Efficacy of Grignard Pure™ Against a Variety of Bioaerosols

Independent Testing Conducted
June 2022

AGENDA

I. Testing Location

- Aerosol Research and Engineering Labs, Inc. Overview

II. Objective

- Show Efficacy of Grignard Pure™ against Multiple Microorganisms (bacteria, viruses, mold endospores)
- Individual Microbial Species Tested

III. Methodology

- Controlled Time Release (see slide 5)
- Room Sized Bioaerosol Chamber

IV. Results

- NET LOG_{10} Reduction at T = 15 minutes
- NET LOG_{10} Reduction at T = 60 minutes
- NET LOG_{10} vs. Time
- Estimated Time to Reach >3.0 NET LOG_{10} Reduction

OVERVIEW:

AEROSOL RESEARCH & ENGINEERING LABORATORIES, INC.

ARE Labs, Inc. was founded 2009 with a focus on Aerosol Sciences

- Bioaerosol Programs
  - Bioaerosol mitigation Reduction
  - Bioaerosol ingress
  - Sensor Triggering
- Respiratory Drug Deliver of Pharmaceuticals
  - Drug Deliver for Traditional API and Biologics
- Inhalational
  - Animal models
- Computation Fluid Dynamic
  - Aerosol Dynamic Molding (Steady and Unsteady State)
  - Validation of Laboratory Scale Testing and Extrapolating to Large Open Environment Modeling
  - Device Design and Performance Improvements

Grignard Pure Bioaerosol Testing Methodology Overview

- Based on Test Protocols developed with FDA in 2012 for Air Purifier Qualification for medical 510(k) Approvals
- Modification based on EPA Testing previously conducted by EPA with Grignard Pure

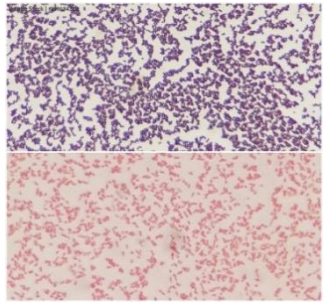
OBJECTIVE

The purpose of this study was to determine efficacy of Grignard Pure™ when applied at a specified concentration utilizing a control time release method against a variety of bioaerosols including the following:

- Gram positive bacteria
- Gram negative bacteria
- Enveloped virus
- Non-enveloped virus
- Mycobacteria
- Mold Spore

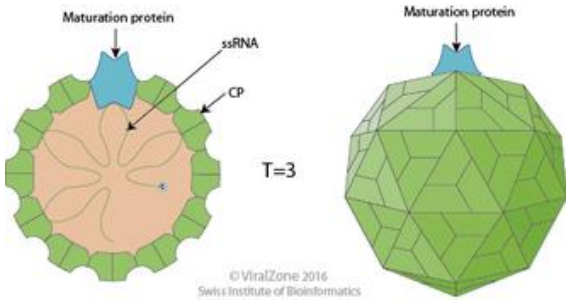
OBJECTIVE: SPECIES TESTED

- **Gram Positive Bacteria**
  - *Listeria innocua* (ATCC# 33090)
  - Methicillin-Resistant *Staphylococcus epidermidis* (ATCC# 12228)
- **Gram Negative Bacteria**
  - *Salmonella typhimurium* (ATCC# 53648)
  - *Klebsiella aerogenes* (ATCC# 51697)
  - *Pseudomonas fluorescens* (ATCC# 13525)
- **Non-Enveloped RNA Virus**
  - *Virus MS2* (*Escherichia* virus, ATCC #15597-B1)
- **Enveloped RNA Virus**
  - *Virus Phi6* (*Pseudomonas* virus, ATCC #21781-B1)
- **Acid-Fast Bacteria**
  - *Mycobacterium smegmatis* (ATCC #607)
- **Mold Endospores**
  - *Aspergillus brasiliensis* (ATCC #16404)



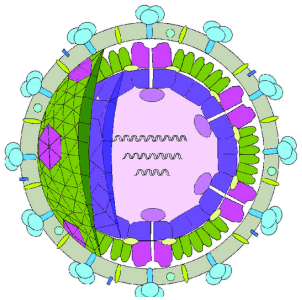
Gram Positive Stained Bacteria

Gram Negative Stained Bacteria



MS2 Virus

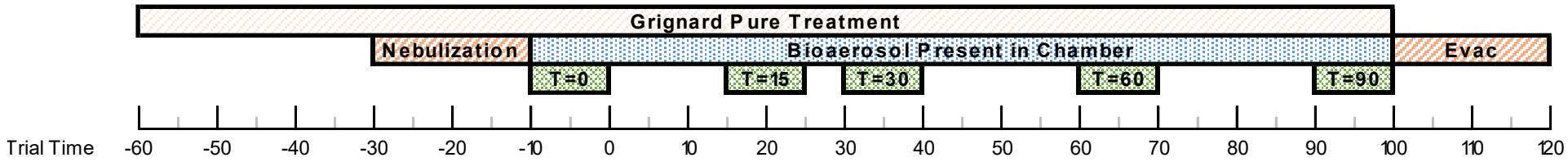
Mycobacterium



Phi6 Virus

METHODOLOGY

General Timeline for Controlled Time Release Testing



1. Testing was conducted in a fully enclosed, environmentally controlled bioaerosol chamber constructed of 304 stainless steel (all sides, floor, ceiling) that has the internal dimensions of 9.1'(L) x 9.1'(W) x 7'(H)

2. For the Grignard Pure™ Trials the dispersion device was turned on to run on a specified dispersal schedule until the target aerosol concentration of Grignard Pure was achieved in the chamber. The Grignard Pure™ concentration was measured on a TSI AM520 with a PM10 particle cutoff. The target concentration for testing was set at 0.04mg/m^3 for all species except for *A.brasiliensis*. The *A.brasiliensis* testing was conducted at 0.16mg/m^3 with an alternative dispersal device.

3. All species used a Collison 24-jet nebulizer for dissemination with the exception of A. brasiliensis which used a dry powder feeder to disseminate dry spores.

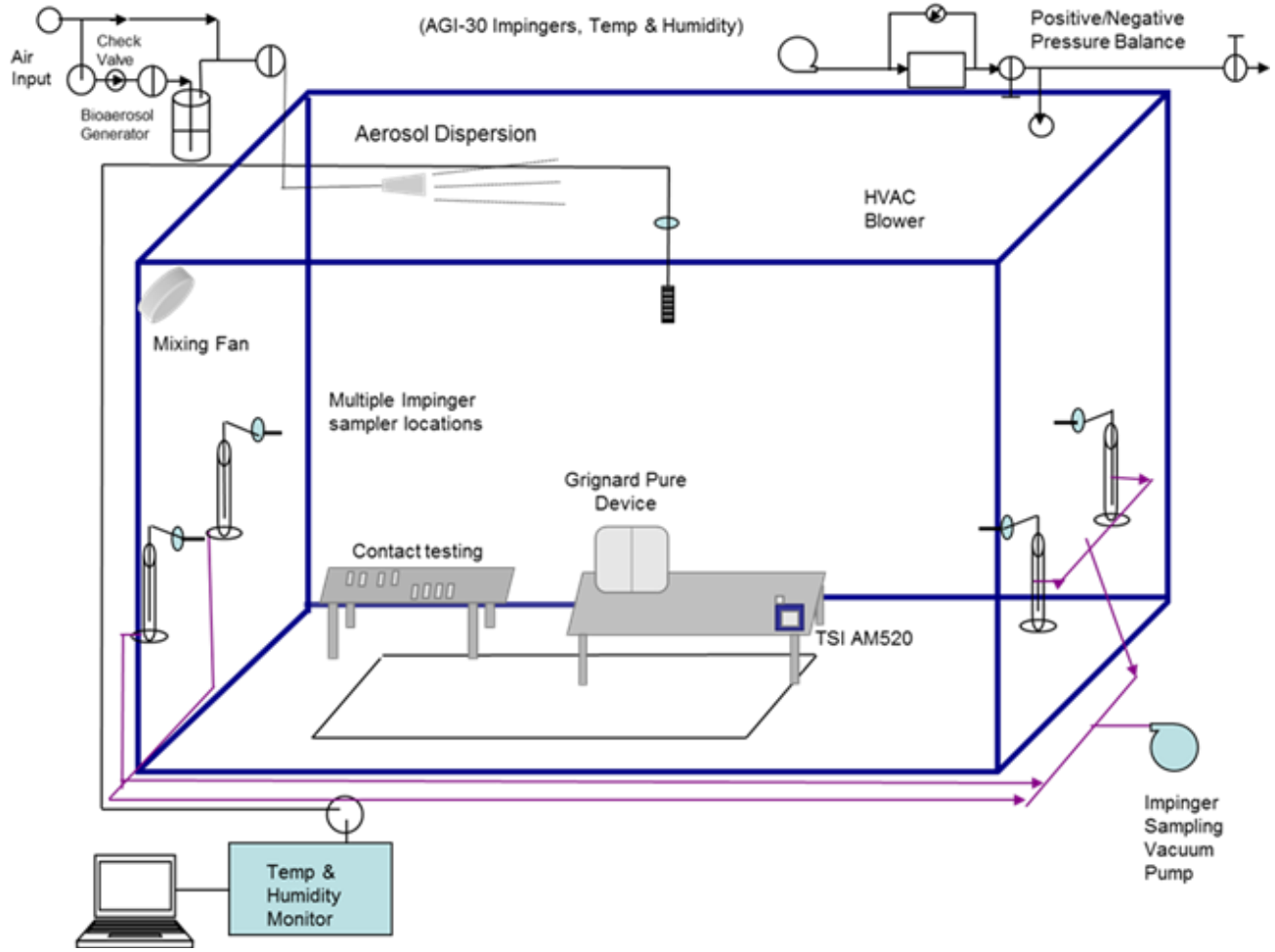
4. Once target aerosol concentration of Grignard Pure in the chamber was achieved and steady, the test pathogen was nebulized in to the chamber for 20 minutes.

