ACCURATUS
LAB SERVICES

FINAL STUDY REPORT

STUDY TITLE

AOAC Use-Dilution Method

Test Organisms:

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

PRODUCT IDENTITY

Gluquat 300
Lot 13-12-03, Lot 14-02-18 and Lot 14-08-28

TEST GUIDELINE

OCSPP 810.2200

AUTHOR

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Study Director

STUDY COMPLETION DATE

March 26, 2015

PERFORMING LABORATORY

Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
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SPONSOR

West Penetone, Inc.
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Anjou, QC H1J 1S5
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PROJECT NUMBER

A18076

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: West Penetone, Inc.

Company Agent: ______________________________

____
Title

______________________________ Date:__________
Signature

GOOD LABORATORY PRACTICE STATEMENT

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

The following studies were not performed following GLP regulations: characterization and stability of the compounds.

Submitter:______________________________ Date:______________

Sponsor:______________________________ Date:______________

Study Director:______________________________ Date: 3-26-15
Joshua Luedtke, M.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: AOAC Use-Dilution Method

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed in accordance to standard operating procedures and the study protocol. In accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to Management and the Study Director.

Phase Inspected	Date of Phase Inspection	Date Reported to Study Director	Date Reported to Management
Critical Phase Audit: Product Preparation	March 17, 2015	March 17, 2015	March 18, 2015
Final Report	March 25, 2015	March 25, 2015	March 26, 2015

Quality Assurance Specialist: Judy Heidemann Date: 3-26-15

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STUDY PERSONNEL

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STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: AOAC Use-Dilution Method

Project Number: A18076

Protocol Number: WPC02013015.UD

Sponsor: West Penetone, Inc.
10900 Secant
Anjou, QC H1J 1S5
Canada

Test Facility: Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance Name: Gluquat 300

Lot/Batch(s): Lot 13-12-03, Lot 14-02-18 and Lot 14-08-28

Test Substance Characterization
Test substance characterization as to identity, strength, purity, solubility and composition, as applicable, according to (40 CFR, Part 160, Subpart F [160.105]) was not documented prior to its use in the study.

STUDY DATES

Date Sample Received: February 23, 2015
Study Initiation Date: February 26, 2015
Experimental Start Date: March 17, 2015 (Start time: 2:54 pm)
Experimental End Date: March 22, 2015 (End time: 11:00 am)
Study Completion Date: March 26, 2015

OBJECTIVE

The objective of this study was to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method is in compliance with the requirements of the U.S. Environmental Protection Agency (EPA) and Health Canada.

SUMMARY OF RESULTS

Test Substance: Gluquat 300 (Lot 13-12-03, Lot 14-02-18 and Lot 14-08-28)

Dilution: 1:200 (defined as 1 part test substance + 200 parts 300 ppm AOAC synthetic hard water)

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

Exposure Time: 10 minutes

Exposure Temperature: 20-22°C (20.0°C)

Organic Soil Load: 5% fetal bovine serum

Number of Carriers: 60 per lot

Efficacy Result: Gluquat 300 demonstrated efficacy of three batches, evaluated on three separate test dates, against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and therefore, meets the performance requirements set forth by the U.S. EPA and Health Canada following a 10 minute exposure time at 20-22°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

Gluquat 300 demonstrated efficacy of three batches against *Salmonella enterica* and and therefore, meets the performance requirements set forth by the U.S. EPA and Health Canada following a 10 minute exposure time at 20-22°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

STUDY MATERIALS

Test System/Growth Media

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

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

Recovery Media
Neutralizing Subculture Medium:
Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 + 1.0% Glycine

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

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

Hard Water Description:
For testing performed 3/17/15, the Sponsor specified 300 ppm AOAC synthetic hard water was made using 12.0 mL of AOAC Solution I and 16.0 mL of AOAC Solution II. The total volume of the solution was brought to approximately 4 L using sterile deionized water. The synthetic hard water was prepared, titrated, and used for testing on the day of preparation. The actual titration result was 397 ppm (See Protocol Deviation).

For testing performed 3/18/15, the Sponsor specified 300 ppm AOAC synthetic hard water was made using 6.0 mL of AOAC Solution I and 8.0 mL of AOAC Solution II. The total volume of the solution was brought to approximately 2 L using sterile deionized water. The synthetic hard water was prepared, titrated, and used for testing on the day of preparation. The actual titration result was 303 ppm.

For testing performed 3/19/15, the Sponsor specified 300 ppm AOAC synthetic hard water was made using 6.0 mL of AOAC Solution I and 8.0 mL of AOAC Solution II. The total volume of the solution was brought to approximately 2 L using sterile deionized water. The synthetic hard water was prepared, titrated, and used for testing on the day of preparation. The actual titration result was 305 ppm.

Carriers
Carriers were screened according to the AOAC Official Method of Analysis and all carriers positive for growth were discarded. Only penicylinders which demonstrated no growth during screening were used in this test. Stainless steel penicylinders were pre-soaked overnight in 1N NaOH, washed in water until neutral and autoclaved in deionized water. Carriers were used within three months of sterilization.

TEST METHOD

To satisfy the requirement to evaluate each test substance lot on separate test dates, Lot 13-12-03 was tested on 3/17/15, Lot 14-08-28 was tested on 3/18/15 and Lot 14-02-18 was tested on 3/19/15.Testing was performed the same on each test date, unless otherwise noted.

Preparation of Test Substance

For testing performed 3/18/15 and 3/19/15, an equivalent dilution of 1:200 (defined as 1 part test substance + 200 parts diluent), was prepared using 7.0 mL of the test substance and 1400 mL of 300 ppm AOAC synthetic hard water. Volumetric glassware was used. The prepared test substance was homogenous as determined by visual observation and was used within three hours of preparation.

For testing of Lot 13-12-03 performed 3/17/15, an equivalent dilution of 1:200 (defined as 1 part test substance + 200 parts diluent), was prepared using 10.0 mL of the test substance and 2000 mL of 397 ppm AOAC synthetic hard water (See Protocol Deviation). Volumetric glassware was used. The prepared test substance was homogenous as determined by visual observation and was used within three hours of preparation.

For *S.enterica* Neutralization Confirmation Control testing of Lot 14-08-28 and Lot 14-02-18 performed 3/17/15, an equivalent dilution of 1:200 (defined as 1 part test substance + 200 parts diluent), was prepared using 3.5 mL of the test substance and 700 mL of 397 ppm AOAC synthetic hard water (See Protocol Deviation). Volumetric glassware was used. The prepared test substance was homogenous as determined by visual observation and was used within three hours of preparation.

Ten (10.0) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a 20-22°C (20.0°C) water bath and allowed to equilibrate for ≥10 minutes prior to testing.

Preparation of Test Organism

A 10 µL aliquot of a thawed, vortex mixed cryovial of stock organism broth culture was transferred to an initial 10 mL tube of growth medium.

The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation, without vortex mixing the *Pseudomonas* culture, a 10 µL aliquot of culture was transferred to sufficient 20 x 150 mm Morton Closure tubes containing 10 mL of culture medium (daily transfer #1). For testing performed 3/17/15, one additional daily transfer was prepared inoculating a sufficient number of tubes for the final test culture. For testing performed 3/18/15, two additional daily transfers were prepared inoculating a sufficient number of tubes for the final test culture. For testing performed 3/19/15, three additional daily transfers were prepared inoculating a sufficient number of tubes for the final test culture. The final test culture was incubated for 48-54 hours at 35-37°C.

On the day of use, the pellicle was carefully aspirated from the *Pseudomonas aeruginosa* culture by vacuum aspiration. Care was taken to avoid disrupting the pellicle and any visible pellicle on the bottom of the tube was not harvested. To avoid harvesting any visible pellicle at the bottom of the tube, the upper portion of the culture was transferred to a sterile tube. Any culture with disrupted pellicle was not used.

Each test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed. The *Pseudomonas* culture was visually inspected to ensure no pellicle fragments were present.

The *Salmonella enterica* culture was diluted using sterile growth medium by combining 32.0 mL of test organism suspension with 160.0 mL of sterile growth medium.

For testing performed 3/18/15, the *Staphylococcus aureus* culture was diluted using sterile growth medium by combining 40.0 mL of test organism suspension with 40.0 mL of sterile growth medium. For testing performed 3/19/15, the *Staphylococcus aureus* culture was diluted using sterile growth medium by combining 35.0 mL of test organism suspension with 35.0 mL of sterile growth medium.

Each final test culture was mixed thoroughly prior to use.

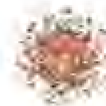
Addition of Organic Soil Load
For *S.aureus* and *P.aeruginosa*, a 3.5 mL aliquot of FBS was added to 66.5 mL of prepared culture to yield a 5% fetal bovine serum organic soil load.

For *S.enterica*, a 9.6 mL aliquot of FBS was added to 182.4 mL of prepared culture to yield a 5% fetal bovine serum organic soil load.

Contamination of Carriers
The culture was transferred to the penicylinders (after siphoning off the water) and the carriers were immersed for 15±2 minutes in a prepared suspension at a ratio of one carrier per one mL of culture. The carriers were completely covered by the culture. A maximum of 100 carriers were inoculated per vessel. The inoculated carriers were transferred to sterile Petri dishes matted with filter paper after tapping the carrier against the side of the container to remove excess inoculum. No more than twelve carriers were placed in each Petri dish. The carriers were dried for 38 minutes at 35-37°C (36.1-36.2°C) and at 53-55.6% relative humidity. Carriers were used in the test procedure within 2 hours of drying.

Exposure Conditions
Each contaminated and dried carrier was placed into a separate tube containing 10.0 mL of the test substance at its use-dilution. Immediately after placing each test carrier in the test tube, the tube was swirled using approximately 2–3 gentle rotations to release any air bubbles trapped in or on the carrier. The carriers were exposed for 10 minutes at 20.0°C. Care was taken to avoid touching the sides of the tubes. The carrier was placed into the test substance within ±5 seconds of the exposure time following a calibrated timer.

Test System Recovery
Following the Sponsor specified exposure time, each medicated carrier was transferred by wire hook at staggered intervals to 10 mL of neutralizing subculture medium and each tube was shaken thoroughly. To accomplish this, the carrier was removed from the disinfectant tube with a sterile hook, tapped against the interior sides of the tube to remove the excess disinfectant and transferred into the subculture tube. Tapping the carrier against the upper third of the tube was avoided. Care was taken to avoid excessive contact with the interior sides of the subculture tubes during transfer.



