

The average $\log_{10}$ value of the test results was determined and used to calculate $\log_{10}$ reduction as more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

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 discarded following study completion. It is the responsibility of the Sponsor to retain a sample of the test substance.

REFERENCES

1. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

RESULTS

For Control and Neutralization Results, see Tables 1-3.
All data measurements/controls including culture purity, neutralizer sterility, test population control, and neutralization confirmation controls performed within acceptance criteria.

For Test Results, see Tables 4-5.

ANALYSIS AND STUDY CONCLUSION

AX250 (Batch # AX-13196-0210) demonstrated a 99.8% (2.65 log_{10}) reduction of *Mycobacterium bovis* - BCG (Organon Teknika) survivors following a 15 second exposure, >99.99% (4.43 log_{10}) reduction of *Mycobacterium bovis* - BCG (Organon Teknika) survivors following a 30 second exposure, >99.999% (>5.21 log_{10}) reduction of *Mycobacterium bovis* - BCG (Organon Teknika) survivors following a 60 second exposure and >99.999% (>5.21 log_{10}) reduction of *Mycobacterium bovis* - BCG (Organon Teknika) survivors following a 90 second exposure when tested at ambient temperature(20.61°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
Purity Control	*Mycobacterium bovis* - BCG (Organon Teknika)	Pure
Neutralizer Sterility Control		No Growth

TABLE 2: TEST POPULATION CONTROL RESULTS

Test Organism	Results	
	CFU/mL	Log_{10}
Mycobacterium bovis - BCG (Organon Teknika)	1.64×10^6	6.21

CFU = Colony Forming Units

Note: *The highest challenge level was achieved for this control based on the use of standard propagation methods.*

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Neutralization Confirmation (CFU)		Pass/Fail ± 1 log_{10} (Log_{10} Difference)
		Numbers Control	Test Substance Results	
AX250 Batch # AX-13196-0210	*Mycobacterium bovis* - BCG (Organon Teknika)	39, 43	41, 35	Pass (0.03)

CFU = Colony Forming Units

TABLE 4: TEST RESULTS

DILUTION (VOLUME PLATED)	Test Organism: *Mycobacterium bovis* - BCG (Organon Teknika)			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)	T, T	5, 7*	0, 0*	0, 0*
10^{-1} (1.00 mL)	35, 36*	1, 0	0, 0	0, 0
10^{-2} (1.00 mL)	5, 9	0, 0	0, 0	0, 0
10^{-3} (1.00 mL)	1, 0	0, 0	0, 0	0, 0
10^{-4} (1.00 mL)	0, 0	0, 0	0, 0	0, 0

T = Too Numerous To Count (>300 colonies)
*Data used to calculate log and percent reduction

TABLE 5: CALCULATED DATA

Test Organism: *Mycobacterium bovis* – BCG (Organon Teknika)					
Exposure Time	**CFU/mL in Test Population Control (Log_{10})**	**CFU/mL of Survivors**	**Log_{10} Survivors**	**Percent Reduction**	**Log_{10} Reduction**
15 seconds	1.64×10^6 (6.21)	3.6×10^3	3.56	99.8%	2.65
30 seconds		6×10^1	1.78	>99.99%	4.43
60 seconds		$<1 \times 10^1$	<1.00	>99.999%	>5.21
90 seconds		$<1 \times 10^1$	<1.00	>99.999%	>5.21

CFU = Colony Forming Units

Note: *For samples with a "<" value sign, a value of <1 was used in place of zero for calculation purposes. For these samples with a "<" value sign, no growth was observed on the duplicate test plates at the lowest dilution plated. The zeros were added together to increase the sensitivity of the test and a value of 2 mL plated was used in the calculation. The limit of detection of this test is a value of <5 CFU/mL.*

Attachment I: Sponsor Test Material Certificate of Analysis

Issued: July 16, 2013
Last Revised: July 29, 2013

FORM-COA-02

AQUAOX INDUSTRIES INC
16155, Sierra Lakes Parkway,
Suite 160-714,
Fontana, CA 92336. USA.

Certificate of Analysis

Date of Manufacture:	07 / 15 / 2013
Product Name:	AX250
Batch / Lot #:	AX-13196-0210
Production Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Testing Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377

TEST	ANALYSIS	UNITS
FAC	226	ppm
pH	6.03	n/a
Conductivity	1225	µS/cm
ORP	943	mV
Osmolality	22	mOsm/kg

This certification states that the intermediate product AX250, bearing the above description and lot number, has been found to conform to the internal specifications established for this product. The above lot was made in accordance with our internal specifications and current good manufacturing practices under controlled procedures.

This lot has been appropriately inspected and tested, and, to the best of our knowledge, conforms to all applicable test methods, standards and internal specifications.

This certification does not constitute any written or expressed warranty or guarantee of any kind.

Rebecca Lei
QA Regulatory Specialist

Date: 7/29/13

EXACT COPY
INITIALS DN DATE 11/6/13

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	October 17, 2013
Sponsor:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Time Kill Assay For Antimicrobial Agents
ATS Labs Protocol Number:	INI01091613.TK.5
ATS Labs Project Number:	A15655

Modifications to Protocol:

Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

Changes to the protocol are acceptable as noted.

Study Director _____ Date 10-17-13

EXACT COPY
INITIALS OM DATE 11/6/13

(For Laboratory Use Only)
ATS Labs Project # A15655 (659-30-13)

ATS LABS

PROTOCOL

Time Kill Assay For Antimicrobial Agents

Test Organism:

Mycobacterium bovis - BCG (Organon Teknika)

PROTOCOL NUMBER

INI01091613.TK.5

PREPARED FOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PERFORMING LABORATORY

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

PREPARED BY

Anne Stemper, B.S.
Senior Microbiologist

DATE

September 16, 2013

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

EXACT COPY
INITIALS JM DATE 11/6/13

Time Kill Assay For Antimicrobial Agents

SPONSOR: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

PURPOSE
The objective of this testing is to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

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. 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 September 24, 2013. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of October 21, 2013. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

A "case-by-case" approach is generally taken by the regulatory authorities and cannot be over-emphasized when considering a testing regimen. While this protocol is based upon our experience in the field of germicidal testing, and the current regulatory guidelines, each product presents a different set of issues to the regulatory authorities. We recommend that you consult with the appropriate agency before finalizing your testing regimen, as ATS Labs cannot guarantee acceptance of this protocol by the regulating authorities.

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 nor 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 regulating 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
Analyzing the efficacy of antimicrobial agents may be performed by various suspension and susceptibility methods. This study is designed to examine the rate-of-kill of a test substance against a pure test culture. This is accomplished by exposing the test culture to the test substance and assaying for survivors following a variety of exposure times. The experimental design in this protocol meets these requirements.

TEST PRINCIPLE

A suspension of the test organism is exposed to the test substance for specified exposure times. After exposure, an aliquot of the suspension is transferred to a neutralizer and assayed for survivors. Appropriate culture purity, sterility, population and neutralization confirmation controls are performed. The current version of Standard Operating Procedure CGT-4130 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	Designation #	Culture Medium	Incubation Parameters
Mycobacterium bovis - BCG	Organon Teknika	Modified Proskauer Beck Broth	35-37°C

The test organism to be used in this study was obtained from the Organon Teknika, Durham, NC.

Preparation of Test Organism

Mycobacterium bovis - BCG will be prepared by inoculating Modified Proskauer-Beck broth (MPB) and incubating at 35-37°C for 19-23 days. Following incubation, the suspension will be homogenized using a sterile tissue grinder. The culture may be standardized to target approximately 1 x 10^8 CFU/mL or greater as necessary. A biosafety cabinet will be utilized when working with *Mycobacterium* cultures.

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

Preparation of Test Substance

The test substance to be tested is prepared according to the directions supplied 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. A 9.5 mL aliquot of the prepared test substance will be transferred to a sterile vessel (glass tube, stomacher bag, etc.) for testing procedures. If necessary, 9.5 g of test substance may be used. Multiple replicate vessels may be set up if requested.

Exposure Conditions

A 0.5 mL aliquot of the standardized inoculum will be added to the test substance representing the start of the test exposure. The inoculated test substance will be immediately mixed thoroughly using a vortex mixer, stirring with a pipette or by any other applicable method. The inoculated and mixed test substance will be held at the Sponsor specified temperature. If the requested exposure temperature lies outside of achievable ambient conditions, the test substance may be placed in a water bath (or other appropriate device) to equilibrate to the desired exposure temperature prior to testing. For very short exposure times or exposure times which are close together, individual test substance vessels may be utilized where necessary.

Test System Recovery

At each Sponsor specified exposure time, the sample will be mixed and a 0.1 mL aliquot of the inoculated test substance will be transferred to 9.9 mL of neutralizer broth (10^0 dilution). Additional ten-fold serial dilutions will be prepared in Butterfield's buffer. Using a standard microbiological spread plate count procedure, 1.0 mL aliquots of the $10^0 - 10^{-4}$ dilutions will be plated in duplicate.

If swarming is a concern, 1.0 mL of 10^0 will be plated in duplicate. In addition, 0.1 mL of $10^0 - 10^{-3}$ will be plated in duplicate.

Incubation and Observation

All *Mycobacterium* plates are incubated at 35-37°C for 17-21 days in a manner to prevent desiccation.

Following incubation, the subcultures will be visually examined for growth and enumerated. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination. Log_{10} and percent reductions will be determined for each time point. Representative subcultures demonstrating growth may 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
If applicable, 1.0 mL of the serum used for soil load will be added to a tube of Fluid Thioglycollate, incubated, and observed for lack of growth. The acceptance criterion for this study control is lack of growth.

Neutralizer Sterility Control
A 1.0 mL aliquot of the neutralizer will be plated as in the test and incubated. The acceptance criterion for this study control is lack of growth.

Test Population Control
In a similar manner as the culture inoculum is added to the test substance, add an equivalent volume of inoculum (0.5 mL) to 9.5 mL Butterfield's buffer (or the same volume as the test substance). This suspension will be neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. If requested, the sample may be exposed as in the test and evaluated at an additional time point. (If requested, the final time point is recommended.) The suspension will be serially diluted and appropriate dilutions plated using standard microbiological techniques. *If swarming is a concern, 0.1 mL aliquots will be plated.*

Following incubation, the organism plates will be observed and enumerated. If more than one time point is evaluated, the geometric mean will be determined prior to reduction calculations. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of test substance will be neutralized as in the test procedure. Only the most concentrated test substance needs to be evaluated in this control. Remove and discard 1.0 mL of the neutralized sample. To the neutralized sample, add 1.0 mL of an organism suspension to target approximately 100-1000 CFU per mL of neutralizer and vortex mix. Plate, in duplicate, 1.0 mL of neutralized mixture to appropriate recovery agar and incubate. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 1.0 mL aliquots, in duplicate, and incubate. This control may be performed prior to or concurrent with testing.

NOTE: If swarming is a concern, add 1.0 mL of an organism suspension containing 1000-10,000 CFU/mL and vortex mix. Plate, in duplicate, 0.1 mL of the neutralized mixture. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 0.1 mL aliquots, in duplicate.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log.

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 test organism 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 subcultures, etc. during the course of the test. Test subcultures 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
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results will be expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol.

REPORT
The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism 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.

TEST SUBSTANCE RETENTION
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. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{(\text{average CFU}) \times (\text{dilution factor}) \times (\text{volume neutralized solution in mL})}{(\text{volume plated in mL})}$$

A value of <1 may be used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros may be added together to increase the sensitivity of the test. (A value of 2 mL plated is used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

If applicable, the geometric mean value for the population control will be determined and used to calculate percent reduction if multiple time points are evaluated in the control. The geometric mean value of the test results will be determined and used to calculate percent reduction if more than one replicate is performed.

$$\text{Geometric mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

If applicable, the average $\log_{10}$ value for the population control will be determined and used to calculate $\log_{10}$ reduction if multiple time points are evaluated in the control. The average $\log_{10}$ value of the test results will be determined and used to $\log_{10}$ reduction if more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

Statistical Analysis: None used.

Study Information

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number - exactly as it should appear on final report):
AX250 Batch # AX-13196-0210

Expiration Date: 07/2015

Test Substance Active Concentration (upon submission to ATS Labs): 0.024% HOCl

Product Description:

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

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:

_____ defined as _____ + _____
(example: 1 oz/gallon) (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.

Exposure Times: 15 seconds, 30 seconds, 60 seconds, and 90 seconds

Number of Test Replicate(s) per sample: 1

Exposure Temperature:

- ☑ Ambient
- ☐ Other _____

Organic Soil Load:

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

Test Organism:

- ☑ *Mycobacterium bovis* - BCG (Organon Teknika))

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: 7/11/13 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

_____

_____

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Dr. Fred Ma TITLE: M.D., Ph.D. Chief Medical Officer

SIGNATURE: Dr. Fred Ma DATE: 09/17/13

PHONE: (909) 822 - 6000 FAX: _____ EMAIL: fma@innovacyn.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

Hannah Carroll (hannahc@innovacyn.com)

ATS Labs:

NAME: Jill Ruhnke
Study Director

SIGNATURE: Jill Ruhnke DATE: 9/26/13
Study Director

NAMSA

REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
9 Morgan
Irvine, CA 92618
949.951.3110

STUDY TITLE

ANTIMICROBIAL SPECIAL - Modified USP<51> Antimicrobial Effectiveness study for AX250 with various concentrations of Free Available Chlorine, i.e. 250ppm, 100ppm, 60ppm, 30ppm and 10ppm

SPONSOR

Michel van Schaik
Aquaox Industries, Inc.
16155 Sierra Lakes Pkwy Suite 160-714
Fontana, CA 92336

TEST ARTICLE NAME

AX250

TEST ARTICLE IDENTIFICATION

AX-130650-0210 (250ppm), AX-13071-0410 (100ppm), AX-13071-0310 (60ppm), AX-13071-0210 (30ppm), AX-13071-0110 (10ppm)

TEST ARTICLE PHYSICAL DESCRIPTION

Aqueous solution, clear and colorless, slightly chlorinated

TEST ARTICLE RECEIVED

April 16, 2013, and April 24, 2013

PURPOSE

The purpose of this study was to demonstrate the antimicrobial effectiveness of AX 250 at various concentrations of free available chlorine, i.e. 250ppm, 100ppm, 60ppm, 30ppm and 10ppm following the methodology describe in USP<51> antimicrobial effectiveness testing.

TEST INFORMATION

Date Initiated: 04-18-13 (bacteria) / 04-24-13 (Fungi and Yeast) Date Completed: 05-28-13

Study was conducted following protocol 13C_29383_02.

Test Article Name: AX 250 (250ppm (AX-130650-0210), 100ppm (AX-13071-0410), 60ppm (AX-13071-0310), 30ppm (AX-13071-0210) and 10ppm (AX-13071-0110))

TEST PROCEDURE

This study was conducted following Protocol 13C_29383_02. For each test article, 20 mL aliquots were transferred into seven separate sterile test tubes. Each tube was inoculated with respective organism to yield a final organism concentration of 1.0×10^5 - 1.0×10^6 CFU per mL of the test article. Inoculum volume was 0.5% to 1.0% of the product volume. Theoretical organism concentration per mL of the test article was calculated by the verification of the inoculum suspension. The inoculum suspension concentration was verified by standard plate count method, using SCDA for bacteria and SDA for yeast and mold.

At each sampling time point each tube was mixed thoroughly and then 1.0 mL of the test article was removed from each tube and added to a separate sterile tube containing 9.0 mL of the D/E neutralizing broth. The neutralized inoculated test article was mixed thoroughly and dilution 10^{-1} to dilution 10^{-5} was plated in duplicate using SCDA for the bacterial plating and SDA for the yeast and mold plating.

All bacterial cultures were incubated at 30-35°C for 3 to 5 days, yeast culture plates were incubated at 20-25°C for 3 to 5 days, and mold plates were incubated at 20-25°C for 3 to 7 days. After the incubation the number of the recoverable viable organism per mL of the test article was verified and the logarithmic change in microbial concentration within the inoculated test article after each storage time point versus the theoretical concentration of microorganisms present at the start of the test was calculated.

Test Facility
9 Morgan
Irvine, CA 92618
949.951.3110

	Verified Organism Concentration per mL of Inoculum (CFUs/mL)	Volume of Inoculum used for the test article inoculation	Theoretical Inoculum Concentration per mL of test article
Staphylococcus aureus (Source No. 6538)*	1.78×10^8	0.2 mL	1.78×10^6
Pseudomonas aeruginosa (Source No. 9027)*	9.95×10^7	0.2 mL	9.95×10^5
Escherichia coli (Source No. 8739)*	7.05×10^7	0.2 mL	7.05×10^5
Serratia marcescens (Source No. 13380)*	7.65×10^7	0.2 mL	7.65×10^5
Klebsiella pneumoniae CRE (Source No. BAA-1706)*	9.55×10^7	0.2 mL	9.55×10^5
Proteus vulgaris (Source No. 6380)*	9.50×10^7	0.2 mL	9.50×10^5
Acinetobacter baumannii. (Source No. 19606)*	1.31×10^8	0.2 mL	1.31×10^6
Candida albicans (Source No. 10231)*	2.40×10^7	0.2 mL	2.40×10^5
Aspergillus brasiliensis (Source No. 16404)*	5.15×10^7	0.2 mL	5.15×10^5

ACCEPTANCE CRITERIA

1: If the USP<1227> study fails to meet acceptance criteria, results collected during this study will be inconclusive.

2: If the initial population of each original inoculum, as indicated by the time zero plate count, is not within the range 1×10^5 - 1×10^6 CFU/mL the test must be repeated.

Note: Because the test article is not classifiable per the Compendial Product Categorization Scheme in USP<51>, there are no acceptance criteria associated with the 15 second, 30 second, 1 minute, 2 minute, 5 minute, 7 day, 14 day and 28 day results.

CONCLUSION

USP <1227> study has passed (13C_29382_03). Initial population of *S. aureus* (1.78×10^6) and *A. baumannii* (1.31×10^6) was slightly higher than the population range. Study will not be repeated.

Results Summary

AX250 (250ppm) product demonstrated greater than 4 log reduction for all the bacterial organisms (gram positive and negative), yeast and mold at each time point(15sec, 30sec, 1minute, 2minute, 5minute, 7, 14 and 28 days).

AX250 (100ppm) product demonstrated greater than 4 log reduction for all the bacterial organisms (gram positive and negative) at each time point (15sec, 30sec, 1minute, 2minute, 5minute, 7, 14 and 28 days). Yeast demonstrated greater than 4 log reduction at 15second, 30 second, 1 minute, 5 minute, 7, 14 and 28 days. 2 minute shows greater than 1 log reduction. Mold at 15 second and 30 second demonstrated greater than 2 log reduction and 1minute, 2 minute, 5 minute, 7 day, 14 day and 28 day shows greater than 4 log reduction.

AX250 (60ppm) product demonstrated greater than 4 log reduction for all the bacterial organisms (gram positive and negative) and yeast at each time point (15sec, 30sec, 1minute, 2minute, 5minute, 7, 14 and 28 days). Mold exhibit greater than 2 log reduction at 15 second, 30 second and 1 minute. At 2 minute greater than 3 log reduction was achieved while 5 minute, 7 day, 14 day and 28 day shows greater than 4 log reduction.

AX250 (30ppm) product demonstrated greater than 4 log reduction for all the bacterial organisms (gram positive and negative), and yeast demonstrated greater than 4 log reduction at 15second, 30 second, 1 minute, 5 minute, 7, 14 and 28 days. Mold

Test Facility
9 Morgan
Irvine, CA 92618
949.951.3110

demonstrated greater than 2 log reduction at 15 second, 30 second, 1 minute, 2 minutes and 5 minutes while demonstrated greater than 4 log reduction at 7, 14 and 28 days time points.

