EPA Office of Research and Development

HOMELAND SECURITY RESEARCH

COVID-19 Research: Ozone and Aerosol Treatment

HSRP Webinar Series

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ORD's Center for Environmental Solutions and Emergency Response

Overview

Regulatory

- Pesticide Registration (FIFRA)
- FIFRA Enforcement
- Test Method Development

Research

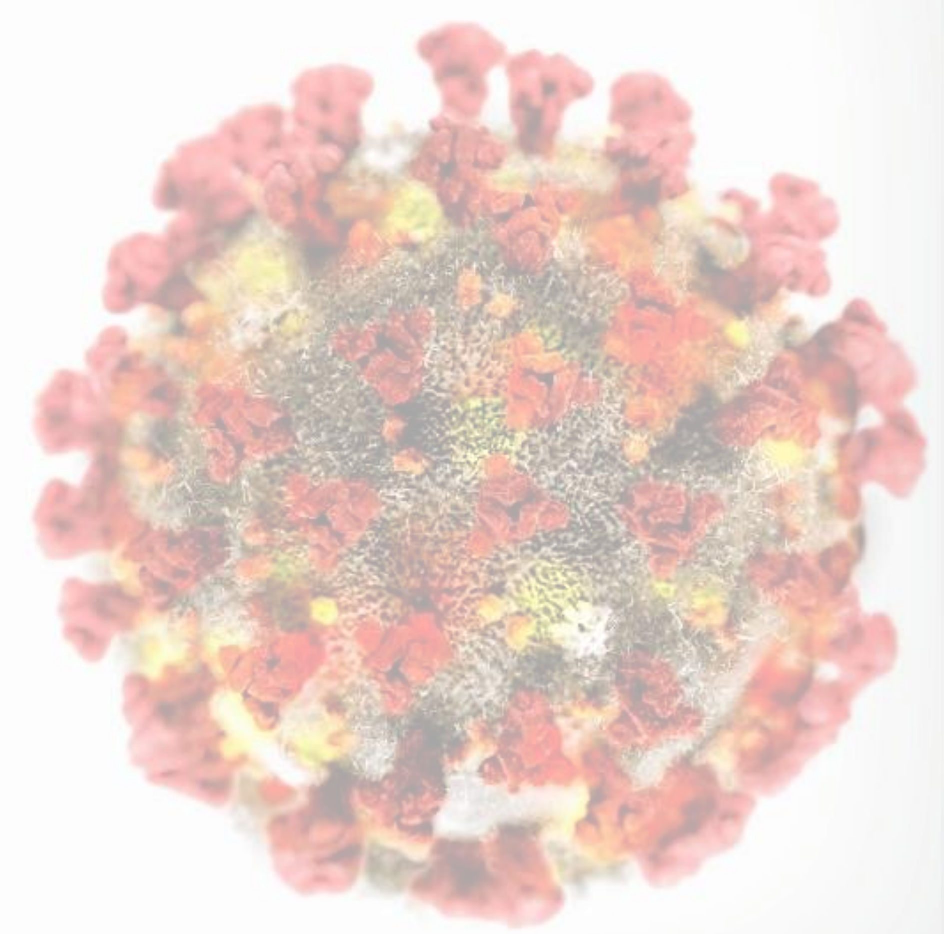
- Aerosol Treatment
- Surface Cleaning and Disinfection
- Pesticide Application
- Pesticide Devices
- Residual Antimicrobial Coatings

Response

- Cleanup Guidance
- Technical Support
- Preparedness/Mitigation

Outline

- Ozone: Disinfection of Surfaces
  - Research Objectives
  - Background
  - Setup and Test Matrix
  - Results
- Aerosol Treatment Research
  - Background and Research Objectives
  - Methods and Test Chamber
  - Results
    - Bipolar Ionization
    - Grignard Pure
    - 3-Stage Air Filtration and Purification System



Research Objectives: Evaluating Alternative Surface Disinfection Methods

Assessment of methods to disinfect challenging materials (e.g., porous surfaces) and application methods suitable for large or complex areas

- Supplemental methods to regular surface disinfection approaches

<u>Initial selection:</u>

1. UV light – *webinar on January 21, 2021*
2. **Ozone – discussed in this webinar**
3. Steam – *research in progress*

https://www.epa.gov/emergency-response-research/covid-19-uv-c-devices-and-methods-surface-disinfection-webinar

