STUDY REPORT

STUDY TITLE

Determination of the Efficacy of the Controlled Time Release
of Grignard Pure™ Antimicrobial Air Treatment with the Aura Dispersion Device
Against *Staphylococcus epidermidis*

TEST ORGANISM(S)

Staphylococcus epidermidis (ATCC # 12228)

PRODUCT IDENTITY

Grignard Pure™

PROTOCOL NUMBER

ARELPRO10948.90

AUTHOR

Braeden Scott

STUDY COMPLETION DATE

27Feb2026

PERFORMING LABORATORY

Aerosol Research and Engineering Laboratories
12880 Metcalf Ave
Overland Park, KS 66213

SPONSOR

BleuGarde™, LLC.
505 Capobianco Plaza
Rahway, NJ, 07065

PROJECT NUMBER

10948.90

REPORT NUMBER

10948.90.01

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: BleuGarde™, LLC.

Company Agent: Grishma Desai

Quality Lab Manager

Title

Grishma Desai Date: 03/23/26

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.

Submitter: _____ Date: 3/20/26

Sponsor: Grishma Desai Date: 03/23/26

Study Director: _____ Date: 3/20/26

QUALITY ASSURANCE UNIT SUMMARY

Study: Determination of the Efficacy of the Controlled Time Release of Grignard Pure™ Antimicrobial Air Treatment with the Aura Dispersion Device Against *Staphylococcus epidermidis.*

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of the studies. This study has been performed in accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol, 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 Study Director and Management.

Phase of Study Inspected	Date of Phase Inspection	Date Reported to Study Director	Date Reported to Management
Bioaerosol Testing	04Feb26	04Feb26	04Feb26
Final Report	26Feb26	26Feb26	26Feb26

Quality Assurance Specialist: _____ Date: 26Feb26

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STUDY PERSONNEL

STUDY DIRECTOR: **Weston Schaper**

Professional personnel involved:	**Braeden Scott**	Title:	**Research Scientist**
	Sean McLeod		**Quality Assurance**

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title:	Determination of the Efficacy of the Controlled Time Release of Grignard Pure™ Antimicrobial Air Treatment with the Aura Dispersion Device against *Staphylococcus epidermidis*
Project Number:	10948.90
Protocol Number:	ARELPRO10948.90
Sponsor:	BleuGarde™, LLC. 505 Capobianco Plaza Rahway, NJ, 07065
Test Facility:	Aerosol Research and Engineering Laboratory 12880 Metcalf Ave Overland Park, KS 66213

DISPERSAL DEVICE IDENTITY

Device Name: Aura Dispersion Device
Device Manufacturer: Air Esscentials

TEST SUBSTANCE IDENTITY

Test Substance Name: Grignard Pure™
Active Ingredient: Triethylene Glycol
Batch/ Lot(s): 012126A, 012126B, 012126C
Batch Concentration: TBD
Manufacture Date(s): January 21, 2026
Expiration Date(s): 1 year after opening

Test Substance Characterization

The substance characterization as to identity, strength, purity, stability, and uniformity, as applicable, according to 40CFR, Part 160, Subpart F (160.105), was documented prior to its use in the study. The Test Substance Certificate(s) of Analysis are provided in attachment I.

STUDY DATES AND TIMES

Sample Received:	30Jan26	10:00am
Study Initiation:	04Feb26	7:25am
Experimental Start:	04Feb26	8:10am
Experimental End:	11Feb26	2:22pm
Study Completion:	27Feb26	12:47pm

OBJECTIVE

The purpose of this study was to execute the "controlled time release" application test protocol for the Grignard Pure™ solution against aerosolized bacteria using the Aura Dispersion Device. For this study the test substance was tested against the gram-positive bacteria, *Staphylococcus epidermidis.*

This report presents organism-specific results for *Staphylococcus epidermidis* generated under Protocol No. ARELPRO10948.90. The governing protocol was written to support testing of multiple microorganisms under the same study design; however, this final report is limited to the methods, data, and conclusions applicable to *Staphylococcus epidermidis* only. Results for other organisms evaluated under the same protocol are documented separately in companion report(s), as applicable.

SUMMARY OF RESULTS

Test Type: Chamber bioaerosol reduction efficacy
Test Substance: Grignard Pure™
Dilution: Ready-to-Use (RTU)

Test Organism(s): *Staphylococcus epidermidis*
Nebulization stock concentration: 6.12E+09 cfu/mL
Nebulization Time: 20 Minutes

Chamber Size: $16m^3$
Exposure Temperature: 22-28°C
Exposure Humidity: 30-50% Relative Humidity

Contact Time: The contact time for this study is assessed as the amount of time of aerosol contact
Aerosol Sampling Duration: 10 minutes
Aerosol Sampling Time Points: 0 -minute, 15-minute, 30-minute, 45-minute, 60-minute, and 90-minute.

Efficacy Result: A reduction of over 4.0 net $\log_{10}$, or 99.99%, in the *Staphylococcus epidermidis* concentration occurred using time-controlled release of Grignard Pure™ in the testing chamber by the 60-minute aerosol sampling time point.

STUDY MATERIALS

Test System/ Growth Media

Test Organism	Designation #	Growth Medium	Incubation Parameters
S. epidermidis	12228	Tryptic Soy Broth	35 +/- 2°C

Recovery Media

Neutralizer: Phosphate buffered saline solution + .005% Tween 80
Agar Plate Medium: Tryptic Soy Agar

TEST METHOD

Chamber Preparation

Prior to the initiation of testing, and between each trial, the chamber was wiped clean with 95% Isopropyl alcohol and a non-fibrous towel. All ports were cleaned with non-fibrous brushes. All surfaces were allowed to dry before the trial commencement. The lot number of the cleaning solution, as well as the time and date of the cleaning, were recorded for each cleaning of the chamber.

Preparation of Test Organism

Pure strain seed stocks were purchased from ATCC for testing. The working stock culture was prepared using aseptic techniques in a class 2 biological safety cabinet and followed standard preparation methodologies. Approximately 250mL of the biological stock was prepared in tryptic soy broth, and incubated for 24 hours at 37°C. Stock cultures were centrifuged for 10 minutes at 3000rpm in an LD-3 centrifuge in sterile 15mL conical tubes, growth media was removed, and the cells re-suspended in sterile PBS buffer for aerosolization. Aliquots of these suspensions were enumerated on agar plates for viable counts. The species were streaked to ensure no contamination was present prior to starting the trials. For each organism, test working stocks were grown in sufficient volume to satisfy use quantities for all tests conducted using the same culture stock material.