5. All microorganism were nebulized in 50% PBS and 50% fresh growth media. Additionally, Albumin, 2%, was added to viral nebulization stock. The additional of the growth media and albumen acts as a protectant to the microorganism simulating mucosal and other protectant found in respiratory generated aerosols.

6. At end of bioaerosol nebulization, the first sample to establish the initial concentration was taken. For figures, this sample is referred to as T=0. Subsequent Samples were then taken at (T) = 15 minutes, 30 minutes and 60 minutes (sample time are sample start times). Methodology for this study was based off of previous testing conducted at an EPA laboratory.

METHODOLOGY

CHAMBER FLOW DIAGRAM



Chamber Flow Diagram

Test Chamber

AGI-30 Impinger

Collison Nebulizer

RESULTS

	NET LOG_{10} Reduction at 15 minutes
1. Gram Positive Bacteria	
Listeria innocua:	2.55 net LOG_{10} reduction in 15 minutes
Staphylococcus epidermidis:	4.76 net LOG_{10} reduction in 15 minutes
2. Gram Negative Bacteria	
Salmonella typhimurium:	2.35 net LOG_{10} reduction in 15 minutes
Klebsiella aerogenes:	2.00 net LOG_{10} reduction in 15 minutes
Pseudomonas fluorescens:	3.10 net LOG_{10} reduction in 15 minutes
3. Non-Enveloped RNA Virus	
Virus *MS2*:	2.61 net LOG_{10} reduction in 15 minutes
4. Enveloped RNA Virus	
Phi 6:	Reduced to LOD* levels in 15 minutes 2.26 net LOG_{10} reduction
5. Acid-Fast Bacteria	
Mycobacterium smegmatis:	3.07 net LOG_{10} reduction in 15 minutes
6. Mold Spores	
Aspergilus Brasiliensis:	0.79 net LOG_{10} reduction in 15 minutes

How Results Were Calculated::

- Master stock was split and used for both control and Grignard Pure trials with the same nebulization pressure and nebulization time for direct 1:1 comparison
- Results were calculated using the uninhibited T=0 sample from the control trial. All data normalized to control T=0.

RESULTS

	NET LOG_{10} Reduction at 60 minutes
1. Gram Positive Bacteria	
Listeria innocua:	3.23 net LOG_{10} reduction in 60 minutes
Staphylococcus epidermidis:	5.88 net LOG_{10} reduction in 60 minutes
2. Gram Negative Bacteria	
Salmonella typhimurium:	3.75 net LOG_{10} reduction in 60 minutes
Klebsiella aerogenes:	3.22 net LOG_{10} reduction in 60 minutes
Pseudomonas fluorescens:	Reduced to LOD* levels in 15 minutes
3. Non-Enveloped RNA Virus	
Virus *MS2*:	3.01 net LOG_{10} reduction in 60 minutes
4. Enveloped RNA Virus	
Phi 6:	Reduced to LOD* levels in 15 minutes
5. Acid-Fast Bacteria	
Mycobacterium smegmatis:	3.54 net LOG_{10} reduction in 60 minutes
6. Mold Spores	
Aspergilus Brasiliensis:	1.50 net LOG_{10} reduction in 60 minutes

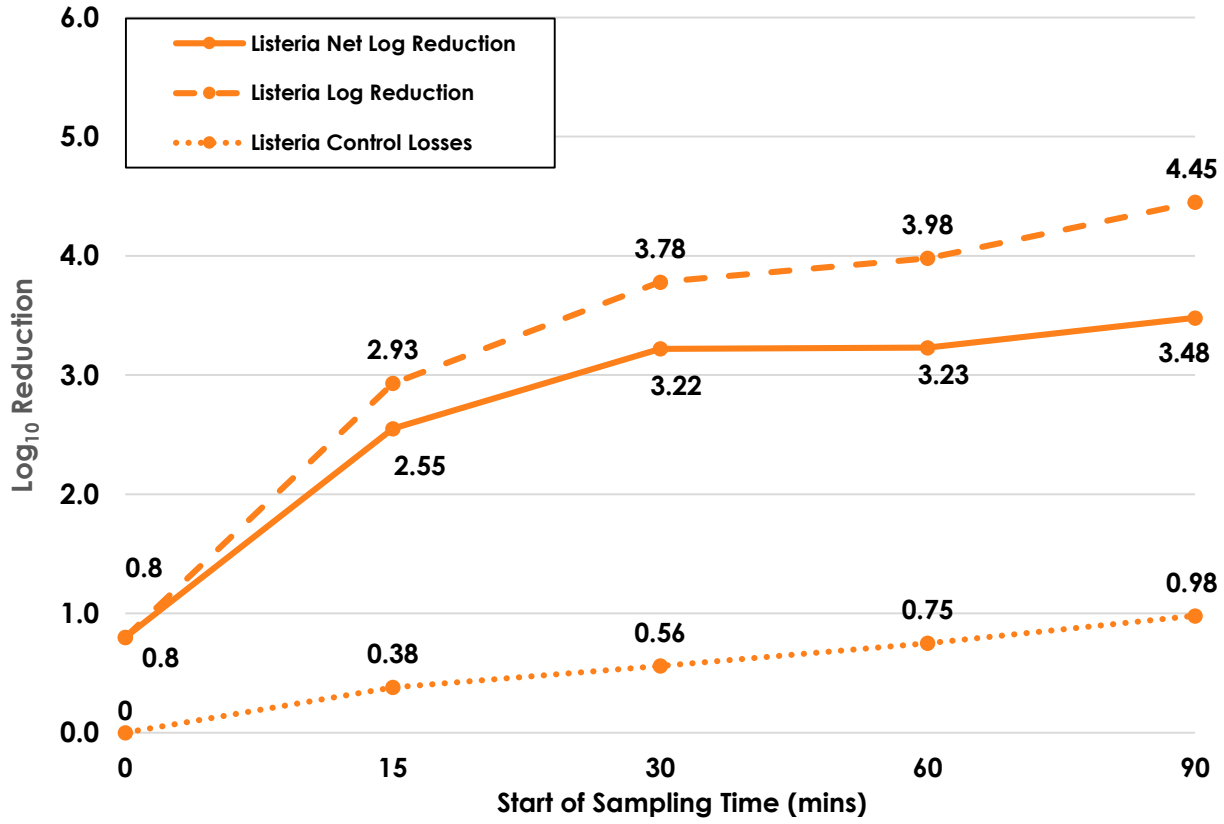
How Results Were Calculated:

- Master stock was split and used for both control and Grignard Pure trials with the same nebulization pressure and nebulization time for direct 1:1 comparison
- Results were calculated using the uninhibited T=0 sample from the control trial. All data normalized to control T=0.
- Net reduction calculated by taking the log reduction from the Grignard Pure trials and subtracting the natural die off from the control trials.