Incubation and Observation
All subculture vessels and control plates were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of growth.

On 3/19/15 and 3/21/15, representative test and positive control subculture tubes showing growth were subcultured to Tryptic Soy Agar + 5% Sheep's blood and incubated at 35-37°C for one day. The resultant growth was visually examined, Gram stained and biochemically assayed to confirm or rule out the presence of the test organism.

STUDY CONTROLS

Study controls were performed the same on each test date, unless otherwise noted.

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

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

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

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

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

Neutralization Confirmation Control
The neutralization of the test substance was confirmed concurrent with testing by exposing at least one sterile carrier to the test substance and transferring the carrier to subcultures containing 10 mL of neutralizing subculture medium as in the test. The subcultures were inoculated with a target of 10-100 colony forming units (CFU) of each test organism, incubated under test conditions and visually examined for the presence of growth. This control was performed with multiple replicates using different dilutions of the test organism. A standardized spread plate procedure was run concurrently in order to enumerate the number of CFU per tube actually added.

The acceptance criterion for this study control is growth in the subculture broth following inoculation with ≤100 CFU per tube.

Carrier Population Control
Two sets of three inoculated carriers (one set prior to testing and one set following testing) for each organism carrier set were assayed. Each inoculated carrier was individually subcultured into a tube containing 10 mL of neutralizing subculture medium and sonicated for 1 minute ±5 seconds. Tubes were contained in a beaker with water suspended in the ultrasonic cleaner such that all fluids were level. Following sonication, the contents of the three subcultured carriers were pooled (30mL) and briefly vortex mixed. For testing performed 3/18/15, following sonication and pooling of the subcultured carriers, the vessels were refrigerated at 2-8°C for no longer than 2 hours prior to dilution. Appropriate serial ten-fold dilutions were prepared and the duplicate aliquots spread plated on agar plate medium and incubated. Following incubation, the resulting colonies were enumerated and the CFU per carrier set was calculated. The individual CFU per carrier set results were calculated and the Log_{10} value of each carrier set was determined. The average Log_{10} value per organism was calculated. For *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the acceptance criterion for this study control is a minimum average Log_{10} value of 6.0. For *Salmonella enterica*, the acceptance criterion for this study control is a minimum average Log_{10} value of 5.0.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
For *Staphylococcus aureus*, the efficacy performance requirements for label claims state that the test substance must kill the microorganism on 57 out of the 60 inoculated carriers.

For *Pseudomonas aeruginosa*, the efficacy performance requirements for label claims state that the test substance must kill the microorganism on 54 out of the 60 inoculated carriers.

For *Salmonella enterica*, the efficacy performance requirements for label claims state that the test substance must kill the microorganism on 59 out of the 60 inoculated carriers.

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

PROTOCOL CHANGES

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

Protocol Deviation:
Day 1 testing of Lot 13-12-03 against *P.aeruginosa* and *S.aureus*, and also testing of all three lots against *S.enterica* was inadvertently performed 3/17/15 using 397 ppm AOAC Synthetic Hard Water as the product diluent instead of 300 ppm Hard Water as specified in the protocol. The use of a higher ppm Hard Water solution when diluting the test substance typically results in a more difficult challenge, however because all test subcultures met the required test acceptance criteria on this day of testing (despite the higher ppm solution), the use of 397 ppm Hard Water had no impact on testing and results remain valid.

DATA ANALYSIS

Calculations

The CFU/Carrier set in the Carrier Population Control was determined using all average counts between 0-300 CFU as follows:

$$\text{CFU/carrier} = \frac{[(\text{avg. CFU for } 10^{-x}) + (\text{avg. CFU for } 10^{-y}) + (\text{avg. CFU for } 10^{-z})] \times (\text{Volume of neutralizer})}{[10^{-x} + 10^{-y} + 10^{-z}] \times (\text{Volume plated}) \times (\text{\# of carriers per set})}$$

Where 10^{-x}, 10^{-y}, and 10^{-z} are example dilutions that may be used

$$\text{Average } Log_{10} \text{ Carrier Population Control} = \frac{Log_{10}X_1 + Log_{10}X_2 + \ldots Log_{10}X_N}{N}$$

Where: X equals CFU/carrier set
N equals number of control carrier sets

Statistical Analysis

None used.

STUDY RETENTION

Record Retention

All of the original raw data developed exclusively for this study shall be archived at Accuratus Lab Services, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121 for a minimum of five years following the study completion date. After this time, the Sponsor (or the Sponsor Representative, if applicable) will be contacted to determine the final disposition. 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. Association of Official Analytical Chemists (AOAC) Official Method 964.02, Testing Disinfectants against *Pseudomonas aeruginosa* - Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
2. Association of Official Analytical Chemists (AOAC) Official Method 955.15, Testing Disinfectants against *Staphylococcus aureus* - Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
3. Association of Official Analytical Chemists (AOAC) Official Method 955.14, Testing Disinfectants against *Salmonella enterica*- Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
4. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of synthetic hard water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
5. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.
6. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.
7. Health Canada, January, 2014. Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.
8. Health Canada, January, 2014. Guidance Document - Disinfectant Drugs.

RESULTS

For Control and Neutralization Results, see Tables 1-5.
All data measurements/controls including the culture purity, viability, organic soil sterility, neutralizing subculture medium sterility, carrier sterility, carrier population, and neutralization confirmation were within acceptance criteria.

For Test Results, see Tables 6 and 7.

ANALYSIS

Gluquat 300 (Lot 14-08-28) diluted 1:200 (defined as 1 part test substance + 200 parts 300 ppm AOAC synthetic hard water), demonstrated no growth of *Pseudomonas aeruginosa* (ATCC 15442) in any of the 60 subculture tubes following a 10 minute exposure time at 20-22°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

Gluquat 300 (Lot 13-12-03) diluted 1:200 (defined as 1 part test substance + 200 parts 397 ppm AOAC synthetic hard water), demonstrated no growth of *Pseudomonas aeruginosa* (ATCC 15442) in any of the 60 subculture tubes following a 10 minute exposure time at 20-22°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

Gluquat 300 (Lot 14-02-18) diluted 1:200 (defined as 1 part test substance + 200 parts 300 ppm AOAC synthetic hard water), demonstrated growth of *Pseudomonas aeruginosa* (ATCC 15442) in 1 of the 60 subculture tubes following a 10 minute exposure time at 20-22°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

Gluquat 300 (Lot 13-12-03) diluted 1:200, defined as 1 part test substance + 200 parts 397 ppm AOAC synthetic hard water, demonstrated no growth of *Salmonella enterica* (ATCC 10708) in any of the 60 subculture tubes following a 10 minute exposure time at 20-22°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

Gluquat 300 (Lot 14-08-28) diluted 1:200, defined as 1 part test substance + 200 parts 300 ppm AOAC synthetic hard water, demonstrated no growth of *Salmonella enterica* (ATCC 10708) in any of the 60 subculture tubes following a 10 minute exposure time at 20-22°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

Gluquat 300 (Lot 14-02-18) diluted 1:200 (defined as 1 part test substance + 200 parts 397 ppm AOAC synthetic hard water), demonstrated growth of *Salmonella enterica* (ATCC 10708) in 1 of the 60 subculture tubes following a 10 minute exposure time at 20-22°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

Gluquat 300 (Lot 14-02-18 and Lot 14-08-28) diluted 1:200 (defined as 1 part test substance + 200 parts 300 ppm AOAC synthetic hard water), demonstrated no growth of *Staphylococcus aureus* (ATCC 6538) in any of the 60 subculture tubes following a 10 minute exposure time at 20-22°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

Gluquat 300 (Lot 13-12-03) diluted 1:200 (defined as 1 part test substance + 200 parts 397 ppm AOAC synthetic hard water), demonstrated no growth of *Staphylococcus aureus* (ATCC 6538) in any of the 60 subculture tubes following a 10 minute exposure time at 20-22°C (20.0°C) in the presence of a 5% fetal bovine serum organic soil load.

STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum organic soil load, Gluquat 300, diluted 1:200 (defined as 1 part test substance + 200 parts 300 ppm AOAC synthetic hard water), evaluated on three separate test dates, demonstrated efficacy against *Pseudomonas aeruginosa* and *Staphylococcus aureus* as required by the U.S. EPA and Health Canada following a 10 minute exposure time at 20-22°C (20.0°C).

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum organic soil load, Gluquat 300, diluted 1:200 (defined as 1 part test substance + 200 parts 300 ppm AOAC synthetic hard water), demonstrated efficacy against *Salmonella enterica* as required by the U.S. EPA and Health Canada following a 10 minute exposure time at 20-22°C (20.0°C).

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

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TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Date Performed: 3/17/15			
Type of Control	**Results**		
	***Pseudomonas aeruginosa* (ATCC 15442)**	***Salmonella enterica* (ATCC 10708)**	***Staphylococcus aureus* (ATCC 6538)**
Purity Control	Pure	Pure	Pure
Viability Control	Growth	Growth	Growth
Organic Soil Load Sterility Control	No Growth		
Neutralizing Subculture Medium Sterility Control	No Growth		
Carrier Sterility Control	No Growth		

Date Performed: 3/18/15		
Type of Control	**Results**	
	***Pseudomonas aeruginosa* (ATCC 15442)**	***Staphylococcus aureus* (ATCC 6538)**
Purity Control	Pure	Pure
Viability Control	Growth	Growth
Organic Soil Load Sterility Control	No Growth	
Neutralizing Subculture Medium Sterility Control	No Growth	
Carrier Sterility Control	No Growth	
Date Performed: 3/19/15		
Purity Control	Pure	Pure
Viability Control	Growth	Growth
Organic Soil Load Sterility Control	No Growth	
Neutralizing Subculture Medium Sterility Control	No Growth	
Carrier Sterility Control	No Growth	

TABLE 2: CARRIER POPULATION CONTROL RESULTS - *Pseudomonas aeruginosa*

Test Date: 3/17/15							
Volume Plated: 0.100 mL							
Carrier set	Dilution Factor				CFU/ carrier	Log_{10}	Average Log_{10}
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			
Pre-testing	T,T	150,159	15,22	1,0	1.58×10^6	6.20	6.47
Post-testing	T,T	T,T	56,49	7,6	5.5×10^6	6.74	
Test Date: 3/18/15							
Volume Plated: 0.100 mL							
Carrier set	Dilution Factor				CFU/ carrier	Log_{10}	Average Log_{10}
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			
Pre-testing	T,T	T,T	35,31	5,2	3.4×10^6	6.53	6.26
Post-testing	T,T	102,84	11,17	1,0	9.7×10^5	5.99	
Test Date: 3/19/15							
Volume Plated: 0.100 mL							
Carrier set	Dilution Factor				CFU/ carrier	Log_{10}	Average Log_{10}
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			
Pre-testing	T,T	T,T	37,34	3,2	3.5×10^6	6.54	6.52
Post-testing	T,T	T,T	32,28	5,2	3.1×10^6	6.49	