Neutralization Verification

Neutralization verification testing was run to assure that all kill assessed to the test device was due to kill in the air and not kill in the neutralizer PBS solution after sampling and during plating. For neutralization verification testing, the amount of Grignard Pure™ was calculated based on a 10-minute sample at the target concentration with an impinger pulling 12.5 l/min. The Grignard Pure™ was diluted in PBS + 0.005% Tween 80 for testing in a 50ml conical tube. To have a comparison to the Grignard Pure™ dilution, a conical tube was filled the PBS + 0.005% Tween alone with no Grignard Pure™ added, and a final conical tube was filled with DI water. After growing *S. epidermidis* stock, it was diluted to a concentration below 1.00×10^3 cfu/mL and added to each conical tube. The contact time in the solutions for testing was 15 minutes. After 15 minutes, all samples were diluted and plated using the sample plating method described in the report, incubated, and enumerated for concentration. The concentration of each sample was compared to that of the control with only PBS solution and a control with the sample diluted in sterile deionized water to confirm neutralization. A range of concentrations was tested to encompass all possible TEG air concentrations. The equivalent air concentrations were 0.04, 0.4. and 4.0 mg/m^3. The table below shows the neutralizer test concentrations based off of the target air concentrations.

Target Air Concentration (mg/m³)	Sampling Time (min)	Flow Rate (L/min)	Total Air Volume Sampled (L)	Total Air Volume Sampled (m³)	Expected Mass Collected (mg)	Expected Impinger Concentration (mg/mL)	Expected Impinger Concentration (µg/mL)
0.04	10	12.5	125	0.125	0.005	0.00025	0.25
0.4	10	12.5	125	0.125	0.05	0.0025	2.5
4	10	12.5	125	0.125	0.5	0.025	25

Note: Expected impinger concentrations were calculated assuming complete capture of triethylene glycol from the sampled air into 20 mL of collection liquid.

Chamber Testing

Bioaerosol testing for Grignard Pure™ against *Staphylococcus epidermidis* consisted of a single control and triplicate trials. The control was run to account for any natural die off of the organism airborne over time in the chamber and to establish a non-treated baseline concentration to compare the trial concentrations to.

The Aura reservoir was filled with Grignard Pure™ solution (lot: 012126A, 012126B, or 012126C) in preparation for testing. Each challenge trial was performed with a separate lot of Grignard Pure™ solution. The Aura was adjusted to the settings determined in the concentration matching section of the protocol. The device was then primed before being placed into the chamber. The priming consisted of running the device outside of the chamber for 20 minutes. This 20-minute prime ensured that the lines of the device were full of fresh Grignard Pure™ solution and that the device was running correctly.

Prior to the introduction of bioaerosol, the device was placed in the center of the chamber on a stainless-steel table and 3 feet off the floor of the chamber. The device was checked to ensure the proper settings were still enabled before the door of the chamber was closed. After the chamber door was locked, all outlets of the chamber were checked to make sure they were sealed, all vacuum pumps were checked to assure they were working, and the pressurized air was verified as functioning. After the checks were complete, the device was switched on remotely and Grignard Pure™ solution was dispersed into the chamber air for 30 minutes prior to the introduction of the test bioaerosol. The 30 minutes served as a pre-conditioning for the room to simulate an environment already having been treated with Grignard Pure™. The nebulization stock was nebulized at 40 psi for a period of 20 minutes prior to testing. After the nebulization of the bioaerosol into the chamber air, the chamber remained sealed until the decontamination and evacuation of the chamber was complete. The temperature and humidity were recorded for the duration of every test.

Aerosol Collection

All samples were collected using AGI-30 impingers filled with 20mL of sterile PBS with the addition of 0.005% Tween 80. The addition of Tween 80 is used in order to increase the impinger collection efficiency and for the de-agglomeration of all microorganisms. The impingers sampled from opposite corners of the chamber simultaneously. After sampling, the impingers were pooled and collected in conical tubes for plating. The impingers sampled the chamber at 12.5 L/min using suction from the vacuum pumps. Each impinger used in the study was flow characterized prior to the initiation of the study. Aerosol samples were collected at 0-minute, 15-minute, 30-minute, 45-

minute, 60-minute, and 90-minute time points. All samples collected were for a duration of 10 minutes.

Sampler Cleaning

Between each sample, the impingers were submerged in a 10% bleach solution and then in DI water. They were then washed with high pressure tap water in the sink a total of six times, followed by a DI water rinse. After the cleaning, the impingers were air dried before being used for another sample.

Sample Plating

For sample plating, 1.5ml micro centrifuge tubes were filled with 900µL of sterile PBS for dilution of the samples. The number of tubes needed was based on the number of samples to be plated and the predicted dilution ranges needed to plate the samples. Using the set of dilution tubes serial dilutions were performed with the test samples. For this study a small drop assay technique was instituted for plating. The countable range for this assay was 5-50 colonies. Counts >50 cfu were labeled as too numerous to count (TNTC). The samples were plated in their respective dilution ranges in triplicate on pre-labeled TSA culture plates. The plates were allowed to dry, then placed in the incubator set at 35 +/- 2 °C for 24 hours.

Incubation and Observation

Plates were allowed to incubate in the 35 +/- 2 °C incubator for 24 hours before being removed to be enumerated. Plate counts were recorded in lab notebooks at the time of enumeration and transferred to Microsoft Excel spreadsheets for analysis.

STUDY CONTROLS

Control Count

The *S. epidermidis* nebulization inoculum yielded acceptable starting concentration counts of >1.0 x 10^7 cfu/m^3.

Sterility Control

The media sterility check revealed no growth of a contaminant.

Culture Purity

The host culture showed no signs of contamination when being streak plated on a tryptic soy agar plate.

Neutralization Control

Neutralizer yielded no signs of contaminant growth after being plated.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria

a. The results must show a reduction of >99.9% reduction in viable microorganism in the listed testing conditions.

b. Test will result in failure if one of the items listed below occur:

- Neutralizer test results deviate outside of the limits outlined in this protocol at any concentration tested.
- Contamination in the sterility streak plate prior to testing.
- Minimum viable bacterial concentrations not achieved for testing.
- Any deviations in the dispersal of the Grignard solution occur.
- Any deviation outside of the relative humidity or temperature occurs at any point during testing.

Control Acceptance Criteria

The media and neutralizer must remain organism free. The working stock must be homogeneous; it must show no signs of another organism.

Protocol Deviation(s)

No protocol deviations occurred during this study.

DATA ANALYSIS

Calculation of % Reduction

- The starting bioaerosol concentration of the challenge trial was compared to the starting concentration of the control trial to calculate the effect of the Grignard Pure™ chamber pre- treatment. The method for nebulization and neb stock remained the same from the control to the challenge trial so the concentration of the challenge trial would be expected to be the same as the control. The change in the starting concentration seen in the challenge trial was therefore attributed to the pretreatment of the chamber.

- To determine the log reduction of the trial at each time point, the percent of viable microorganism remaining airborne in the chamber was compared to the initial bioaerosol concentration of the uninhibited control trial. Log_{10} reduction was calculated as the difference between the log_{10} concentration of the control and the log_{10} concentration of the treated sample at each time point.

- For net reduction, the natural losses from the control trial were subtracted from the test trial to account for natural losses not caused by the test solution.