RESULTS

GRAM POSITIVE BACTERIA: LISTERIA

Reduction of *Listeria Innocua* when treated with Control Time Release of Grignard Pure™

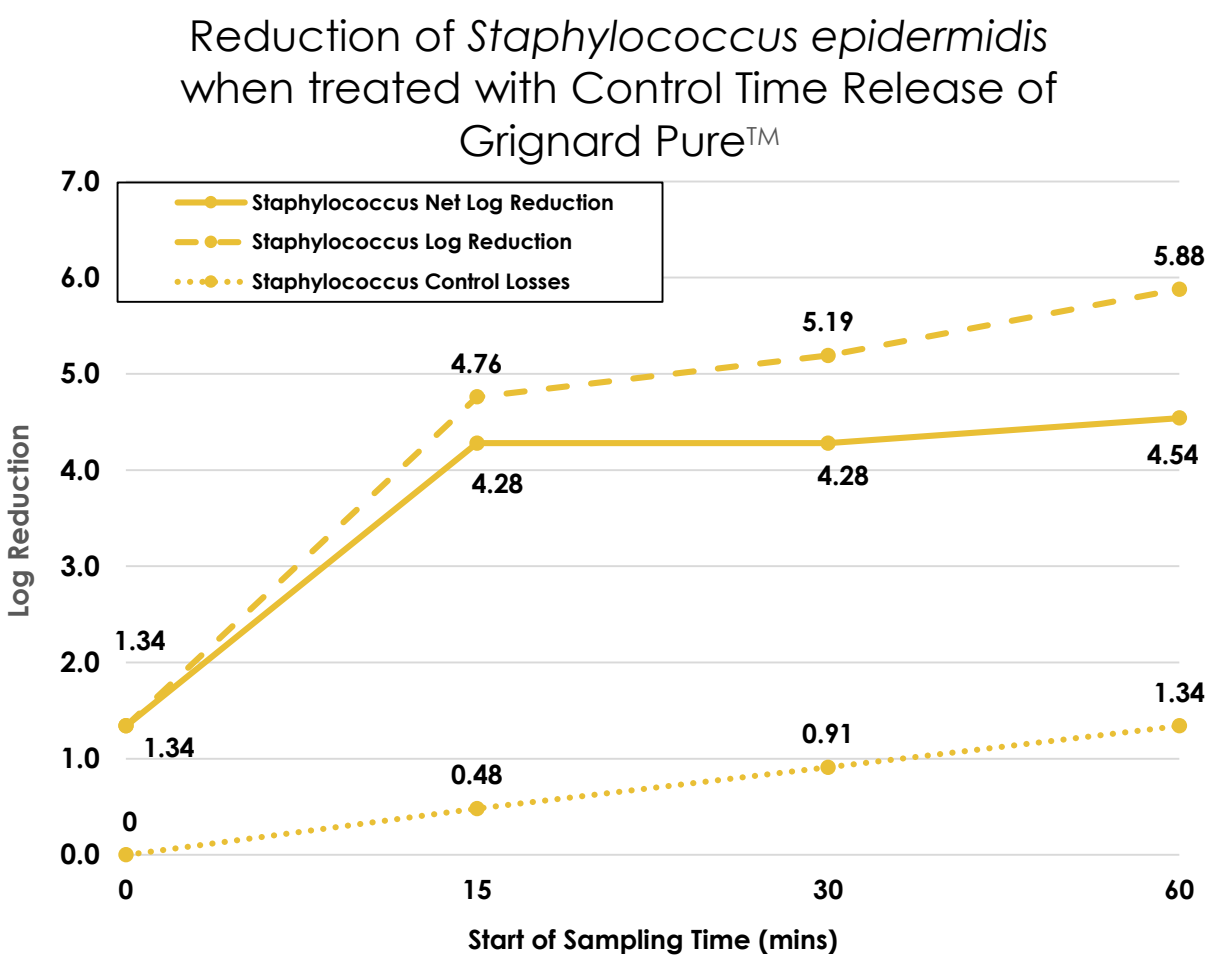


Listeria innocua:

- Pretreating the chamber with Grignard Pure™ resulted in a reduction in the starting concentration of the trial of 0.80 LOG_{10} from the uninhibited control.
- The trial is estimated to reach a net LOG_{10} reduction of >3.0 in about 24 minutes.

RESULTS

GRAM POSITIVE BACTERIA: STAPHYLOCOCCUS EPIDERMIS

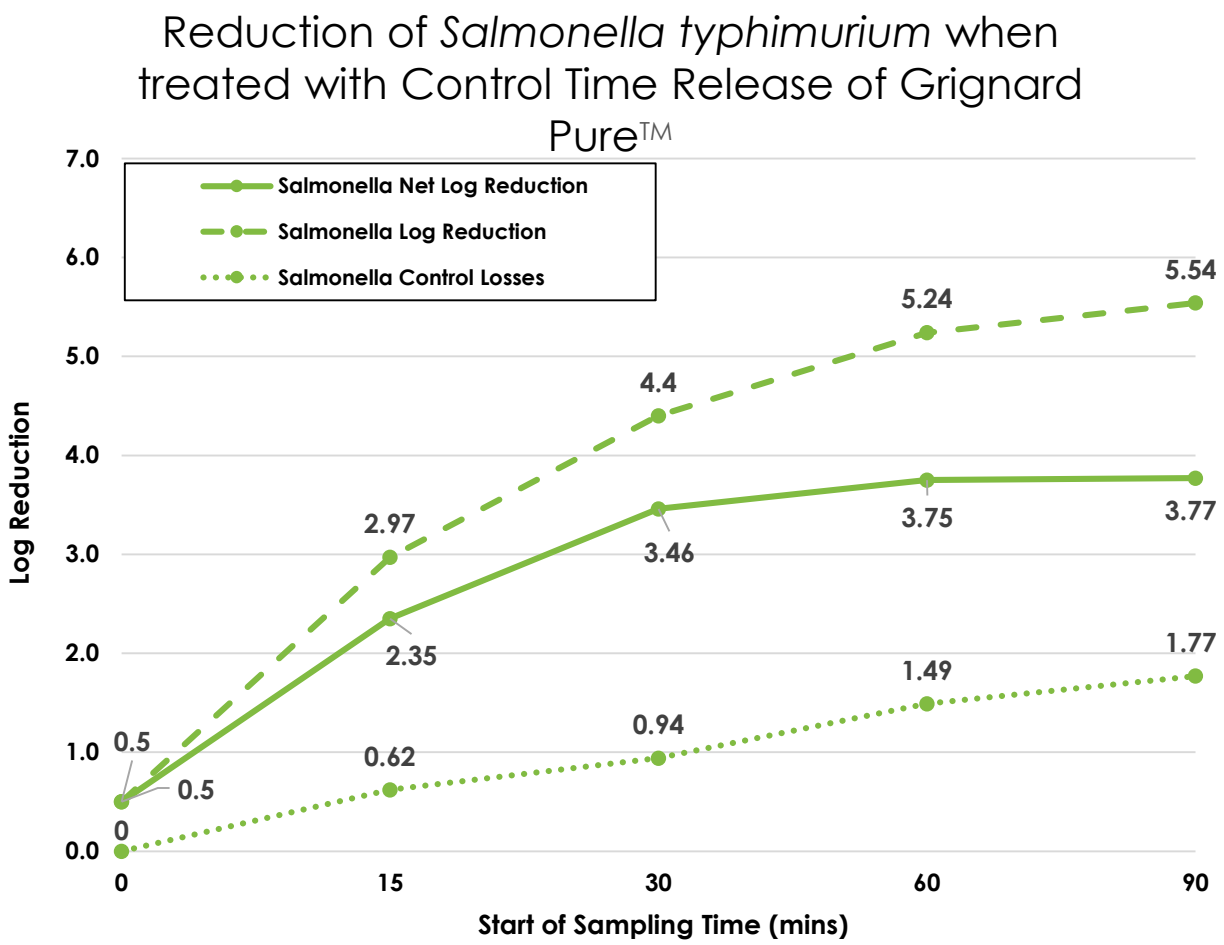


Staphylococcus epidermidis:

- After the pretreat of the chamber prior the Grignard Pure™ trial concentration was 1.34 LOG_{10} lower from the uninhibited control.
- The trial is estimated to reach a net LOG_{10} reduction of >3.0 in about 8 minutes.