AX250 (10ppm) product demonstrated greater than 4 log reduction for all the bacterial organisms (gram positive and negative) demonstrated greater than 4 log reduction at 15second, 30 second, 1 minute, 5 minute, 7, 14 and 28 days. Yeast demonstrated 1 log reduction at 15 second and 30 second and greater than 4 log reduction at 1minute, 2 minutes, 5 minutes, 7, 14 and 28 days. Mold at 15 second, 30 second and 1 minute demonstrated greater than 1 log reduction, 2 minute, 5 minute demonstrated greater than 2 log reduction, 7, 14 and 28 days shows greater than 4 log reduction.

Calculation:

For each organism, at each time point, calculate the number of microorganisms per mL of inoculated test article as follows:

$$\text{Total Recoverable Viable Microorganisms Per mL of Test Article} = \text{Total Number of CFU Recovered} \times \frac{1}{\text{Dilution Factor}}$$

For each organism, calculate the logarithmic change in microbial concentration within the inoculated test article after each storage time point versus the theoretical concentration of microorganisms present at the start of the test as follows:

Log Reduction = Log (A) – Log (B)

Where: A = the initial challenge organisms theoretical concentration (CFU/mL)

B = the inoculated test article population at the specified time point (CFU/mL)

RESULTS

250 ppm (AX-130650-0210)	15 second % Reduction / Log reduction		30 second % Reduction / Log reduction		1 minute % Reduction / Log reduction		2 minute % Reduction / Log reduction		5 minute % Reduction / Log reduction	
S. aureus	>99.99	>5.25	>99.99	>5.25	>99.99	>5.25	>99.99	>5.25	>99.99	>5.25
P. aeruginosa	>99.99	>5.00	>99.99	>5.00	>99.99	>5.00	>99.99	>5.00	>99.99	>5.00
E. coli	>99.99	>4.85	>99.99	>4.85	>99.99	>4.85	>99.99	>4.85	>99.99	>4.85
S. marcescens	>99.99	>4.88	>99.99	>4.88	>99.99	>4.88	>99.99	>4.88	>99.99	>4.88
K .pneumoniae	>99.99	>4.98	>99.99	>4.98	>99.99	>4.98	>99.99	>4.98	>99.99	>4.98
P. vulgaris	>99.99	>4.98	>99.99	>4.98	>99.99	>4.98	>99.99	>4.98	>99.99	>4.98
A.. baumannii	>99.99	>5.12	>99.99	>5.12	>99.99	>5.12	>99.99	>5.12	>99.99	>5.12
C. albicans	>99.99	>4.38	>99.99	>4.38	>99.99	>4.38	>99.99	>4.38	>99.99	>4.38
A. brasiliensis	99.99	4.11	99.99	4.11	99.99	4.11	99.99	4.11	99.99	4.11

250 ppm (AX-130650-0210)	7 Day % Reduction / Log reduction		14 day % Reduction / Log reduction		28 day % Reduction / Log reduction	
S. aureus	>99.99	>5.25	>99.99	>5.25	>99.99	>5.25
P. aeruginosa	>99.99	>5.00	>99.99	>5.00	>99.99	>5.00
E. coli	>99.99	>4.85	>99.99	>4.85	>99.99	>4.85
S.marcescens	>99.99	>4.88	>99.99	>4.88	>99.99	>4.88
K.pneumoniae	>99.99	>4.98	>99.99	>4.98	>99.99	>4.98
P.vulgaris	>99.99	>4.98	>99.99	>4.98	>99.99	>4.98
A. baumannii	>99.99	>5.12	>99.99	>5.12	>99.99	>5.12
C. albicans	>99.99	>4.38	>99.99	>4.38	>99.99	>4.38
A. brasiliensis	>99.99	>4.71	>99.99	>4.71	>99.99	>4.71

FINAL STUDY REPORT

STUDY TITLE

Time Kill Assay For Antimicrobial Agents

Test Organisms:

Acinetobacter baumannii – Multi Drug Resistant (ATCC 19606)
Enterococcus faecium - Multi Drug Resistant (ATCC 51559)
Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)
Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)

PRODUCT IDENTITY

AX250
Batch # AX-13196-0210

AUTHOR

Gracia Schroeder, B.S.
Study Director

STUDY COMPLETION DATE

November 7, 2013

PERFORMING LABORATORY

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

SPONSOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PROJECT NUMBER

A15627

EXACT COPY
INITIALS KS DATE 11-7-13

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

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 and antibiotic sensitivity testing performed at the University of Minnesota Physicians Outreach Laboratory.

Submitter:_____ Date:_____

Sponsor:_____ Date:_____

Study Director: Gracia Schroeder, B.S. Date: 11/7/13

QUALITY ASSURANCE UNIT SUMMARY

Study: Time Kill Assay For Antimicrobial Agents

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 (21 CFR Part 58) 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 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	October 2, 2013	October 2, 2013	October 2, 2013
Draft Report	October 15, 2013	October 15, 2013	October 17, 2013
Final Report	November 7, 2013	November 7, 2013	November 7, 2013

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

Quality Assurance Auditor: _____ Date: 11/7/13

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STUDY PERSONNEL

STUDY DIRECTOR:	Gracia Schroeder, B.S.
Professional personnel involved:	
Scott R. Steinagel, B.S.	- Director, Microbiology Operations
Becky Lien, B.A.	- Manager, Microbiology Operations
Peter Toll, B.S.	- Supervisor, Microbiology Laboratory Operations
Anne Stemper, B.S.	- Senior Microbiologist
Matthew Sathe, B.S.	- Senior Microbiologist
Philip Lange, B.S.	- Associate Microbiologist
Rebecca Astrup, B.S.	- Associate Microbiologist
Nicole Zroka, B.A.	- Associate Microbiologist
Kathryn Thomas, B.S.	- Lab Technician

STUDY REPORT

GENERAL STUDY INFORMATION

Protocol Title: Time Kill Assay For Antimicrobial Agents

Project Number: A15627

Protocol Number: INI01091613.TK.1

Sponsor: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

TEST SUBSTANCE IDENTITY

Test Substance Name: AX250

Batch Number: Batch # AX-13196-0210

Test Substance Characterization
Test substance characterization as to content, stability, etc., (21 CFR, Part 58, Subpart F [58.105]) is the responsibility of the Sponsor. The Sponsor Test Material Certificate of Analysis Report may be found in Attachment III.

STUDY DATES

Date Sample Received: September 11, 2013
Study Initiation Date: September 24, 2013
Experimental Start Date: October 2, 2013
Experimental End Date: October 3, 2013
Study Completion Date: November 7, 2013

OBJECTIVE

The objective of this testing was to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

SUMMARY OF RESULTS

Test Substance:	AX250, Batch # AX-13196-0210
Dilution:	Ready to use (RTU)
Test Organisms:	*Acinetobacter baumannii* - Multi Drug Resistant (ATCC 19606) *Enterococcus faecium* - Multi Drug Resistant (ATCC 51559) Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592) Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)
Exposure Times:	15 seconds, 30 seconds, 60 seconds, and 90 seconds
Exposure Temperature:	Ambient temperature (21°C)
Organic Soil Load:	No organic soil load required
Efficacy Result:	AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.45 $\log_{10}$) reduction of *Acinetobacter baumannii* - Multi Drug Resistant(ATCC 19606) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at ambient temperature (21°C). AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.30 $\log_{10}$) reduction of *Enterococcus faecium* - Multi Drug Resistant (ATCC 51559) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at ambient temperature (21°C). AX250, Batch # AX-13196-0210 demonstrated a >99.999% (>5.36 $\log_{10}$) reduction of Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at ambient temperature (21°C). AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.56 $\log_{10}$) reduction of Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575) survivors following a 15 exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at ambient temperature (21°C).

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Acinetobacter baumannii – Multi Drug Resistant	19606	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Enterococcus faecium – Multi Drug Resistant	51559		
Methicillin Resistant *Staphylococcus aureus* - MRSA	33592		
Vancomycin Resistant *Enterococcus faecalis* - VRE	51575		

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

Recovery Media

Neutralizer: Letheen Broth + 0.1% Sodium Thiosulfate
Agar Plate Medium: Tryptic Soy Agar with 5% Sheep Blood (BAP)

TEST METHOD

Preparation of Test Organisms
Using a stock slant, each test organism culture was streaked onto an appropriate growth medium. The bacterial cultures were incubated for two days at 35-37°C.

On the day of test, a sufficient amount of organism growth was transferred into Butterfield's Buffer to create a uniform suspension targeting approximately 1×10^8 CFU/mL where possible. *Acinetobacter baumannii* - Multi Drug Resistant was adjusted to a 2.0 McFarland Turbidity Standard. *Enterococcus faecium* - Multi Drug Resistant, Methicillin Resistant *Staphylococcus aureus* - MRSA and Vancomycin Resistant *Enterococcus faecalis* - VRE were each adjusted to a 1.0 McFarland Turbidity Standard.

Antibiotic susceptibility testing was performed by ATS Labs for Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592) to verify the antimicrobial resistance pattern stated. The Kirby Bauer susceptibility assay was performed utilizing a representative culture from the day of testing. Each appropriate Quality Control (QC) and test organism was subcultured onto Tryptic Soy + 5% Sheep Blood Agar and was incubated overnight at 35-37°C. Following incubation, a sterile cotton-tipped swab was used to make suspensions equal to a 0.5 McFarland Turbidity Standard in 0.85% sterile saline. The suspension was vortex mixed within 15 minutes of preparing the suspension turbidity. A sterile cotton-tipped applicator swab was dipped into each suspension, rotated several times in the suspension and the excess inoculum was removed by rotating the swab several times above the fluid line pressing firmly against the inside of the tube wall. The swab was removed from the tube and the entire surface of a sterile, moisture-free Mueller Hinton agar plate was streaked. The inoculation procedure was repeated twice, rotating the plate approximately 60° each time to evenly distribute the inoculum. This was performed for the test and QC plates. Using a single disk ejector, each antibiotic disk was ejected into the lid of the agar plate Petri dish lid. Each 1 µg oxacillin disk was carefully placed in the center of each of the inoculated plates using a sterile forceps. Once contact was made, the disk was not moved. Each disk was then pressed down upon using sterile forceps. Within 15 minutes of application, the plates were inverted and incubated at 35-37°C for ≥24 hours. Following incubation, the zone (diameter) of inhibition showing no visible growth was measured. If no zone was present, the size of the disc was reported (6 mm). Refer to table 6 for results.

Antibiotic susceptibility testing was performed by ATS Labs for Vancomycin Resistant *Enterococcus faecalis* – VRE (ATCC 51575) to verify the antimicrobial resistance pattern stated. The Kirby Bauer susceptibility assay was performed utilizing a representative culture from the day of testing. Each appropriate Quality Control (QC) and test organism was subcultured onto Tryptic Soy + 5% Sheep Blood Agar and was incubated overnight at 35-37°C. Following incubation, a sterile cotton-tipped swab was used to make suspensions equal to a 0.5 McFarland Turbidity Standard in 0.85% sterile saline. The suspension was vortex mixed within 15 minutes of preparing the suspension turbidity. A sterile cotton-tipped applicator swab was dipped into each suspension, rotated several times in the suspension and the excess inoculum was removed by rotating the swab several times above the fluid line pressing firmly against the inside of the tube wall. The swab was removed from the tube and the entire surface of a sterile, moisture-free Mueller Hinton agar plate was streaked. The inoculation procedure was repeated twice, rotating the plate approximately 60° each time to evenly distribute the inoculum. This was performed for the test and QC plates. Using a single disk ejector, each antibiotic disk was ejected into the lid of the agar plate Petri dish lid. Each 30 µg vancomycin disk was carefully placed in the center of each of the inoculated plates using a sterile forceps. Once contact was made, the disk was not moved. Each disk was then pressed down upon using sterile forceps. Within 15 minutes of application, the plates were inverted and incubated at 35-37°C for ≥24 hours. Following incubation, the zone (diameter) of inhibition showing no visible growth was measured. If no zone was present, the size of the disc was reported (6 mm). Refer to table 7 for results.

Antibiotic sensitivity testing was performed for *Acinetobacter baumannii* – Multi Drug Resistant and *Enterococcus faecium* Multi Drug Resistant using a representative culture from the day of testing to verify the stated antibiotic resistance pattern. This testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. This testing was not performed under FDA Good Laboratory Practices (21 CFR Part 58). See Attachments I and II for results.

Preparation of Test Substance

The test substance was ready to use (RTU), as received from the Sponsor. A 9.5 mL aliquot of the test substance was transferred to a sterile vessel for use in testing. The test substance was homogenous as determined by visual observation.

Exposure Conditions

A 0.50 mL aliquot of each standardized inoculum was added to 9.5 mL test substance representing the start of the test exposure. The inoculated test substance was immediately mixed thoroughly using a vortex mixer. Each inoculated and mixed test substance was exposed for the exposure times of 15 seconds, 30 seconds, 60 seconds, and 90 seconds at ambient temperature (21°C).

Test System Recovery
At each Sponsor specified exposure time, each sample was mixed and a 0.100 mL aliquot of the inoculated test substance was transferred to 9.9 mL of neutralizer representing a 10^0 dilution. Additional ten-fold serial dilutions were prepared from the 10^0 neutralized material in Butterfield's Buffer.

Using standard microbiological spread plate procedures, 1.00 mL aliquots of the 10^0 dilution and 0.100 mL aliquots of the 10^0-10^{-3} dilutions were plated in duplicate on appropriate recovery medium.

Incubation and Observation
The bacterial subculture plates were incubated for 24-48 hours at 35-37°C. Following incubation, the agar plates were visually examined for the presence of growth and enumerated. Log_{10} and percent reductions were determined for each exposure time.

STUDY 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.

Neutralizer Sterility Control
A 1.00 mL aliquot of the neutralizer was plated as in the test and incubated. The acceptance criterion for this study control is a lack of growth.

Test Population Control
In a similar manner as the culture inoculum was added to the test substance, an equivalent volume of inoculum, 0.5 mL, was added to 9.5 mL Butterfield's buffer. This suspension was neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. The suspension was serially diluted and appropriate dilutions were plated using standard microbiological techniques and 0.100 mL aliquots. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of the test substance was neutralized as in the test procedure. A 1.0 mL aliquot of the neutralized sample was then removed and discarded. To the neutralized sample, 1.00 mL of each organism suspension containing approximately 100-1000 CFU/mL was added and the suspension was vortex mixed. A 1.00 mL aliquot of the neutralized mixture was plated in duplicate on appropriate recovery agar and incubated. A numbers control was performed by adding 1.00 mL of the same organism suspension to 9 mL of untreated neutralizer. A 0.100 mL aliquot was plated in duplicate and incubated.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log. The most appropriate dilution was reported.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results are expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

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

PROTOCOL CHANGES

Protocol Amendment:

1. Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

    a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

    b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

    c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

2. This protocol is amended to change study directors due to the departure of the original study director from ATS Labs. The study director has been changed from Anne Stemper to Gracia Schroeder

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{(\text{average CFU}) \times (\text{dilution factor}) \times (\text{volume neutralized solution in mL})}{(\text{volume plated in mL})}$$

A value of <1 was used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros were added together to increase the sensitivity of the test. (A value of 2 mL plated was used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

The geometric mean value for the population control was determined and used to calculate percent reduction as multiple time points were evaluated in the control.

The geometric mean value of the test results were determined and used to calculate percent reduction as more than one replicate is performed.

$$\text{Geometric mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

The average $\log_{10}$ value for the population control was determined and used to calculate $\log_{10}$ reduction as multiple time points are evaluated in the control.

The average $\log_{10}$ value of the test results was determined and used to calculate $\log_{10}$ reduction as more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

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 discarded following study completion. It is the responsibility of the Sponsor to retain a sample of the test substance.

REFERENCES

1. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

RESULTS

For Control and Neutralization Results, see Tables 1-3, and 6-7.
All data measurements/controls including culture purity, neutralizer sterility, test population control, and neutralization confirmation controls performed within acceptance criteria. Furthermore, the test organism antibiotic resistance profile met the established criteria.

For Test Results, see Tables 4-5.

ANALYSIS AND STUDY CONCLUSION

AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.45 log_{10}) reduction of *Acinetobacter baumannii* - Multi Drug Resistant (ATCC 19606) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at ambient temperature (21°C)

AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.30 log_{10}) reduction of *Enterococcus faecium* - Multi Drug Resistant (ATCC 51559) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at ambient temperature (21°C).

AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.36 log_{10}) reduction of Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at ambient temperature (21°C).

AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.56 log_{10}) reduction of Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at ambient temperature (21°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
Purity Control	*Acinetobacter baumannii* - Multi Drug Resistant (ATCC 19606)	Pure
	Enterococcus faecium - Multi Drug Resistant (ATCC 51559)	Pure
	Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)	Pure
	Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)	Pure
Neutralizer Sterility Control		No Growth

TABLE 2: TEST POPULATION CONTROL RESULTS

Test Organism	Results	
	CFU/mL	Log_{10}
Acinetobacter baumannii - Multi Drug Resistant (ATCC 19606)	1.41×10^6	6.15
Enterococcus faecium - Multi Drug Resistant (ATCC 51559)	1.00×10^6	6.00
Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)	1.14×10^6	6.06
Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)	1.82×10^6	6.26

CFU = Colony Forming Units

Note: *The highest challenge level was achieved for this control based on the use of standard propagation methods.*

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Neutralization Confirmation (CFU)		Pass/Fail ± 1 log_{10} (Log_{10} Difference)
		Numbers Control	Test Substance Results	
AX250 Batch # AX-13196-0210	*Acinetobacter baumannii* - Multi Drug Resistant (ATCC 19606)	42, 47	41, 36	Pass (0.06)
	Enterococcus faecium - Multi Drug Resistant (ATCC 51559)	20, 26	23, 26	Pass (-0.04)
	Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)	22, 25	34, 28	Pass (-0.11)
	Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)	43, 31	35, 22	Pass (0.11)

CFU = Colony Forming Units

TABLE 4: TEST RESULTS FOR AX250 Batch # AX-13196-0210

DILUTION (VOLUME PLATED)	Test Organism: *Acinetobacter baumannii* - Multi Drug Resistant (ATCC 19606)			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^{0} (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^{0} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: *Enterococcus faecium* - Multi Drug Resistant (ATCC 51559)**			
	Exposure Time			
	15 seconds	**30 seconds**	**60 seconds**	**90 seconds**
	Number of Survivors			
10^{0} (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^{0} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)**			
	Exposure Time			
	15 seconds	**30 seconds**	**60 seconds**	**90 seconds**
	Number of Survivors			
10^{0} (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^{0} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)**			
	Exposure Time			
	15 seconds	**30 seconds**	**60 seconds**	**90 seconds**
	Number of Survivors			
10^{0} (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^{0} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0

* Indicates dilution used for calculation purposes.