CFU = Colony Forming Unit
T = Too Numerous To Count (>300 colonies)

TABLE 3: CARRIER POPULATION CONTROL RESULTS -
Salmonella enterica

Test Date: 3/17/15							
Volume Plated: 0.100 mL							
Carrier set	**Dilution Factor**				**CFU/ carrier**	**Log_{10}**	**Average Log_{10}**
	10^{-1}	**10^{-2}**	**10^{-3}**	**10^{-4}**			
Pre-testing	T,T	T,T	42,47	1,9	4.5×10^6	6.65	6.08
Post-testing	T,T	28,40	2,2	0,0	3.2×10^5	5.51	

CFU = Colony Forming Unit
T = Too Numerous To Count (>300 colonies)

TABLE 4: CARRIER POPULATION CONTROL RESULTS - *Staphylococcus aureus*

Test Date: 3/17/15							
Volume Plated: 0.100 mL							
Carrier set	Dilution Factor				CFU/ carrier	Log_{10}	Average Log_{10}
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			
Pre-testing	T,T	T,T	31,30	4,5	3.3×10^6	6.52	6.70
Post-testing	T,T	T,T	67,79	7,9	7.4×10^6	6.87	
Test Date: 3/18/15							
Volume Plated: 0.100 mL							
Carrier set	Dilution Factor				CFU/ carrier	Log_{10}	Average Log_{10}
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			
Pre-testing	T,T	T,T	30,30	2,4	3.0×10^6	6.48	6.43
Post-testing	T,T	230,238	23,25	4,4	2.36×10^6	6.37	
Test Date: 3/19/15							
Volume Plated: 0.100 mL							
Carrier set	Dilution Factor				CFU/ carrier	Log_{10}	Average Log_{10}
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			
Pre-testing	T,T	T,T	51,56	8,6	5.5×10^6	6.74	6.56
Post-testing	T,T	244,235	18,26	5,1	2.39×10^6	6.38	

CFU = Colony Forming Unit
T = Too Numerous To Count (>300 colonies)

TABLE 5: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Dilution	CFU Added	Average CFU	Number of Subcultures	
					Tested	Positive
Gluquat 300 Lot 13-12-03	*Pseudomonas aeruginosa* (ATCC 15442)	10^{-5}	164,147	156	1	1
		10^{-6}	18,16	17	1	1
		10^{-7}	3,1	2	1	1
	Salmonella enterica (ATCC 10708)	10^{-5}	T,T	>300	1	1
		10^{-6}	134,125	130	1	1
		10^{-7}	12,9	11	1	1
	Staphylococcus aureus (ATCC 6538)	10^{-5}	T,T	>300	1	1
		10^{-6}	41,29	35	1	1
		10^{-7}	4,2	3	1	1
Gluquat 300 Lot 14-02-18	*Pseudomonas aeruginosa* (ATCC 15442)	10^{-5}	204,211	208	1	1
		10^{-6}	26,15	21	1	1
		10^{-7}	5,4	5	1	0
	Salmonella enterica (ATCC 10708)	10^{-5}	T,T	>300	1	1
		10^{-6}	134,125	130	1	1
		10^{-7}	12,9	11	1	1
	Staphylococcus aureus (ATCC 6538)	10^{-5}	T,T	>300	1	1
		10^{-6}	59,63	61	1	1
		10^{-7}	9,7	8	1	1

CFU = Colony Forming Unit
T = Too Numerous To Count (>300 colonies)

TABLE 5: NEUTRALIZATION CONFIRMATION CONTROL RESULTS (Continued)

Test Substance	Test Organism	Dilution	CFU Added	Average CFU	Number of Subcultures	
					Tested	Positive
Gluquat 300 Lot 14-08-28	*Pseudomonas aeruginosa* (ATCC 15442)	10^{-5}	115,128	122	1	1
		10^{-6}	12,15	14	1	1
		10^{-7}	2,0	1	1	1
	Salmonella enterica (ATCC 10708)	10^{-5}	T,T	>300	1	1
		10^{-6}	134,125	130	1	1
		10^{-7}	12,9	11	1	1
	Staphylococcus aureus (ATCC 6538)	10^{-5}	T,T	>300	1	1
		10^{-6}	62,57	60	1	1
		10^{-7}	4,3	4	1	1

CFU = Colony Forming Unit
T = Too Numerous To Count (>300 colonies)

TABLE 6: TEST RESULTS – *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Test Substance	Test Organism	Sample Dilution	Number of Carriers		
			Exposed	Showing Growth	Confirmed As Test Organism
Date Performed: 3/17/15					
Gluquat 300 Lot 13-12-03	*Pseudomonas aeruginosa* (ATCC 15442)	1:200	60	0	0
	Staphylococcus aureus (ATCC 6538)		60	0	0
Date Performed: 3/18/15					
Gluquat 300 Lot 14-08-28	*Pseudomonas aeruginosa* (ATCC 15442)	1:200	60	0	0
	Staphylococcus aureus (ATCC 6538)		60	0	0
Date Performed: 3/19/15					
Gluquat 300 Lot 14-02-18	*Pseudomonas aeruginosa* (ATCC 15442)	1:200	60	1	1
	Staphylococcus aureus (ATCC 6538)		60	0	0

TABLE 7: TEST RESULTS – *Salmonella enterica*

Date Performed: 3/17/15					
Test Substance	Test Organism	Sample Dilution	Number of Carriers		
			Exposed	Showing Growth	Confirmed As Test Organism
Gluquat 300 Lot 13-12-03	*Salmonella enterica* (ATCC 10708)	1:200	60	0	0
Gluquat 300 Lot 14-02-18			60	1	1
Gluquat 300 Lot 14-08-28			60	0	0

PROTOCOL

AOAC Use-Dilution Method

Test Organisms:

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

PROTOCOL NUMBER

WPC02013015.UD

PREPARED FOR

West Penetone, Inc.
10900 Secant
Anjou, QC H1J 1S5
Canada

PERFORMING LABORATORY

Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

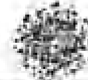
DATE

January 30, 2015

3-26-15

PROPRIETARY INFORMATION

THIS DOCUMENT IS THE PROPERTY OF AND CONTAINS PROPRIETARY INFORMATION OF ACCURATUS LAB SERVICES. NEITHER THIS DOCUMENT, NOR INFORMATION CONTAINED HEREIN IS TO BE REPRODUCED OR DISCLOSED TO OTHERS IN WHOLE OR IN PART, NOR USED FOR ANY PURPOSE OTHER THAN THE PERFORMANCE OF THIS WORK ON BEHALF OF THE SPONSOR, WITHOUT PRIOR WRITTEN PERMISSION OF ACCURATUS LAB SERVICES.



AOAC Use-Dilution Method

SPONSOR: West Penetone, Inc.
10900 Secant
Anjou, QC H1J 1S5
Canada

TEST FACILITY: Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE
The purpose of this study is to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method is in compliance with the requirements of the following: The U.S. Environmental Protection Agency (EPA) and Health Canada.

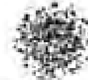
TEST SUBSTANCE CHARACTERIZATION
According to (40 CFR, Part 160, Subpart F [160.105]) test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with 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 Accuratus Lab Services. Accuratus Lab Services will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once Accuratus Lab Services receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is February 12, 2015. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of March 12, 2015. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Accuratus Lab Services.

If a test must be repeated, or a portion of it, due to failure by Accuratus Lab Services 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 Accuratus Lab Services nor any of its employees are to be used in advertising or other promotion without written consent from Accuratus Lab Services.

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 Accuratus Lab Services final report and notify Accuratus Lab Services of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Accuratus Lab Services will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

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

Protocol Number: WPC02013015.UD
West Penetone, Inc
Page 3 of 12

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TEST PRINCIPLE

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

TEST METHOD

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

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

Recovery Agar Medium: Tryptic Soy Agar + 5% Sheep's Blood

Carriers

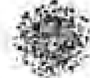
Carriers will be screened according to AOAC Official Method of Analysis and any carrier positive for growth will be discarded. Only penicylinders showing no growth may be used. Stainless steel penicylinders will be pre-soaked overnight in 1N NaOH, washed in water until neutral and autoclaved in deionized water. Carriers shall be used within three months of sterilization.

Preparation of Test Organism

Transfer 10 µL of a thawed, vortex mixed, cryovial of stock organism broth culture to an initial 10 mL tube of growth medium.

Mix and incubate the initial culture for 24±2 hours at 35-37°C. Following incubation, and without vortex mixing the *Pseudomonas* culture, transfer 10 µL of culture to sufficient 20 x 150 mm Morton closure tubes containing 10 mL of culture medium (daily transfer #1). One daily transfer is required but up to four additional daily transfers may be prepared. Incubate the final test culture for 48-54 hours at 35-37°C. On the day of use, the pellicle will be carefully aspirated from the *Pseudomonas aeruginosa* culture by vacuum aspiration. Care will be taken to avoid disrupting the pellicle and any visible pellicle on the bottom of the tube will not be harvested. To avoid harvesting any visible pellicle at the bottom of the tube, the upper portion of the culture may be transferred to a sterile tube. Any disruption of the pellicle resulting in dropping or breaking up of the pellicle before or during removal renders that culture tube unusable.

The test culture will be vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture will be removed, leaving behind any clumps or debris and will be pooled in a sterile vessel and mixed. The *Pseudomonas* culture will be visually inspected to ensure no pellicle fragments are present. The culture may be diluted or centrifuge-concentrated. Applicable culture dilutions shall be performed using sterile growth medium. An organic soil load will be added to the test culture per Sponsor's request. The final test culture will be mixed thoroughly prior to use.