RESULTS

GRAM NEGATIVE BACTERIA: SALMONELLA TYPHIMUNIUM



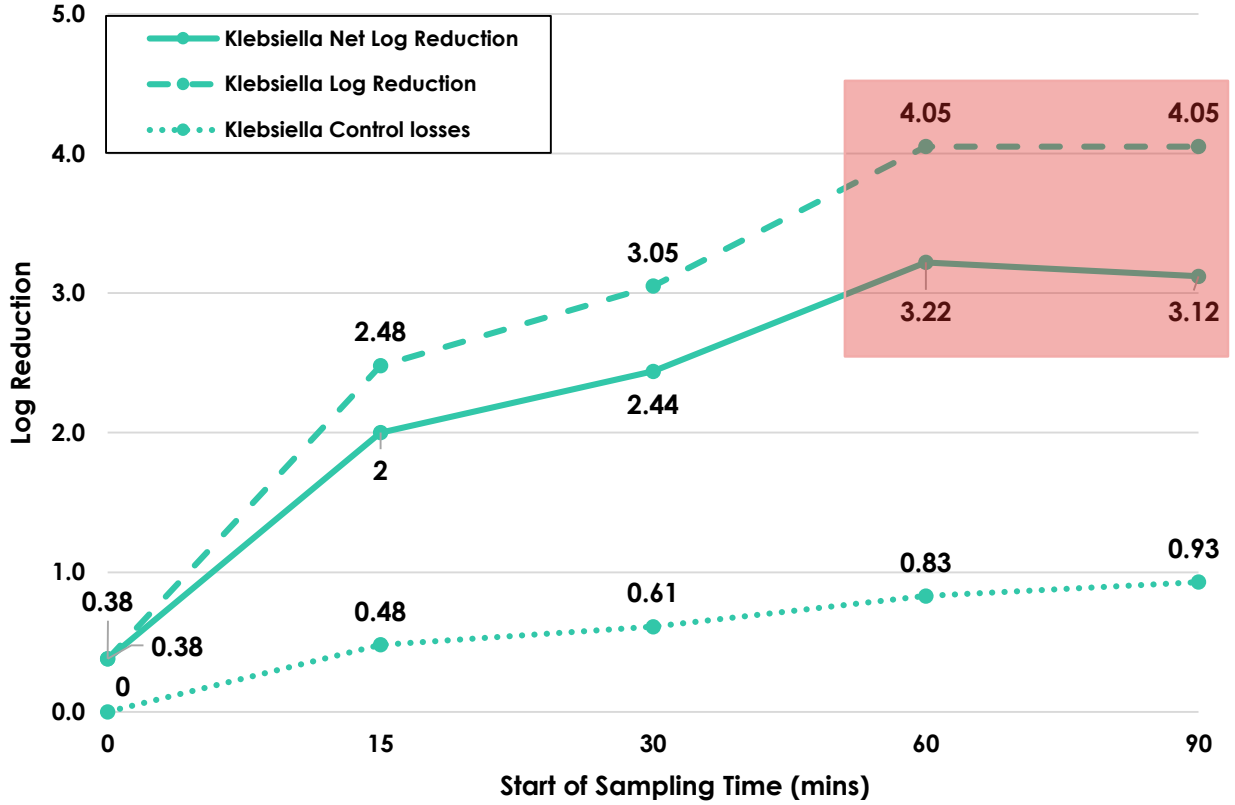
Salmonella typhimurium :

- Pretreating the chamber with Grignard Pure™ resulted in a reduction in the starting concentration of the trial of 0.50 LOG_{10} from the uninhibited control.
- The trial is estimated to reach a net LOG_{10} reduction of >3.0 in about 24 minutes.

RESULTS

GRAM NEGATIVE BACTERIA: KLEBSIELLA AEROGENES

Reduction of *Klebsiella aerogenes* when treated with Control Time Release of Grignard Pure™



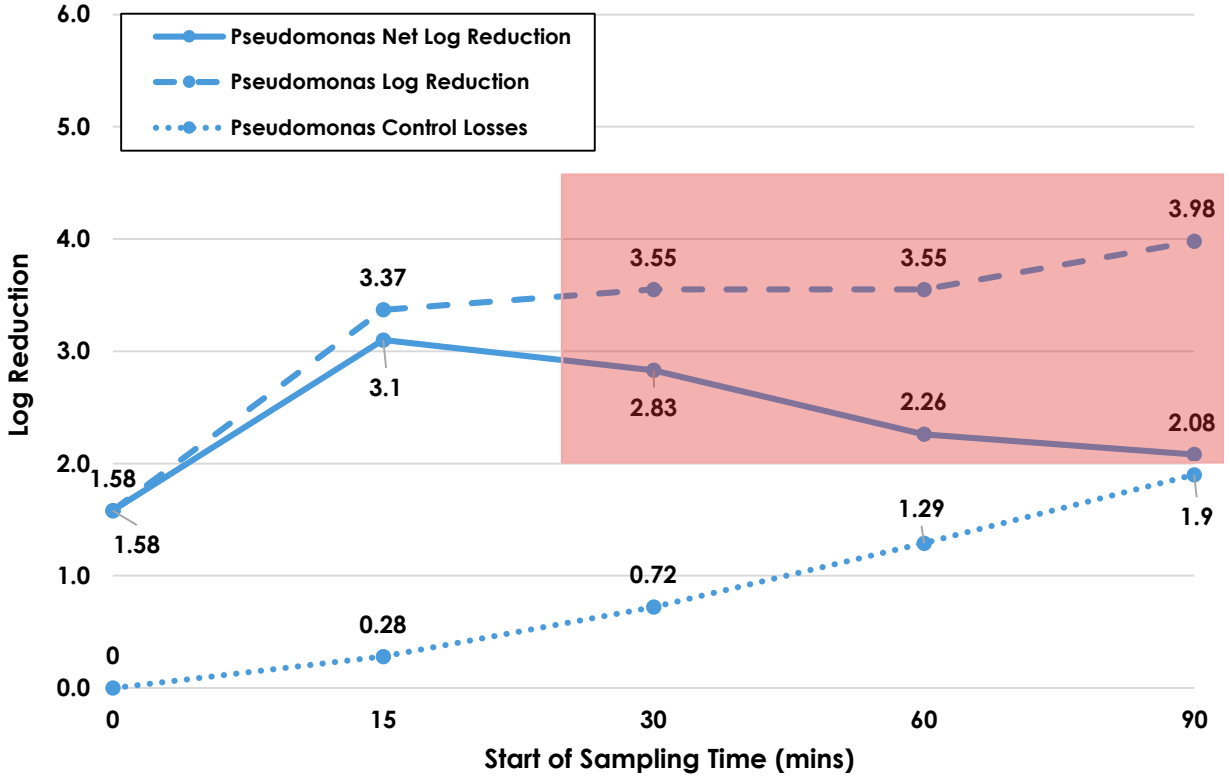
Klebsiella aerogenes:

- Pretreating the chamber with Grignard Pure™ resulted in a reduction in the starting concentration of the trial of 0.38 LOG_{10} from the uninhibited control.
- The trial is estimated to reach a net LOG_{10} reduction of >3.0 in about 28 minutes.
- The final two timepoint are highlighted in red to show that the value at these timepoint was effected by the Grignard Pure trial reaching the method limit of detection for the trial.