TABLE 5: CALCULATED DATA FOR AX250 Batch # AX-13196-0210

Test Organism	Exposure Time	CFU/mL in Test Population Control (Log_{10})	CFU/mL of Survivors	Log_{10} Survivors	Percent Reduction	Log_{10} Reduction
Acinetobacter baumannii – Multi Drug Resistant (ATCC 19606)	15 seconds	1.41×10^6 (6.15)	<5	<0.70	>99.999%	>5.45
	30 seconds		<5	<0.70	>99.999%	>5.45
	60 seconds		<5	<0.70	>99.999%	>5.45
	90 seconds		<5	<0.70	>99.999%	>5.45
Enterococcus faecium Multi Drug Resistant (ATCC 51559)	15 seconds	1.00×10^6 (6.00)	<5	<0.70	>99.999%	>5.30
	30 seconds		<5	<0.70	>99.999%	>5.30
	60 seconds		<5	<0.70	>99.999%	>5.30
	90 seconds		<5	<0.70	>99.999%	>5.30
Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)	15 seconds	1.14×10^6 (6.06)	<5	<0.70	>99.999%	>5.36
	30 seconds		>5	<0.70	>99.999%	>5.36
	60 seconds		<5	<0.70	>99.999%	>5.36
	90 seconds		<5	<0.70	>99.999%	>5.36
Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)	15 seconds	1.82×10^6 (6.26)	<5	<0.70	>99.999%	>5.56
	30 seconds		<5	<0.70	>99.999%	>5.56
	60 seconds		<5	<0.70	>99.999%	>5.56
	90 seconds		<5	<0.70	>99.999%	>5.56

CFU = Colony Forming Units

Note: *A value of <1 was used in place of zero for calculation purposes. No growth was observed on the duplicate test plates at the lowest dilution plated. The zeros were added together to increase the sensitivity of the test and a value of 2 mL plated was used in the calculation. The limit of detection of this test is a value of <5 CFU/mL.*

TABLE 6: VERIFICATION OF ANTIBIOTIC RESISTANCE FOR Methicillin Resistant *Staphylococcus aureus* - MRSA

Quality Control Organism	Zone of Inhibition (mm)	CLSI* Acceptable Range (mm)
Staphylococcus aureus (ATCC 25923)	6	18 – 24
Test Organism	**Zone of Inhibition (mm)**	**CLSI* Resistant Range (mm)**
Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)	19	≤10

*CLSI = Clinical and Laboratory Standards Institute
Interpretation of result and acceptable range are from the Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Information Supplement January 2012, Volume 31 Number 1, Approved Standard M02-A11 and M07-A9, Wayne, Pennsylvania.

TABLE 7: VERIFICATION OF ANTIBIOTIC RESISTANCE FOR Vancomycin Resistant *Enterococcus faecalis* - VRE

Quality Control Organism	Zone of Inhibition (mm)	CLSI* Acceptable Range (mm)
Staphylococcus aureus (ATCC 25923)	6	17-21
Test Organism	**Zone of Inhibition (mm)**	**CLSI* Resistant Range (mm)**
Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)	17	≤14

*CLSI = Clinical and Laboratory Standards Institute
Interpretation of result and acceptable range are from the Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Information Supplement January 2012, Volume 31 Number 1, Approved Standard M02-A11 and M07-A9, Wayne, Pennsylvania.

ATTACHMENT I: Antibiotic Sensitivity Testing Results *Acinetobacter baumannii* – Multi Drug Resistant

University of Minnesota Physicians
Outreach Laboratories

Mayo Building D-293 (MMC 198)
420 Delaware St. S.E.
Minneapolis, MN 55455
Tel: 612-273-7838
888-318-3627 ext. 3-7838
Fax: 612-273-0183

ATS Labs
Attn: Chris Slitts
1285 Corporate Ctr Dr, Suite 110
Eagan, MN 55121
A15627 m 10-16-13

PATIENT NAME	PATIENT ID	DOB	SEX	STATUS	DESTINATION
ATS LABS, A15627 AB	Z1590-174	01/01/1900	U	Final	DZ1590

PHYSICIAN	COLLECT DATE & TIME	DATE OF SERVICE	EXTRACT DATE/TIME	PAGE
UNKNOWN, PHYSICIAN	10/02/2013 12:27	10/03/2013 12:28	10/07/2013 03:33	1

REQUISITION NO.	EXTERNAL ID
10202.Z1590	A15627AB

COMMENTS: ATCC 19606

Diagnostic Procedure	Result In Range	Result Out of Range	Units	Reference Range
Referral sensitivity				
TRANSPORT TIME	24.0			
MICRO LAB SETUP	10/03/2013 1309			
Specimen Description	Culture plate			
Culture	Acinetobacter baumannii A15627			
Report status	FINAL 10/06/2013			
Susceptibility				
Organism:	Acinetobacter baumannii			
Method	MIC			
Amikacin	16.0 Susceptible			
Ampicillin	>16.0 Resistant			
Ampicillin/Sulbactam	<=8.0 Susceptible			
Cefazolin	>16.0 Resistant			
Cefepime	16.0 Intermediate			
Ceftazidime	4.0 Susceptible			
Ceftriaxone	32.0 Intermediate			
Ciprofloxacin	<=0.5 Susceptible			
Gentamicin	>8.0 Resistant			
Imipenem	<=1.0 Susceptible			
Levofloxacin	<=1.0 Susceptible			
Piperacillin/Tazo	<=8.0 Susceptible			
Ticarcillin/Clav	<=8.0 Susceptible			
Tobramycin	4.0 Susceptible			
Trimethoprim/Sulfa	>2.0/38.0 Resistant			
Meropenem	<=1.0 Susceptible			
End of Report				

ATS LABS, A15627 AB 10/07/2013 03:33 DZ1590

EXACT COPY
INITIALS JMV DATE 11/7/13

ATTACHMENT II: Antibiotic Sensitivity Testing Results *Enterococcus faecium* – Multi Drug Resistant

To: 6513795549 07 Oct 2013 03:34 Page 3 of 5

University of Minnesota Physicians
Outreach Laboratories

Mayo Building D-293 (MMC 198)
420 Delaware St. S.E.
Minneapolis, MN 55455

Tel: 612-273-7838
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Fax: 612-273-0183

ATS Labs
Attn: Chris Slitts
1285 Corporate Ctr Dr, Suite 110
Eagan, MN 55121

A15627 JH 10-16-13

PATIENT NAME	PATIENT ID	DOB	SEX	STATUS	DESTINATION
ATS LABS, A15627 EF	Z1590-173	01/01/1900	U	Final	DZ1590

PHYSICIAN	COLLECT DATE & TIME	DATE OF SERVICE	EXTRACT DATE/TIME	PAGE
UNKNOWN, PHYSICIAN	10/02/2013 12:25 (a)	10/03/2013 12:26	10/07/2013 03:33	1

REQUISITION NO.		EXTERNAL ID
10201.Z1590		A15627EF

COMMENTS: ATIC 51559

Diagnostic Procedure	Result: In Range	Result: Out of Range	Units	Reference Range
Referral sensitivity Collected on: 10/02/2013 12:25				
TRANSPORT TIME	24.0			
MICRO LAB SETUP	10/03/2013 1307			
Specimen Description	Other			
Culture	Enterococcus faecium VRE A15627			
Report status	FINAL 10/06/2013			
Susceptibility Collected on: 10/02/2013 12:25				
Organism:	Enterococcus faecium VRE			
Method	E Test			
Daptomycin	4.0 Susceptible			
Susceptibility Collected on: 10/02/2013 12:25				
Organism:	Enterococcus faecium VRE			
Method	MIC			
Ampicillin	>256.0 Resistant			
Penicillin	>=64 Resistant			
Vancomycin	>256.0 Resistant VRE- Requires Contact Precautions			
Gentamicin Screen	Resistant High level gentamicin resistance was found and this is predictive of resistance to tobramycin and amikacin.			
Quinupristin/Dalfopr	0.5 Susceptible			
Linezolid	1.5 Susceptible			
End of Report				

ATS LABS, A15627 EF **10/07/2013 03:33** **DZ1590**

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INITIALS JH DATE 11/7/13

Attachment III: Sponsor Test Material Certificate of Analysis - Batch AX-13196-0210

Issued: July 15, 2013
Last Revised: July 29, 2013

TCMA-COA-02

AQUAOX INDUSTRIES INC
16155, Sierra Lakes Parkway,
Suite 160-714,
Fontana, CA 92336, USA.

Certificate of Analysis

Date of Manufacture:	07 / 15 / 2013
Product Name:	AX250
Batch / Lot #:	AX-13196-0210
Production Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Testing Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377

TEST	ANALYSIS	UNITS
FAC	226	ppm
pH	6.03	n/a
Conductivity	1225	µS/cm
ORP	943	mV
Osmolality	22	mOsm/kg

This certification states that the intermediate product AX250, bearing the above description and lot number, has been found to conform to the internal specifications established for this product. The above lot was made in accordance with our internal specifications and current good manufacturing practices under controlled procedures.

This lot has been appropriately inspected and tested, and, to the best of our knowledge, conforms to all applicable test methods, standards and internal specifications.

This certification does not constitute any written or expressed warranty or guarantee of any kind.

Rebecca Lei
QA Regulatory Specialist

Date: 7/29/13

EXACT COPY
INITIALS: DATE 11/7/13

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	10/11/13
Sponsor:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Time Kill Assay For Antimicrobial Agents
ATS Labs Protocol Number:	INI01091613.TK.1
ATS Labs Project Number:	A15627

Modifications to Protocol:

Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

Changes to the protocol are acceptable as noted.

Study Director _____ Date 10-11-13

EXACT COPY
INITIAL _____ DATE 11/7/13

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	2
Effective Date:	10/29/13
Sponsor:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Time Kill Assay For Antimicrobial Agents
ATS Labs Protocol Number:	INI01091613.TK.1
ATS Labs Project Number:	A15627

Modifications to Protocol:

This protocol is amended to change study directors due to the departure of the original study director from ATS Labs. The study director has been changed from Anne Stemper to Gracia Schroeder.

Changes to the protocol are acceptable as noted.

Gracia Schroeder
Study Director

10/29/13
Date

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INITIALS GMS DATE 11/7/13

(For Laboratory Use Only)
ATS Labs Project # A15627
9/25/13

ATS LABS

PROTOCOL

Time Kill Assay For Antimicrobial Agents

Test Organisms:

Acinetobacter baumannii - Multi Drug Resistant (ATCC 19606)
Enterococcus faecium – Multi Drug Resistant (ATCC 51559)
Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)
Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)

PROTOCOL NUMBER

INI01091613.TK.1

PREPARED FOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PERFORMING LABORATORY

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

PREPARED BY

Anne Stemper, B.S.
Senior Microbiologist

DATE

September 16, 2013

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

EXACT COPY
INITIALS gms DATE 11/7/13

Time Kill Assay For Antimicrobial Agents

SPONSOR: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

PURPOSE
The objective of this testing is to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

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. 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 September 24, 2013. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of October 21, 2013. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

A "case-by-case" approach is generally taken by the regulatory authorities and cannot be over-emphasized when considering a testing regimen. While this protocol is based upon our experience in the field of germicidal testing, and the current regulatory guidelines, each product presents a different set of issues to the regulatory authorities. We recommend that you consult with the appropriate agency before finalizing your testing regimen, as ATS Labs cannot guarantee acceptance of this protocol by the regulating authorities.

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 nor 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 regulating 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
Analyzing the efficacy of antimicrobial agents may be performed by various suspension and susceptibility methods. This study is designed to examine the rate-of-kill of a test substance against a pure test culture. This is accomplished by exposing the test culture to the test substance and assaying for survivors following a variety of exposure times. The experimental design in this protocol meets these requirements.

TEST PRINCIPLE

A suspension of the test organism is exposed to the test substance for specified exposure times. After exposure, an aliquot of the suspension is transferred to a neutralizer and assayed for survivors. Appropriate culture purity, sterility, population and neutralization confirmation controls are performed. The current version of Standard Operating Procedure CGT-4130 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Culture Medium	Incubation Parameters
Acinetobacter baumannii Multi Drug Resistant	19606	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Enterococcus faecium Multi Drug Resistant	51559	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Methicillin Resistant *Staphylococcus aureus* - MRSA	33592	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Vancomycin Resistant *Enterococcus faecalis* - VRE	51575	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic

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

Preparation of Test Organism

From a stock plate or stock slant culture, streak a culture of each test organism onto the culture medium listed above. This represents the second culture transfer. Incubate the second culture transfer for 1-5 days at the incubation parameters listed above. (Alternate or extended incubation may be required for certain strains). Transfer a sufficient amount of organism growth into a sterile diluent to create a uniform suspension targeting approximately 1 x 10^8 CFU/mL or greater where possible. This may be achieved by comparison to McFarland standards, by spectrophotometric means or by any other appropriate method.

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

Antibiotic sensitivity testing will be performed using a representative organism from the day of testing to verify resistance to at least two antibiotics. This testing may be performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. If not performed by ATS Labs, testing will not be performed under EPA Good Laboratory Practices (40 CFR Part 160) and will be exempt from the GLP compliance statement.

Antimicrobial susceptibility testing will be performed utilizing a representative culture from the day of testing to verify the antimicrobial resistance pattern stated.

Preparation of Test Substance

The test substance to be tested is prepared according to the directions supplied 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. A 9.5 mL aliquot of the prepared test substance will be transferred to a sterile vessel (glass tube, stomacher bag, etc.) for testing procedures. If necessary, 9.5 g of test substance may be used. Multiple replicate vessels may be set up if requested.

Exposure Conditions

A 0.5 mL aliquot of the standardized inoculum will be added to the test substance representing the start of the test exposure. The inoculated test substance will be immediately mixed thoroughly using a vortex mixer, stirring with a pipette or by any other applicable method. The inoculated and mixed test substance will be held at the Sponsor specified temperature. If the requested exposure temperature lies outside of achievable ambient conditions, the test substance may be placed in a water bath (or other appropriate device) to equilibrate to the desired exposure temperature prior to testing. For very short exposure times or exposure times which are close together, individual test substance vessels may be utilized where necessary.

Test System Recovery
At each Sponsor specified exposure time, the sample will be mixed and a 0.1 mL aliquot of the inoculated test substance will be transferred to 9.9 mL of neutralizer broth (10^0 dilution). Additional ten-fold serial dilutions will be prepared in Butterfield's buffer. Using a standard microbiological spread plate count procedure, 1.0 mL aliquots of the $10^0 - 10^{-4}$ dilutions will be plated in duplicate.

If swarming is a concern, 1.0 mL of 10^0 will be plated in duplicate. In addition, 0.1 mL of $10^0 - 10^{-3}$ will be plated in duplicate.

Incubation and Observation
All bacterial subculture plates are incubated for 24-48 hours at 35-37°C. Alternate or extended incubation may be required for certain strains.

Following incubation, the subcultures will be visually examined for growth and enumerated. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination. Log_{10} and percent reductions will be determined for each time point. Representative subcultures demonstrating growth may 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
If applicable, 1.0 mL of the serum used for soil load will be added to a tube of Fluid Thioglycollate, incubated, and observed for lack of growth. The acceptance criterion for this study control is lack of growth.

Neutralizer Sterility Control
A 1.0 mL aliquot of the neutralizer will be plated as in the test and incubated. The acceptance criterion for this study control is lack of growth.

Test Population Control
In a similar manner as the culture inoculum is added to the test substance, add an equivalent volume of inoculum (0.5 mL) to 9.5 mL Butterfield's buffer (or the same volume as the test substance). This suspension will be neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. If requested, the sample may be exposed as in the test and evaluated at an additional time point. (If requested, the final time point is recommended.) The suspension will be serially diluted and appropriate dilutions plated using standard microbiological techniques. *If swarming is a concern, 0.1 mL aliquots will be plated.*

Following incubation, the organism plates will be observed and enumerated. If more than one time point is evaluated, the geometric mean will be determined prior to reduction calculations. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of test substance will be neutralized as in the test procedure. Only the most concentrated test substance needs to be evaluated in this control. Remove and discard 1.0 mL of the neutralized sample. To the neutralized sample, add 1.0 mL of an organism suspension to target approximately 100-1000 CFU per mL of neutralizer and vortex mix. Plate, in duplicate, 1.0 mL of neutralized mixture to appropriate recovery agar and incubate. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 1.0 mL aliquots, in duplicate, and incubate. This control may be performed prior to or concurrent with testing.

NOTE: If swarming is a concern, add 1.0 mL of an organism suspension containing 1000-10,000 CFU/mL and vortex mix. Plate, in duplicate, 0.1 mL of the neutralized mixture. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 0.1 mL aliquots, in duplicate.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log.

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 test organism 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 subcultures, etc. during the course of the test. Test subcultures 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
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results will be expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol.

REPORT
The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism 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.

TEST SUBSTANCE RETENTION

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. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

DATA ANALYSIS

Calculations

CFU/mL = $\frac{\text{(average CFU) x (dilution factor) x (volume neutralized solution in mL)}}{\text{(volume plated in mL)}}$

A value of <1 may be used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros may be added together to increase the sensitivity of the test. (A value of 2 mL plated is used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

If applicable, the geometric mean value for the population control will be determined and used to calculate percent reduction if multiple time points are evaluated in the control. The geometric mean value of the test results will be determined and used to calculate percent reduction if more than one replicate is performed.

Geometric mean = Antilog of $\frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

If applicable, the average $\log_{10}$ value for the population control will be determined and used to calculate $\log_{10}$ reduction if multiple time points are evaluated in the control. The average $\log_{10}$ value of the test results will be determined and used to $\log_{10}$ reduction if more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

Statistical Analysis: None used.

Study Information

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number - exactly as it should appear on final report):
AX250 Batch # AX-13196-0210

Expiration Date: 07/2015

Test Substance Active Concentration (upon submission to ATS Labs): 0.024% HOCl

Product Description:

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

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:

_____ defined as _____ + _____
(example: 1 oz/gallon) (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.*

Exposure Times: 15 seconds, 30 seconds, 60 seconds, and 90 seconds

Number of Test Replicate(s) per sample: 1

Exposure Temperature:

- ☑ Ambient
- ☐ Other _____

Organic Soil Load:

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

Test Organisms:

- ☑ *Acinetobacter baumannii* – Multi Drug Resistant (ATCC 19606)
- ☑ Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)
- ☑ *Enterococcus faecium* - Multi Drug Resistant (ATCC 51559)
- ☑ Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)

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: 7/11/13 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

_____

_____

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Dr. Fred Ma TITLE: M.D., Ph.D. Chief Medical Officer

SIGNATURE: Dr. Fred Ma DATE: 09/17/13

PHONE: (909) 822 - 6000 FAX: _____ EMAIL: fma@innovacyn.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

Hannah Carroll (hannahc@innovacyn.com)

ATS Labs:

NAME: Anne Stemper
Study Director

SIGNATURE: Anne Stemper DATE: 9-24-13
Study Director

FINAL STUDY REPORT

STUDY TITLE

Time Kill Assay For Antimicrobial Agents

Test Organisms:

Bacteroides fragilis (ATCC 25285)
Haemophilus influenzae (ATCC 10211)
Streptococcus pyogenes (ATCC 19615)

PRODUCT IDENTITY

AX250
Batch # AX-13196-0210

AUTHOR

Jill Ruhme, B.S.
Study Director

STUDY COMPLETION DATE

November 7, 2013

PERFORMING LABORATORY

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

SPONSOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PROJECT NUMBER

A15669

EXACT COPY
INITIALS KT DATE 11-7-13

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

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:_____

Sponsor:_____ Date:_____

Study Director: Jill Ruhme Date: 11-7-13
Jill Ruhme, B.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: Time Kill Assay For Antimicrobial Agents

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 (21 CFR Part 58) 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 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	October 7, 2013	October 7, 2013	October 14, 2013
Draft Report	October 24, 2013	October 25, 2013	October 29, 2013
Final Report	November 7, 2013	November 7, 2013	November 7, 2013

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

Quality Assurance Auditor: _____ Date: 11/7/13

TABLE OF CONTENTS

STUDY PERSONNEL

STUDY DIRECTOR:	Jill Ruhme, B.S.

Professional personnel involved:

Scott R. Steinagel, B.S.	- Director, Microbiology Operations
Becky Lien, B.A.	- Manager, Microbiology Operations
Peter Toll, B.S.	- Supervisor, Microbiology Laboratory Operations
Anne Stemper, B.S.	- Senior Microbiologist
Adam W. Pitt, B.S.	- Senior Microbiologist
Rebecca Astrup, B.S.	- Associate Microbiologist
Elizabeth Schwandt, B.S.	- Associate Microbiologist
Kathryn Thomas, B.S.	- Laboratory Technician

STUDY REPORT

GENERAL STUDY INFORMATION

Protocol Title: Time Kill Assay For Antimicrobial Agents

Project Number: A15669

Protocol Number: INI01091613.TK.3

Sponsor: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

TEST SUBSTANCE IDENTITY

Test Substance Name: AX250

Batch Number: Batch # AX-13196-0210

Test Substance Characterization
Test substance characterization as to content, stability, etc., (21 CFR, Part 58, Subpart F [58.105]) is the responsibility of the Sponsor. The Sponsor Test Material Certificate of Analysis Report may be found in Attachment I.