Contamination of Carriers
The culture will be transferred to the penicylinders (after siphoning off the water) and the carriers will be immersed for 15±2 minutes in a prepared suspension at a ratio of one carrier per one mL of culture to completely cover the carriers. A maximum of 100 carriers will be inoculated per vessel and each vessel inoculated may be considered a part of one total inoculation run per organism. The inoculated carriers will be transferred to sterile Petri dishes matted with filter paper after tapping the carrier against the side of the container to remove excess inoculum. No more than twelve carriers will be placed in each Petri dish. The carriers will be dried for 40±2 minutes at 35-37°C. *NOTE: Organisms not specifically mentioned in the AOAC methodology may require modified drying conditions for the purpose of obtaining maximum survival following drying. The actual drying conditions will be clearly documented.* Carriers will be used in the test procedure within 2 hours of drying. Carriers that touch during drying or have fallen over will not be used in the test.

Preparation of Test Substance
The test substance(s) to be assayed will be used as directed by the Sponsor. If a dilution of the test substance is requested by the Sponsor, the diluted test substance(s) shall be used within three hours of preparation. For products requiring dilution, use ≥1.0 mL or ≥1.0 g of test substance and volumetric glassware when preparing the dilution unless otherwise specified by the Sponsor. Ten (10) mL of the test substance at its use-dilution will be aliquotted into the required number of sterile 25 x 150 mm or 25 x 100 mm tubes. The tubes will be placed into a waterbath at the specified exposure temperature, and allowed to equilibrate for ≥10 minutes prior to testing.

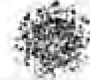
Exposure Conditions
Each contaminated and dried carrier will be placed into a separate tube containing 10 mL of the test substance at its use-dilution for the desired exposure time and temperature. Immediately after placing each test carrier in the test tube, swirl the tube using approximately 2–3 gentle rotations to release any air bubbles trapped in or on the carrier. Care will be taken to avoid touching the sides of the tubes which may compromise exposure. The carrier will be placed into the test substance within ±5 seconds of the exposure time for exposure times above 1 minute following a calibrated timer. The carrier will be placed into the test substance within ±3 seconds of the exposure time for exposure times of ≤1 minute. If the exposure conditions are compromised in any way for a given carrier, a new carrier may be treated in its place. If this cannot be done, the carrier will be marked and the compromised carrier will be identified in the raw data. If a marked carrier demonstrates a positive result, the carrier set may be invalidated and repeated by Sponsor request.

Test System Recovery
Following the Sponsor specified exposure time, each medicated carrier will be transferred by wire hook at staggered intervals to 10 mL of primary neutralizing subculture medium and each tube will be shaken thoroughly. To accomplish this, the carrier is removed from the disinfectant tube with a sterile hook, tapped against the interior sides of the tube to remove the excess disinfectant, avoiding the upper one-third of the tube, and transferred into the subculture tube. Care will be taken to avoid excessive contact to the interior sides of the subculture tubes during transfer. If secondary neutralization is requested by the Sponsor or deemed necessary due to test substance active and/or concentration, carriers will be transferred into individual secondary subculture tubes containing 10 mL of neutralizing broth beginning approximately 25-60 minutes after subculture of the carrier into the primary neutralizing subculture medium. Shake each tube thoroughly. If neutralization is a concern, 20 mL of subculture medium may be used.

Incubation and Observation
All subculture vessels and control plates are incubated for 48±2 hours at 35-37°C.

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

Representative subculture tubes showing growth will be subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism.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STUDY CONTROLS

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

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

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

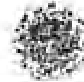
Neutralizing Subculture Medium Sterility Control
A representative sample of uninoculated neutralizing subculture medium will be incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control
One representative inoculated carrier will be added to a vessel containing each type of subculture medium. If secondary subcultures are performed using a different media type, one carrier will be placed in the primary subculture medium and one carrier will be placed in the secondary subculture medium. The vessels containing each carrier will be incubated and visually examined for growth. The acceptance criterion for this study control is growth in the subculture media.

Neutralization Confirmation Control
Prior to testing or concurrent with testing, the neutralization of the test substance will be confirmed by exposing at least one sterile carrier to the test substance and transferring the carrier to primary subcultures containing 10-20 mL of neutralizing subculture medium as in the test. If performed in the test procedure, each carrier will then be transferred from primary subcultures into individual secondary subcultures beginning approximately 25-60 minutes following the primary transfer. The subcultures (primary and secondary as applicable) will be inoculated with a target of 10-100 colony forming units (CFU) of each test organism, incubated under test conditions and visually examined for the presence of growth. This control will be performed with multiple replicates using different dilutions of the test organism. A standardized spread plate procedure will be run concurrently in order to enumerate the number of CFU actually added per tube. NOTE: Only the most concentrated test substance dilution and/or shortest exposure time needs to be evaluated in this control.

The acceptance criterion for this study control is growth in the final subculture broth, minimally, following inoculation with ≤100 CFU per tube. If all the organism dilution(s) used in this control fail to provide adequate numbers (10-100 CFU) which coincides in a failure to meet the acceptance criterion for this study control, the control may be repeated in its entirety.

Carrier Population Control
Two sets of three inoculated carriers (one set prior to testing and one set following testing) for each organism carrier set will be assayed. Each inoculated carrier will be individually subcultured into a tube containing 10 mL of neutralizing subculture medium and sonicated for 1 minute±5 seconds. Tubes will be contained in a beaker with water suspended in the ultrasonic cleaner such that all fluids will be level. Following sonication, the contents of the three subcultured carriers will be pooled (30 mL) and briefly vortex mixed. Appropriate serial ten-fold dilutions will be prepared and the duplicate 0.1 mL aliquots spread plated on agar plate medium, and incubated. If serial dilutions are not performed and plated immediately following sonication, the vessels may be refrigerated at 2-8°C for up to 2 hours prior to dilution. Following incubation, the resulting colonies will be enumerated and the CFU per carrier set calculated. The individual CFU per carrier set results will be calculated, and the Log_{10} value of each carrier set determined. The average Log_{10} value per organism will be calculated. For *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the acceptance criterion for this study control is a minimum average Log_{10} value of 6.0. For *Salmonella enterica*, the acceptance criterion for this study control is a minimum average Log_{10} value of 5.0.



PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM
Accuratus Lab Services 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
For *Staphylococcus aureus*, the efficacy performance requirements for label claims state that the test substance must kill the microorganism on 57 out of the 60 inoculated carriers.

For *Pseudomonas aeruginosa*, the efficacy performance requirements for label claims state that the test substance must kill the microorganism on 54 out of the 60 inoculated carriers.

For *Salmonella enterica*, the efficacy performance requirements for label claims state that the test substance must kill the microorganism on 59 out of the 60 inoculated carriers.

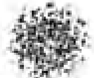
Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any control acceptance criteria are not met, the test may be repeated under the current protocol number. If the population control exceeds an average $\log_{10}$ value of 7.0 for *Staphylococcus aureus* and/or *Pseudomonas aeruginosa* or 6.0 for *Salmonella enterica*, and the test substance does not meet the performance criteria, the Sponsor may invalidate the study and repeat testing.

Any positive test carriers confirmed as a contaminant will be reported. Any test carrier set that demonstrates a number of contaminated tubes that contributes to results that exceed the product performance/success criteria may be invalidated per Sponsor's request and may be re-tested. For sixty carrier studies, only one contaminant is tolerated per carrier set.

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

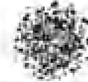
All of the original raw data developed exclusively for this study shall be archived at Accuratus Lab Services for a minimum of five years for GLP studies or a minimum of six months for all other studies following the study completion date. After this time, the Sponsor (or the Sponsor Representative, if applicable) will be contacted to determine the final disposition. 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 Accuratus Lab Services. These documents include, but are not limited to, the following:

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



REFERENCES

1. Association of Official Analytical Chemists (AOAC) Official Method 964.02, Testing Disinfectants against *Pseudomonas aeruginosa* - Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
2. Association of Official Analytical Chemists (AOAC) Official Method 955.15, Testing Disinfectants against *Staphylococcus aureus* - Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
3. Association of Official Analytical Chemists (AOAC) Official Method 955.14, Testing Disinfectants against *Salmonella enterica*- Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
4. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
5. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.
6. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.
7. Health Canada, January, 2014. Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.
8. Health Canada, January, 2014. Guidance Document - Disinfectant Drugs.

DATA ANALYSIS

Calculations

Determine the CFU/Carrier set in the Carrier Population Control using all average counts between 0-300 CFU as follows:

$$\text{CFU/carrier} = \frac{[(\text{avg. CFU for } 10^{-6}) + (\text{avg. CFU for } 10^{-7}) + (\text{avg. CFU for } 10^{-8})] \times (\text{Volume of neutralizer})}{[10^{-6} + 10^{-7} + 10^{-8}] \times (\text{Volume plated}) \times (\text{\# of carriers per set})}$$

where 10^{-6}, 10^{-7}, and 10^{-8} are example dilutions that may be used.

$$\text{Average } Log_{10} \text{ Carrier Population Control} = \frac{Log_{10}X_1 + Log_{10}X_2 + \ldots Log_{10}X_N}{N}$$

Where: X equals CFU/carrier set
N equals number of control carrier sets

Statistical Analysis
None used

STUDY INFORMATION

(All blank sections are completed by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)

Test Substance (Name & Batch Numbers) exactly as it should appear on final report:

Test Date #1: Gluquat 300 lot 13-12-03
Test Date #2: Gluquat 300 lot 14-08-28
Test Date #3: Gluquat 300 lot 14-02-18

Testing at the lower certified limit (LCL) is required for registration, no aged batch is necessary.

Product Description:

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

Approximate Test Substance Active Concentration (upon submission to Accuratus Lab Services):
6.7% Quat and 12.5% glutaraldehyde

(This value is used for neutralization planning only. This value is not intended to represent characterization values.)

Neutralization/Subculture Broth: ☐

(NOTE: All broth must also serve as an appropriate growth medium for the test organism)

☑ Accuratus Lab Services' Discretion. By checking, the Sponsor authorizes Accuratus Lab Services, 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: 1:200 defined as 1 part (amount of test substance) + 200 parts (amount of diluent)
  (example: 1 oz/gallon)
  - ☐ Deionized Water (Filter or Autoclave Sterilized)
  - ☐ Soft Tap Water (Filter or Autoclave Sterilized)
  - ☑ AOAC Synthetic Hard Water: 300 PPM
  - ☐ Other

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

Test Organisms:

- ☑ *Pseudomonas aeruginosa* (ATCC 15442)
- ☑ *Staphylococcus aureus* (ATCC 6538)
- ☑ *Salmonella enterica* (ATCC 10708) – *all batches may be tested on one test date*

Carrier Number: 60 per batch

Exposure Time: 10 minutes

Exposure Temperature: 20-22 °C

Organic Soil Load:

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

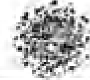
Clarified per email 2-25-15. JR 2-25-15

Template: 2HI-11G

- Proprietary Information -

Protocol Number: WPC02013015.UD

West Penetone, Inc.
Page 10 of 12



TEST SUBSTANCE SHIPMENT STATUS

(This section is for informational purposes only.)