RESULTS

GRAM NEGATIVE BACTERIA: PSEUDOMONAS FLUORESCENS

Reduction of *Pseudomonas fluorescens* when treated with Control Time Release of Grignard Pure™

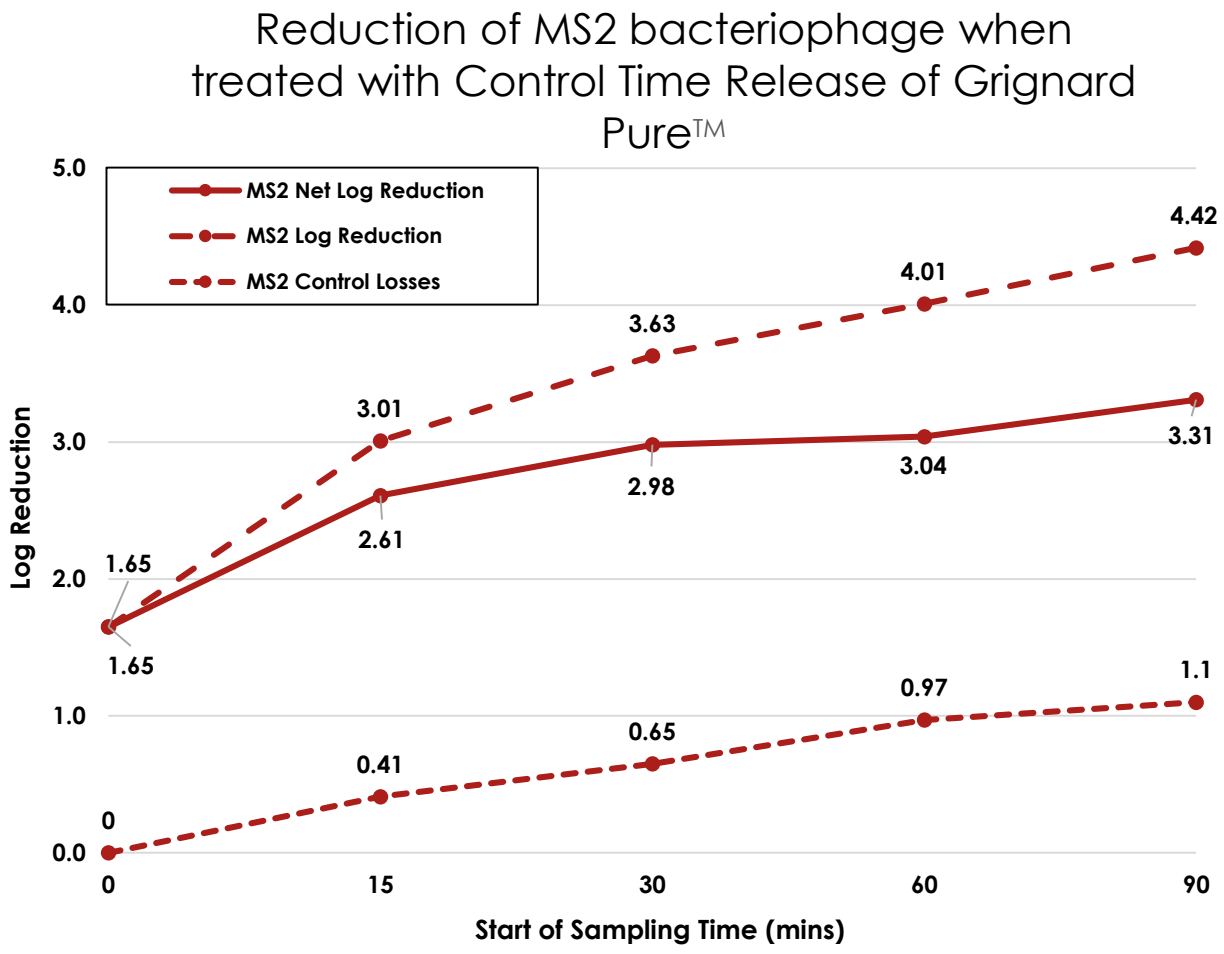


Pseudomonas fluorescens :

- Pretreating the chamber with Grignard Pure™ resulted in a reduction in the starting concentration of the trial of 1.58 LOG_{10} from the uninhibited control.
- The trial is estimated to reach a net LOG_{10} reduction of >3.0 in about 14 minutes.
- Each timepoint after the T-15 sample is highlighted in red to show that the values at these timepoint were effected by the Grignard Pure trial reaching the method limit of detection for the trial.

RESULTS

NON-ENVELOPED RNA VIRUS: MS2 BACTERIOPHAGE



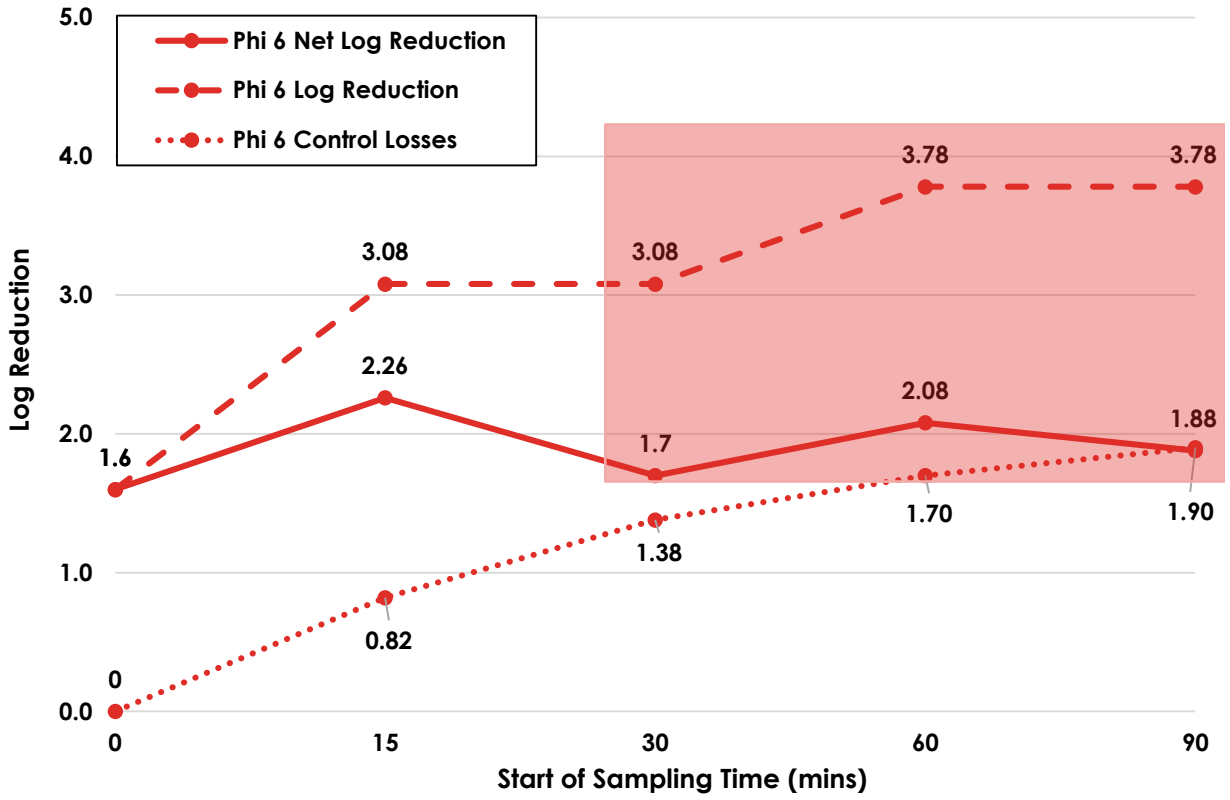
MS2 bacteriophage:

- Pretreating the chamber with Grignard Pure™ resulted in a reduction in the starting concentration of the trial of 1.65 LOG_{10} from the uninhibited control.
- The trial is estimated to reach a net LOG_{10} reduction of >3.0 in about 45 minutes.