STUDY DATES

Date Sample Received: September 11, 2013
Study Initiation Date: October 3, 2013
Experimental Start Date: October 7, 2013
Experimental End Date: October 10, 2013
Study Completion Date: November 7, 2013

OBJECTIVE

The objective of this testing was to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

SUMMARY OF RESULTS

Test Substance:	AX250 (Batch # AX-13196-0210)
Dilution:	Ready to use (RTU)
Test Organisms:	*Bacteroides fragilis* (ATCC 25285) *Haemophilus influenzae* (ATCC 10211) *Streptococcus pyogenes* (ATCC 19615)
Exposure Times:	15 seconds, 30 seconds, 60 seconds, and 90 seconds
Exposure Temperature:	Ambient (21°C)
Organic Soil Load:	No organic soil load required
Efficacy Result:	AX250 (Batch # AX-13196-0210) demonstrated >99.999% (>5.89 $\log_{10}$) reduction of *Bacteroides fragilis* (ATCC 25285) survivors following a 15 second, 30 second 60 second and 90 second exposure when tested at ambient temperature (21°C). AX250 (Batch # AX-13196-0210) demonstrated >99.99% (>4.44 $\log_{10}$) reduction of *Haemophilus influenzae* (ATCC 10211) survivors following a 15 second, 30 second 60 second and 90 second exposure when tested at ambient temperature (21°C). AX250 (Batch # AX-13196-0210) demonstrated >99.999% (>5.79 $\log_{10}$) reduction of *Streptococcus pyogenes* (ATCC 19615) survivors following a 15 second, 30 second 60 second and 90 second exposure when tested at ambient temperature (21°C).

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Bacteroides fragilis	25285	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, anaerobic
Haemophilus influenzae	10211	Chocolate Agar	35-37°C, in CO_2
Streptococcus pyogenes	19615	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, in CO_2

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

Recovery Media

Neutralizer:	Letheen Broth + 0.1% Sodium Thiosulfate
Agar Plate Medium:	Tryptic Soy Agar with 5% Sheep Blood (BAP) for *Bacteroides fragilis* and *Streptococcus pyogenes* Chocolate Agar for *Haemophilus influenzae*

TEST METHOD

Preparation of Test Organisms

Using a stock plate for *Bacteroides fragilis* and *Haemophilus influenzae* and a stock slant for *Streptococcus pyogenes*, each test organism culture was streaked onto an appropriate growth medium. *Streptococcus pyogenes* and *Haemophilus influenzae* cultures were incubated for 3 days at 35-37°C in 6.0% CO_2. *Bacteroides fragilis* culture was incubated for 3 days at 35-37°C under anaerobic conditions.

On the day of test, a sufficient amount of organism growth was transferred into Butterfield's Buffer to create a uniform suspension targeting approximately 1×10^8 CFU/mL where possible. *Bacteroides fragilis* was adjusted to a 1.0 McFarland Turbidity Standard. *Haemophilus influenzae* was adjusted to >4.0 McFarland Turbidity Standard. *Streptococcus pyogenes* was adjusted to >4.0 McFarland Turbidity Standard.

Preparation of Test Substance

The test substance was ready to use (RTU), as received from the Sponsor. A 9.5 mL aliquot of the test substance was transferred to a sterile vessel for use in testing. The test substance was homogenous as determined by visual observation.

One replicate sample was set up and evaluated per test organism.

Exposure Conditions

A 0.50 mL aliquot of each standardized inoculum was added to 9.5 mL test substance representing the start of the test exposure. The inoculated test substance was immediately mixed thoroughly using a vortex mixer. Each inoculated and mixed test substance was exposed for the exposure times of 15 seconds, 30 seconds, 60 seconds, and 90 seconds at ambient temperature (21°C).

Test System Recovery

At each Sponsor specified exposure time, each sample was mixed and a 0.100 mL aliquot of the inoculated test substance was transferred to 9.9 mL of neutralizer representing a 10^0 dilution. Additional ten-fold serial dilutions were prepared from the 10^0 neutralized material in Butterfield's Buffer.

Using standard microbiological spread plate procedures, 1.00 mL aliquots of the 10^0 dilution and 0.100 mL aliquots of the 10^0-10^{-3} dilutions were plated in duplicate on appropriate recovery medium.

Incubation and Observation

The subculture plates for *Streptococcus pyogenes* and *Haemophilus influenzae* were incubated for 3 days at 35-37°C in 6.0% CO_2. The subculture plates for *Bacteroides fragilis* were incubated for 3 days at 35-37°C under anaerobic conditions. Following incubation, the agar plates were visually examined for the presence of growth and enumerated. Log_{10} and percent reductions were determined for each exposure time.

STUDY 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.

Neutralizer Sterility Control

A 1.00 mL aliquot of the neutralizer was plated as in the test and incubated. The acceptance criterion for this study control is a lack of growth.

Test Population Control

In a similar manner as the culture inoculum was added to the test substance, an equivalent volume of inoculum (0.50 mL) was added to 9.5 mL Butterfield's buffer. This suspension was neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. The suspension was serially diluted and appropriate dilutions were plated using standard microbiological techniques and 0.100 mL aliquots. Following incubation, the organism plates were observed and enumerated. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control

An aliquot of the test substance was neutralized as in the test procedure. A 1.00 mL aliquot of the neutralized sample was then removed and discarded. To the neutralized sample, 1.00 mL of the organism suspension containing approximately 1000-10,000 CFU/mL was added and the suspension was vortex mixed. A 0.100 mL aliquot of the neutralized mixture was plated in duplicate on appropriate recovery agar and incubated. A numbers control was performed by adding 1.00 mL of the same organism suspension to 9 mL of untreated neutralizer. A 0.100 mL aliquot was plated in duplicate and incubated.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log. The most appropriate dilution was reported.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results are expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

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

PROTOCOL CHANGES

Protocol Amendment:
Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{(\text{average CFU}) \times (\text{dilution factor}) \times (\text{volume neutralized solution in mL})}{(\text{volume plated in mL})}$$

A value of <1 was used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros were added together to increase the sensitivity of the test. (A value of 2 mL plated was used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

The geometric mean value for the population control was determined and used to calculate percent reduction as multiple time points were evaluated in the control.

The geometric mean value of the test results were determined and used to calculate percent reduction as more than one replicate is performed.

$$\text{Geometric mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

The average $\log_{10}$ value for the population control was determined and used to calculate $\log_{10}$ reduction as multiple time points are evaluated in the control.

The average $\log_{10}$ value of the test results was determined and used to calculate $\log_{10}$ reduction as more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

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 discarded following study completion. It is the responsibility of the Sponsor to retain a sample of the test substance.

REFERENCES

1. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

RESULTS

For Control and Neutralization Results, see Tables 1-3.
All data measurements/controls including culture purity, neutralizer sterility, test population control, and neutralization confirmation controls performed within acceptance criteria.

For Test Results, see Tables 4-5.

ANALYSIS AND STUDY CONCLUSION

AX250 (Batch # AX-13196-0210) demonstrated >99.999% (>5.89 $\log_{10}$) reduction of *Bacteroides fragilis* (ATCC 25285) survivors following a 15 second, 30 second 60 second and 90 second exposure when tested at ambient temperature (21°C).

AX250 (Batch # AX-13196-0210) demonstrated >99.99% (>4.44 $\log_{10}$) reduction of *Haemophilus influenzae* (ATCC 10211) survivors following a 15 second, 30 second 60 second and 90 second exposure when tested at ambient temperature (21°C).

AX250 (Batch # AX-13196-0210) demonstrated >99.999% (>5.79 $\log_{10}$) reduction of *Streptococcus pyogenes* (ATCC 19615) survivors following a 15 second, 30 second 60 second and 90 second exposure when tested at ambient temperature (21°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
Purity Control	*Bacteroides fragilis* (ATCC 25285)	Pure
	Haemophilus influenzae (ATCC 10211)	Pure
	Streptococcus pyogenes (ATCC 19615)	Pure
Neutralizer Sterility Control		No Growth

TABLE 2: TEST POPULATION CONTROL RESULTS

Test Organism	Results	
	CFU/mL	Log_{10}
Bacteroides fragilis (ATCC 25285)	3.9×10^6	6.59
Haemophilus influenzae (ATCC 10211)	1.38×10^5	5.14
Streptococcus pyogenes (ATCC 19615)	3.1×10^6	6.49

CFU = Colony Forming Units

Note: *The highest challenge level was achieved for this control based on the use of standard propagation methods.*

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Neutralization Confirmation (CFU)		Pass/Fail ± 1 log_{10} (Log_{10} Difference)
		Numbers Control	Test Substance Results	
AX250 Batch # AX-13196-0210	*Bacteroides fragilis* (ATCC 25285)	64, 62	46, 54	Pass (0.10)
	Haemophilus influenzae (ATCC 10211)	29, 42	25, 34	Pass (0.08)
	Streptococcus pyogenes (ATCC 19615)	61, 94	82, 70	Pass (0.01)

CFU = Colony Forming Units

TABLE 4: TEST RESULTS

DILUTION (VOLUME PLATED)	Test Organism: *Bacteroides fragilis* (ATCC 25285)			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: *Haemophilus influenzae* (ATCC 10211)**			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: *Streptococcus pyogenes* (ATCC 19615)**			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0

*Data used to calculate log and percent reduction

TABLE 5: CALCULATED DATA

Test Organism	Exposure Time	CFU/mL in Test Population Control (Log_{10})	CFU/mL of Survivors	Log_{10} Survivors	Percent Reduction	Log_{10} Reduction
Bacteroides fragilis (ATCC 25285)	15 seconds	3.9×10^6 (6.59)	<5	<0.70	>99.999%	>5.89
	30 seconds		<5	<0.70	>99.999%	>5.89
	60 seconds		<5	<0.70	>99.999%	>5.89
	90 seconds		<5	<0.70	>99.999%	>5.89
Haemophilus influenzae (ATCC 10211)	15 seconds	1.38×10^5 (5.14)	<5	<0.70	>99.99%	>4.44
	30 seconds		<5	<0.70	>99.99%	>4.44
	60 seconds		<5	<0.70	>99.99%	>4.44
	90 seconds		<5	<0.70	>99.99%	>4.44
Streptococcus pyogenes (ATCC 19615)	15 seconds	3.1×10^6 (6.49)	<5	<0.70	>99.999%	>5.79
	30 seconds		<5	<0.70	>99.999%	>5.79
	60 seconds		<5	<0.70	>99.999%	>5.79
	90 seconds		<5	<0.70	>99.999%	>5.79

CFU = Colony Forming Units

Note: *For samples with a "<" value sign, a value of <1 was used in place of zero for calculation purposes. For these samples with a "<" value sign, no growth was observed on the duplicate test plates at the lowest dilution plated. The zeros were added together to increase the sensitivity of the test and a value of 2 mL plated was used in the calculation. The limit of detection of this test is a value of <5 CFU/mL.*

Attachment I: Sponsor Test Material Certificate of Analysis

Issued: July 16, 2013
Last Revised: July 29, 2013

FORM-COA-02

AQUAOX INDUSTRIES INC
16155, Sierra Lakes Parkway,
Suite 160-714,
Fontana, CA 92336, USA.

Certificate of Analysis

Date of Manufacture:	07 / 15 / 2013
Product Name:	AX250
Batch / Lot #:	AX-13196-0210
Production Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Testing Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377

TEST	ANALYSIS	UNITS
FAC	226	ppm
pH	6.03	n/a
Conductivity	1225	µS/cm
ORP	943	mV
Osmolality	22	mOsm/kg

This certification states that the intermediate product AX250, bearing the above description and lot number, has been found to conform to the internal specifications established for this product. The above lot was made in accordance with our internal specifications and current good manufacturing practices under controlled procedures.

This lot has been appropriately inspected and tested, and, to the best of our knowledge, conforms to all applicable test methods, standards and internal specifications.

This certification does not constitute any written or expressed warranty or guarantee of any kind.

Rebecca Lei
QA Regulatory Specialist

Date: 7/29/13

Page 1 of 1

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AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	October 14, 2013
Sponsor:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Time Kill Assay For Antimicrobial Agents
ATS Labs Protocol Number:	INI01091613.TK.3
ATS Labs Project Number:	A15669

Modifications to Protocol:

Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

Changes to the protocol are acceptable as noted.

Study Director _____ Date 10/14/13

EXACT COPY
INITIALS _____ DATE 11-7-13

(For Laboratory Use Only)
ATS Labs Project # A15669
KAS 10-4-13

ATS LABS

PROTOCOL

Time Kill Assay For Antimicrobial Agents

Test Organisms:

Bacteroides fragilis (ATCC 25285)
Haemophilus influenzae (ATCC 10211)
Streptococcus pyogenes (ATCC 19615)

PROTOCOL NUMBER

INI01091613.TK.3

PREPARED FOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PERFORMING LABORATORY

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

PREPARED BY

Anne Stemper, B.S.
Senior Microbiologist

DATE

September 16, 2013

1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax 651.379.5548

EXACT COPY
INITIALS DM DATE 11/7/13

Time Kill Assay For Antimicrobial Agents

SPONSOR: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

PURPOSE
The objective of this testing is to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

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. 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 September 24, 2013. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of October 21, 2013. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

A "case-by-case" approach is generally taken by the regulatory authorities and cannot be over-emphasized when considering a testing regimen. While this protocol is based upon our experience in the field of germicidal testing, and the current regulatory guidelines, each product presents a different set of issues to the regulatory authorities. We recommend that you consult with the appropriate agency before finalizing your testing regimen, as ATS Labs cannot guarantee acceptance of this protocol by the regulating authorities.

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 nor 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 regulating 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
Analyzing the efficacy of antimicrobial agents may be performed by various suspension and susceptibility methods. This study is designed to examine the rate-of-kill of a test substance against a pure test culture. This is accomplished by exposing the test culture to the test substance and assaying for survivors following a variety of exposure times. The experimental design in this protocol meets these requirements.

TEST PRINCIPLE

A suspension of the test organism is exposed to the test substance for specified exposure times. After exposure, an aliquot of the suspension is transferred to a neutralizer and assayed for survivors. Appropriate culture purity, sterility, population and neutralization confirmation controls are performed. The current version of Standard Operating Procedure CGT-4130 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Culture Medium	Incubation Parameters
Bacteroides fragilis	25285	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, anaerobic
Haemophilus influenzae	10211	Chocolate agar	35-37°C in CO_2
Streptococcus pyogenes	19615	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C in CO_2

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

Preparation of Test Organism

From a stock plate or stock slant culture, streak a culture of each test organism onto the culture medium listed above. This represents the second culture transfer. Incubate the second culture transfer for 1-5 days at the incubation parameters listed above. (Alternate or extended incubation may be required for certain strains). Transfer a sufficient amount of organism growth into a sterile diluent to create a uniform suspension targeting approximately 1 x 10^8 CFU/mL or greater where possible. This may be achieved by comparison to McFarland standards, by spectrophotometric means or by any other appropriate method.

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

Preparation of Test Substance

The test substance to be tested is prepared according to the directions supplied 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. A 9.5 mL aliquot of the prepared test substance will be transferred to a sterile vessel (glass tube, stomacher bag, etc.) for testing procedures. If necessary, 9.5 g of test substance may be used. Multiple replicate vessels may be set up if requested.

Exposure Conditions

A 0.5 mL aliquot of the standardized inoculum will be added to the test substance representing the start of the test exposure. The inoculated test substance will be immediately mixed thoroughly using a vortex mixer, stirring with a pipette or by any other applicable method. The inoculated and mixed test substance will be held at the Sponsor specified temperature. If the requested exposure temperature lies outside of achievable ambient conditions, the test substance may be placed in a water bath (or other appropriate device) to equilibrate to the desired exposure temperature prior to testing. For very short exposure times or exposure times which are close together, individual test substance vessels may be utilized where necessary.

Test System Recovery

At each Sponsor specified exposure time, the sample will be mixed and a 0.1 mL aliquot of the inoculated test substance will be transferred to 9.9 mL of neutralizer broth (10^0 dilution). Additional ten-fold serial dilutions will be prepared in Butterfield's buffer. Using a standard microbiological spread plate count procedure, 1.0 mL aliquots of the $10^0 - 10^{-4}$ dilutions will be plated in duplicate.

If swarming is a concern, 1.0 mL of 10^0 will be plated in duplicate. In addition, 0.1 mL of $10^0 - 10^{-3}$ will be plated in duplicate.

Incubation and Observation
Bacteroides fragilis plates are incubated for 2-5 days at 35-37°C under anaerobic conditions. *Haemophilus influenzae* plates are incubated for 2-5 days at 35-37°C in CO_2. *Streptococcus pyogenes* plates are incubated for 2-3 days at 35-37°C in CO_2. Alternate or extended incubation may be required for certain strains.

Following incubation, the subcultures will be visually examined for growth and enumerated. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination. Log_{10} and percent reductions will be determined for each time point. Representative subcultures demonstrating growth may 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
If applicable, 1.0 mL of the serum used for soil load will be added to a tube of Fluid Thioglycollate, incubated, and observed for lack of growth. The acceptance criterion for this study control is lack of growth.

Neutralizer Sterility Control
A 1.0 mL aliquot of the neutralizer will be plated as in the test and incubated. The acceptance criterion for this study control is lack of growth.

Test Population Control
In a similar manner as the culture inoculum is added to the test substance, add an equivalent volume of inoculum (0.5 mL) to 9.5 mL Butterfield's buffer (or the same volume as the test substance). This suspension will be neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. If requested, the sample may be exposed as in the test and evaluated at an additional time point. (If requested, the final time point is recommended.) The suspension will be serially diluted and appropriate dilutions plated using standard microbiological techniques. *If swarming is a concern, 0.1 mL aliquots will be plated.*

Following incubation, the organism plates will be observed and enumerated. If more than one time point is evaluated, the geometric mean will be determined prior to reduction calculations. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of test substance will be neutralized as in the test procedure. Only the most concentrated test substance needs to be evaluated in this control. Remove and discard 1.0 mL of the neutralized sample. To the neutralized sample, add 1.0 mL of an organism suspension to target approximately 100-1000 CFU per mL of neutralizer and vortex mix. Plate, in duplicate, 1.0 mL of neutralized mixture to appropriate recovery agar and incubate. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 1.0 mL aliquots, in duplicate, and incubate. This control may be performed prior to or concurrent with testing.

NOTE: If swarming is a concern, add 1.0 mL of an organism suspension containing 1000-10,000 CFU/mL and vortex mix. Plate, in duplicate, 0.1 mL of the neutralized mixture. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 0.1 mL aliquots, in duplicate.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log.

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 test organism 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 subcultures, etc. during the course of the test. Test subcultures 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

This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results will be expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

Control Acceptance Criteria

The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol.

REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism 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.

TEST SUBSTANCE RETENTION

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. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

DATA ANALYSIS

Calculations

CFU/mL = $\frac{\text{(average CFU) x (dilution factor) x (volume neutralized solution in mL)}}{\text{(volume plated in mL)}}$

A value of <1 may be used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros may be added together to increase the sensitivity of the test. (A value of 2 mL plated is used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

If applicable, the geometric mean value for the population control will be determined and used to calculate percent reduction if multiple time points are evaluated in the control. The geometric mean value of the test results will be determined and used to calculate percent reduction if more than one replicate is performed.