☐ Test Substance is already present at Accuratus Lab Services.
☑ Test Substance has been or will be shipped to Accuratus Lab Services.
Date of expected receipt at Accuratus Lab Services: __________
☐ Test Substance to be hand-delivered (must arrive by noon at least one day prior to testing or other arrangements made with the Study director)

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 or Development Study)

PROTOCOL MODIFICATIONS

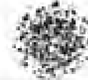
☑ Approved without modification
☐ Approved with modification

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

Template: 210-11G – Proprietary Information –

1285 Corporate Center Drive, Suite 110 · Eagan, MN 55121 · 877.287.8378 · 651.379.5510 · www.accuratuslabs.com



TEST SUBSTANCE CHARACTERIZATION & STABILITY TESTING
[Verification required per 40 CFR Part 160 Subpart B (160.31(d))].

☐ Characterization/Stability testing is not required (For Non-GLP or Development testing only)

OR

Physical and Chemical Characterization (identity, purity, strength, solubility, as applicable) of the test lots

☐ **Physical & Chemical Characterization has been or will be completed prior to efficacy testing.**

GLP compliance status of physical & chemical characterization testing:
- ☐ Testing was or will be performed following 40 CFR Part 160 GLP regulations
- ☐ Characterization has not been or will not be performed following GLP regulations

Check and complete the following that apply:
- ☐ A Certificate of Analysis (C of A) has been or will be provided for each lot of test substance to be appended to the report.
- ☐ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #: ____________
- ☐ Test has been or will be conducted by another facility under protocol or study #: ____________

☑ **Physical & Chemical Characterization was not or will not be performed prior to efficacy testing.**

Stability Testing of the formulation

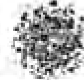
☐ **Stability testing has been or will be completed prior to or concurrent with efficacy testing.**

GLP compliance status of stability testing:
(GLP compliance is required by 40 CFR Part 160)
- ☐ Testing was or will be performed following 40 CFR Part 160 GLP regulations
- ☐ Stability testing has not been or will not be performed following GLP regulations

Check and complete the following that apply:
- ☐ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #: ____________
- ☐ Test has been or will be conducted by another facility under protocol or study #: ____________

☑ **Stability testing was not or will not be performed prior to or concurrent with efficacy testing.**

If test substance characterization or stability testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.



APPROVAL SIGNATURES

SPONSOR:

NAME: Mr. Pierre Stewart TITLE: Manager of Technical Services

SIGNATURE: [signature] DATE: FEB 17 2015

PHONE: 1 (514) 355 - 6060 Ext: 3007 FAX: 1 (514) 355 – 2319 EMAIL: pstewart@westpenetoneinc.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

Accuratus Lab Services:

NAME: Joshua Luedtke
Study Director

SIGNATURE: [signature] DATE: 2-26-15
Study Director

element

FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Porcine Epidemic Diarrhea Virus

PRODUCT IDENTITY

Gluquat 300
Lot 2211051 and Lot 2302013

TEST GUIDELINE

OCSPP 810.2200

PROTOCOL NUMBER

WPC02020123.PEDV

AUTHOR

Mary J. Miller, M.T.
Study Director

STUDY COMPLETION DATE

April 24, 2023

PERFORMING LABORATORY

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

PROJECT NUMBER

A37720

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: West Penetone, Inc.

Company Agent: ______________________________

____
Title

______________________________ Date:__________
Signature

GOOD LABORATORY PRACTICE STATEMENT

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

Stability testing of the compounds was not performed by the Sponsor prior to use in the study or concurrent with the study per 40 CFR Part 160.

The following study was not performed following GLP regulations: characterization of the compounds.

Submitter:____________________________ Date:__________

Sponsor:____________________________ Date:__________

Study Director: Mary J. Miller (signature) Date: 4-24-23
Mary J. Miller, M.T.

QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed in accordance to standard operating procedures and the study protocol. In accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to Management and the Study Director.

Phase Inspected	Date of Phase Inspection	Date Reported to Study Director	Date Reported to Management
Critical Phase Audit: Preparation of Test Substance	April 4, 2023	April 4, 2023	April 24, 2023
Final Report	April 20, 2023	April 20, 2023	

Quality Assurance Specialist: Date: 4/24/23

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STUDY PERSONNEL

STUDY DIRECTOR: Mary J. Miller, M.T.

Professional Personnel Involved:

Kelleen Lauer, M.S.	- General Manager
Nicole Felicelli, B.A.	- Manager, Study Director Operations
Miranda Peskar, B.S.	- Core Services Laboratory Operations Manager
Joseph Artus, B.S.	- Virologist
Maria Cullen, B.A.	- Virologist
Sydney Sawatzke, B.S.	- Associate Virologist

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Project Number: A37720

Protocol Number: WPC02020123.PEDV

Sponsor: West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

Testing Facility: Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance Name: Gluquat 300

Lot/Batch(s): Lot 2211051 and Lot 2302013

Manufacture Date: Lot 2211051 – November 8, 2022
Lot 2302013 – January 17, 2023

Test Substance Characterization

Test substance characterization as to identity, strength, purity, and uniformity, as applicable, was performed and documented prior to its use in this study, however not in accordance with 40 CFR Part 160, Subpart F (160.105). Test substance stability testing was not performed prior to or concurrent with its use in this study. The Test Substance Certificate of Analysis Report(s) may be found in Attachment I.

Test substance manufacturing information such as the manufacture and expiration date(s), chemical identity, characterization, and stability included in this report was provided by the Sponsor unless otherwise indicated. Element Materials Technology Eagan is not involved in manufacturing of the test substance(s) used in this study, and therefore, the Sponsor is responsible for ensuring the accuracy of the information for the test substance(s) used in this study.

STUDY DATES

Date Sample Received: March 16, 2023
Study Initiation Date: March 30, 2023
Experimental Start Date: April 4, 2023 (Start time: 11:25 a.m.)
Experimental End Date: April 11, 2023 (End time: 1:14 p.m.)
Study Completion Date: See Page 1 of Report

OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure was to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to Health Canada.

SUMMARY OF RESULTS

Test Substance: Gluquat 300, Lot 2211051 and Lot 2302013

Dilution: 1:200 defined as 1 part of test substance + 199 parts of 300 ppm AOAC Synthetic Hard Water

Virus: Porcine Epidemic Diarrhea Virus, Strain Colorado 2013 Isolate

Exposure Time: 10 minutes

Exposure Temperature: 20±2°C (21.0°C)

Exposure Humidity: 20.00%

Organic Soil Load: 5% fetal bovine serum

Efficacy Result:

Test Organism	Test Results		Efficacy Performance
	Gluquat 300		
	Lot 2211051	Lot 2302013	
Porcine Epidemic Diarrhea Virus	A ≥3 log_{10} reduction in titer was demonstrated. **(PASS)**	A ≥3 log_{10} reduction in titer was demonstrated. **(PASS)**	Requirements met

TEST SYSTEM

1. Virus

The Colorado 2013 Isolate strain of Porcine Epidemic Diarrhea Virus used for this study was obtained from the National Veterinary Services Laboratories, Ames, IA. The stock virus was prepared by collecting the supernatant culture fluid from 50-75% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at ≤-70°C until the day of use. On the day of use, an aliquot of stock virus (Lot PED-66) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The Stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Porcine Epidemic Diarrhea Virus on Vero 76 cells.

2. Indicator Cell Cultures

Cultures of Vero 76 cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CRL-1587). The cells were propagated by Element Materials Technology Eagan personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO_2. On the day of testing, the cells were observed as having proper cell integrity and confluency, and therefore, were acceptable for use in this study.

All cell culture documentation was retained for the cell cultures used in the assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

3. Test Medium

The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-Trypsin, 10% Tryptose Phosphate Broth, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B.

TEST METHOD

1. Preparation of Test Substance

Two lots of Gluquat 300 (Lot 2211051 and Lot 2302013) were tested at 1:200 defined as 1 part of test substance + 199 parts of 300 ppm AOAC Synthetic Hard Water (1.00 mL product + 199.0 mL water) as requested by the Sponsor. The test substance was in solution as determined by visual observation and used on the day of preparation. The prepared test substance was at the exposure temperature prior to use.

The 300 ppm AOAC Synthetic Hard Water was prepared using 3.0 mL of Solution I and 4.0 mL of Solution II. The total volume of hard water was brought to approximately 1 liter using sterile deionized water. The 300 ppm hard water was prepared, titrated (at 297 ppm) and used on the day of testing.

2. Preparation of Virus Films

Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 20.0°C in a relative humidity of 30% until visibly dry (20 minutes).

3. Preparation of Sephadex Gel Filtration Columns

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus was separated from the test substance by filtration through Sephadex LH-20 gel. On the day of testing, Sephadex columns were prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns were then ready to be used in the assay.

4. Input Virus Control

On the day of testing, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

5. Treatment of Virus Films with the Test Substance

For each lot of test substance, one dried virus film was individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for 10 minutes at 20±2°C (21.0°C) and 20.00% relative humidity. The virus films were completely covered with the test substance. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

6. Treatment of Dried Virus Control Film

One virus film was prepared as previously described (paragraph 2). The virus control film was exposed to 2.00 mL of test medium in lieu of the test substance and held covered for 10 minutes at 20±2°C (21.0°C) and 20.00% relative humidity. Just prior to the end of the exposure time, the virus control was scraped with a cell scraper and at the end of the exposure time the virus mixture was immediately passed through a Sephadex column in the same manner as the test virus (paragraph 5). The filtrate (10^{-1} dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

7. Cytotoxicity Controls

A 2.00 mL aliquot of the use dilution of each lot of the test substance was filtered through a Sephadex column and the filtrate was diluted serially in medium and inoculated into Vero 76 cell cultures. Cytotoxicity of the Vero 76 cell cultures was scored at the same time as the virus-test substance and virus control cultures.