To evaluate the viable aerosol delivery efficiency and define operation parameters of the system, calculations based on (theoretical) 100% efficacy of aerosol dissemination were derived using the following steps:

- Plating and enumeration of the biological to derive the concentration of the stock suspension (C_s) cfu/ml
- Collison 24 jet nebulizer use rate (R_{neb}) volume of biological stock dispersed at 40 psi air supply pressure.
- Aerosol Generation time (t)

- Chamber volume (V_c) = 15,993 Liters

Assuming 100% efficiency, the quantity of aerosolized viable particles (V_P) per liter of air in the chamber for a given biological stock concentration (C_s) is calculated as:

$$V_P = \frac{C_s \cdot R_{neb}}{V_c} t$$

AGI – 30 impinger collection calculation:

- Viable aerosol concentration collection (C_a) = cfu/L of chamber air.
- Viable Impinger concentration collection (C_{Imp}) = cfu/mL from enumeration of impinger sample
- Impinger sample collection volume (I_{vol}) = 20 mL collection fluid/impinger
- AGI–30 impinger sample flow rate (Q_{imp}) = 12.5 L/min.
- AGI–30 impinger sample time (t) = 5 or 10 minutes, test dependent.

For viable impinger aerosol concentration collection (C_a) = cfu/L of chamber air:

$$C_a = \frac{\mathrm{C_{Imp} \cdot I_{vol}}}{\mathrm{Q_{imp}}} \mathrm{t}$$

- The aerosol system viable delivery efficiency (expressed as %) is:

$$Efficiency = \frac{\mathrm{C_a}}{\mathrm{V_p}} \cdot 100$$

All runs will be averaged to obtain a mean and standard deviation for each trial.
Total viable biological concentrations along with reductions over time will be log reductions of aerosolized biologicals will be calculated and graphed for each trial.

RECORD RETENTION

Data and records to be maintained by ARE Labs include, but are not limited to, the study protocol, amendments and deviations, test article receipt and handling, chamber temperature and humidity records, laboratory notebooks, study correspondence, and study final report. All data, records, documents, notes, or other materials generated by the study will be collected and securely stored on site at the ARE facility.

NOTE: All recording of data will be made directly, promptly, and legibly in indelible ink. All data entries will be dated on the date of entry and signed or initialed by the person responsible for the entry. Any changes to data entries will be made so as to not obscure the original entries. The

changes will be justified and then checked and signed by the study director and the QA representative.

Test Substance Retention

The test substance will be discarded following study completion. It is the responsibility of the Sponsor to retain samples of the test-solutions.

REFERENCES

1. 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 Pesticides – Guidance for Efficacy Testing. February 2018.

2. T. Reponen, K. Willeke, V. Ulevicius et al. *Techniques of Dispersion of Microorganisms in Air.* Aerosol Science and Technology. 27: 1997. pp. 405-421.

3. Ding and Wing. *Effects of Sampling Time on the Total Recovery rate of AGI-30 Impingers for E. coli*. Aerosol and Air Quality Research, Vol. 1, No. 1, 2001, pp. 31-36.

RESULTS

Results: Neutralization Testing

Neutralization Test Set	Test Substance	Test Organism	Concentration (cfu/mL)	% Concentration from DI Water Control
DI Control	N/A	*S. epidermidis* (ATCC# 12228)	5.47E+01	100.0%
PBS Control	N/A	*S. epidermidis* (ATCC# 12228)	5.87E+01	107.3%
0.04 mg/m^3 chamber sample	Grignard Pure	*S. epidermidis* (ATCC# 12228)	5.33E+01	97.6%
0.40 mg/m^3 chamber sample	Grignard Pure	*S. epidermidis* (ATCC# 12228)	5.43E+01	99.4%
4.0 mg/m^3 chamber sample	Grignard Pure	*S. epidermidis* (ATCC# 12228)	5.53E+01	101.2%

Figure 1: Neutralization testing concentrations and percent comparison to the PBS control concentration.

Results: Bioaerosol Testing

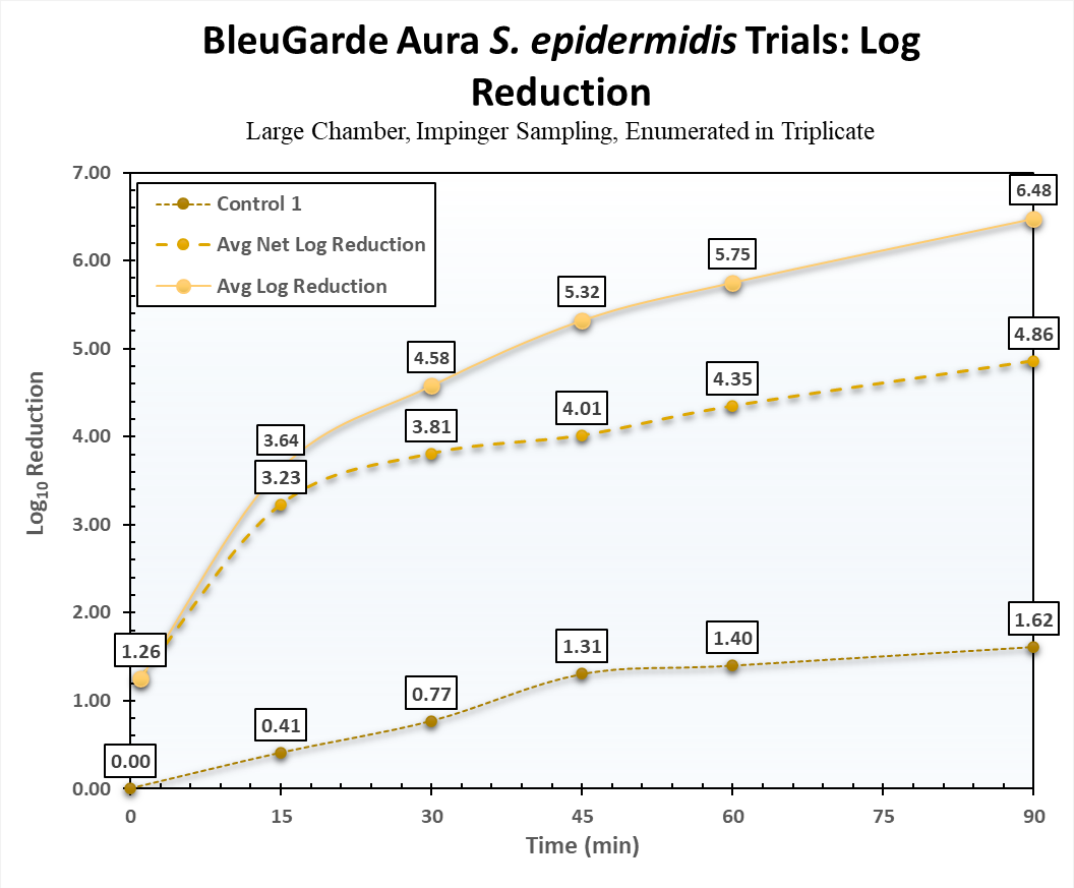

Figure 2: Graph depicting the log_{10} reduction, the control reduction, and the net log_{10} reduction after the control was accounted for.