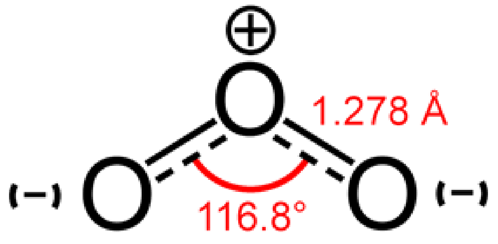
<u>Disinfection Performance Goal</u>:

- Three (3)-log reduction* (99.9%) in viable/infective virus post-treatment

**: Virucidal Claim: A product should demonstrate a ≥3 log_{10} reduction on every surface in the presence or absence of cytotoxicity.*
- EPA 810.2200 Disinfectants for Use on Environmental Services

Ozone as a Disinfectant

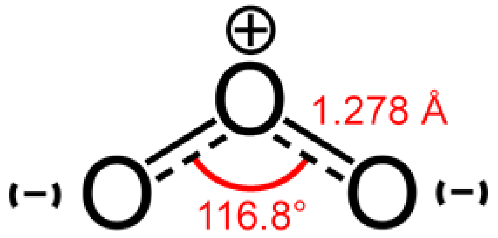
- Ozone is a strong oxidizing agent and inhalation carries a health risk (damage to respiratory system)
- Ozone Immediately Dangerous to Life and Health (IDLH) is 5 ppm
- NIOSH recommended exposure limit (REL) for ozone is 0.1 ppm [OSHA PEL 8h-TWA is also 0.1 ppm]
- Ozone is our friend in the stratosphere but a pollutant in the air we breathe



Ozone

Ozone as a Disinfectant

- Antiviral and antimicrobial properties of ozone are well studied
  - Mostly with ozone in water
- At start of the study, data on disinfection ability of ozone against SARS-CoV-2 was limited to small number of studies
- Need more information for more realistic field conditions (e.g., on various materials, inoculum types, realistic exposure conditions)
- Large variability in reported inactivation rates due to different test conditions

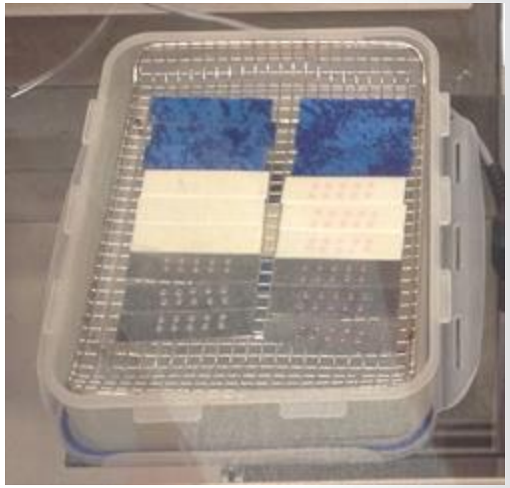


Ozone

Ozone Setup and Generation

Test chamber within Bio Safety Level (BSL)-3 facility

Rack to hold coupons; added fan to promote air circulation



Example of layout Coupons

Ozone Generator
Queenaire QT Storm
(Corona discharge)

Ozone Test Matrix

	SARS-CoV-2
Ozone Concentrations	10 ppm or 20 ppm
Relative Humidity	50% or 70-80%
Contact Times	30 and 60 minutes
Materials (2.5 cm x 4.0 cm)	301 Stainless Steel ABS Plastic Bus Seat Fabric (pile; 85% wool, 15% nylon)
Inoculum Application	10 x 10 µL Droplets
Inoculum Matrix	Tissue Culture (TC) Media with 5% FBS or Simulated Saliva (ASTM)
Inoculum Presence	Wet / Dry Droplets

Virus Recovery

- Aseptic retrieval of material coupon followed by placement into sterile conical tubes with extraction buffer
- Vortexing (2 min)
- For SARS-CoV-2, samples were split with one part stored for RV-PCR analysis (*in progress*)
- Culture on a well plate titer with varying dilutions of the testing fluid are added to the wells.
- SARS-CoV-2 Eluents were tested for viable virus by cell culture (Median Tissue Culture Infectious Dose, TCID50 assay)

Efficacy:

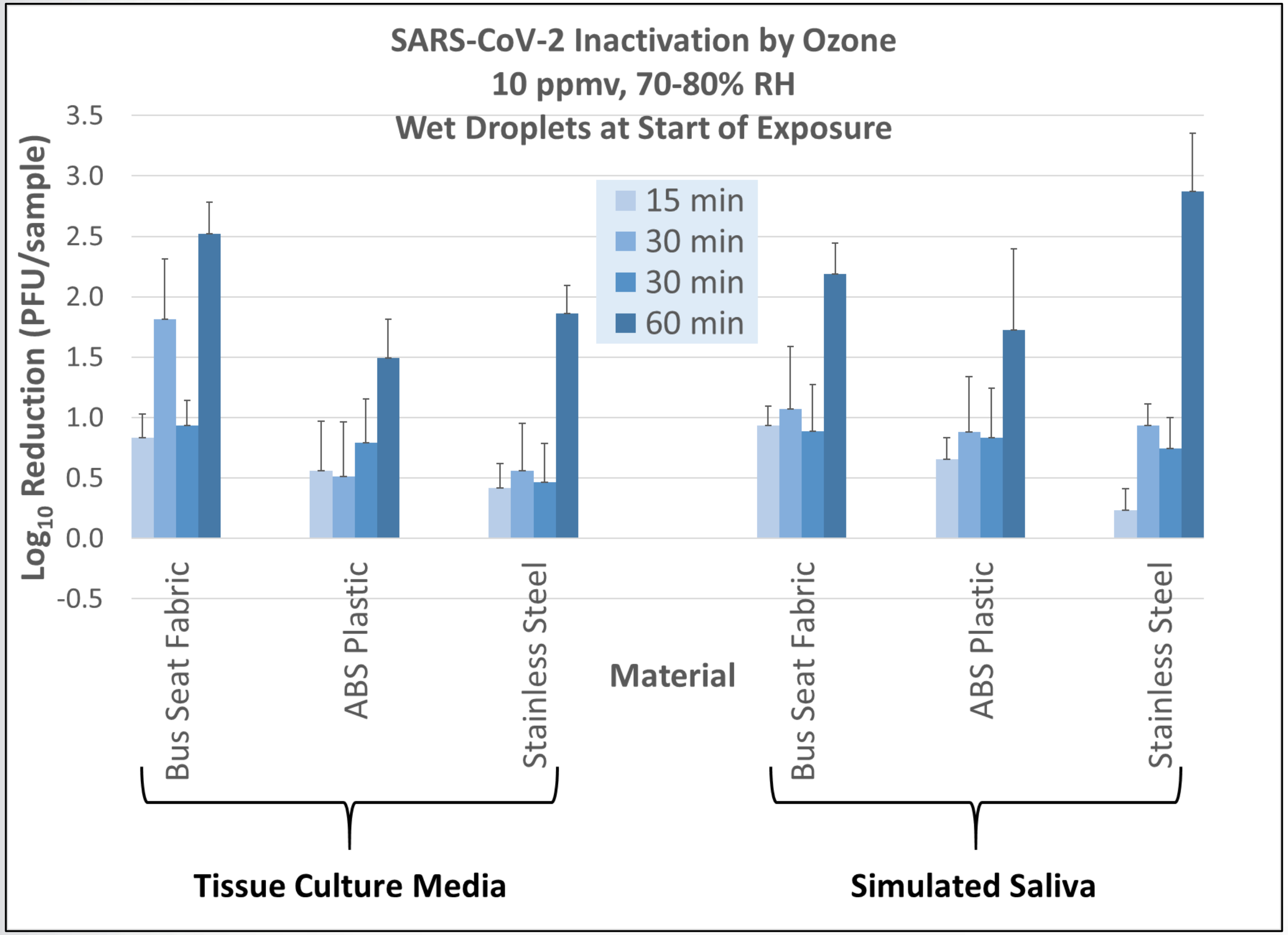
Log Reduction (LR) = Mean $\log_{10}$ recovery (Positive Controls) - Mean $\log_{10}$ recovery (Test Coupons)

Method Development

Prior to the ozone testing, we demonstrated that:

- Sufficient SARS-CoV-2 high recoveries (low 10^5 PFU/coupon) can be obtained from all materials
  - Typically, lower recoveries for SARS-CoV-2 in simulated saliva (high 10^3 PFU/coupon)
- No cytotoxicity of materials observed
- Based on TCID50 procedures, a detection limit of 6.3 virions/coupon was established