8. Assay of Non-Virucidal Level of Test Substance (Neutralization Control)
The neutralization control was performed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) was challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, was retained. Dilutions that showed virucidal activity were not considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures was inoculated with a 200 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus (approximately 16 infectious units) was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates. Although the calculated value for infectious units used in the neutralization control was lower than the typical target range, the low inoculum increases the stringency of this control. Growth of virus in the neutralization control indicates neutralization of the test substance was demonstrated as required. Therefore, the neutralization control is valid.

9. Infectivity Assays
The Vero 76 cell line, which exhibits cytopathic effect (CPE) in the presence of Porcine Epidemic Diarrhea Virus, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 200 µL of the dilutions prepared from test and control groups. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C (37.0°C) in a humidified atmosphere of 5-7% CO_2 (6.0% CO_2) in sterile disposable cell culture labware. The cell cultures were examined approximately 20 hours post incubation for cytotoxicity, at which time no dilutions were demonstrating cytotoxicity. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.

10. Statistical Methods: Not applicable

PLANNED PROTOCOL CHANGES

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

Planned Protocol Deviations:
No planned protocol deviations occurred during this study.

UNFORESEEN CIRCUMSTANCES

No unforeseen circumstances occurred during this study.

DATA ANALYSIS

Calculation of Titers

Viral and cytotoxicity titers will be expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity (TCD_{50}), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier) :

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

Log_{10} of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 Log_{10})

*$TCID_{50}$ value calculated based on the volume inoculated per well

Calculation of Log Reduction

The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control Log_{10} $TCID_{50}$ – Test Substance Log_{10} $TCID_{50}$ = Log Reduction

Calculation of Infectious Units

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus: $10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

STUDY ACCEPTANCE CRITERIA

Health Canada Submission

A valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032.

REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-12 (Reapproved 2017).
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-02, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 2019.

RESULTS

For Input Virus Control, Treatment of Virus Films with the Test Substance, and Treatment of Dried Virus Control Film Results, see Table 1.

For Cytotoxicity Control Results, see Table 2.

For Assay of Non-Virucidal Level of Test Substance (Neutralization Control) Results, see Table 3.

Key for Control and Results Tables:

(+) = Positive for the presence of test virus

(0) = No test virus recovered and/or no cytotoxicity present

(NT) = Not tested

(NA) = Not applicable

ANALYSIS

Results of tests with two lots of Gluquat 300 (Lot 2211051 and Lot 2302013), diluted 1:200 defined as 1 part of test substance + 199 parts of 300 ppm AOAC Synthetic Hard Water, exposed to Porcine Epidemic Diarrhea Virus in the presence of a 5% fetal bovine serum organic soil load at 20±2°C (21.0°C) and 20.00% relative humidity for 10 minutes were as described below. All cell controls were negative for test virus infectivity.

The titer of the input virus control was 6.00 log_{10}/200 µL. The titer of the dried virus control was 5.50 log_{10}/200 µL (5.50 log_{10}/carrier). Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested [≤0.50 log_{10}/200 µL (≤0.50 log_{10}/carrier)]. Test substance cytotoxicity was not observed in either lot at any dilution tested (≤0.50 log_{10}/200 µL). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤0.50 log_{10}/200 µL for both lots.

Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer, per volume inoculated per well and per carrier, was ≥5.00 log_{10} for both lots.

STUDY CONCLUSION

Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, Gluquat 300, diluted 1:200 defined as 1 part of test substance + 199 parts of 300 ppm AOAC Synthetic Hard Water, demonstrated a ≥3 log_{10} reduction in titer of Porcine Epidemic Diarrhea Virus following a 10 minute exposure time at 20±2°C (21.0°C) and 20.00% relative humidity as required by Health Canada.

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data. Results pertain only to the items tested.

The use of the Element Materials Technology Eagan name, logo or any other representation of Element Materials Technology Eagan without the written approval of Element Materials Technology Eagan is prohibited. In addition, Element Materials Technology Eagan may not be referred to in any form of promotional materials, press releases, advertising or similar materials (whether by print, broadcast, communication or electronic means) without the expressed written permission of Element Materials Technology Eagan.

TABLE 1: Virus Controls and Test Results

Effects of Gluquat 300 (Lot 2211051 and Lot 2302013) Following a 10 Minute Exposure to Porcine Epidemic Diarrhea Virus Dried on an Inanimate Surface

Dilution	Input Virus Control	Dried Virus Control	Porcine Epidemic Diarrhea Virus + Lot 2211051	Porcine Epidemic Diarrhea Virus + Lot 2302013
Cell Control	0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	+ +	+ + + +	0 0 0 0	0 0 0 0
10^{-2}	+ +	+ + + +	0 0 0 0	0 0 0 0
10^{-3}	+ +	+ + + +	0 0 0 0	0 0 0 0
10^{-4}	+ +	+ + + +	0 0 0 0	0 0 0 0
10^{-5}	+ +	+ + + +	0 0 0 0	0 0 0 0
10^{-6}	+ 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-7}	0 0	0 0 0 0	NT	NT
10^{-8}	0 0	0 0 0 0	NT	NT
10^{-9}	0 0	NT	NT	NT
$TCID_{50}$/200 µL	$10^{6.00}$	$10^{5.50}$	$\leq 10^{0.50}$	$\leq 10^{0.50}$
$TCID_{50}$/carrier	NA	$10^{5.50}$	$\leq 10^{0.50}$	$\leq 10^{0.50}$
Log Reduction[1]	NA	NA	≥5.00	≥5.00

[1] This reduction is both per volume inoculated per well and per carrier.

TABLE 2: Cytotoxicity Control Results

Cytotoxicity of Gluquat 300 on Vero 76 Cell Cultures

Dilution	Cytotoxicity Control Lot 2211051	Cytotoxicity Control Lot 2302013
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	0 0 0 0	0 0 0 0
10^{-2}	0 0 0 0	0 0 0 0
10^{-3}	0 0 0 0	0 0 0 0
10^{-4}	0 0 0 0	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0
TCD_{50}/200 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$

TABLE 3: Neutralization Control Results

Non-Virucidal Level of the Test Substance (Neutralization Control)

Dilution	Test Virus + Cytotoxicity Control Lot 2211051	Test Virus + Cytotoxicity Control Lot 2302013
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	+ + + +	+ + + +
10^{-2}	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +
10^{-4}	+ + + +	+ + + +
10^{-5}	+ + + +	+ + + +
10^{-6}	+ + + +	+ + + +

Results of the non-virucidal level control indicate that the test substance was neutralized at a $TCID_{50}$/200 µL of ≤0.50 log_{10} for both lots.

ATTACHMENT I: Test Substance Certificate(s) of Analysis
Lot 2211051

West Penetone
[illegible]
[illegible]
H1J 1S5
(800) 361-5921
[illegible]
[illegible]

Certificate of analysis

West Penetone

Product name	Gluquat 300
Drug identification number (DIN)	02449234
Product code	111900
Lot no.	2211051
Batch size (litres)	25,000
Batch tank no	2
Filtration	Yes
Certificate issue date	March 8, 2023
Manufacturing date	November 8, 2022
Certification date	November 8, 2022
Expiration date	November 1, 2024

Contact
Title
Email
Purchase order
Shipping date
Transport company

Description	Method	Unit	Results	Limits	
				Lower	Upper
Appearance - Clear pale yellow liquid	Visual		Pass		
Odour - Light aldehyde			Pass		
pH (100%)	WM 30	pH unit	4.45	4.00	5.00
Brix		%	26.9	25.0	27.0
Glutaraldehyde	WM 74 - s2.0	% w/w	11.4	11.0	6.0
Quat	Taylor	% w/w	6.8	6.0	7.5

Quality control was performed during manufacturing. Samples were taken from the mixing tank.

Fiche de sécurité en français
Safety data sheets in French
Code QR

Fiche de sécurité en anglais
Safety data sheet in English
Code QR

Product testing was performed by *Kahina Zehrand*
Quality control technician

Approved by
Éric Lepage
Chemist, M.Sc., OCQ 2013-090
Technical Services Main Director

Version 1.5

The information contained in this document is based on analytical tests of samples taken from the mixing tank. West Penetone certifies the accuracy of the information cited in the quality certificate. For any additional information, please contact customer service at the telephone number or email address indicated above.

World leader in the development of cleaning and sanitation products that are environmentally friendly

Test Substance Certificate(s) of Analysis
Lot 2302013

West Penetone
10900, Sécant
Anjou, Québec
H1J 1S3
(800) 361-8327
[illegible]
[illegible]

Certificate of analysis

West Penetone

Product name	Gluquat 300
Drug identification number (DIN)	02449234
Product code	111900
Lot no.	2302013
Batch size (litres)	25,000
Batch tank no	2
Filtration	Yes
Certificate issue date	March 8, 2023
Manufacturing date	January 17, 2023
Certification date	January 17, 2023
Expiration date	January 3, 2025

Contact
Title
Email
Purchase order
Shipping date
Transport company

Description	Method	Unit	Results	Limits	
				Lower	Upper
Appearance - Clear pale yellow liquid	Visual		Pass		
Odour - Light aldehyde			Pass		
pH (100%)	WM 20	pH unit	4.35	4.00	5.00
Brix		%	26.8	25.0	27.0
Glutaraldehyde	WM 74 - v2.0	% w/w	11.3	11.0	6.0
Quat	Taylor	% w/w	6.4	6.0	7.5

Quality control was performed during manufacturing. Samples were taken from the mixing tank.

Fiche de sécurité en français
Safety data sheets in French
Code QR

Fiche de sécurité en anglais
Safety data sheet in English
Code QR

Product testing was performed by *Kahina Zakraoui*
Quality control technician

Approved by
Éric Lepage
Chemist, M.Sc., OCQ 2013-090
Technical Services Main Director

Version 1.5

The information contained in this document is based on analytical tests of samples taken from the mixing tank. West Penetone certifies the accuracy of the information cited in the quality certificate. For any additional information, please contact customer service at the telephone number or email address indicated above.

[illegible]

(For Laboratory Use Only)
Element Materials Technology Eagan Project # A37720
Test Substance Tracking # TS031623.WPC02 3-2023

PROTOCOL

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Porcine Epidemic Diarrhea Virus

PROTOCOL NUMBER

WPC02020123.PEDV

SPONSOR

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

PERFORMING LABORATORY

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

DATE

February 1, 2023

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

PURPOSE

The purpose of this study is to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure is to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA) and Health Canada.