RESULTS

ENVELOPED RNA VIRUS: PHI 6

Reduction of Phi 6 when treated with Control Time Release of Grignard Pure™



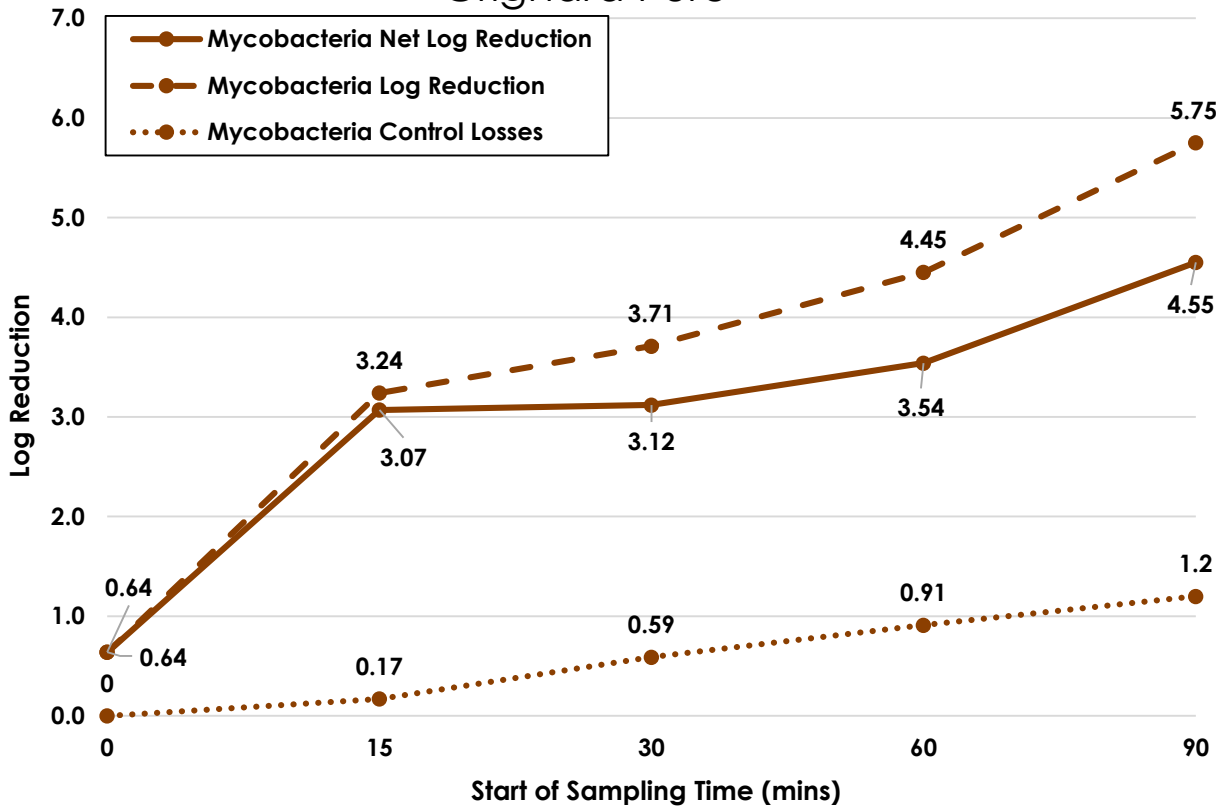
Phi 6 bacteriophage:

- After the pretreat of the chamber prior the Grignard Pure™ trial concentration was 1.60 LOG_{10} lower from the uninhibited control.
- If not for the limit of detection being reached the trial would've been expected to hit over >3.0 net LOG_{10} in 30 minutes
- Each timepoint after the T-15 sample is highlighted in red to show that the values at these timepoint were effected by the Grignard Pure trial reaching the method limit of detection for the trial.
- The increase in LOG_{10} reduction from T-30 to T-60 was due to a change in plating volumes

RESULTS

MYCOBACTERIUM SMEGMATIS

Reduction of *Mycobacterium smegmatis* when treated with Control Time Release of Grignard Pure™



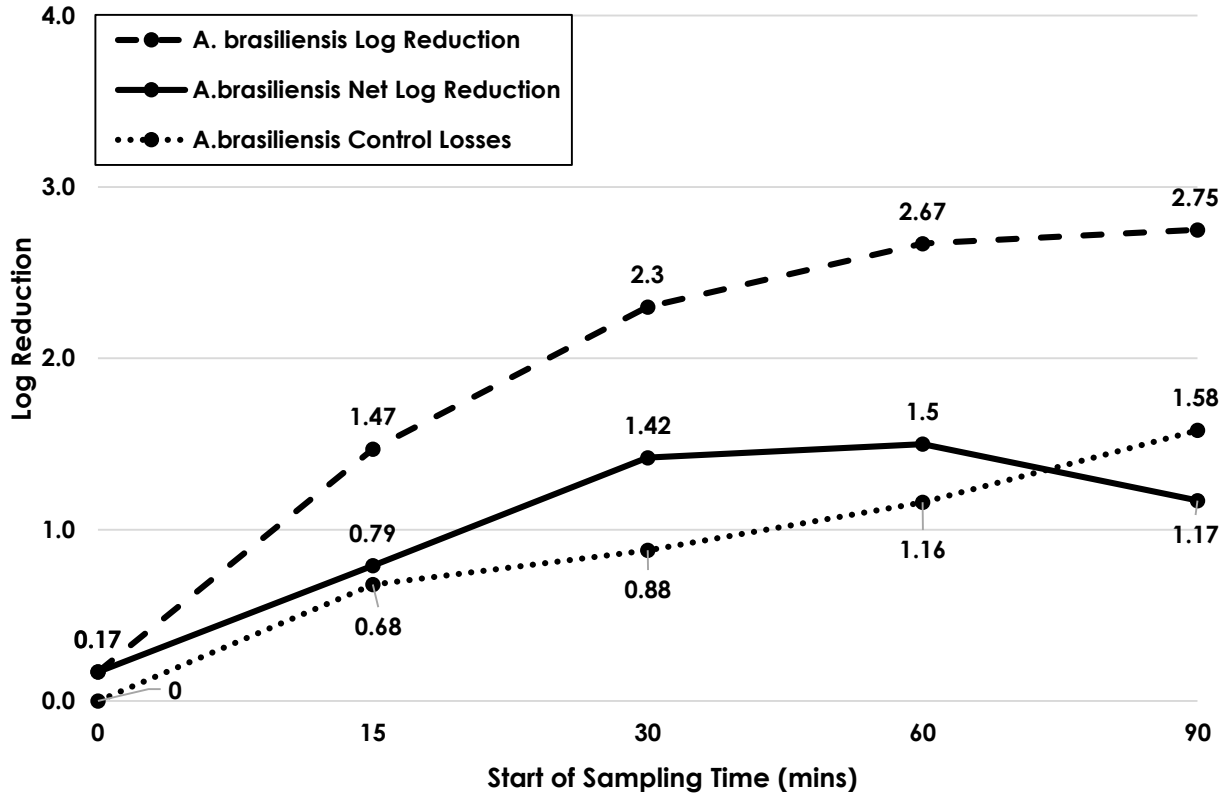
Mycobacterium smegmatis:

- Pretreating the chamber with Grignard Pure™ resulted in a reduction in the starting concentration of the trial of 0.64 LOG_{10} from the uninhibited control.
- The trial is estimated to reach a net LOG_{10} reduction of >3.0 in about 14 minutes.