Chamber Concentration Data Summary

Sample Time Point	Control 1	Staph EP Trial 1 (W5/P30)	Staph EP Trial 2 (W5/P30)	Staph EP Trial 3 (W5/P30)	Control Log Reduction	Triplicate Average Trial Log	Triplicate Average Net Log Reduction
0	2.71E+06	1.12E+05	1.90E+05	1.58E+05	0.00	-1.26	-1.26
15	1.05E+06	6.93E+02	5.12E+02	6.83E+02	-0.41	-3.64	-3.23
30	4.59E+05	6.40E+01	5.92E+01	9.62E+01	-0.77	-4.58	-3.81
45	1.34E+05	1.55E+01	1.32E+01	1.07E+01	-1.31	-5.32	-4.01
60	1.07E+05	5.28E+00	3.81E+00	5.55E+00	-1.40	-5.75	-4.35
90	6.56E+04	1.12E+00	1.04E+00	6.40E-01	-1.62	-6.48	-4.86

*Concentration values are in cfu/L

Figure 3: Table showing the control and test trial concentrations and the amount of reduction yielded.

Results: Quality Assurance Controls

QA Check Performed for	Trial Controls	Pass/Fail	Date/Time of Confirmation
Staph Control	PBS Control	Pass	04Feb2026 7:00 AM
Staph Control	Media Control	Pass	04Feb2026 7:00 AM
Staph Control	Purity Check	Pass	04Feb2026 7:00 AM
Staph Control	Neutralizer Control	Pass	04Feb2026 7:00 AM

Figure 4: Results from the controls performed prior to the control trial.

QA Check Performed for	Trial Controls	Pass/Fail	Date/Time of Confirmation
Staph Trials	PBS Control	Pass	11Feb2026 7:00 AM
Staph Trials	Media Control	Pass	11Feb2026 7:00 AM
Staph Trials	Purity Check	Pass	11Feb2026 7:00 AM
Staph Trials	Neutralizer Control	Pass	11Feb2026 7:00 AM

Figure 5: Results from the controls performed prior to the Grignard Pure™ bioaerosol trial.

Conclusion:

The neutralization verification testing showed that all reduction assessed in this study was due to the effects of the Grignard Pure™ solution in the aerosolized state and no additional kill occurred in the liquid impinger samples.

The results of this testing show the efficacy of the Grignard Pure™ solution, when being aerosolized by the Aura Dispersion Device, against *Staphylococcus epidermidis*. The device yielded over a 4.0 net log, or 99.99%, reduction at the 60-minute time point.

No deviations from the protocol were noted during testing. All trials remained within the guidelines outlined in the protocol.

ATTACHMENT I TEST SUBSTANCE CERTIFICATE OF ANALYSIS – LOT 012126

ATTACHMENT II STUDY PROTOCOL

Determination of the Efficacy of Grignard Pure Technology Using a Controlled Time Release against an Aerosolized Microorganism

Test Microorganism(s)

Gram Positive Bacteria
Staphylococcus epidermidis ATCC 12228

Gram Negative Bacteria
Pseudomonas fluorescens ATCC 13525

Protocol Number

ARE Lab Protocol # ARELPRO10948.90

SPONSOR

Bleu Garde LLC.
505 Capobianco Plaza
Rahway, NJ, 07065

PERFORMING LABORATORY

Aerosol Research and Engineering Laboratories
12880 Metcalf Ave
Overland Park, KS 66213

DATE

26Feb26

Determination of the Efficacy of Grignard Pure Technology Using a Controlled Time Release against an Aerosolized Microorganism

1.0 Purpose

1.1. The purpose of this study is to evaluate the reduction of Grignard Pure solution against an airborne pathogen in an aerosol chamber to support air sanitization for efficacy label claims.

2.0 Good Laboratory Practice

2.1. This study will be conducted in accordance with Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) regulation 40 CFR Part 160, Subpart F (160.05). Any deviations from the EPA GLP regulations will be noted in the final study report. The quality assurance team will review vital steps throughout the testing process and will sign off as they are reviewing.

2.2. If necessary, an external Quality Assurance Unit may be provided for this study. The following exceptions to EPA Good Laboratory Practice may be noted in the final study report.

2.3. A datalogger placed in the chamber monitors the air temperature and relative humidity throughout testing. The captured data from each trial will be exported and archived in the study file.

2.4. All calibration certificates for the equipment used throughout the study will be copied and then archived into the study file.

3.0 Scope

3.1. This protocol outlines the stepwise procedure for assessing the bioaerosol reduction efficacy of Grignard Pure when being used in a controlled time release manner at reducing viable microorganism concentrations in an indoor environment.

3.2. This protocol will be strictly adhered to during testing. Any deviations to the protocol will be justified and recorded in the final report for review.

4.0 Test Substance Characterization and Handling

4.1. According to 40 CFR, Part 160, Subpart F (160.105): Each batch of test substance shall be characterized as to identity, strength, purity, composition, and solubility (as applicable), and shall be documented prior to use in this assay. The stability of the test substance shall be determined prior to or concurrently with this study.

4.2. The test substance is handled as follows:

4.2.1. The test substance is stored at ambient temperature.

4.2.2. The test substance is well mixed prior to use (if applicable).

4.2.3. The test substance is handled safely in accordance with the Chemical risks stated in the SDS or by the Study Sponsor.

4.2.4. The Grignard Pure solution will be utilized undiluted when loaded into the aerosolization device for testing purposes.

4.2.4.1. This approach ensures the application method closely mimics real-world usage scenarios, providing a reliable basis for evaluating the efficacy of Grignard Pure in reducing airborne pathogens within enclosed spaces.

4.2.5. Prior to the commencement of efficacy testing, pre-test trials will be conducted to determine the optimal settings on the aerosolization device.

4.2.5.1. These settings will ensure appropriate aerosol concentrations of Grignard Pure within the chamber, replicating conditions relevant to the intended use of the product. The determination of these settings is crucial for achieving the desired concentration levels of Grignard Pure in the air, thereby ensuring the validity and relevance of the efficacy results.

4.2.6. Throughout all trial runs, the concentration of triethylene glycol vapor in the air will be meticulously monitored using a Fourier Transform Infrared (FTIR) gas analyzer. The FTIR gas analyzer provides precise and continuous monitoring of the air quality within the test chamber, specifically measuring the concentration of triethylene glycol vapors emanated from the Grignard Pure solution.

4.2.6.1. This real-time monitoring is essential for maintaining the test conditions within the specified parameters and for documenting the exposure levels experienced by the test microorganisms.