TEST SUBSTANCE CHARACTERIZATION

According to 40 CFR, Part 160, Subpart F [160.105] test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with 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 Element Materials Technology Eagan. Element Materials Technology Eagan will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

SCHEDULING AND DISCLAIMER OF WARRANTY

Experimental start dates are generally scheduled on a first-come/first-serve basis once Element Materials Technology Eagan receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is February 17, 2023. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of March 17, 2023. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Element Materials Technology Eagan.

If a test must be repeated, or a portion of it, because of failure by Element Materials Technology Eagan 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 Element Materials Technology Eagan nor any of its employees are to be used in advertising or other promotion without written consent from Element Materials Technology Eagan.

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

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Regulatory agencies require that a specific virucidal claim for a disinfectant intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed virus. Each agency will accept adequate data generated by any appropriate technique in support of a virucidal efficacy claim. This is accomplished by treating the target virus with the disinfectant (test substance) under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the disinfectant is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting virological data. The Vero 76 cell line, which supports the growth of the Porcine Epidemic Diarrhea Virus, will be used in this study. The experimental design in this protocol meets these requirements and is guided by ASTM E1053-20.

TEST PRINCIPLE

A film of virus, dried on a glass surface, is exposed to the test substance for a specified exposure time. At the end of the exposure time, the virucidal and cytotoxic activities are removed from the virus-test substance mixture, and the mixture is assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls are run concurrently.

STUDY DESIGN

Dried virus films will be prepared in parallel and used as follows:

The appropriate number of films for each batch of test substance assayed per exposure time requested.

The appropriate number of films for virus control titration (titer of virus after drying) per exposure time requested.

The inoculated carriers are exposed to the test substance for the Sponsor specified exposure time. At the end of the specified exposure time, resuspended virus-test substance mixtures will be detoxified and made non-virucidal by immediately adding the contents to a Sephadex gel filtration column followed by 10-fold serial dilutions in test medium. Each dilution is inoculated into indicator cell cultures. The resuspended virus control film and each batch of test substance alone will be treated in exactly the same manner. For analysis of infectivity, cultures will be held for the appropriate incubation period at the end of which time cultures will be scored for the presence of the test virus. Cultures will be monitored at that time for cell viability. Uninfected indicator cell cultures will be carried in parallel and similarly monitored. For analysis of cytotoxicity, the viability of cultures inoculated with dilutions of each test and cytotoxicity control will be determined. In addition to the above titrations for infectivity and cytotoxicity, the residual virucidal activity of the test substance after neutralization will be determined by adding a low titer of stock virus to each dilution of the test substance (cytotoxicity control dilutions). The resulting mixtures of dilutions are assayed for infectivity in order to determine the dilution(s) of test substance at which virucidal activity, if any, is retained.

VIRUS

The Colorado 2013 isolate strain of Porcine Epidemic Diarrhea Virus to be used for this study was obtained from the ~~American Type Culture Collection, Manassas, VA (ATCC Colorado 2013 Isolate)~~.① Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at ≤ -70°C until the day of use. Alternate methods of viral propagation may be utilized based on the growth requirements of the virus. The propagation method will be specified in the raw data and in the report. On the day of use the appropriate number of aliquots are removed, thawed, combined (if applicable) and maintained at a refrigerated temperature until used in the assay. Note: If the Sponsor requests an organic soil load challenge, fetal bovine serum (FBS) or the requested organic soil will be incorporated into the stock virus aliquot. The stock virus aliquot will be adjusted to yield the percent organic soil load requested.

INDICATOR CELL CULTURES

Cultures of Vero 76 cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CRL-1587). The cells are propagated by Element Materials Technology Eagan personnel. The cells are seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO_2. The confluency of the cells will be appropriate for the test virus. Vero 76 cells obtained from an alternate, reputable source may be used. The source of the cells will be specified in the final report.

All cell culture documentation is retained for the cell cultures used in this assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

① Per 3-27-23 email, corrected to National Veterinary Services Laboratories, Ames, IA. mm 3-30-23

TEST MEDIUM
The test medium used for this assay is Minimum Essential Medium (MEM) supplemented with 0-10% (v/v) heat inactivated fetal bovine serum. The medium may also be supplemented with one or more of the following: 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 1.0-2.0 mM L-glutamine, and 0.5 – 5 µg/mL trypsin. The composition of the test medium may be altered based on the virus and/or cells. The composition of the medium will be specified in the raw data and in the report.

PREPARATION OF OECD HARD WATER (if applicable)
Sterile OECD hard water will be prepared by adding 6.0 mL of European hard water stock solution A to approximately 600 mL of sterile deionized water. Eight (8.0) mL of European hard water stock solution B will be added. The total volume will be adjusted to 1000 mL using deionized water. (Equivalent dilutions may be made). The pH of the hard water will be adjusted to 7.0 ± 0.2. The prepared water must be used within 24 hours of preparation. On the day of test, the water will be titrated and must demonstrate 338-394 ppm hardness. Appropriate solution adjustments may be made to target the final hardness concentration.

PREPARATION OF UN-SOFTENED TAP WATER (if applicable)
Place the required amount of un-softened tap water in a sterile vessel. Titrate for water hardness per CGT-0001 (Section 6.3.1-6.3.11). The acceptable range is 180-210 ppm. If the un-softened tap water falls above 210 ppm the water will be diluted with deionized water and re-titrated per CGT-0001 (Section 6.3.1-6.3.11).

PREPARATION OF TEST SUBSTANCE
The dilution of test substance(s) will be used as recommended by the Sponsor. The product will be pre-equilibrated to the desired test temperature if applicable.

PREPARATION OF VIRUS FILMS
Films of virus will be prepared by spreading 200 µL of virus inoculum uniformly over the bottom of the appropriate number of 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus will be air-dried at 10°C-30°C until visibly dry (≥20 minutes). A calibrated timer will be used for timing the drying. The drying conditions (temperature and humidity) will be appropriate for the test virus for the purpose of obtaining maximum survival following drying. The actual drying conditions, drying time and calibrated timer used will be clearly documented.

One dried virus film per batch of test substance will be assayed unless otherwise requested.

TEST METHOD

Preparation of Sephadex Gel Filtration Columns
To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus is separated from the test substance by filtration through Sephadex gel. The type of Sephadex used will be specified in the final report. On the day of testing, Sephadex columns are prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns are now ready to be used in the assay.

Input Virus Control
On the day of testing, the stock virus utilized in the assay will be titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

Treatment of Virus Films with the Test Substance

For each batch of test substance assayed, the appropriate number of dried virus films are individually exposed to a 2.0 mL aliquot of the use dilution of the test substance (liquid products), or to the amount of spray released under use conditions (spray products) and held covered for the specified exposure time(s) and temperature. A calibrated timer will be used for timing the exposure. The actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the plates are individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrate (10^{-1} dilution) is then titered by serial dilution and assayed for infectivity and/or cytotoxicity. To further aid in the removing of the cytotoxic effects of the test substance to the indicator cell cultures, individual dilutions may be passed through additional individual Sephadex columns.

Treatment of Dried Virus Control Film

The appropriate number of virus films are prepared as described previously for each exposure time assayed. The virus control films are run in parallel to the test virus but a 2.0 mL aliquot of test medium is added in lieu of the test substance. The virus control films are held covered and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films are exposed to the test substance. A calibrated timer will be used for timing the exposure and the actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the virus films are individually scraped as previously described and at the end of the exposure time the mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger. The filtrate (10^{-1} dilution) is then titered by serial dilution and assayed for infectivity. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the virus control will be passed through additional individual Sephadex columns.

Cytotoxicity Control

A 2.0 mL aliquot of each batch of test substance (liquid products) or the amount of the test substance recovered when sprayed onto a sterile petri dish (spray products), is filtered through a Sephadex column utilizing the syringe plunger and the filtrate is diluted serially in medium and inoculated into cell cultures for assay of cytotoxicity concurrently with the virus control and test substance-treated virus samples. For spray products, the cytotoxicity control will be held covered for the longest requested exposure time at the requested exposure temperature. A calibrated timer will be used for timing the exposure. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the cytotoxicity control will be passed through additional individual Sephadex columns.

Assay of Non-Virucidal Level of Test Substance (Neutralization Control)

The neutralization control will be confirmed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) will be challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures will be inoculated with a 200 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus will be inoculated into each cell culture well and the indicator cell cultures will be incubated along with the test and virus control plates. The infectious units of the low titer stock virus will be calculated and included in the final report.

Infectivity Assays
The Vero 76 cell line, which exhibits cytopathic effect (CPE) in the presence of Porcine Epidemic Diarrhea Virus, will be used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes will be inoculated in quadruplicate with 200 µL of the dilutions prepared from test and control groups. The input virus control will be inoculated in duplicate. Uninfected indicator cell cultures (cell controls) will be inoculated with test medium alone. The cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware for approximately seven days.

The cell cultures will be examined approximately 1-36 hours post incubation for cytotoxicity. Test medium will be aspirated from any dilutions demonstrating cytotoxicity and replaced with fresh test medium. Additional dilutions may be treated in the same manner to mitigate risk of additional cytotoxicity. On the final day of incubation, the cell cultures will be microscopically observed for the absence or presence of CPE (virus infectivity), cytotoxicity and for viability. The observations will be recorded on the raw data worksheets. The cell cultures may be observed at other times during incubation; only the results from the final observations will be reported.

DATA ANALYSIS

Calculation of Titers
Viral and cytotoxicity titers will be expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity (TCD_{50}), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier):

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

Log_{10} of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 Log_{10})

*$TCID_{50}$ value calculated based on the volume inoculated per well

Calculation of Log Reduction
The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control Log_{10} $TCID_{50}$ – Test Substance Log_{10} $TCID_{50}$ = Log Reduction

If multiple dried virus control replicates are performed, the average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

Calculation of Infectious Units

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus: $10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

Statistical Methods
None used.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

The specialized virucidal testing section of Element Materials Technology Eagan maintains Standard Operating Procedures (SOPs) relative to virucidal efficacy testing studies. Virucidal 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 virus and cell stocks for purposes of identification, receipt and use of chemical reagents including cell culture medium components, etc. These procedures are designed to document each step of virucidal 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 virucidal efficacy test is assigned a unique Project Number when the Study Director initiates the protocol for the study. This number is used for identification of the test culture plates, etc. during the course of the test. Test culture plates are also labeled with reference to the test virus, experimental start date, and test product. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: N/A

STUDY ACCEPTANCE CRITERIA

Only the applicable acceptance criteria and references for the regulatory agency reviewing the data will be included in the final report.