RESULTS

ASPERGILIUS BRASILIENSIS

Reduction of Aspergillus brasiliensis when treated with Control Time Release of Grignard Pure™

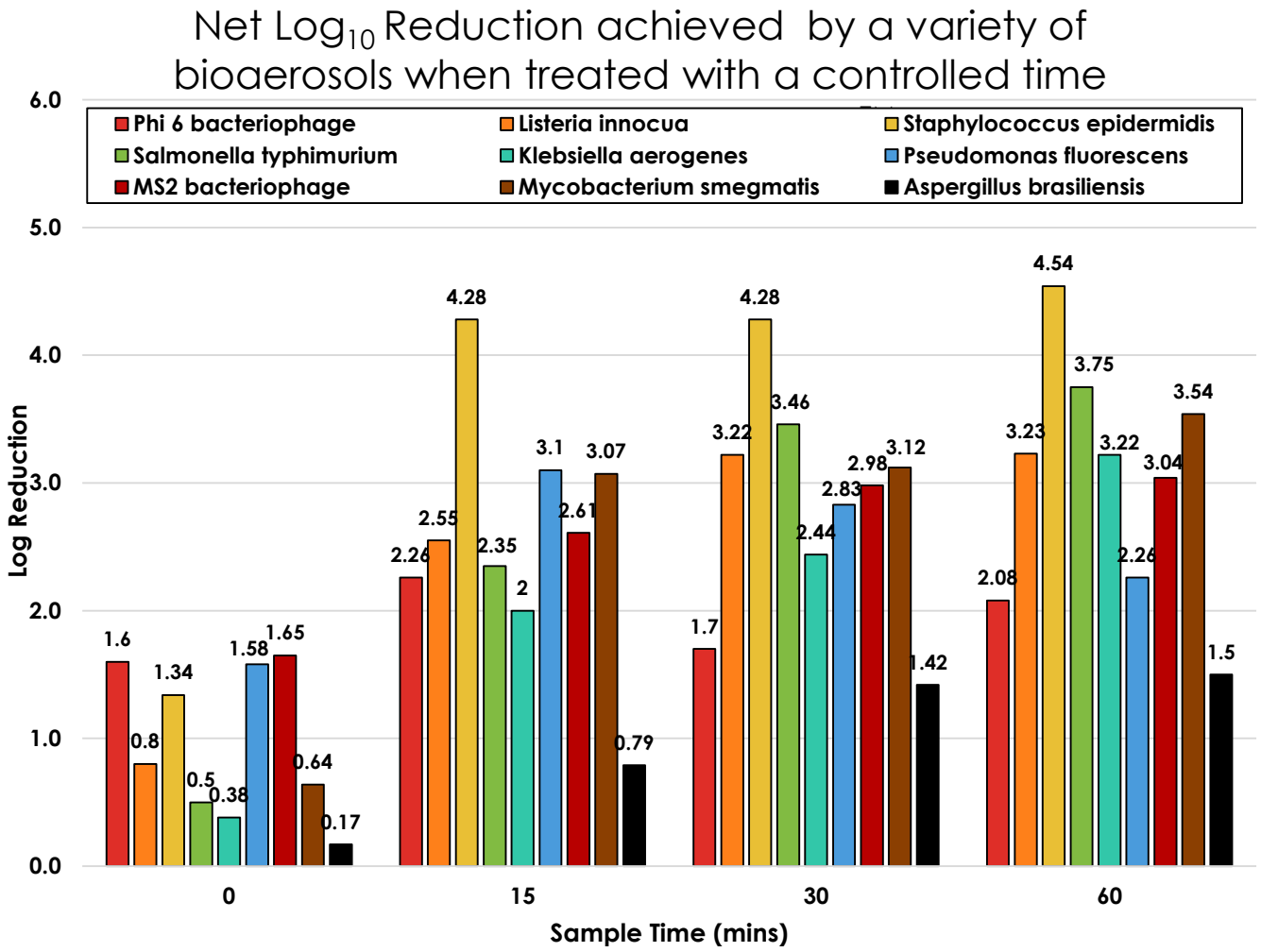


Aspergillus brasiliensis:

- Pretreating the chamber with Grignard Pure™ resulted in a reduction in the starting concentration of the trial of 0.17 LOG_{10} from the uninhibited control.
- With the T=90 time point being less net reduction than the T=60 time point it is unclear when the device would achieve a >3.0 net LOG_{10} reduction

RESULTS

CONTROLLED TIME RELEASE BIOAEROSOL SUMMARY



Controlled Time Release Summary:

- The graph shows the net LOG_{10} value of each organism at each time point out to 60 minutes
- Staphylococcus epidermidis yielded the highest amount of reduction when treated with the Grignard Pure solution.
- Multiple species were effected by reaching the method limit of detection during the Grignard Pure treatment trial

RESULTS

TIME CONTROLLED RELEASE BIOAEROSOL SUMMARY

The Grignard Pure™ Solution, when tested using a controlled time release method, showed efficacy against all of the tested microorganisms

The starting concentration of the species has an impact on the testing results

- A relatively low starting concentration of some species prevented from observing high level LOG_{10} reduction due to organism reaching LOD before 60 minute time point
- Example: *Pseudomonas fluorescens*
  - Starting concentrations was lower than other bacterial species
  - LOD reach at T=15 minutes sample point, however Net LOG_{10} reduction was still measured >3.0 at 15min.

All organisms hit >3.0 Net LOG_{10} with the following exceptions:

- RNA Virus *Phi6*
  - Low initial starting concentration
  - LOD reached at T=15 (or sooner)
  - Re-testing with higher concentrations is expected to improve results
- Mold Spore *Aspergillus brasiliensis*
  - Very hardy species for all methods of antimicrobial action
  - Maximum Net LOG_{10} reduction reached at 60 minutes
  - Not at the LOD for the trial

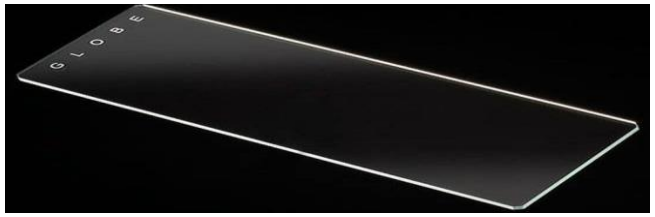
SETTLING SLIDE METHODOLOGY

Overview

- Settling Slides during bioaerosol trials were used to assess reduction of viable microorganism deposition onto surfaces
- Settling slides show net accumulation of organisms due to deposition
- Comparison between Control Trials (no Grignard Pure solution) and Grignard Pure trials shows net reduction of deposition between control and treatment trails.
- A net reduction validates that the microorganism are inactivated and that reduction in bioaerosol results are not due to agglomeration and settling