5.0 Lab Ware and Consumables

5.1. 200µl Micropipette + tips for dispensing 20-200µl volumes
5.2. 1000µl Micropipette + tips for dispensing 100-1000µl volumes
5.3. Sterile 1.7mL microcentrifuge tubes for sample processing and serial dilutions
5.4. Sterile 50mL conical tubes for sample processing
5.5. Sterile 15mL conical tubes for sample processing
5.6. 250mL suspension culture flask with vent cap for growth of bacterial and viral cultures & stocks
5.7. Serological Pipettor and pipettes (25mL) for dispersing PBS into the AGI-30 impingers

6.0 Lab Equipment

6.1. 3 separate lots of test substance (Grignard Pure™) being assessed for performance.
6.2. Dispersal device used to disperse the test substance during testing
6.3. A closed, 16 cubic meter bioaerosol test chamber with negative pressure capabilities
6.4. Magnehelic differential pressure gauge to monitor the pressure within the test chamber
6.5. Calibrated mass flow meters to monitor the flow of air or vacuum air flow during sampling
6.6. 24-jet Collison Nebulizer to aerosolize the selected test microorganism
6.7. Dry powder eductor used for dispersal of mold spores
6.8. Ace Glass Inc. Impingers (AGI-30) to capture the bioaerosol samples during testing
6.9. Calibrated Scale to measure out reagents or samples as appropriate during the experiment.

6.10. SKC Biostage Single Stage Impactor used for low concentration sampling of bacteria and spores
6.11. Incubator to facilitate growth of plated microorganisms at specified temperatures
6.12. A closed, 16 cubic meter bioaerosol test chamber with negative pressure capabilities
6.13. Vortex laboratory mixer for equilibration of biological samples
6.14. Centrifuge for spinning down cultures to be used in testing
6.15. 1/3 HP Gast Rotary Vane Vacuum Pump for sampling to be done at a proper air flow
6.16. Biological Safety Cabinet
6.17. TSI AM520 for monitoring particle concentration within the test chamber during testing
6.18. -20+/-2°C Freezer for storage of certain biological stocks, samples, and reagents
6.19. -80+/-2°C Freezer for storage of certain biological stocks, samples, and reagents
6.20. 6+/-2°C Refrigerator for storage of certain biological stocks, samples, and reagents
6.21. Steam Sterilizer for sterilization of biological buffers
6.22. Temperature and Humidity Sensor for monitoring temperature and humidity throughout the trials
6.23. Sorbent tubes for quantification of test substance present in the air
6.24. ThermoFisher MAX-FTiR for measuring test substance present in the air

7.0 Solutions and Reagents

7.1. Deionized water for preparation of PBS and other stock solutions
7.2. Tryptic Soy Broth (TSB) for growth of microorganism stocks
7.3. Tryptic Soy Agar (TSA) for plating test microorganisms
7.4. Brain infusion broth for growth of microorganism stocks
7.5. Brain infusion agar for plating of test microorganisms
7.6. Potato dextrose agar for plating of the mold test samples
7.7. Antifoam A concentrate for reducing the foaming that occurs upon nebulization with solutions containing surfactants or other foam-inducing excipients
7.8. Phosphate buffered saline solution (PBS) growth and maintenance of stocks as well as capture of test microorganisms during sampling
7.9. Polysorbate 80 (Tween 80) as an additive to PBS to enhance the survival of microorganisms
7.10. Polystyrene Latex Microspheres for PSL device characterization
7.11. Artificial Saliva for Pharmaceutical Research SAE0149 for adding a soil load to all test species

8.0 Personal Protective Equipment

8.1. Lab coats for protection of skin from contact with various solutions and reagents being used
8.2. Lab gloves for personal protection as well as reducing risk of contamination
8.3. Safety glasses for personal protection from contact with various solutions and reagents being used

8.4. Personal respirator for personal protection from contact with various aerosolized particles

9.0 Experimental Design

9.1 A general sequence of the main aerosolization procedure of the testing representing normal operation (Figure 1a) is given below.

General Timeline for Occupied Space Chamber Testing

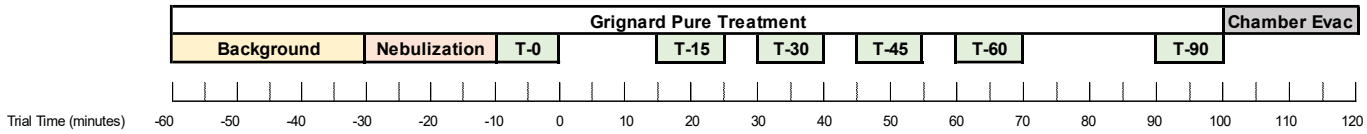

Figure 1a: *Tentative General Timeline for Bioaerosol Chamber Testing*

10.0 Test System Identification

10.1. When applicable, all test and control tube racks will be labeled with the microorganism, test substance, lot identifier, and project number prior to initiation of the study and during incubation. Petri dishes will be labeled prior to initiation of the study with microorganism and project number during incubation. The test substance and usage will be traced according to SOP's extant in the laboratory.

11.0 Maintenance, passage, and storage of test species

11.1. Obtain strains of each organism to be used in testing from a reputable source such as the American Type Culture Collection (ATCC). Every 12 months (or sooner if the quality of the culture stock is compromised or if the culture stock runs out) prepare new stock cultures of the test species from the original source. If the original stock is unavailable order a new batch of the beginning stock from the same source for replacement.

12.0 Test Microorganisms

Test Organism	ATCC Reference Number	Growth Medium	Incubation Parameters
Pseudomonas fluorescens	13525	Tryptic Soy Broth	28 ± 2°C
Staphylococcus epidermidis	12228	Tryptic Soy Broth	37 ± 2°C

13.0 Culture Initiation

13.1. To start any culture, wipe the outside of all ampules/vials of the freeze-dried cultures with a towelette prewetted with 91% isopropyl alcohol and open inside a class 2 biosafety cabinet.

For bacterial species

13.2. Open vial according to enclosed instructions that comes with the species.
13.3. Rehydrate the entire pellet with approximately 0.5 mL of the recommended broth
13.4. Aseptically transfer the entire contents to a 5-6 mL tube of the recommended broth
13.5. Additional test tubes can be inoculated by transferring 0.5 mL of the primary broth tube to these secondary tubes.
13.6. Streak a loopful of the suspension onto two recommended agar plates to test for purity of the culture.
13.7. Incubate the plates at 37°C for 24-48 hours and inspect the colonies for typical colony morphology.
13.8. If any secondary species are present on the streak plate a new stock must be ordered and prepared.

14.0 Cryopreservation of cultures

14.1. Prepare a culture of the desired species with the instructions provided in the culture initiation section of the protocol.
14.2. Add 1.0ml of sterilized glycerol to the culture, shake well and incubate for 2 hours.
14.3. Mix well and aliquot into labelled vials displaying the source, name, date, passage number and lot number. Store Species for no longer than 12 months.