Geometric mean = Antilog of $\frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

If applicable, the average log_{10} value for the population control will be determined and used to calculate log_{10} reduction if multiple time points are evaluated in the control. The average log_{10} value of the test results will be determined and used to log_{10} reduction if more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

Statistical Analysis: None used.

Study Information

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number - exactly as it should appear on final report):
AX250 Batch # AX-13196-0210

Expiration Date: 07/2015

Test Substance Active Concentration (upon submission to ATS Labs): 0.024% HOCl

Product Description:

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

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:

_____ defined as _____ + _____
(example: 1 oz/gallon) (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.

Exposure Times: 15 seconds, 30 seconds, 60 seconds, and 90 seconds

Number of Test Replicate(s) per sample: 1

Exposure Temperature:

- ☑ Ambient
- ☐ Other _____

Organic Soil Load:

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

Test Organisms:

- ☑ *Bacteroides fragilis* (ATCC 25285)
- ☑ *Haemophilus influenzae* (ATCC 10211)
- ☑ *Streptococcus pyogenes* (ATCC 19615)

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: 7/11/13 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

(1) ☑ ~~Approved without modification~~
(1) ☑ Approved with modification
To confirm ~~at 5~~ * growth/identity of Bacteroides fragilis, it is a gram negative bacilli, indole negative and ~~catalase~~ catalase positive.
* cc VM 9-25-13 (1) added per email VM 9-25-13

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Dr. Fred Ma TITLE: M.D., Ph.D. Chief Medical Officer

SIGNATURE: DR. Fred Ma DATE: 09/17/13

PHONE: (909) 822 - 6000 FAX: _____ EMAIL: fma@Innovacyn.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

Hannah Carroll (hannahc@Innovacyn.com)

ATS Labs:

NAME: Jill Ruhnke
Study Director

SIGNATURE: Jill Ruhnke DATE: 10-3-13
Study Director

FINAL STUDY REPORT

STUDY TITLE

Time Kill Assay For Antimicrobial Agents

Test Organisms:

Staphylococcus epidermidis (ATCC 12228)
Staphylococcus haemolyticus (ATCC 29970)
Staphylococcus hominis (ATCC 25615)
Staphylococcus saprophyticus (ATCC 15305)

PRODUCT IDENTITY

AX250
Batch # AX-13196-0210

AUTHOR

Gracia Schroeder, B.S.
Study Director

STUDY COMPLETION DATE

November 6, 2013

PERFORMING LABORATORY

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

SPONSOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PROJECT NUMBER

A15629

EXACT COPY
INITIALS KT DATE 11-6-13

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

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:_____

Sponsor:_____ Date:_____

Study Director: Gracia Schroeder, B.S. Date: 11/10/13

QUALITY ASSURANCE UNIT SUMMARY

Study: Time Kill Assay For Antimicrobial Agents

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 (21 CFR Part 58) 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 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	September 30, 2013	September 30, 2013	September 30, 2013
Draft Report	October 10, 2013	October 10, 2013	October 11, 2013
Final Report	November 6, 2013	November 6, 2013	November 6, 2013

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

Quality Assurance Auditor: Judy Heidemann Date: 11-6-13

TABLE OF CONTENTS

STUDY PERSONNEL

STUDY DIRECTOR:	Gracia Schroeder, B.S.
Professional personnel involved:	
Scott R. Steinagel, B.S.	- Director, Microbiology Operations
Becky Lien, B.A.	- Manager, Microbiology Operations
Peter Toll, B.S.	- Supervisor, Microbiology Laboratory Operations
Matthew Sathe, B.S.	- Senior Microbiologist
Philip Lange, B.S.	- Associate Microbiologist
Kristen Niehaus, B.A.	- Microbiologist
Nicole Zroka, B.A.	- Associate Microbiologist

STUDY REPORT

GENERAL STUDY INFORMATION

Protocol Title: Time Kill Assay For Antimicrobial Agents

Project Number: A15629

Protocol Number: INI01091613.TK.4

Sponsor: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

TEST SUBSTANCE IDENTITY

Test Substance Name: AX250

Batch Number: Batch # AX-13196-0210

Test Substance Characterization
Test substance characterization as to content, stability, etc., (21 CFR Part 58, Subpart F [58.105]) is the responsibility of the Sponsor. The Sponsor Test Material Certificate of Analysis Report may be found in Attachment I.

STUDY DATES

Date Sample Received: September 11, 2013
Study Initiation Date: September 24, 2013
Experimental Start Date: September 30, 2013
Experimental End Date: October 2, 2013
Study Completion Date: November 6, 2013

OBJECTIVE

The objective of this testing was to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

SUMMARY OF RESULTS

Test Substance:	AX250 (Batch # AX-13196-0210)
Dilution:	Ready to use (RTU)
Test Organism:	*Staphylococcus epidermidis* (ATCC 12228) *Staphylococcus haemolyticus* (ATCC 29970) *Staphylococcus hominis* (ATCC 25615) *Staphylococcus saprophyticus* (ATCC 15305)
Exposure Times:	15 seconds, 30 seconds, 60 seconds, and 90 seconds
Exposure Temperature:	Ambient Temperature (22°C)
Efficacy Result:	AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.08 $\log_{10}$) reduction of *Staphylococcus epidermidis* (ATCC 12228) survivors following a 15 second exposure, a >99.999% (>5.08 $\log_{10}$) reduction of *Staphylococcus epidermidis* (ATCC 12228) survivors following a 30 second exposure, a >99.999% (>5.08 $\log_{10}$) reduction of *Staphylococcus epidermidis* (ATCC 12228) survivors following a 60 second exposure and a >99.999% (>5.08 $\log_{10}$) reduction of *Staphylococcus epidermidis* (ATCC 12228) survivors following a 90 second exposure when tested at ambient temperature (22°C). AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.01 $\log_{10}$) reduction of *Staphylococcus haemolyticus* (ATCC 29970) survivors following a 15 second exposure, a >99.999% (>5.01 $\log_{10}$) reduction of *Staphylococcus haemolyticus* (ATCC 29970) survivors following a 30 second exposure, a >99.999% (>5.01 $\log_{10}$) reduction of *Staphylococcus haemolyticus* (ATCC 29970) survivors following a 60 second exposure and a >99.999% (>5.01 $\log_{10}$) reduction of *Staphylococcus haemolyticus* (ATCC 29970) survivors following a 90 second exposure when tested at ambient temperature (22°C). AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.32 $\log_{10}$) reduction of *Staphylococcus hominis* (ATCC 25615) survivors following a 15 second exposure, a >99.999% (>5.32 $\log_{10}$) reduction of *Staphylococcus hominis* (ATCC 25615) survivors following a 30 second exposure, a >99.999% (>5.32 $\log_{10}$) reduction of *Staphylococcus hominis* (ATCC 25615) survivors following a 60 second exposure and a >99.999% (>5.32 $\log_{10}$) reduction of *Staphylococcus hominis* (ATCC 25615) survivors following a 90 second exposure when tested at ambient temperature (22°C).

Efficacy Result (continued):

AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.15 $\log_{10}$) reduction of *Staphylococcus saprophyticus* (ATCC 15305) survivors following a 15 second exposure, a >99.999% (>5.15 $\log_{10}$) reduction of *Staphylococcus saprophyticus* (ATCC 15305) survivors following a 30 second exposure, a >99.999% (>5.15 $\log_{10}$) reduction of *Staphylococcus saprophyticus* (ATCC 15305) survivors following a 60 second exposure and a >99.999% (>5.15 $\log_{10}$) reduction of *Staphylococcus saprophyticus* (ATCC 15305) survivors following a 90 second exposure when tested at ambient temperature (22°C).

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Culture Medium	Incubation Parameters
Staphylococcus epidermidis	12228	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Staphylococcus haemolyticus	29970	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Staphylococcus hominis	25615	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Staphylococcus saprophyticus	15305	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic

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

Recovery Media

Neutralizer: Letheen Broth + 0.1% Sodium Thiosulfate
Agar Plate Medium: Tryptic Soy Agar + 5% Sheep Blood Agar

TEST METHOD

Preparation of Test Organism

Using a stock slant, each test organism culture was streaked onto an appropriate growth medium. The bacterial cultures were incubated for two days at 35-37°C.

On the day of test, a sufficient amount of organism growth was transferred into Butterfield's Buffer to create a uniform suspension targeting approximately 1 x 10^8 CFU/mL where possible. *Staphylococcus epidermidis* and *Staphylococcus hominis* were adjusted to a 3.0 McFarland Turbidity Standard. *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus* were adjusted to a 2.0 McFarland Turbidity Standard.

Preparation of Test Substance
The test substance was ready to use (RTU), as received from the Sponsor. A 9.5 mL aliquot of the test substance was transferred to a sterile vessel for use in testing. The test substance was homogenous as determined by visual observation.

One replicate sample was set up and evaluated.

Exposure Conditions
A 0.50 mL aliquot of each standardized inoculum was added to 9.5 mL test substance representing the start of the test exposure. The inoculated test substance was immediately mixed thoroughly using a vortex mixer. Each inoculated and mixed test substance was exposed for the exposure times of 15 seconds, 30 seconds, 60 seconds, and 90 seconds at ambient temperature (22°C).

Test System Recovery
At each Sponsor specified exposure time, each sample was mixed and a 0.100 mL aliquot of the inoculated test substance was transferred to 9.9 mL of neutralizer representing a 10^0 dilution. Additional ten-fold serial dilutions were prepared from the 10^0 neutralized material in Butterfield's Buffer.

Using standard microbiological spread plate procedures, 1.00 mL aliquots of the 10^0 dilution and 0.100 mL aliquots of the 10^0-10^{-3} dilutions were plated in duplicate on appropriate recovery medium for each test organism.

Incubation and Observation
The bacterial subculture plates were incubated for 24-48 hours at 35-37°C. Following incubation, the agar plates were visually examined for the presence of growth and enumerated. Log_{10} and percent reductions were determined for each exposure time.

STUDY 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.

Neutralizer Sterility Control
A 1.00 mL aliquot of the neutralizer was plated as in the test and incubated. The acceptance criterion for this study control is a lack of growth.

Test Population Control
In a similar manner as the culture inoculum was added to the test substance, an equivalent volume of inoculum (0.50 mL) was added to 9.5 mL Butterfield's buffer). This suspension was neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. The suspension was serially diluted and appropriate dilutions were plated using standard microbiological techniques and 0.100 mL aliquots. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of the test substance was neutralized as in the test procedure. A 1.00 mL aliquot of the neutralized sample was then removed and discarded. To the neutralized sample, 1.00 mL of each organism suspension containing approximately 1000-10,000 CFU/mL was added and the suspension was vortex mixed. A 0.100 mL aliquot of the neutralized mixture was plated in duplicate on appropriate recovery agar and incubated. A numbers control was performed by adding 1.00 mL of the same organism suspension to 9.0 mL of untreated neutralizer. A 0.100 mL aliquot was plated in duplicate and incubated.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log. The most appropriate dilution was reported.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results are expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

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

PROTOCOL CHANGES

Protocol Amendments:
Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{\text{(average CFU) x (dilution factor) x (volume neutralized solution in mL)}}{\text{(volume plated in mL)}}$$

A value of <1 was used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros were added together to increase the sensitivity of the test. (A value of 2 mL plated was used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

The geometric mean value for the population control was determined and used to calculate percent reduction as multiple time points were evaluated in the control.

The geometric mean value of the test results were determined and used to calculate percent reduction as more than one replicate is performed.

$$\text{Geometric mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

The average $\log_{10}$ value for the population control was determined and used to calculate $\log_{10}$ reduction as multiple time points are evaluated in the control.

The average $\log_{10}$ value of the test results was determined and used to calculate $\log_{10}$ reduction as more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

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 discarded following study completion. It is the responsibility of the Sponsor to retain a sample of the test substance.

REFERENCES

1. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

RESULTS

For Control and Neutralization Results, see Tables 1-3.
All data measurements/controls including culture purity, neutralizer sterility, test population control, and neutralization confirmation controls performed within acceptance criteria.

For Test Results, see Tables 4-5.

ANALYSIS AND STUDY CONCLUSION

AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.08 log_{10}) reduction of *Staphylococcus epidermidis* (ATCC 12228) survivors following a 15 second exposure, a >99.999% (>5.08 log_{10}) reduction of *Staphylococcus epidermidis* (ATCC 12228) survivors following a 30 second exposure, a >99.999% (>5.08 log_{10}) reduction of *Staphylococcus epidermidis* (ATCC 12228) survivors following a 60 second exposure and a >99.999% (>5.08 log_{10}) reduction of *Staphylococcus epidermidis* (ATCC 12228) survivors following a 90 second exposure when tested at ambient temperature (22°C).

AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.01 log_{10}) reduction of *Staphylococcus haemolyticus* (ATCC 29970) survivors following a 15 second exposure, a >99.999% (>5.01 log_{10}) reduction of *Staphylococcus haemolyticus* (ATCC 29970) survivors following a 30 second exposure, a >99.999% (>5.01 log_{10}) reduction of *Staphylococcus haemolyticus* (ATCC 29970) survivors following a 60 second exposure and a >99.999% (>5.01 log_{10}) reduction of *Staphylococcus haemolyticus* (ATCC 29970) survivors following a 90 second exposure when tested at ambient temperature (22°C).

AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.32 log_{10}) reduction of *Staphylococcus hominis* (ATCC 25615) survivors following a 15 second exposure, a >99.999% (>5.32 log_{10}) reduction of *Staphylococcus hominis* (ATCC 25615) survivors following a 30 second exposure, a >99.999% (>5.32 log_{10}) reduction of *Staphylococcus hominis* (ATCC 25615) survivors following a 60 second exposure and a >99.999% (>5.32 log_{10}) reduction of *Staphylococcus hominis* (ATCC 25615) survivors following a 90 second exposure when tested at ambient temperature (22°C).

AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.15 log_{10}) reduction of *Staphylococcus saprophyticus* (ATCC 15305) survivors following a 15 second exposure, a >99.999% (>5.15 log_{10}) reduction of *Staphylococcus saprophyticus* (ATCC 15305) survivors following a 30 second exposure, a >99.999% (>5.15 log_{10}) reduction of *Staphylococcus saprophyticus* (ATCC 15305) survivors following a 60 second exposure and a >99.999% (>5.15 log_{10}) reduction of *Staphylococcus saprophyticus* (ATCC 15305) survivors following a 90 second exposure when tested at ambient temperature (22°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
Purity Control	*Staphylococcus epidermidis* (ATCC 12228)	Pure
	Staphylococcus haemolyticus (ATCC 29970)	Pure
	Staphylococcus hominis (ATCC 25615)	Pure
	Staphylococcus saprophyticus (ATCC 15305)	Pure
Neutralizer Sterility Control		No Growth

TABLE 2: TEST POPULATION CONTROL RESULTS

Test Organism	Results	
	CFU/mL	Log_{10}
Staphylococcus epidermidis (ATCC 12228)	6.0×10^5	5.78
Staphylococcus haemolyticus(ATCC 29970)	5.1×10^5	5.71
Staphylococcus hominis (ATCC 25615)	1.04×10^6	6.02
Staphylococcus saprophyticus (ATCC 15305)	7.0×10^5	5.85

CFU = Colony Forming Units

Note: *The highest challenge level was achieved for this control based on the use of standard propagation methods.*

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Neutralization Confirmation (CFU)		Pass/Fail ± 1 log_{10} (Log_{10} Difference)
		Numbers Control	Test Substance Results	
AX250 Batch # AX-13196-0210	*Staphylococcus epidermidis* (ATCC 12228)	10, 11	13, 15	Pass (-0.11)
	Staphylococcus haemolyticus (ATCC 29970)	18, 9	13, 14	Pass (0.00)
	Staphylococcus hominis (ATCC 25615)	30, 38	30, 20	Pass (0.13)
	Staphylococcus saprophyticus (ATCC 15305)	24, 16	10, 14	Pass (0.22)

CFU = Colony Forming Units

TABLE 4: TEST RESULTS FOR AX250 Batch # AX-13196-0210

DILUTION (VOLUME PLATED)	Test Organism: *Staphylococcus epidermidis* (ATCC 12228)			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: *Staphylococcus haemolyticus* (ATCC 29970)**			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: *Staphylococcus hominis* (ATCC 25615)**			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: *Staphylococcus saprophyticus* (ATCC 15305)**			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0

* Indicates dilution used for calculation purposes.

TABLE 5: CALCULATED DATA FOR AX250 Batch # AX-13196-0210

Test Organism	Exposure Time	CFU/mL in Test Population Control (Log_{10})	CFU/mL of Survivors	Log_{10} Survivors	Percent Reduction	Log_{10} Reduction
Staphylococcus epidermidis (ATCC 12228)	15 seconds	6.0×10^5 (5.78)	<5	<0.70	>99.999%	>5.08
	30 seconds		<5	<0.70	>99.999%	>5.08
	60 seconds		<5	<0.70	>99.999%	>5.08
	90 seconds		<5	<0.70	>99.999%	>5.08
Staphylococcus haemolyticus (ATCC 29970)	15 seconds	5.1×10^5 (5.71)	<5	<0.70	>99.999%	>5.01
	30 seconds		<5	<0.70	>99.999%	>5.01
	60 seconds		<5	<0.70	>99.999%	>5.01
	90 seconds		<5	<0.70	>99.999%	>5.01
Staphylococcus hominis (ATCC 25615)	15 seconds	1.04×10^6 (6.02)	<5	<0.70	>99.999%	>5.32
	30 seconds		<5	<0.70	>99.999%	>5.32
	60 seconds		<5	<0.70	>99.999%	>5.32
	90 seconds		<5	<0.70	>99.999%	>5.32
Staphylococcus saprophyticus (ATCC 15305)	15 seconds	7.0×10^5 (5.85)	<5	<0.70	>99.999%	>5.15
	30 seconds		<5	<0.70	>99.999%	>5.15
	60 seconds		<5	<0.70	>99.999%	>5.15
	90 seconds		<5	<0.70	>99.999%	>5.15

CFU = Colony Forming Units

Note: *No growth was observed on the duplicate test plates at the lowest dilution plated. The zeros were added together to increase the sensitivity of the test and a value of 2 mL plated was used in the calculation. The limit of detection of this test is a value of <5 CFU/mL.*

Attachment I: Sponsor Test Material Certificate of Analysis - Batch AX-13196-0210

Issued: July 16, 2013
Last Revised: September 10, 2013

FORM-COA-02

AQUAOX INDUSTRIES INC
16155, Sierra Lakes Parkway,
Suite 160-714,
Fontana, CA 92336, USA.

Certificate of Analysis

Date of Manufacture:	07 / 15 / 2013
Product Name:	AX250
Batch / Lot #:	AX-13196-0210
Production Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Testing Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377

TEST	ANALYSIS	UNITS
FAC	207	ppm
pH	5.91	n/a
Conductivity	1230	µS/cm
ORP	966	mV
Osmolality	22	mOsm/kg

This certification states that the intermediate product AX250, bearing the above description and lot number, has been found to conform to the internal specifications established for this product. The above lot was made in accordance with our internal specifications and current good manufacturing practices under controlled procedures.

This lot has been appropriately inspected and tested, and, to the best of our knowledge, conforms to all applicable test methods, standards and internal specifications.

This certification does not constitute any written or expressed warranty or guarantee of any kind.

Rebecca Lei
QA Regulatory Specialist

Date: 9/10/13

EXACT COPY
INITIALS JM DATE 11/6/13

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	10/10/13
Sponsor:	Innovacyn, Inc. 3546 N. Riverside Ave, Rialto, CA 92377
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Time Kill Assay For Antimicrobial Agents
ATS Labs Protocol Number:	INI01091613.TK.4
ATS Labs Project Number:	A15629

Modifications to Protocol:

Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

Changes to the protocol are acceptable as noted.