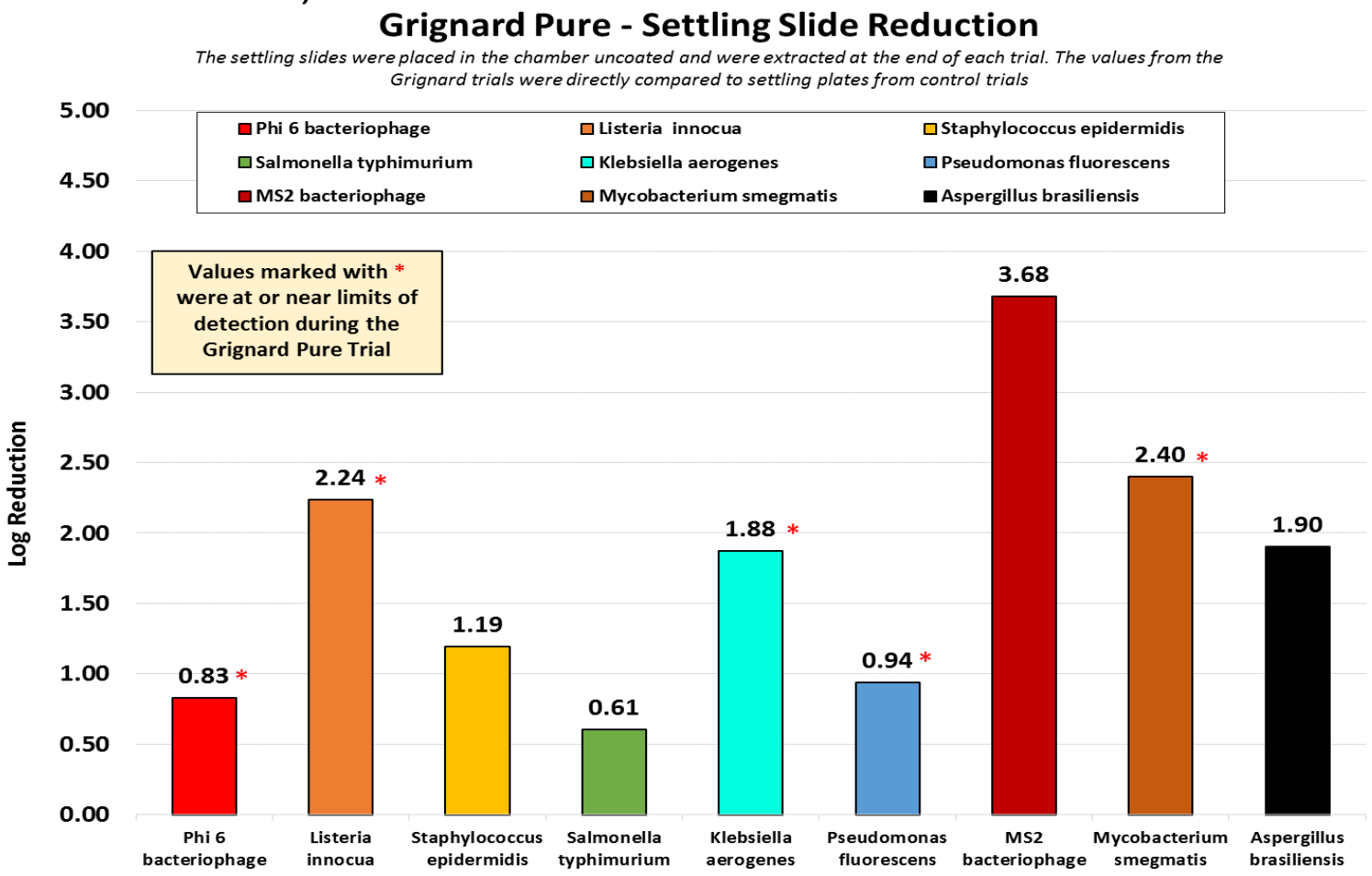
Methodology

1. Slides were placed on a stainless steel table at a height of 3 ft. off of the ground and 3 ft. away from the Grignard dispersal device prior to the pretreatment of the testing chamber.
2. Slides remained in the chamber throughout each trial. They were pulled from the chamber at the conclusion of the trial after all bioaerosol was evacuated from the chamber.
3. Settling slides show net accumulation of organisms due to deposition
4. The slides were placed into 50mL conical tubes containing buffer solution for extraction, sampling, and plating
5. The average concentration of the microorganism on the slides collected from the control trials were compared to the average concentration of the microorganism on the slides collected from the Grignard Pure trials to determine the overall amount of reduction.



RESULTS

CONTROLLED TIME RELEASE SETTLING (REDUCTON FROM CONTROLS)

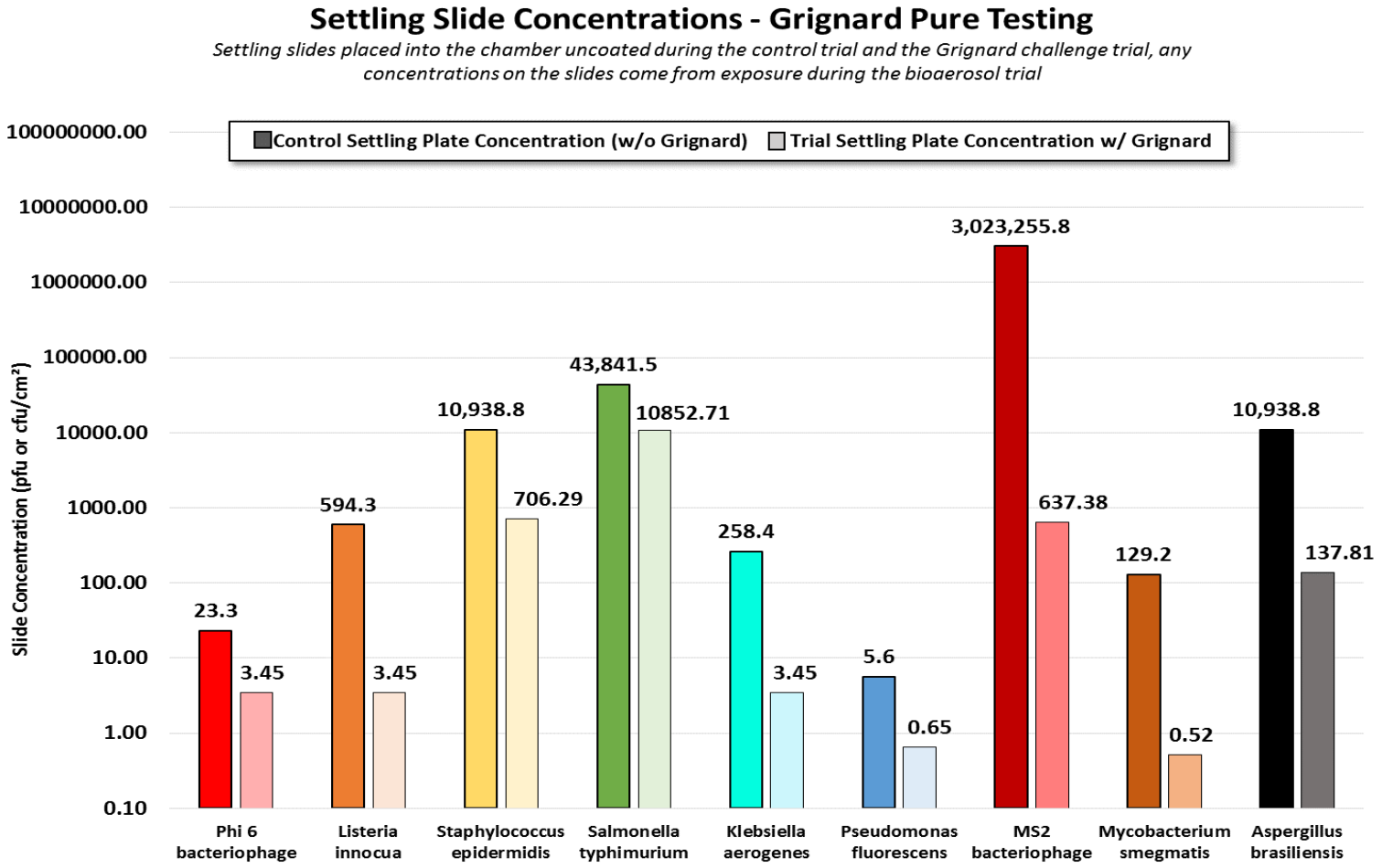


Settling Slide Results:

- MS2 yielded the most reduction during the Grignard Pure multiple species testing
- The amount of reduction observed in the trials depended heavily on the amount of settling yielded in the control trials. The species with less settling in the control trial had less resolution for reduction.
- The species marked with a red star were at or close to the limit of detection for the sampling method used during testing

RESULTS

CONTROLLED TIME RELEASE SETTLING SLIDE: CONTROL VS. AGENT SLIDE CONCENTRATIONS, CFU/PFU PER AREA (CM2)



Settling Slide Results:

- The MS2 bacteriophage control trial yielded a significantly higher settling concentration than the rest of the species tested.
- Species such as *Pseudomonas fluorescens* and *Phi 6* had concentrations under 25 cfu (or pfu) per cm^2
  - *This low control concentration led to less reduction resolution*

RESULTS

CONTROLLED TIME RELEASE – SETTLING SLIDE SUMMARY

The Grignard Pure™ Solution, when tested using a controlled time release method, showed efficacy against all of the tested microorganisms when examining the settling slide data from the trials

- The most settling reduction was observed during MS2 bacteriophage testing
- All organisms yielded settling slide reductions from the control slides of over 75%

The control concentration of the species has an impact on the testing results

- A relatively low control concentration of some species prevented from observing high level LOG_{10} reduction due to organism being at or near level of detection for the sampling method

The reduction in settling concentration for every species verifies that the Grignard Pure solution is not agglomerating particles causing increased settling.

- The reduction of the Grignard Pure solution can be attributed to kill or inactivation.

WANT TO LEARN MORE?

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