U.S. EPA and Health Canada Submission

A valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

If the test substance fails to meet the test acceptance criteria and the dried virus control fails to meet the control acceptance criteria, the study is considered valid and no repeat testing is necessary, unless requested by the Sponsor.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number.

For any studies with presence of contamination in subculture media, a control failure, system failure, technician error, etc. the Repeat Testing Policy from the Series 810 Guidelines FAQ document will be followed.

FINAL REPORT

The report will include, but not be limited to, identification of the sample and date received, dates on which the test was initiated and completed, identification of the virus strain used and composition of the inoculum, description of cells, medium and reagents, description of the methods employed, tabulated results, calculated titers for infectivity and cytotoxicity, a conclusion as it relates to the purpose of the test and all other items required by 40 CFR Part 160.185. A draft report may be requested by the Sponsor. The final report will be prepared once the Sponsor has reviewed the draft report and notified the Study Director to complete the study.

PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for change 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.

TEST SUBSTANCE RETENTION

Test substance retention shall be the responsibility of the Sponsor. Unused test substance will be discarded following study completion unless otherwise requested.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032. 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 the final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at Element Materials Technology Eagan. 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.

PROPOSED STATISTICAL METHODS: N/A

REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-12 (Reapproved 2017).
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-02, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 2019.

Protocol Number: WPC02020123.PEDV
West Penetone, Inc.
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STUDY INFORMATION

(All blank sections are verified by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)

Test Substance (Name and Lot/Batch Number exactly as it should appear on final report)

Test Substance Name	Lot/Batch Number	Manufacture Date
~~GLUQUAT 300~~ Gluquat 300 ①	2211051	Nov. 8, 2022
~~GLUQUAT 300~~ Gluquat 300 ①	2302012	JAN 17, 2023

Testing at the lower certified limit (LCL) for the hardest-to-kill virus on your label is required for registration.

Product Description

- ☐ Quaternary ammonia
- ☐ Iodophor
- ☐ Peracetic acid
- ☐ Peroxide
- ☐ Sodium hypochlorite
- ☑ Other GLUTARALDEHYDE + QUATERNARY AMMONIA

Approximate Test Substance Active Concentration (upon submission to Element Materials Technology Eagan): 15.5% GLUTARALDEHYDE + 6.7% QUATERNARY AMMONIA w/w ①

(This value is used for neutralization planning only. This value is not intended to represent characterization values.)

Storage Conditions

- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other ____________

Hazards

- ☐ None known: Use Standard Precautions
- ☑ ① Material Safety Data Sheet, Attached for each product
- ☐ As Follows: ____________

Product Preparation

- ☐ No dilution required, Use as received (RTU)
- ☑ *Dilution(s) to be tested:

1:200 (example: 1 oz/gallon) defined as 1 part (amount of test substance) + 199 parts (amount of diluent)

- ☐ AOAC Synthetic Hard Water: 400 ppm (360-420 ppm)
- ☐ Un-softened Tap Water: 200 ppm (180-210 ppm)
- ☐ OECD Hard Water: 375 ppm (338-394 ppm)
- ☑ Other 300 ppm AOAC Synthetic Hard Water

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

Test Virus: Porcine Epidemic Diarrhea Virus

Exposure Time: 10 minutes

Exposure Temperature: ☐ Room temperature ☑ Other: 20±2 °C (please specify range)

Directions for application of aerosol/spray products:

① ☑ Spray instructions are not applicable.

Trigger spray application:

- ☐ Spray carriers using 3 sprays, or until thoroughly wet, at a distance of 6 to 8 inches.
- ☐ Spray carriers using ______ sprays at a distance of ______ to ____ inches/cm. (circle one)

Aerosol spray application:

- ☐ Spray carriers for ______ seconds, or until thoroughly wet, at a distance of ______ to ____ inches/cm

Organic Soil Load

- ☐ 0% fetal bovine serum *(only for Human Rotavirus, Porcine Epidemic Diarrhea Virus and most Influenza viruses)*
- ☐ 1% fetal bovine serum *(minimum level that can be tested for all other viruses)*
- ☑ 5% fetal bovine serum
- ☐ Other ____________

Number of Carriers to be Tested

- ☑ One (typical for U.S. EPA submission)
- ☐ Five (required for broad-spectrum virucidal claims for Health Canada submission)

① Corrections and additions made per 3-27-23 email. pm 3-30-23

SPRAY BOTTLES USED IN TESTING (section only applicable for spray products)

To ensure expected levels of product are delivered, it is recommended that the Sponsor provide the spray bottles used in testing. Please indicate the desired source of the sprayer bottles used in testing:

☐ Sprayer(s) and bottle(s) are provided by the Sponsor
☐ General purpose spray bottle(s) are to be provided by Element Materials Technology Eagan
☐ The spray nozzle(s) are provided by the Sponsor and general purpose bottle(s) will be provided by Element Materials Technology Eagan

REGULATORY AGENCY(S) THAT MAY REVIEW DATA

☐ U.S. EPA
☑ Health Canada
☐ Not applicable - For internal/other use only (Efficacy result will be based on U.S. EPA requirements)

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 or Development Study)

PROTOCOL MODIFICATIONS

①☑ Approved without modification
☐ Approved with modification

____

____

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

TEST SUBSTANCE SHIPMENT STATUS

(This section is for informational purposes only.)
①☑ Test Substance is already present at Element Materials Technology Eagan.
☐ Test Substance has been or will be shipped to Element Materials Technology Eagan.
Date of expected receipt at Element Materials Technology Eagan: ______________

TESTING FACILITY MANAGEMENT VERIFICATION OF 40 CFR PART 160 SUBPART B (160.31(D))

Identity, strength, purity, and uniformity, as applicable, of the test lots has been or will be completed prior to efficacy testing: ☑ **Yes** ① ☐ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☑ **No*** ①

Stability testing of the formulation has been or will be completed prior to or concurrent with efficacy testing:
☐ **Yes** ☑ **No*** ① ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☐ **No***

**If testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.*

① Added per 3-27-23 email. mm 3-30-23

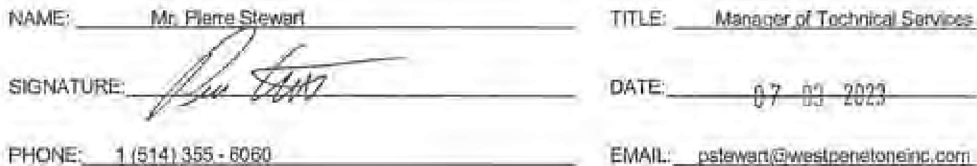
PROPRIETARY INFORMATION

THIS DOCUMENT IS THE PROPERTY OF AND CONTAINS PROPRIETARY INFORMATION OF ELEMENT MATERIALS TECHNOLOGY EAGAN. NEITHER THIS DOCUMENT, NOR INFORMATION CONTAINED HEREIN IS TO BE REPRODUCED OR DISCLOSED TO OTHERS, IN WHOLE OR IN PART, NOR USED FOR ANY PURPOSE OTHER THAN THE PERFORMANCE OF THIS WORK ON BEHALF OF THE SPONSOR, WITHOUT PRIOR WRITTEN PERMISSION OF ELEMENT MATERIALS TECHNOLOGY EAGAN.

APPROVAL SIGNATURES

SPONSOR:

NAME: Mr. Pierre Stewart TITLE: Manager of Technical Services

SIGNATURE:  DATE: 07-03-2023

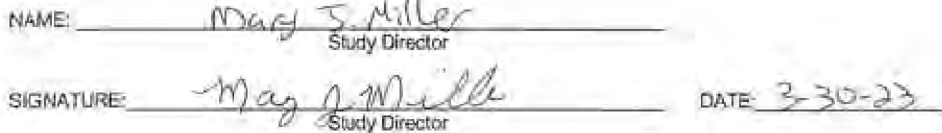
PHONE: 1 (514) 355 - 6060 EMAIL: pstewart@westpenetoneinc.com

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

Element Materials Technology Eagan:

NAME: Mary J. Miller
Study Director

SIGNATURE:  DATE: 3-30-23
Study Director

GLUQUAT 300 EFFICACY AGAINST AVIAN INFLUENZA H5N1 – PRELIMINARY REPORT

The preliminary unaudited test results for your Virucidal Efficacy testing performed at Element Materials Technology Eagan on 4/4/23 are shown below.

Project #: A37721
Protocol #: WPC02020123.AFLU
Test Substance: Gluquat 300, Lot 2211051 and Lot 2302013
Dilution: 1:200 defined as 1 part of test substance + 199 parts of 300 ppm AOAC Synthetic Hard Water
Virus: Avian Influenza A (H5N1) virus, Strain VNH5N1-PR8/CDC-RG, obtained from the Centers for Disease Control and Prevention (CDC) Atlanta, Georgia (CDC-2006719965)
Organic Soil Load: 5% fetal bovine serum
Exposure Temperature: 20±2°C (19.86°C)
Exposure Humidity: 24.51%
Exposure Time: 10 minutes

Dried Virus Control Results

Avian Influenza A (H5N1) virus = 5.50 log_{10}/100 µL (5.80 log_{10}/carrier)

Cytotoxicity Control Results

Lot 2211051 = ≤0.50 log_{10}/100 µL
Lot 2302013 = ≤0.50 log_{10}/100 µL

Neutralization Control Results

Lot 2211051 = Neutralized at ≤0.50 log_{10}/100 µL
Lot 2302013 = Neutralized at ≤0.50 log_{10}/100 µL

Test Results

Lot 2211051

Complete inactivation of the test virus was demonstrated [≤0.50 log_{10}/100 µL (≤0.80 log_{10}/carrier)]. A ≥5.00 log_{10} reduction in viral titer was demonstrated per volume inoculated per well and per carrier. **(PASSED)**

Lot 2302013

Complete inactivation of the test virus was demonstrated [≤0.50 log_{10}/100 µL (≤0.80 log_{10}/carrier)]. A ≥5.00 log_{10} reduction in viral titer was demonstrated per volume inoculated per well and per carrier. **(PASSED)**

All test control results met acceptance criteria for a valid test.

Test results meet Health Canada criteria for a virucidal label claim.

Mary Miller
Principle Study Director
Element Materials Technology
1285 Corporate Center Dr., Suite 110
Eagan, Minnesota 55121, United States of America