Study Director

10/10/13
Date

EXACT COPY
INITIALS [illegible] DATE 11/6/13

(For Laboratory Use Only)
ATS Labs Project # A15629 9-25-13

PROTOCOL

Time Kill Assay For Antimicrobial Agents

Test Organisms:

Staphylococcus epidermidis (ATCC 12228)
Staphylococcus haemolyticus (ATCC 29970)
Staphylococcus hominis (ATCC 25615)
Staphylococcus saprophyticus (ATCC 15305)

PROTOCOL NUMBER

INI01091613.TK.4

PREPARED FOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PERFORMING LABORATORY

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

PREPARED BY

Anne Stemper, B.S.
Senior Microbiologist

DATE

September 16, 2013

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

EXACT COPY
INITIALS CMS DATE 11/01/13

Time Kill Assay For Antimicrobial Agents

SPONSOR: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

PURPOSE
The objective of this testing is to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

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. 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 September 24, 2013. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of October 21, 2013. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

A "case-by-case" approach is generally taken by the regulatory authorities and cannot be over-emphasized when considering a testing regimen. While this protocol is based upon our experience in the field of germicidal testing, and the current regulatory guidelines, each product presents a different set of issues to the regulatory authorities. We recommend that you consult with the appropriate agency before finalizing your testing regimen, as ATS Labs cannot guarantee acceptance of this protocol by the regulating authorities.

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 nor 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 regulating 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
Analyzing the efficacy of antimicrobial agents may be performed by various suspension and susceptibility methods. This study is designed to examine the rate-of-kill of a test substance against a pure test culture. This is accomplished by exposing the test culture to the test substance and assaying for survivors following a variety of exposure times. The experimental design in this protocol meets these requirements.

TEST PRINCIPLE

A suspension of the test organism is exposed to the test substance for specified exposure times. After exposure, an aliquot of the suspension is transferred to a neutralizer and assayed for survivors. Appropriate culture purity, sterility, population and neutralization confirmation controls are performed. The current version of Standard Operating Procedure CGT-4130 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Culture Medium	Incubation Parameters
Staphylococcus epidermidis	12228	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Staphylococcus haemolyticus	29970	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Staphylococcus hominis	25615	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Staphylococcus saprophyticus	15305	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic

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

Preparation of Test Organism

From a stock plate or stock slant culture, streak a culture of each test organism onto the culture medium listed above. This represents the second culture transfer. Incubate the second culture transfer for 1-5 days at the incubation parameters listed above. (Alternate or extended incubation may be required for certain strains). Transfer a sufficient amount of organism growth into a sterile diluent to create a uniform suspension targeting approximately 1 x 10^8 CFU/mL or greater where possible. This may be achieved by comparison to McFarland standards, by spectrophotometric means or by any other appropriate method.

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

Preparation of Test Substance

The test substance to be tested is prepared according to the directions supplied 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. A 9.5 mL aliquot of the prepared test substance will be transferred to a sterile vessel (glass tube, stomacher bag, etc.) for testing procedures. If necessary, 9.5 g of test substance may be used. Multiple replicate vessels may be set up if requested.

Exposure Conditions

A 0.5 mL aliquot of the standardized inoculum will be added to the test substance representing the start of the test exposure. The inoculated test substance will be immediately mixed thoroughly using a vortex mixer, stirring with a pipette or by any other applicable method. The inoculated and mixed test substance will be held at the Sponsor specified temperature. If the requested exposure temperature lies outside of achievable ambient conditions, the test substance may be placed in a water bath (or other appropriate device) to equilibrate to the desired exposure temperature prior to testing. For very short exposure times or exposure times which are close together, individual test substance vessels may be utilized where necessary.

Test System Recovery

At each Sponsor specified exposure time, the sample will be mixed and a 0.1 mL aliquot of the inoculated test substance will be transferred to 9.9 mL of neutralizer broth (10^0 dilution). Additional ten-fold serial dilutions will be prepared in Butterfield's buffer. Using a standard microbiological spread plate count procedure, 1.0 mL aliquots of the 10^0 – 10^{-4} dilutions will be plated in duplicate.

If swarming is a concern, 1.0 mL of 10^0 will be plated in duplicate. In addition, 0.1 mL of 10^0 – 10^{-3} will be plated in duplicate.

Incubation and Observation
All bacterial subculture plates are incubated for 24-48 hours at 35-37°C. Alternate or extended incubation may be required for certain strains.

Following incubation, the subcultures will be visually examined for growth and enumerated. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination. Log_{10} and percent reductions will be determined for each time point. Representative subcultures demonstrating growth may 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
If applicable, 1.0 mL of the serum used for soil load will be added to a tube of Fluid Thioglycollate, incubated, and observed for lack of growth. The acceptance criterion for this study control is lack of growth.

Neutralizer Sterility Control
A 1.0 mL aliquot of the neutralizer will be plated as in the test and incubated. The acceptance criterion for this study control is lack of growth.

Test Population Control
In a similar manner as the culture inoculum is added to the test substance, add an equivalent volume of inoculum (0.5 mL) to 9.5 mL Butterfield's buffer (or the same volume as the test substance). This suspension will be neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. If requested, the sample may be exposed as in the test and evaluated at an additional time point. (If requested, the final time point is recommended.) The suspension will be serially diluted and appropriate dilutions plated using standard microbiological techniques. *If swarming is a concern, 0.1 mL aliquots will be plated.*

Following incubation, the organism plates will be observed and enumerated. If more than one time point is evaluated, the geometric mean will be determined prior to reduction calculations. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of test substance will be neutralized as in the test procedure. Only the most concentrated test substance needs to be evaluated in this control. Remove and discard 1.0 mL of the neutralized sample. To the neutralized sample, add 1.0 mL of an organism suspension to target approximately 100-1000 CFU per mL of neutralizer and vortex mix. Plate, in duplicate, 1.0 mL of neutralized mixture to appropriate recovery agar and incubate. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 1.0 mL aliquots, in duplicate, and incubate. This control may be performed prior to or concurrent with testing.

NOTE: If swarming is a concern, add 1.0 mL of an organism suspension containing 1000-10,000 CFU/mL and vortex mix. Plate, in duplicate, 0.1 mL of the neutralized mixture. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 0.1 mL aliquots, in duplicate.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log.

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 test organism 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 subcultures, etc. during the course of the test. Test subcultures 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

This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results will be expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

Control Acceptance Criteria

The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol.

REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism 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.

TEST SUBSTANCE RETENTION

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. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbicidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

DATA ANALYSIS

Calculations

CFU/mL = $\frac{\text{(average CFU) x (dilution factor) x (volume neutralized solution in mL)}}{\text{(volume plated in mL)}}$

A value of <1 may be used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros may be added together to increase the sensitivity of the test. (A value of 2 mL plated is used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

If applicable, the geometric mean value for the population control will be determined and used to calculate percent reduction if multiple time points are evaluated in the control. The geometric mean value of the test results will be determined and used to calculate percent reduction if more than one replicate is performed.

Geometric mean = Antilog of $\frac{Log_{10}X_1 + Log_{10}X_2 + Log_{10}X_N}{N}$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

If applicable, the average log_{10} value for the population control will be determined and used to calculate log_{10} reduction if multiple time points are evaluated in the control. The average log_{10} value of the test results will be determined and used to log_{10} reduction if more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

Statistical Analysis: None used.

Study Information

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number - exactly as it should appear on final report):
AX250 Batch # AX-13196-0210

Expiration Date: 07/2015

Test Substance Active Concentration (upon submission to ATS Labs): 0.024% HOCl

Product Description:
- ☐ Quaternary ammonia
- ☐ Iodophor
- ☑ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☑ Other Hypochlorous acid

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:

_____ defined as _____ ÷ _____
(example: 1 oz/gallon) (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.

Exposure Times: 15 seconds, 30 seconds, 60 seconds, and 90 seconds

Number of Test Replicate(s) per sample: 1

Exposure Temperature:
- ☑ Ambient
- ☐ Other _____

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

Test Organisms:
- ☑ *Staphylococcus epidermidis* (ATCC 12228)
- ☑ *Staphylococcus haemolyticus* (ATCC 29970)
- ☑ *Staphylococcus saprophyticus* (ATCC 15305)
- ☑ *Staphylococcus hominis* (ATCC 25615)

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: 7/11/13 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

* Staphylococcus hominus: colony morphology- medium- large, smooth, opaque, butyrous. cream- yellow/orange. Gram positive cocci. Biochemical tests: latex agglutination: negative, catalase positive.

* Staphylococcus saprophyticus: colony morphology- large, very glossy, smooth, opaque, convex. usually white but can be yellow or orange. Gram positive cocci. Biochemical tests: catalase: positive latex agglutination: negative

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Dr. Fred Ma TITLE: M.D., Ph.D. Chief Medical Officer

SIGNATURE: Dr. Fred Ma DATE: 09/17/13

PHONE: (909) 822 - 6000 FAX: _____ EMAIL: fma@innovacyn.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

Hannah Carroll (hannahc@innovacyn.com)

ATS Labs:

NAME: Gracia Schroeder
Study Director

SIGNATURE: Gracia M Schr DATE: 9/24/13
Study Director

*added organism confirmation procedures for those not in the SOP qms 9/24/13

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

FINAL STUDY REPORT

STUDY TITLE

Time Kill Assay For Antimicrobial Agents

Test Organisms:

Enterobacter aerogenes (ATCC 13048)
Escherichia coli (ATCC 8739)
Klebsiella pneumoniae (ATCC 4352)
Micrococcus luteus (ATCC 49732)
Proteus mirabilis (ATCC 9240)
Pseudomonas aeruginosa (ATCC 9027)
Serratia marcescens (ATCC 13880)

PRODUCT IDENTITY

AX250
Batch # AX-13196-0210

AUTHOR

Gracia Schroeder, B.S.
Study Director

STUDY COMPLETION DATE

November 6, 2013

PERFORMING LABORATORY

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

SPONSOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PROJECT NUMBER

A15630

EXACT COPY
INITIALS KT DATE 11-6-13

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with the U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

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 compounds.

Submitter:_____ Date:_____

Sponsor:_____ Date:_____

Study Director: Gracia Schroeder, B.S. Date: 11/12/13

QUALITY ASSURANCE UNIT SUMMARY

Study: Time Kill Assay For Antimicrobial Agents

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 (21 CFR Part 58) 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 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	October 1, 2013	October 1, 2013	October 2, 2013
Draft Report	October 14, 2013	October 14, 2013	October 17, 2013
Final Report	November 6, 2013	November 6, 2013	November 6, 2013

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

Quality Assurance Auditor: Judy Heidemann Date: 11-6-13

TABLE OF CONTENTS

STUDY PERSONNEL

STUDY DIRECTOR:	Gracia Schroeder, B.S.
Professional personnel involved:	
Scott R. Steinagel, B.S.	- Director, Microbiology Operations
Becky Lien, B.A.	- Manager, Microbiology Operations
Peter Toll, B.S.	- Supervisor, Microbiology Laboratory Operations
Anne Stemper, B.S.	- Senior Microbiologist
Matthew Sathe, B.S.	- Senior Microbiologist
Joshua Luedtke, M.S.	- Microbiologist
Philip Lange, B.S.	- Associate Microbiologist
Kristen Niehaus, B.A.	- Microbiologist
Elizabeth Schwandt, B.S.	- Associate Microbiologist
Nicole Zroka, B.A.	- Associate Microbiologist

STUDY REPORT

GENERAL STUDY INFORMATION

Protocol Title: Time Kill Assay For Antimicrobial Agents

Project Number: A15630

Protocol Number: INI01091613.TK.6

Sponsor: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

TEST SUBSTANCE IDENTITY

Test Substance Name: AX250

Batch Number: Batch # AX-13196-0210

Test Substance Characterization
Test substance characterization as to content, stability, etc., (21 CFR, Part 58, Subpart F [58.105]) is the responsibility of the Sponsor. The Sponsor Test Material Certificate of Analysis Report may be found in Attachment I.

STUDY DATES

Date Sample Received: September 11, 2013
Study Initiation Date: September 24, 2013
Experimental Start Date: October 1, 2013
Experimental End Date: October 3, 2013
Study Completion Date: November 6, 2013

OBJECTIVE

The objective of this testing was to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

SUMMARY OF RESULTS

Test Substance:	AX250, Batch # AX-13196-0210
Dilution:	Ready to use (RTU)
Test Organism:	*Enterobacter aerogenes* (ATCC 13048) *Escherichia coli* (ATCC 8739) *Klebsiella pneumoniae* (ATCC 4352) *Micrococcus luteus* (ATCC 49732) *Proteus mirabilis* (ATCC 9240) *Pseudomonas aeruginosa* (ATCC 9027) *Serratia marcescens* (ATCC 13880)
Exposure Times:	15 seconds, 30 seconds, 60 seconds, and 90 seconds
Exposure Temperature:	Ambient (21°C)
Efficacy Result:	AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.88 $\log_{10}$) reduction of *Enterobacter aerogenes* (ATCC 13048) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C). AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.61 $\log_{10}$) reduction of *Escherichia coli* (ATCC 8739) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C). AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.42 $\log_{10}$) reduction of *Klebsiella pneumoniae* (ATCC 4352) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C). AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>4.46 $\log_{10}$) reduction of *Micrococcus luteus* (ATCC 49732) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C). AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.92 $\log_{10}$) reduction of *Proteus mirabilis* (ATCC 9240) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C). AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.65 $\log_{10}$) reduction of *Pseudomonas aeruginosa* (ATCC 9027) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C).

AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.43 log_{10}) reduction of *Serratia marcescens* (ATCC 13880) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C).

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Culture Medium	Incubation Parameters
Enterobacter aerogenes	13048	Tryptic Soy Agar with 5% Sheep Blood (BAP)	25-30°C, aerobic
Escherichia coli	8739	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Klebsiella pneumoniae	4352	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Micrococcus luteus	49732	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Proteus mirabilis	9240	MacConkey Agar	35-37°C, aerobic
Pseudomonas aeruginosa	9027	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Serratia marcescens	13880	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic

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

Recovery Media

Neutralizer: Letheen Broth + 0.1% Sodium Thiosulfate
Agar Plate Medium: Tryptic Soy + 5% Sheep Blood Agar
MacConkey Agar

TEST METHOD

Preparation of Test Organism

Using a stock slant, the *Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa* and *Serratia marcescens* cultures was streaked onto an appropriate growth medium. Using a stock plate, the *Proteus mirabilis* culture was streaked onto an appropriate growth medium. The *Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa* and *Serratia marcescens* cultures were incubated for two days at 35-37°C. The *Proteus mirabilis* culture was incuabted for one day at 35-37°C. The *Enterobacter aerogenes* culture was incubated for two days at 25-30°C.

On the day of test, a sufficient amount of organism growth was transferred into Butterfield's Buffer to create a uniform suspension targeting approximately 1 x 10^8 CFU/mL where possible. *Enterobacter aerogenes* was adjusted to a 1.0 McFarland Turbidity Standard. *Escherichia coli* was adjusted to a 2.0 McFarland Turbidity Standard. *Klebsiella pneumoniae* was adjusted to a 1.0 McFarland Turbidity Standard. *Micrococcus luteus* was adjusted to a 4.0 McFarland Turbidity Standard. *Proteus mirabilis, Pseudomonas aeruginosa* and *Serratia marcescens* were each adjusted to a 1.0 McFarland Turbidity Standard.

Preparation of Test Substance

The test substance was ready to use (RTU), as received from the Sponsor. A 9.5 mL aliquot of the test substance was transferred to a sterile vessel for use in testing. The test substance was homogenous as determined by visual observation.

One replicate sample was set up and evaluated for each organism.

Exposure Conditions

A 0.50 mL aliquot of the standardized inoculum was added to 9.5 mL test substance representing the start of the test exposure. The inoculated test substance was immediately mixed thoroughly using a vortex mixer. The inoculated and mixed test substance was exposed for the exposure times of 15 seconds, 30 seconds, 60 seconds, and 90 seconds at room temperature (21°C).

Test System Recovery

At each Sponsor specified exposure time, the sample was mixed and a 0.100 mL aliquot of the inoculated test substance was transferred to 9.9 mL of neutralizer representing a 10^0 dilution. Additional ten-fold serial dilutions were prepared from the 10^0 neutralized material in Butterfield's Buffer.

Using standard microbiological spread plate procedures, 1.00 mL aliquots of the 10^0 dilution and 0.100 mL aliquots of the 10^0-10^{-3} dilutions were plated in duplicate on appropriate recovery medium.

Incubation and Observation

The bacterial subculture plates were incubated for 24-48 hours at 35-37°C. The *Enterobacter aerogenes* subculture plates were incubated for 24-48 hours at 25-30°C. Following incubation, the agar plates were visually examined for the presence of growth and enumerated. Log_{10} and percent reductions were determined for each exposure time.

STUDY 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.

Neutralizer Sterility Control
A 1.00 mL aliquot of the neutralizer was plated as in the test and incubated. The acceptance criterion for this study control is a lack of growth.

Test Population Control
In a similar manner as the culture inoculum was added to the test substance, an equivalent volume of inoculum (0.5 mL) was added to 9.5 mL Butterfield's buffer). This suspension was neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. The suspension was serially diluted and appropriate dilutions were plated using standard microbiological techniques and 0.100 mL aliquots. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of the test substance was neutralized as in the test procedure. A 1.00 mL aliquot of the neutralized sample was then removed and discarded. To the neutralized sample, 1.00 mL of the organism suspension containing approximately 1000-10,000 CFU/mL was added and the suspension was vortex mixed. A 0.100 mL aliquot of the neutralized mixture was plated in duplicate on appropriate recovery agar and incubated. A numbers control was performed by adding 1.00 mL of the same organism suspension to 9.0 mL of untreated neutralizer. A 0.100 mL aliquot was plated in duplicate and incubated.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log. The most appropriate dilution was reported.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results are expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

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

PROTOCOL CHANGES

Protocol Amendment:

1. Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

   a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

   b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

   c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

2. This protocol is amended to change study directors due to the departure of the original study director from ATS Labs. The study director has been changed from Anne Stemper to Gracia Schroeder.

Protocol Deviations:

No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{(\text{average CFU}) \times (\text{dilution factor}) \times (\text{volume neutralized solution in mL})}{(\text{volume plated in mL})}$$

A value of <1 was used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros were added together to increase the sensitivity of the test. (A value of 2 mL plated was used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

The geometric mean value for the population control was determined and used to calculate percent reduction as multiple time points were evaluated in the control.

The geometric mean value of the test results were determined and used to calculate percent reduction as more than one replicate is performed.

$$\text{Geometric mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

The average $\log_{10}$ value for the population control was determined and used to calculate $\log_{10}$ reduction as multiple time points are evaluated in the control.

The average $\log_{10}$ value of the test results was determined and used to calculate $\log_{10}$ reduction as more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

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 discarded following study completion. It is the responsibility of the Sponsor to retain a sample of the test substance.

REFERENCES

1. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

RESULTS

For Control and Neutralization Results, see Tables 1-3.
All data measurements/controls including culture purity, neutralizer sterility, test population control, and neutralization confirmation controls performed within acceptance criteria.

For Test Results, see Tables 4-5.

ANALYSIS AND STUDY CONCLUSION

AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.88 log_{10}) reduction of *Enterobacter aerogenes* (ATCC 13048) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C).

AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.61 log_{10}) reduction of *Escherichia coli* (ATCC 8739) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C).

AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.42 log_{10}) reduction of *Klebsiella pneumoniae* (ATCC 4352) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C).

AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>4.46 log_{10}) reduction of *Micrococcus luteus* (ATCC 49732) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C).

AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.92 log_{10}) reduction of *Proteus mirabilis* (ATCC 9240) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C).

AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.65 log_{10}) reduction of *Pseudomonas aeruginosa* (ATCC 9027) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°C).