15.0 Test Chamber Specifications

15.1. Structurally, the test chamber has dimensions of 16 cubic meters, or approximately 565 ft^3, with the width deliberately maintained within 85 to 100% of its length.
15.1.1. This dimensional consistency ensures a uniform testing space, allowing reliable experimentation.
15.2. Constructed from a non-porous material, the chamber's walls emit minimal volatile organic compounds (VOCs), is non-reactive, non-reflective, and has a non-ionizing quenching nature. This creates an environment conducive to reliable and repeatable testing conditions.
15.3. The airtight integrity is monitored and controlled within the chamber, achieving a controlled air change rate (ACH) below 0.05, as per the benchmark set by ASTME 241.
15.4. The chamber is designed to prevent external microbial contamination while maintaining internal atmospheric conditions.
15.4.1. These features include an aseptic maintenance system, HEPA filtration, cross-contamination-free item transfer mechanisms, external power control, real-time observation facilitated by multiple viewing windows, and the capability to produce and evenly disperse aerosolized microbes.
15.5. Sampling ports, positioned approximately 48 inches from the floor and 12 inches from the walls, ensure optimal sample collection while maintaining prescribed device

separation. A programmable controller will maintain the chamber's temperature and humidity within the limits described in this protocol.

15.6. Incorporating negative pressure airflow allows for controlled purging, and a HEPA filter adds an additional layer of protection, inhibiting potential contamination.

15.7. A general flow diagram of the test chamber can be found at the top of the next page in **Figure 2.**

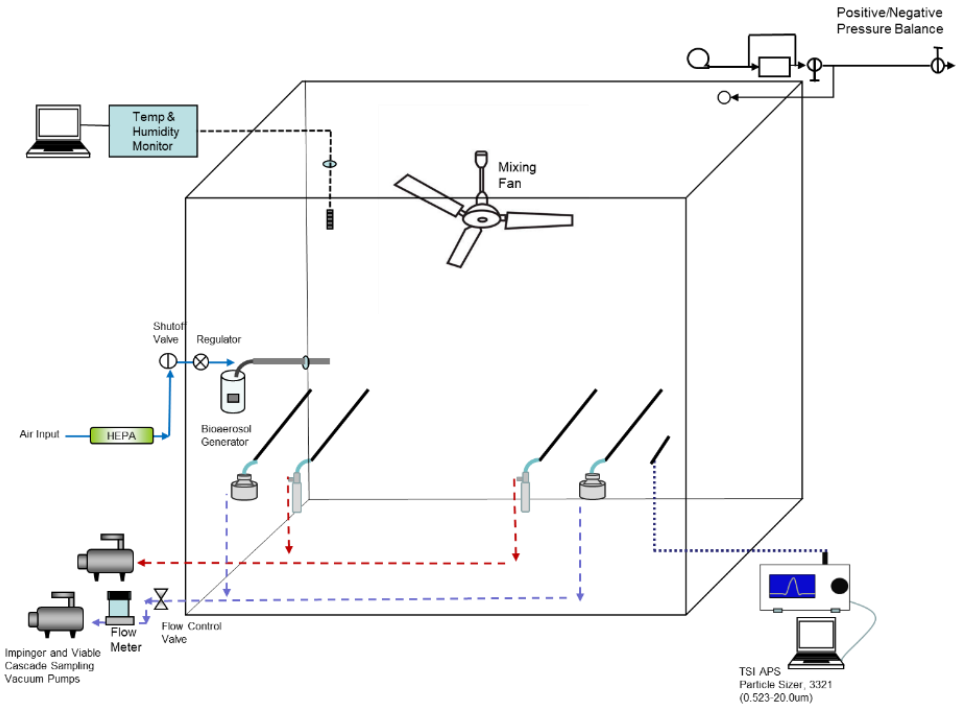

Figure 2: *General bioaerosol testing chamber configuration of the 16m^3 chamber.*

16.0 Test Requirements & Bioaerosol Chamber Set-Up

16.1. Bioaerosol testing will be conducted using a dynamically balanced bioaerosol chamber with integrated decontamination systems.

16.2. For air sampling, the chamber is equipped with four 3/8-inch diameter stainless steel probes that are positioned 12” from the nearest sidewall. (Figure 1).

16.3. Temperature and relative humidity will be monitored throughout each trial by a remote sensor to ensure the proper levels are maintained. The levels will be recorded in the lab notebook at the beginning of each bioaerosol sample.

16.3.1. Temperature range: 22°C – 28°C

16.3.2. Relative humidity range: 30-50%

16.4. The reservoir of the device will be filled with Grignard solution to the fill line on the reservoir before each trial.

16.5. The device will be turned on to the proper settings using the work instructions provided by the study sponsor.

16.6. The device will be primed by allowing the device to run on the selected controlled time release settings for 20 minutes to allow for lines to fill with Grignard Pure solution.

16.7. After priming the device, it will be placed in the center of the chamber, 3 ft. off of the ground on a stainless-steel table.

16.8. A Collison 24-jet nebulizer will be filled with approximately 40 mL of prepared biological stock and operated at 40 psi for a period of 20 minutes.

16.9. The nebulization stock for each trial will be prepared according to the Preparation of Test Organisms section of this protocol. Once the culture is resuspended in PBS 100µL of antifoam A will be added to the stock to reduce the shearing of the microorganism observed during nebulization

16.10. The AGI-30 impingers will be filled with 20 mL of sterilized PBS with an addition of 0.005% v/v Tween 80 for bioaerosol collection.

16.10.1. The addition of Tween 80 will be used in order to increase the impinger collection efficiency and de-agglomeration of all microorganisms.

16.11. The chamber mixing fan, located at the top of the chamber in the center of the area, will be turned on for the duration of the trial to ensure a homogenous mixture of bioaerosol is present throughout the chamber.

16.12. To ensure the uniform distribution of bioaerosols and Grignard Pure within the test chamber, the mixing fan set to operate at a constant rate of 20 Air Changes per Hour (ACH). This specification applies equally to both control trials and those involving the application of Grignard Pure, ensuring a standardized approach across all testing phases. The adoption of a 20 ACH rate is grounded in extensive validation testing, which indicates that this specific air change rate is optimal for achieving rapid homogeneity within the test environment—typically within minutes of initiation.

16.12.1. Continued application of this air change rate has been proven to maintain chamber homogeneity throughout the duration of the test, which is crucial for the accurate assessment of Grignard Pure's efficacy against targeted pathogens.

17.0 Soil Load

17.1. A sterile artificial soil will be added to the nebulization stock of each species to simulate the particles (mucosal or equivalent) that these organisms would be found with when airborne in a real-life scenario.

17.2. The soils to be used in the aerosolization study will be Artificial Saliva for Pharmaceutical Research SAE0149 purchased from Sigma-Aldrich

17.3. The soil will be plated before each test as a control to ensure that it is not a point of contamination in any trials.

18.0 Stock Preparation Procedure

Bacterial Stock Preparation

18.1. Thaw the freezer stock for use in the trial by removing it from the freezer and running it under 40°C water until the stock is completely thawed.

18.2. Incubate 250 ml of tryptic soy broth (30g/l) containing the thawed freezer stock of the desired organism overnight at the appropriate temperature listed in the species table.

18.3. If necessary, centrifuge stock cultures for 10 minutes at 3000rpm in an LD-3 centrifuge in sterile 15 mL conical tubes, remove growth media, and re-suspended the cells in sterile PBS buffer for aerosolization.

18.4. Extract 100ul from the organism stock and serially dilute it in sterile PBS in 1.5mL microcentrifuge tubes, and then plate it on the recommended agar plates and place it in the incubator for stock concentration determination.

18.5. Streak the culture on tryptic soy agar plates to ensure no contamination was present prior to starting the trials.

18.6. Add in required prepared soil solution prior to use in testing.

Note: Test working stocks will be grown in sufficient volume to satisfy use quantities for all tests conducted using the same culture stock material.

19.0 Test Matrix

20.1. The test matrix for the study will include 3 lots of the test substance tested for each bacterial species.

20.2. The test matrix will also include a single control trial to determine the organisms baseline concentration and the natural losses that occur with the species over time in the chamber with no product introduced.

20.2.1. These control losses will be accounted for when determining the net reduction attributable to the test substance during each trial.

20.3. A sample test matrix for the study can be found below.

Trial	Run	Test Substance Lot	Pathogenic Organism Represented	Surrogate Species (gram, description)	ATCC Ref	Target Particle Size	Challenge Conc. (#/L)	Trial Time (min)	Sample Time (min)	Sampling	Plating and Enumeration
1	Control	N/A	*Pseudomonas aeruginosa*	*Pseudomonas (G -, vegetative)*	13525	2.5-3.0um	1.00E+04	90	0, 15, 30, 45, 60, 90	AGI-30 Impingers, TSI AM520, Max iR FTIR	All samples in triplicate
2	Challenge	Lot 1									
3	Challenge	Lot 2									
4	Challenge	Lot 3									
5	Control	N/A	*Methicillin resistant Staphylococcus aureus*	*Staphylococcus (G +, vegetative)*	12228	2.5-3.0um	1.00E+04	90	0, 15, 30, 45, 60, 90	AGI-30 Impingers, TSI AM520, Max iR FTIR	All samples in triplicate
6	Challenge	Lot 1									
7	Challenge	Lot 2									
8	Challenge	Lot 3									

20.0 Neutralization Testing

20.1. Previously the Grignard Pure formulation was tested in liquid form to ensure that all reduction yielded in the trial is due to the performance in the chamber and not because of kill occurring in the impinger sample fluid.

20.2. This data will be included in the final report.

21.0 Bioaerosol Sampling

21.1. Either AGI-30 impingers or SKC BioStage single stage impactor are used for bioaerosol collection to determine the chamber concentration during testing.

21.2. These bioaerosols are connected to the bioaerosol chamber via sample ports located at opposite corners of the chamber.

21.3. The aerosol samplers vacuum source is maintained at a minimum negative pressure of 17 inches of Hg during all sampling to ensure critical flow conditions of the samplers.

21.4. All air samplers are characterized using a calibrated TSI model 4040 mass flow meters

22.0 Testing Timeline

22.1. During Grignard Pure challenge trials, prior to the introduction of bioaerosol, the Grignard Pure dispersal device will be allowed to disperse the Grignard Pure solution into the chamber air on a predetermined setting for 30 minutes to simulate a room being continuously treated with Grignard Pure.

22.2. After 30 minutes of pre-treatment with Grignard Pure the bioaerosol will be introduced into the chamber through nebulization using a 24-jet Collison nebulizer.

22.3. Following bioaerosol generation, baseline concentrations will be established for each pilot challenge test by sampling simultaneously with two AGI-30 impingers located at opposite corners of the chamber. This sample will be known as T-0. For this study T-0 will be when the first sample is taken after the nebulization of the bioaerosol.

22.4. Control trials will begin with the nebulization of the bioaerosol and will not involve pre-treatment in the room.

22.5. Collected impinger chamber samples will be collected separately at each sample interval for each test and placed into two specifically marked 15mL conical tubes.

22.6. The conical tubes containing the samples will immediately be taken to a biosafety cabinet to be serially diluted in PBS and plated on tryptic soy agar plates

22.7. In between samples the impingers will be taken to the lab sink to be rinsed 6x with DI water and then dried prior to being used for the next sample

22.8. The Grignard Pure solution will continue to disperse on a set schedule throughout the trial using the same predetermined setting throughout the entirety of the trial.

22.9. Subsequent impinger samples will be taken at the 15 minute, 30 minute, 45 minute, 60 minute, and 90 minute time points during the trial.

22.10. These samples will be enumerated for viable concentration to measure the effective viable bioaerosol reduction achieved during operation of the device dispersing the Grignard solution over time.

22.11. All samples will be plated in triplicate on the appropriate media over a minimum 3 log dilution range.

22.12. Plates will be incubated for the recommended amount of time at the recommended temperature and enumerated for viable colony forming units (cfu) to calculate aerosol challenge concentrations in the chamber and reduction of viable microorganism

23.0 Statistical Analysis

23.1. The data will be subjected to appropriate statistical analyses for the preparation of the final report.

23.2. Analysis of the test data will include, at minimum, the average of the data sets as well as the standard deviation of the data sets.

24.0 Data Analysis/Calculations

24.1. **Calculation of % Reduction and LOG Reduction during testing**

24.2. The starting bioaerosol concentration of the challenge trial will be compared to the starting concentration of the control trial to calculate the effect of the Grignard Pure™ chamber pre- treatment. The method for nebulization and nebulization stock will remain the same from the control to the challenge trial so the concentration of the challenge trial would be expected to be the same as the control. The change in the starting concentration seen in the challenge trial will therefore be attributed to the pretreatment of the chamber.

24.3. To determine the log reduction of the trial at each time point, the percentage of viable microorganism remaining airborne in the chamber will be compared to the initial bioaerosol concentration of the uninhibited control trial. This remaining percent will then be multiplied by the log10 function to yield log reduction.

24.4. For net reduction, the natural losses from the control trial will be subtracted from the test trial to account for natural losses not caused by the test solution.

24.5. AGI – 30 impinger collection calculation:

24.5.1. Viable aerosol concentration collection (C_a) = cfu/L of chamber air.

24.5.2. Viable Impinger concentration collection (C_{Imp}) = cfu/mL

24.5.3. Sampler collection volume (I_{vol}) = X ml collection fluid (impinger), sampler dependent

24.5.4. Sample flow rate (Q_{imp}) = Y L/min, sampler dependent

24.5.5. Sample time (t) = Z minutes, test dependent.

24.5.6. For viable impinger aerosol concentration collection (C_a) = cfu/L of chamber air:

$$C_a = \frac{\mathrm{C_{Imp} \cdot I_{vol}}}{\mathrm{Q_{imp}}} \mathrm{t}$$

24.5.7. To evaluate the viable aerosol delivery efficiency and define operation parameters of the system, calculations based on (theoretical) 100% efficacy of aerosol dissemination will be derived using the following steps:

24.5.8.