AX250, Batch # AX-13196-0210, demonstrated a >99.999% (>5.43 log_{10}) reduction of *Serratia marcescens* (ATCC 13880) survivors following a 15 second exposure, a 30 second exposure, a 60 second exposure and a 90 second exposure when tested at room temperature (21°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
Purity Control	*Enterobacter aerogenes* (ATCC 13048)	Pure
	Escherichia coli (ATCC 8739)	Pure
	Klebsiella pneumoniae (ATCC 4352)	Pure
	Micrococcus luteus (ATCC 49732)	Pure
	Proteus mirabilis (ATCC 9240)	Pure
	Pseudomonas aeruginosa (ATCC 9027)	Pure
	Serratia marcescens (ATCC 13880)	Pure
Neutralizer Sterility Control		No Growth

TABLE 2: TEST POPULATION CONTROL RESULTS

Test Organism	Results	
	CFU/mL	Log_{10}
Enterobacter aerogenes (ATCC 13048)	3.8×10^6	6.58
Escherichia coli (ATCC 8739)	2.06×10^6	6.31
Klebsiella pneumoniae (ATCC 4352)	1.31×10^6	6.12
Micrococcus luteus (ATCC 49732)	1.46×10^5	5.16
Proteus mirabilis (ATCC 9240)	4.2×10^6	6.62
Pseudomonas aeruginosa (ATCC 9027)	2.24×10^6	6.35
Serratia marcescens (ATCC 13880)	1.34×10^6	6.13

CFU = Colony Forming Units

Note: *The highest challenge level was achieved for this control based on the use of standard propagation methods.*

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Neutralization Confirmation (CFU)		Pass/Fail ± 1 log_{10} (Log_{10} Difference)
		Numbers Control	Test Substance Results	
AX250 Batch # AX-13196-0210	*Enterobacter aerogenes* (ATCC 13048)	54, 65	59, 49	Pass (-0.05)
	Escherichia coli (ATCC 8739)	47, 46	41, 53	Pass (0.00)
	Klebsiella pneumoniae (ATCC 4352)	30, 32	37, 42	Pass (-0.11)
	Micrococcus luteus (ATCC 49732)	64, 79	57, 74	Pass (0.04)
	Proteus mirabilis (ATCC 9240)	40, 52	47, 35	Pass (0.05)
	Pseudomonas aeruginosa (ATCC 9027)	45, 48	45, 44	Pass (0.02)
	Serratia marcescens (ATCC 13880)	34, 31	30, 38	Pass (-0.01)

CFU = Colony Forming Units

TABLE 4: TEST RESULTS FOR AX250 Batch # AX-13196-0210

DILUTION (VOLUME PLATED)	Test Organism: *Enterobacter aerogenes* (ATCC 13048)			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: *Escherichia coli* (ATCC 8739)**			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: *Klebsiella pneumoniae* (ATCC 4352)**			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: *Micrococcus luteus* (ATCC 49732)**			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0

* Indicates dilution used for calculation purposes.

TABLE 4: TEST RESULTS FOR AX250 Batch # AX-13196-0210 (continued)

DILUTION (VOLUME PLATED)	Test Organism: Proteus mirabilis (ATCC 9240)			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: *Pseudomonas aeruginosa* (ATCC 9027)**			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
DILUTION (VOLUME PLATED)	**Test Organism: *Serratia marcescens* (ATCC 13880)**			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0

* Indicates dilution used for calculation purposes.

TABLE 5: CALCULATED DATA FOR AX250 Batch # AX-13196-0210

Test Organism	Exposure Time	CFU/mL in Test Population Control (Log_{10})	CFU/mL of Survivors	Log_{10} Survivors	Percent Reduction	Log_{10} Reduction
Enterobacter aerogenes (ATCC 13048)	15 seconds	3.8×10^6 (6.58)	<5	<0.70	>99.999%	>5.88
	30 seconds		<5	<0.70	>99.999%	>5.88
	60 seconds		<5	<0.70	>99.999%	>5.88
	90 seconds		<5	<0.70	>99.999%	>5.88
Escherichia coli (ATCC 8739)	15 seconds	2.06×10^6 (6.31)	<5	<0.70	>99.999%	>5.61
	30 seconds		<5	<0.70	>99.999%	>5.61
	60 seconds		<5	<0.70	>99.999%	>5.61
	90 seconds		<5	<0.70	>99.999%	>5.61
Klebsiella pneumoniae (ATCC 4352)	15 seconds	1.31×10^6 (6.12)	<5	<0.70	>99.999%	>5.42
	30 seconds		<5	<0.70	>99.999%	>5.42
	60 seconds		<5	<0.70	>99.999%	>5.42
	90 seconds		<5	<0.70	>99.999%	>5.42
Micrococcus luteus (ATCC 49732)	15 seconds	1.46×10^5 (5.16)	<5	<0.70	>99.999%	>4.46
	30 seconds		<5	<0.70	>99.999%	>4.46
	60 seconds		<5	<0.70	>99.999%	>4.46
	90 seconds		<5	<0.70	>99.999%	>4.46

CFU = Colony Forming Units
Note: A value of <1 was used in place of zero for calculation purposes. No growth was observed on the duplicate test plates at the lowest dilution plated. The zeros were added together to increase the sensitivity of the test and a value of 2 mL plated was used in the calculation. The limit of detection of this test is a value of <5 CFU/mL.

TABLE 5: CALCULATED DATA FOR AX250 Batch # AX-13196-0210 (continued)

Test Organism	Exposure Time	CFU/mL in Test Population Control (Log_{10})	CFU/mL of Survivors	Log_{10} Survivors	Percent Reduction	Log_{10} Reduction
Proteus mirabilis (ATCC 9240)	15 seconds	4.2×10^6 (6.62)	<5	<0.70	>99.999%	>5.92
	30 seconds		<5	<0.70	>99.999%	>5.92
	60 seconds		<5	<0.70	>99.999%	>5.92
	90 seconds		<5	<0.70	>99.999%	>5.92
Pseudomonas aeruginosa (ATCC 9027)	15 seconds	2.24×10^6 (6.35)	<5	<0.70	>99.999%	>5.65
	30 seconds		<5	<0.70	>99.999%	>5.65
	60 seconds		<5	<0.70	>99.999%	>5.65
	90 seconds		<5	<0.70	>99.999%	>5.65
Serratia marcescens (ATCC 13880)	15 seconds	1.34×10^6 (6.13)	<5	<0.70	>99.999%	>5.43
	30 seconds		<5	<0.70	>99.999%	>5.43
	60 seconds		<5	<0.70	>99.999%	>5.43
	90 seconds		<5	<0.70	>99.999%	>5.43

CFU = Colony Forming Units
Note: A value of <1 was used in place of zero for calculation purposes. No growth was observed on the duplicate test plates at the lowest dilution plated. The zeros were added together to increase the sensitivity of the test and a value of 2 mL plated was used in the calculation. The limit of detection of this test is a value of <5 CFU/mL.

Attachment I: Sponsor Test Material Certificate of Analysis - Batch AX-13196-0210

Issued: July 15, 2013
Last Revised: July 29, 2013

FORM-COA 02

AQUAOX INDUSTRIES INC
16165, Sierra Lakes Parkway,
Suite 160-714,
Fontana, CA 92336, USA.

Certificate of Analysis

Date of Manufacture:	07 / 15 / 2013
Product Name:	AX250
Batch / Lot #:	AX-13196-0210
Production Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Testing Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377

TEST	ANALYSIS	UNITS
FAC	226	ppm
pH	6.03	n/a
Conductivity	1225	µS/cm
ORP	943	mV
Osmolality	22	mOsm/kg

This certification states that the intermediate product AX250, bearing the above description and lot number, has been found to conform to the internal specifications established for this product. The above lot was made in accordance with our internal specifications and current good manufacturing practices under controlled procedures.

This lot has been appropriately inspected and tested, and, to the best of our knowledge, conforms to all applicable test methods, standards and internal specifications.

This certification does not constitute any written or expressed warranty or guarantee of any kind.

Rebecca Lei
QA Regulatory Specialist

Date: 7/29/13

EXACT COPY
INITIALS DATE 11/6/13

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	10/15/13
Sponsor:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Time Kill Assay For Antimicrobial Agents
ATS Labs Protocol Number:	INI01091613.TK.6
ATS Labs Project Number:	A15630

Modifications to Protocol:

Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

Changes to the protocol are acceptable as noted.

Study Director

10-15-13
Date

EXACT COPY
INITIALS DATE 11/6/13

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	2
Effective Date:	10/29/13
Sponsor:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Time Kill Assay For Antimicrobial Agents
ATS Labs Protocol Number:	INI01091613.TK.6
ATS Labs Project Number:	A15630

Modifications to Protocol:

This protocol is amended to change study directors due to the departure of the original study director from ATS Labs. The study director has been changed from Anne Stemper to Gracia Schroeder.

Changes to the protocol are acceptable as noted.

Gracia N Schr — Study Director

10/29/13 — Date

EXACT COPY
INITIALS CJD DATE 11/6/13

(For Laboratory Use Only)
ATS Labs Project # A15630
A15 9-25-13

ATS LABS

PROTOCOL

Time Kill Assay For Antimicrobial Agents

Test Organisms:

Enterobacter aerogenes (ATCC 13048)
Escherichia coli (ATCC 8739)
Klebsiella pneumoniae (ATCC 4352)
Micrococcus luteus (ATCC 49732)
Proteus mirabilis (ATCC 9240)
Pseudomonas aeruginosa (ATCC 9027)
Serratia marcescens (ATCC 13880)

PROTOCOL NUMBER

INI01091613.TK.6

PREPARED FOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PERFORMING LABORATORY

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

PREPARED BY

Anne Stemper, B.S.
Senior Microbiologist

DATE

September 16, 2013

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

EXACT COPY
INITIALS CH DATE 11/6/13

Time Kill Assay For Antimicrobial Agents

SPONSOR: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

PURPOSE
The objective of this testing is to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

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. 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 September 24, 2013. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of October 21, 2013. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

A "case-by-case" approach is generally taken by the regulatory authorities and cannot be over-emphasized when considering a testing regimen. While this protocol is based upon our experience in the field of germicidal testing, and the current regulatory guidelines, each product presents a different set of issues to the regulatory authorities. We recommend that you consult with the appropriate agency before finalizing your testing regimen, as ATS Labs cannot guarantee acceptance of this protocol by the regulating authorities.

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 nor 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 regulating 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
Analyzing the efficacy of antimicrobial agents may be performed by various suspension and susceptibility methods. This study is designed to examine the rate-of-kill of a test substance against a pure test culture. This is accomplished by exposing the test culture to the test substance and assaying for survivors following a variety of exposure times. The experimental design in this protocol meets these requirements.

TEST PRINCIPLE

A suspension of the test organism is exposed to the test substance for specified exposure times. After exposure, an aliquot of the suspension is transferred to a neutralizer and assayed for survivors. Appropriate culture purity, sterility, population and neutralization confirmation controls are performed. The current version of Standard Operating Procedure CGT-4130 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Culture Medium	Incubation Parameters
Enterobacter aerogenes	13048	Tryptic Soy Agar with 5% Sheep Blood (BAP)	25-30°C, aerobic
Escherichia coli	8739	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Klebsiella pneumoniae	4352	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Micrococcus luteus	49732	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Proteus mirabilis	9240	MacConkey Agar	35-37°C, aerobic
Pseudomonas aeruginosa	9027	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic
Serratia marcescens	13880	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic

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

Preparation of Test Organism

From a stock plate or stock slant culture, streak a culture of each test organism onto the culture medium listed above. This represents the second culture transfer. Incubate the second culture transfer for 1-5 days at the incubation parameters listed above. (Alternate or extended incubation may be required for certain strains). Transfer a sufficient amount of organism growth into a sterile diluent to create a uniform suspension targeting approximately 1 x 10^8 CFU/mL or greater where possible. This may be achieved by comparison to McFarland standards, by spectrophotometric means or by any other appropriate method.

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

Preparation of Test Substance

The test substance to be tested is prepared according to the directions supplied 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. A 9.5 mL aliquot of the prepared test substance will be transferred to a sterile vessel (glass tube, stomacher bag, etc.) for testing procedures. If necessary, 9.5 g of test substance may be used. Multiple replicate vessels may be set up if requested.

Exposure Conditions

A 0.5 mL aliquot of the standardized inoculum will be added to the test substance representing the start of the test exposure. The inoculated test substance will be immediately mixed thoroughly using a vortex mixer, stirring with a pipette or by any other applicable method. The inoculated and mixed test substance will be held at the Sponsor specified temperature. If the requested exposure temperature lies outside of achievable ambient conditions, the test substance may be placed in a water bath (or other appropriate device) to equilibrate to the desired exposure temperature prior to testing. For very short exposure times or exposure times which are close together, individual test substance vessels may be utilized where necessary.

Test System Recovery
At each Sponsor specified exposure time, the sample will be mixed and a 0.1 mL aliquot of the inoculated test substance will be transferred to 9.9 mL of neutralizer broth (10^0 dilution). Additional ten-fold serial dilutions will be prepared in Butterfield's buffer. Using a standard microbiological spread plate count procedure, 1.0 mL aliquots of the $10^0 - 10^{-4}$ dilutions will be plated in duplicate.

If swarming is a concern, 1.0 mL of 10^0 will be plated in duplicate. In addition, 0.1 mL of $10^0 - 10^{-3}$ will be plated in duplicate.

Incubation and Observation
All bacterial subculture plates are incubated for 24-48 hours at 35-37°C. Alternate or extended incubation may be required for certain strains.

Following incubation, the subcultures will be visually examined for growth and enumerated. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination. Log_{10} and percent reductions will be determined for each time point. Representative subcultures demonstrating growth may 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
If applicable, 1.0 mL of the serum used for soil load will be added to a tube of Fluid Thioglycollate, incubated, and observed for lack of growth. The acceptance criterion for this study control is lack of growth.

Neutralizer Sterility Control
A 1.0 mL aliquot of the neutralizer will be plated as in the test and incubated. The acceptance criterion for this study control is lack of growth.

Test Population Control
In a similar manner as the culture inoculum is added to the test substance, add an equivalent volume of inoculum (0.5 mL) to 9.5 mL Butterfield's buffer (or the same volume as the test substance). This suspension will be neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. If requested, the sample may be exposed as in the test and evaluated at an additional time point. (If requested, the final time point is recommended.) The suspension will be serially diluted and appropriate dilutions plated using standard microbiological techniques. *If swarming is a concern, 0.1 mL aliquots will be plated.*

Following incubation, the organism plates will be observed and enumerated. If more than one time point is evaluated, the geometric mean will be determined prior to reduction calculations. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of test substance will be neutralized as in the test procedure. Only the most concentrated test substance needs to be evaluated in this control. Remove and discard 1.0 mL of the neutralized sample. To the neutralized sample, add 1.0 mL of an organism suspension to target approximately 100-1000 CFU per mL of neutralizer and vortex mix. Plate, in duplicate, 1.0 mL of neutralized mixture to appropriate recovery agar and incubate. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 1.0 mL aliquots, in duplicate, and incubate. This control may be performed prior to or concurrent with testing.

NOTE: If swarming is a concern, add 1.0 mL of an organism suspension containing 1000-10,000 CFU/mL and vortex mix. Plate, in duplicate, 0.1 mL of the neutralized mixture. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 0.1 mL aliquots, in duplicate.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log.

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 test organism 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 subcultures, etc. during the course of the test. Test subcultures 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
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results will be expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol.

REPORT
The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism 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.

TEST SUBSTANCE RETENTION

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. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{(\text{average CFU}) \times (\text{dilution factor}) \times (\text{volume neutralized solution in mL})}{(\text{volume plated in mL})}$$

A value of <1 may be used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros may be added together to increase the sensitivity of the test. (A value of 2 mL plated is used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

If applicable, the geometric mean value for the population control will be determined and used to calculate percent reduction if multiple time points are evaluated in the control. The geometric mean value of the test results will be determined and used to calculate percent reduction if more than one replicate is performed.

$$\text{Geometric mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

If applicable, the average $\log_{10}$ value for the population control will be determined and used to calculate $\log_{10}$ reduction if multiple time points are evaluated in the control. The average $\log_{10}$ value of the test results will be determined and used to $\log_{10}$ reduction if more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

Statistical Analysis: None used.

Study Information

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number - exactly as it should appear on final report):
AX250 Batch # AX-13196-0210

Expiration Date: 07/2015

Test Substance Active Concentration (upon submission to ATS Labs): 0.024% HOCl

Product Description:
- ☐ Quaternary ammonia
- ☐ Iodophor
- ☑ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☑ Other Hypochlorous acid

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:

_____ defined as _____ + _____
(example: 1 oz/gallon) (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.

Exposure Times: 15 seconds, 30 seconds, 60 seconds, and 90 seconds

Number of Test Replicate(s) per sample: 1

Exposure Temperature:
- ☑ Ambient
- ☐ Other _____

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

Test Organisms:
- ☑ *Enterobacter aerogenes* (ATCC 13048)
- ☑ *Escherichia coli* (ATCC 8739)
- ☑ *Klebsiella pneumoniae* (ATCC 4352)
- ☑ *Serratia marcescens* (ATCC 13880)
- ☑ *Micrococcus luteus* (ATCC 49732)
- ☑ *Proteus mirabilis* (ATCC 9240)
- ☑ *Pseudomonas aeruginosa* (ATCC 9027)

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: 7/11/13 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

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Dr. Fred Ma TITLE: M.D., Ph.D. Chief Medical Officer

SIGNATURE: DR. Fred Ma DATE: 09/17/13

PHONE: (909) 822 - 6000 FAX: _____ EMAIL: fma@Innovacyn.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

Hannah Carroll (hannahc@Innovacyn.com)

ATS Labs:

NAME: Anne Stenger
Study Director

SIGNATURE: Anne Stenger DATE: 9-24-13
Study Director

ATS LABS

FINAL STUDY REPORT

STUDY TITLE

Time Kill Assay For Antimicrobial Agents

Test Organism:

Candida albicans (ATCC 10231)

PRODUCT IDENTITY

AX250
Batch # AX-13196-0210

AUTHOR

Gracia Schroeder, B.S.
Study Director

STUDY COMPLETION DATE

November 6, 2013

PERFORMING LABORATORY

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

SPONSOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PROJECT NUMBER

A15631

EXACT COPY
INITIALS KT DATE 11-6-13

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

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:_____

Sponsor:_____ Date:_____

Study Director: Gracia Schroeder, B.S. Date: 11/12/13

QUALITY ASSURANCE UNIT SUMMARY

Study: Time Kill Assay For Antimicrobial Agents

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 (21 CFR Part 58) 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 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	September 27, 2013	September 27, 2013	September 27, 2013
Draft Report	October 10, 2013	October 10, 2013	October 10, 2013
Final Report	November 6, 2013	November 6, 2013	November 6, 2013

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

Quality Assurance Auditor: Judy Heidemann Date: 11-6-13

TABLE OF CONTENTS

STUDY PERSONNEL

STUDY DIRECTOR:	Gracia Schroeder, B.S.
Professional personnel involved:	
Scott R. Steinagel, B.S.	- Director, Microbiology Operations
Becky Lien, B.A.	- Manager, Microbiology Operations
Peter Toll, B.S.	- Supervisor, Microbiology Laboratory Operations
Matthew Sathe, B.S.	- Senior Microbiologist
Joshua Luedtke, M.S.	- Microbiologist
Philip Lange, B.S.	- Associate Microbiologist
Rebecca Astrup, B.S.	- Associate Microbiologist
Nicole Zroka, B.A.	- Associate Microbiologist
Kathryn Thomas, B.S.	- Laboratory Technician

STUDY REPORT

GENERAL STUDY INFORMATION

Protocol Title: Time Kill Assay For Antimicrobial Agents

Project Number: A15631

Protocol Number: INI01091613.TK.7

Sponsor: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

TEST SUBSTANCE IDENTITY

Test Substance Name: AX250

Batch Number: Batch # AX-13196-0210

Test Substance Characterization
Test substance characterization as to content, stability, etc., (21 CFR Part 58, Subpart F [58.105]) is the responsibility of the Sponsor. The Sponsor Test Material Certificate of Analysis Report may be found in Attachment I.