24.5.9. Concentration of the stock suspension (C_s) cfu/mL

24.5.10. Collison 24 jet nebulizer use rate (R_{neb}) volume of biological stock dispersed at X psi air supply pressure.

24.5.11. Aerosol Generation time (t)

24.5.12. Chamber volume (V_c) = 16,000 Liters

24.5.13. Assuming 100% efficiency, the quantity of aerosolized viable particles (V_P) per liter of air in the chamber for a given biological stock concentration (Cs) is calculated:

$$V_P = \frac{C_s \cdot R_{neb}}{V_c} t$$

24.6. The aerosol system viable delivery efficiency (expressed as %) is:

$$Efficiency = \frac{C_a}{V_p} \cdot 100$$

24.7. All triplicate runs will be averaged to obtain a mean and standard deviation for each trial.

24.8. Total viable biological concentrations along with reductions over time will be Log reductions of aerosolized biologicals will be calculated and graphed for each trial.

25.0 Quality Control

25.1. Sterility Control: One plate of growth medium will be incubated alongside the test plates. All reagents will be evaluated by plating 1.0 ml of growth media and incubated alongside the test. The acceptance criterion for this control is lack of growth.

25.2. Viability Control: The growth/neutralizing media will be challenged in duplicate with <100 CFU the test bacterium and incubated alongside the test to confirm the media can support growth at low concentration levels.

25.3. Purity Control: A streak plate will be prepared for each test culture or host culture and incubated alongside the test to confirm the purity of the test culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism

26.0 Control Acceptance and Criteria

26.1. All sterility controls must be free of any visible growth.

26.2. Viability Control must demonstrate growth in all media with <100 CFU/plate.

26.3. Purity Control must demonstrate a pure culture.

26.4. Neutralization Validation: The mean number of CFU on the plate unexposed to the test substance and those on the plate exposed to the test substance must be within 50%.

26.5. Temperature and RH readings must stay within range required for the test.

27.0 Study Acceptance and Criteria

27.1. Test Substance Performance Criteria: After correction for bacterial settling and natural biological decay, the test substance must demonstrate ≥99.9% (≥3.0 $\log_{10}$) reduction in the viability of the bacterial, mold, mycobacterium, and bacteriophages over the parallel untreated controls.

27.2. If cytotoxicity is present, the virus/bacteriophage titer should be increased if necessary to demonstrate ≥ $\log_{10}$ reduction in CFU/m^3 beyond the cytotoxic level.

27.3. Baseline Acceptance criteria: The control recovery must demonstrate a minimum of 4.2 Log_{10} to a maximum of 5.0 $\log_{10}$ CFU/m^3 at the start of the treatment for a valid test.

28.0 Retesting

28.1. If any controls or results fail to meet the criteria outlined in this study, the failure will be noted and a retest will occur.

28.2. If the retest of the same species results in a failure the test will be aborted to launch an investigation to find the root cause.

28.3. Upon the results of the investigation a CAPA will be established to ensure the failure does not happen again.

29.0 Report

29.1. Results are reported accurately and fully, in accordance with Environmental Protection Agency GLP 40 CFR Part 160.

29.2. Report will include but not limited to: Purpose, scope, background device and device pictures, organisms tested, test condition, all methods, calculations, statistical analysis, results and conclusions.

29.3. Appendices with additional data, figures and raw plates counts will be provided

30.0 Protocol Amendments and Deviations

30.1. All amendments to and/or deviations from this protocol and the reasons for such shall be documented, approved by management (as may be required), signed by the Study Director and at the study sponsor, dated and maintained with the protocol.

30.2. Any amendments to and/or deviations from this protocol will be recorded in the raw data and documented in the final report.

31.0 Record Retention

31.1. Data and records to be maintained by ARE Labs include, but are not limited to, the study protocol, amendments and deviations, test article receipt and handling, chamber temperature and humidity records, laboratory notebooks, study correspondence, and study final report.

31.2. All data, records, documents, notes, or other materials generated by the study will be collected and securely stored on site at the ARE facility.

31.3. All recordings of data will be made directly, concurrently, and legibly in indelible ink. All data entries will be dated on the date of entry and signed or initialed by the person responsible for the entry. Any changes to data entries will be made to not obscure the original entries. The changes will be justified and then checked and signed by the study director and the QA representative.

32.0 References

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STUDY INFORMATION

Testing parameters to be completed by the Study Sponsor/ Representative

Test Substance Name	
Test Substance Batch Numbers	
Manufacture Date(s)	
Expiration Date(s)	
Test substance active Ingredient(s)	□ Alcohol □ Peroxide □ Quaternary Ammonia □ Sodium Hypochlorite
Test Substance Active Concentration as submitted (for Neutralization information)	
Active Ingredient Level	□ At or below Lower Certified Limit (LCL) □ At or below nominal
Test Substance Storage Conditions	□ Room Temperature □ 2-8˚C □ Other:
Test Substance Hazards	□ None Known: Use Standard Precautions □ Material Safety Data Sheet, attached □ Other:
Test Substance Dilution	□ Ready to Use (RTU) □ Dilution ratio: (e.g., 1 oz per gallon, 1:255)
Dilution to be made	□ N/A, RTU □ Dilution(s) to be tested: _____ defined as _____ + _____ (e.g. :1 oz/gallon) (amount of test substance) (amount of diluent)
Test Substance Diluent	Not applicable for RTU Product

Organic Soil Load	□ None □ 5% fetal bovine serum □ Other:

Contact time (s)	
Exposure Temperature	□ Room Temperature □ Other:
No Number of Test Carriers / Replicates	
Spray Instructions	
Neutralization / Subculture Broth	ARE Lab to determine. Sponsor authorizes pre-test neutralization confirmation assay to be conducted to determine appropriate neutralizer if need. □ Use:
EPA 40 CFR Part 160.31(d) requires testing facility management to assure that the test, control, and reference substances have been appropriately tested for identity, strength, purity, stability, and uniformity, as applicable	Identity, strength, purity, and uniformity, as applicable, of the test lots has been or will be completed prior to efficacy testing □ YES □ No ▪ Performed following 40 CFR Part 160 GLP regulations: □ Yes □ No Stability testing has been or will be completed prior to efficacy testing or concomitantly with efficacy testing: ▪ Performed following 40 CFR Part 160 GLP regulations: □ Yes □ No
Certificate of Analysis (CoA)	□ CoA for each batch provided. CoA will be appended in the final report.
Test Substance Shipment Status	□ Use test substance already present at AER LAB. □ Test substance will be shipped. Estimated arrival date, if known:
Protocol Modification	□ Testing to be performed as outlined in the protocol. □ The following protocol modifications are to be performed:
Regulatory Agency(s) that report may be submitted to	□ EPA □ Health Canada