STUDY DATES

Date Sample Received: September 11, 2013
Study Initiation Date: September 24, 2013
Experimental Start Date: September 27, 2013
Experimental End Date: October 1, 2013
Study Completion Date: November 6, 2013

OBJECTIVE

The objective of this testing was to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

SUMMARY OF RESULTS

Test Substance: AX250 (Batch # AX-13196-0210)

Dilution: Ready to use (RTU)

Test Organism: *Candida albicans* (ATCC 10231)

Exposure Times: 15 seconds, 30 seconds, 60 seconds, and 90 seconds

Exposure Temperature: Ambient Temperature (21°C)

Efficacy Result: AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.31 $\log_{10}$) reduction of *Candida albicans* (ATCC 10231) survivors following a 15 second exposure, a >99.999% (>5.31 $\log_{10}$) reduction of *Candida albicans* (ATCC 10231) survivors following a 30 second exposure, a >99.999% (>5.31 $\log_{10}$) reduction of *Candida albicans* (ATCC 10231) survivors following a 60 second exposure and a >99.999% (>5.31 $\log_{10}$) reduction of *Candida albicans* (ATCC 10231) survivors following a 90 second exposure when tested at ambient temperature (21°C).

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Candida albicans	10231	Sab dex agar	25-30°C, aerobic

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

Recovery Media

Neutralizer: Letheen Broth + 0.1% Sodium Thiosulfate
Agar Plate Medium: Sabouraud Dextrose Agar

TEST METHOD

Preparation of Test Organism

Using a stock slant, the test organism culture was streaked onto an appropriate growth medium. The culture was incubated for two days at 25-30°C.

On the day of test, a sufficient amount of organism growth was transferred into Butterfield's Buffer to create a uniform suspension targeting approximately 1 x 10^8 CFU/mL where possible. *Candida albicans* was adjusted to a >4.0 McFarland Turbidity Standard.

Preparation of Test Substance

The test substance was ready to use (RTU), as received from the Sponsor. A 9.5 mL aliquot of the test substance was transferred to a sterile vessel for use in testing. The test substance was homogenous as determined by visual observation.

One replicate sample was set up and evaluated.

Exposure Conditions

A 0.50 mL aliquot of the standardized inoculum was added to 9.5 mL test substance representing the start of the test exposure. The inoculated test substance was immediately mixed thoroughly using a vortex mixer. The inoculated and mixed test substance was exposed for the exposure times of 15 seconds, 30 seconds, 60 seconds, and 90 seconds at ambient temperature (21°C).

Test System Recovery

At each Sponsor specified exposure time, the sample was mixed and a 0.100 mL aliquot of the inoculated test substance was transferred to 9.9 mL of neutralizer representing a 10^0 dilution. Additional ten-fold serial dilutions were prepared from the 10^0 neutralized material in Butterfield's Buffer.

Using standard microbiological spread plate procedures, 1.00 mL aliquots of the 10^0 dilution and 0.100 mL aliquots of the 10^0-10^{-3} dilutions were plated in duplicate on appropriate recovery medium for *Candida albicans.*

Incubation and Observation

The fungal subculture plates were incubated for 44-76 hours at 25-30°C. Subcultures were stored at 2-8°C for two days prior to examination. Following incubation and storage, the agar plates were visually examined for the presence of growth and enumerated. Log_{10} and percent reductions were determined for each exposure time.

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.

Neutralizer Sterility Control
A 1.00 mL aliquot of the neutralizer was plated as in the test and incubated. The acceptance criterion for this study control is a lack of growth.

Test Population Control
In a similar manner as the culture inoculum was added to the test substance, an equivalent volume of inoculum (0.50 mL) was added to 9.5 mL Butterfield's buffer). This suspension was neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. The suspension was serially diluted and appropriate dilutions were plated using standard microbiological techniques and 0.100 mL aliquots. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of the test substance was neutralized as in the test procedure. A 1.00 mL aliquot of the neutralized sample was then removed and discarded. To the neutralized sample, 1.00 mL of the organism suspension containing approximately 1000-10,000 CFU/mL was added and the suspension was vortex mixed. A 0.100 mL aliquot of the neutralized mixture was plated in duplicate on appropriate recovery agar and incubated. A numbers control was performed by adding 1.00 mL of the same organism suspension to 9.0 mL of untreated neutralizer. A 0.100 mL aliquot was plated in duplicate and incubated.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log. The most appropriate dilution was reported.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results are expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

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

PROTOCOL CHANGES

Protocol Amendment:
Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{\text{(average CFU) x (dilution factor) x (volume neutralized solution in mL)}}{\text{(volume plated in mL)}}$$

A value of <1 was used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros were added together to increase the sensitivity of the test. (A value of 2 mL plated was used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

The geometric mean value for the population control was determined and used to calculate percent reduction as multiple time points were evaluated in the control.

The geometric mean value of the test results were determined and used to calculate percent reduction as more than one replicate is performed.

$$\text{Geometric mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

The average $\log_{10}$ value for the population control was determined and used to calculate $\log_{10}$ reduction as multiple time points are evaluated in the control.

The average $\log_{10}$ value of the test results was determined and used to calculate $\log_{10}$ reduction as more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

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 discarded following study completion. It is the responsibility of the Sponsor to retain a sample of the test substance.

REFERENCES

1. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

RESULTS

For Control and Neutralization Results, see Tables 1-3.
All data measurements/controls including culture purity, neutralizer sterility, test population control, and neutralization confirmation controls performed within acceptance criteria.

For Test Results, see Tables 4-5.

ANALYSIS AND STUDY CONCLUSION

AX250 (Batch # AX-13196-0210) demonstrated a >99.999% (>5.31 log_{10}) reduction of *Candida albicans* (ATCC 10231) survivors following a 15 second exposure, a >99.999% (>5.31 log_{10}) reduction of *Candida albicans* (ATCC 10231) survivors following 30 second exposure, a >99.999% (>5.31 log_{10}) reduction of *Candida albicans* (ATCC 10231) survivors following a 60 second exposure and a >99.999% (>5.31 log_{10}) reduction of *Candida albicans* (ATCC 10231) survivors following a 90 second exposure when tested at ambient temperature (21°C) in the presence.

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
Purity Control	*Candida albicans* (ATCC 10231)	Pure
Neutralizer Sterility Control		No Growth

TABLE 2: TEST POPULATION CONTROL RESULTS

Test Organism	Results	
	CFU/mL	Log_{10}
Candida albicans (ATCC 10231)	1.02×10^6	6.01

CFU = Colony Forming Units

Note: *The highest challenge level was achieved for this control based on the use of standard propagation methods.*

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Neutralization Confirmation (CFU)		Pass/Fail ± 1 log_{10} (Log_{10} Difference)
		Numbers Control	Test Substance Results	
AX250 Batch # AX-13196-0210	*Candida albicans* (ATCC 10231)	26, 19	24, 27	Pass (-0.05)

CFU = Colony Forming Units

TABLE 4: TEST RESULTS FOR AX250 Batch # AX-13196-0210

DILUTION (VOLUME PLATED)	Test Organism: *Candida albicans* (ATCC 10231)			
	Exposure Time			
	15 seconds	30 seconds	60 seconds	90 seconds
	Number of Survivors			
10^0 (1.00 mL)*	0, 0	0, 0	0, 0	0, 0
10^0 (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-1} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-2} (0.100 mL)	0, 0	0, 0	0, 0	0, 0
10^{-3} (0.100 mL)	0, 0	0, 0	0, 0	0, 0

* Indicates dilution used for calculation purposes.

TABLE 5: CALCULATED DATA FOR AX250 Batch # AX-13196-0210

Test Organism	Exposure Time	CFU/mL in Test Population Control (Log_{10})	CFU/mL of Survivors	Log_{10} Survivors	Percent Reduction	Log_{10} Reduction
Candida albicans (ATCC 10231)	15 second	1.02×10^6 (6.01)	<5	<0.70	>99.999%	>5.31
	30 seconds		<5	<0.70	>99.999%	>5.31
	60 seconds		<5	<0.70	>99.999%	>5.31
	90 seconds		<5	<0.70	>99.999%	>5.31

CFU = Colony Forming Units

Note: *No growth was observed on the duplicate test plates at the lowest dilution plated. The zeros were added together to increase the sensitivity of the test and a value of 2 mL plated was used in the calculation. The limit of detection of this test is a value of <5 CFU/mL.*

Attachment I: Sponsor Test Material Certificate of Analysis - Batch AX-13196-0210

Issued: July 16, 2013
Last Revised: September 10, 2013

FORM-COA-02

AQUAOX

AQUAOX INDUSTRIES INC
16155, Sierra Lakes Parkway,
Suite 160-714,
Fontana, CA 92336, USA.

Certificate of Analysis

Date of Manufacture:	07 / 15 / 2013
Product Name:	AX250
Batch / Lot #:	AX-13196-0210
Production Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Testing Facility:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377

TEST	ANALYSIS	UNITS
FAC	207	ppm
pH	5.91	n/a
Conductivity	1230	µS/cm
ORP	966	mV
Osmolality	22	mOsm/kg

This certification states that the intermediate product AX250, bearing the above description and lot number, has been found to conform to the internal specifications established for this product. The above lot was made in accordance with our internal specifications and current good manufacturing practices under controlled procedures.

This lot has been appropriately inspected and tested, and, to the best of our knowledge, conforms to all applicable test methods, standards and internal specifications.

This certification does not constitute any written or expressed warranty or guarantee of any kind.

Rebecca Lei
QA Regulatory Specialist

Date: 9/10/13

EXACT COPY
INITIALS DATE 11/6/13

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	10/10/13
Sponsor:	Innovacyn, Inc. 3546 N. Riverside Ave. Rialto, CA 92377
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Time Kill Assay For Antimicrobial Agents
ATS Labs Protocol Number:	INI01091613.TK.7
ATS Labs Project Number:	A15631

Modifications to Protocol:

Per Sponsor's request, the protocol is amended to change the Regulatory Agency Code of Federal Regulations as follows.

a. Under Test Substance Characterization, (40 CFR, Part 160, Subpart F [160.105]) is changed to (21 CFR Part 58, Subpart F [58.105]).

b. Under Report, 40 CFR Part 160.185 is changed to 21 CFR Part 58.185.

c. Under Compliance in the Study Information Pages, EPA Good Laboratory Practice regulations (40 CFR Part 160) is changed to FDA Good Laboratory Practice regulations (21 CFR Part 58).

Changes to the protocol are acceptable as noted.

Study Director

10/10/13
Date

EXACT COPY
INITIAL DATE 11/12/13

(For Laboratory Use Only)
ATS Labs Project # A15631
KAS 9-25-13

ATS LABS

PROTOCOL

Time Kill Assay For Antimicrobial Agents

Test Organism:

Candida albicans (ATCC 10231)

PROTOCOL NUMBER

INI01091613.TK.7

PREPARED FOR

Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

PERFORMING LABORATORY

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

PREPARED BY

Anne Stemper, B.S.
Senior Microbiologist

DATE

September 16, 2013

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

EXACT COPY
INITIALS CJH DATE 11/6/13

Time Kill Assay For Antimicrobial Agents

SPONSOR: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

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

PURPOSE
The objective of this testing is to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

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. 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 September 24, 2013. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of October 21, 2013. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

A "case-by-case" approach is generally taken by the regulatory authorities and cannot be over-emphasized when considering a testing regimen. While this protocol is based upon our experience in the field of germicidal testing, and the current regulatory guidelines, each product presents a different set of issues to the regulatory authorities. We recommend that you consult with the appropriate agency before finalizing your testing regimen, as ATS Labs cannot guarantee acceptance of this protocol by the regulating authorities.

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 nor 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 regulating 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
Analyzing the efficacy of antimicrobial agents may be performed by various suspension and susceptibility methods. This study is designed to examine the rate-of-kill of a test substance against a pure test culture. This is accomplished by exposing the test culture to the test substance and assaying for survivors following a variety of exposure times. The experimental design in this protocol meets these requirements.

TEST PRINCIPLE

A suspension of the test organism is exposed to the test substance for specified exposure times. After exposure, an aliquot of the suspension is transferred to a neutralizer and assayed for survivors. Appropriate culture purity, sterility, population and neutralization confirmation controls are performed. The current version of Standard Operating Procedure CGT-4130 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Culture Medium	Incubation Parameters
Candida albicans	10231	Sab dex agar and/or Potato Dextrose agar	25-30°C, aerobic

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

Preparation of Test Organism

From a stock plate or stock slant culture, streak a culture of each test organism onto the culture medium listed above. This represents the second culture transfer. Incubate the second culture transfer for 1-5 days at the incubation parameters listed above. (Alternate or extended incubation may be required for certain strains). Transfer a sufficient amount of organism growth into a sterile diluent to create a uniform suspension targeting approximately 1 x 10^8 CFU/mL or greater where possible. This may be achieved by comparison to McFarland standards, by spectrophotometric means or by any other appropriate method.

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

Preparation of Test Substance

The test substance to be tested is prepared according to the directions supplied 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. A 9.5 mL aliquot of the prepared test substance will be transferred to a sterile vessel (glass tube, stomacher bag, etc.) for testing procedures. If necessary, 9.5 g of test substance may be used. Multiple replicate vessels may be set up if requested.

Exposure Conditions

A 0.5 mL aliquot of the standardized inoculum will be added to the test substance representing the start of the test exposure. The inoculated test substance will be immediately mixed thoroughly using a vortex mixer, stirring with a pipette or by any other applicable method. The inoculated and mixed test substance will be held at the Sponsor specified temperature. If the requested exposure temperature lies outside of achievable ambient conditions, the test substance may be placed in a water bath (or other appropriate device) to equilibrate to the desired exposure temperature prior to testing. For very short exposure times or exposure times which are close together, individual test substance vessels may be utilized where necessary.

Test System Recovery

At each Sponsor specified exposure time, the sample will be mixed and a 0.1 mL aliquot of the inoculated test substance will be transferred to 9.9 mL of neutralizer broth (10^0 dilution). Additional ten-fold serial dilutions will be prepared in Butterfield's buffer. Using a standard microbiological spread plate count procedure, 1.0 mL aliquots of the $10^0 - 10^{-4}$ dilutions will be plated in duplicate.

If swarming is a concern, 1.0 mL of 10^0 will be plated in duplicate. In addition, 0.1 mL of $10^0 - 10^{-3}$ will be plated in duplicate.

Incubation and Observation
All fungal subculture plates are incubated for 44-76 hours at 25-30°C. Additional incubation may be required if colony growth is difficult to detect visually.

Following incubation, the subcultures will be visually examined for growth and enumerated. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination. Log_{10} and percent reductions will be determined for each time point. Representative subcultures demonstrating growth may 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
If applicable, 1.0 mL of the serum used for soil load will be added to a tube of Fluid Thioglycollate, incubated, and observed for lack of growth. The acceptance criterion for this study control is lack of growth.

Neutralizer Sterility Control
A 1.0 mL aliquot of the neutralizer will be plated as in the test and incubated. The acceptance criterion for this study control is lack of growth.

Test Population Control
In a similar manner as the culture inoculum is added to the test substance, add an equivalent volume of inoculum (0.5 mL) to 9.5 mL Butterfield's buffer (or the same volume as the test substance). This suspension will be neutralized as in the test procedure to represent the concentration of test organism present in the test substance at the time of inoculation. If requested, the sample may be exposed as in the test and evaluated at an additional time point. (If requested, the final time point is recommended.) The suspension will be serially diluted and appropriate dilutions plated using standard microbiological techniques. *If swarming is a concern, 0.1 mL aliquots will be plated.*

Following incubation, the organism plates will be observed and enumerated. If more than one time point is evaluated, the geometric mean will be determined prior to reduction calculations. The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Neutralization Confirmation Control
An aliquot of test substance will be neutralized as in the test procedure. Only the most concentrated test substance needs to be evaluated in this control. Remove and discard 1.0 mL of the neutralized sample. To the neutralized sample, add 1.0 mL of an organism suspension to target approximately 100-1000 CFU per mL of neutralizer and vortex mix. Plate, in duplicate, 1.0 mL of neutralized mixture to appropriate recovery agar and incubate. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 1.0 mL aliquots, in duplicate, and incubate. This control may be performed prior to or concurrent with testing.

NOTE: If swarming is a concern, add 1.0 mL of an organism suspension containing 1000-10,000 CFU/mL and vortex mix. Plate, in duplicate, 0.1 mL of the neutralized mixture. Perform a numbers control by adding 1.0 mL of the same organism suspension to 9 mL of untreated neutralizer. Plate 0.1 mL aliquots, in duplicate.

The acceptance criterion for this study control requires the neutralization confirmation control and corresponding numbers control results to be within 1.0 Log.

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 test organism 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 subcultures, etc. during the course of the test. Test subcultures 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

This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results will be expressed in percent and $\log_{10}$ reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

Control Acceptance Criteria

The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol.

REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism 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.

TEST SUBSTANCE RETENTION

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. American Society for Testing and Materials (ASTM). Standard Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, E2315-03 (Reapproved 2008).

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

DATA ANALYSIS

Calculations

CFU/mL = (average CFU) x (dilution factor) x (volume neutralized solution in mL)
(volume plated in mL)

A value of <1 may be used in place of zero for calculation purposes. In the event that no growth is observed on both plates in the lowest dilution plated in the test, the zeros may be added together to increase the sensitivity of the test. (A value of 2 mL plated is used in the calculation when one mL is plated in duplicate.)

Percent reduction = [(a - b) / a] x 100

where:
a =CFU/mL in the population control
b =CFU/mL surviving in the test following exposure

If applicable, the geometric mean value for the population control will be determined and used to calculate percent reduction if multiple time points are evaluated in the control. The geometric mean value of the test results will be determined and used to calculate percent reduction if more than one replicate is performed.

Geometric mean = Antilog of $\frac{Log_{10}X_1 + Log_{10}X_2 + Log_{10}X_N}{N}$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the population control) – Log_{10} (CFU/mL surviving in the test following exposure)

If applicable, the average log_{10} value for the population control will be determined and used to calculate log_{10} reduction if multiple time points are evaluated in the control. The average log_{10} value of the test results will be determined and used to log_{10} reduction if more than one replicate is performed.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

Statistical Analysis: None used.

Study Information

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number - exactly as it should appear on final report):
AX250 Batch # AX-13196-0210

Expiration Date: 07/2015

Test Substance Active Concentration (upon submission to ATS Labs): 0.024% HOCl

Product Description:
- ☐ Quaternary ammonia
- ☐ Iodophor
- ☑ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☑ Other Hypochlorous acid

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:

_____ defined as _____ + _____
(example: 1 oz/gallon) (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.*

Exposure Times: 15 seconds, 30 seconds, 60 seconds, and 90 seconds

Number of Test Replicate(s) per sample: 1

Exposure Temperature:
- ☑ Ambient
- ☐ Other _____

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

Test Organism:
- ☑ *Candida albicans* (ATCC 10231)

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: 7/11/13 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

_____

_____

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Dr. Fred Ma TITLE: M.D., Ph.D. Chief Medical Officer

SIGNATURE: Dr. Fred Ma DATE: 09/17/13

PHONE: (909) 822 - 6000 FAX: _____ EMAIL: fma@innovacyn.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

Hannah Carroll (hannahc@innovacyn.com)

ATS Labs:

NAME: Gracia Schroeder
Study Director

SIGNATURE: _____ DATE: 9/24/13
Study Director