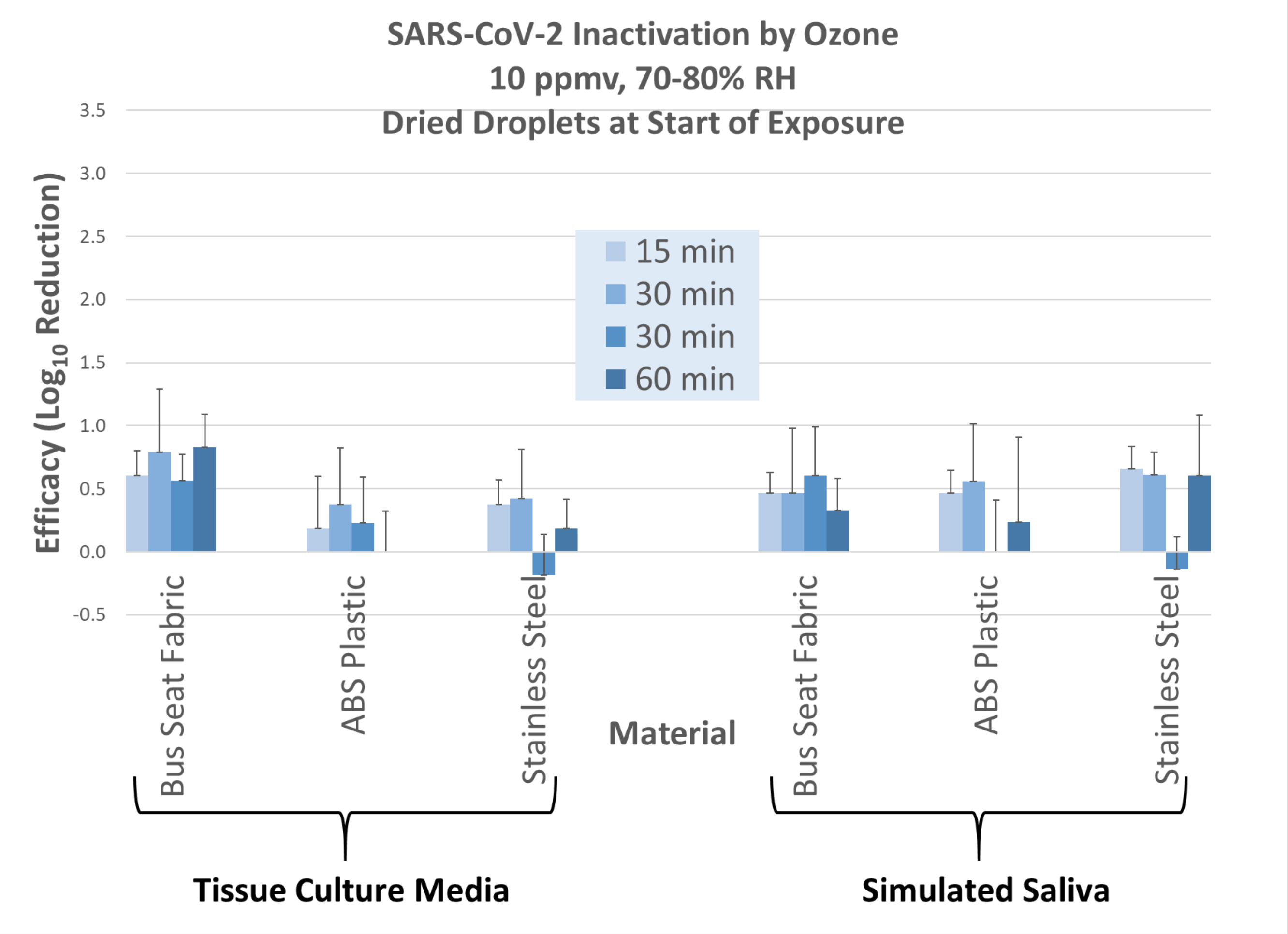
Ozone Results at High RH



Wet droplets at start of ozone exposure; (24 °C; 70-80% RH)

- Positive control recoveries were >10^5 PFU/sample (lower [10^4] for sim. saliva, 60 min)
- Ozone can inactivate the SARS-CoV-2 virus
- A 2- to 3- log reduction can be obtained
  - Residual virus remaining on surfaces except for 60 min Stainless steel, simulated saliva
- No noticeable material dependence
- 30 min timepoint appears twice as it was the longest time for Test1 and shortest for Test2
  - High reproducibility of tests (except for one datapoint – bus seat fabric)

Ozone Results at High RH

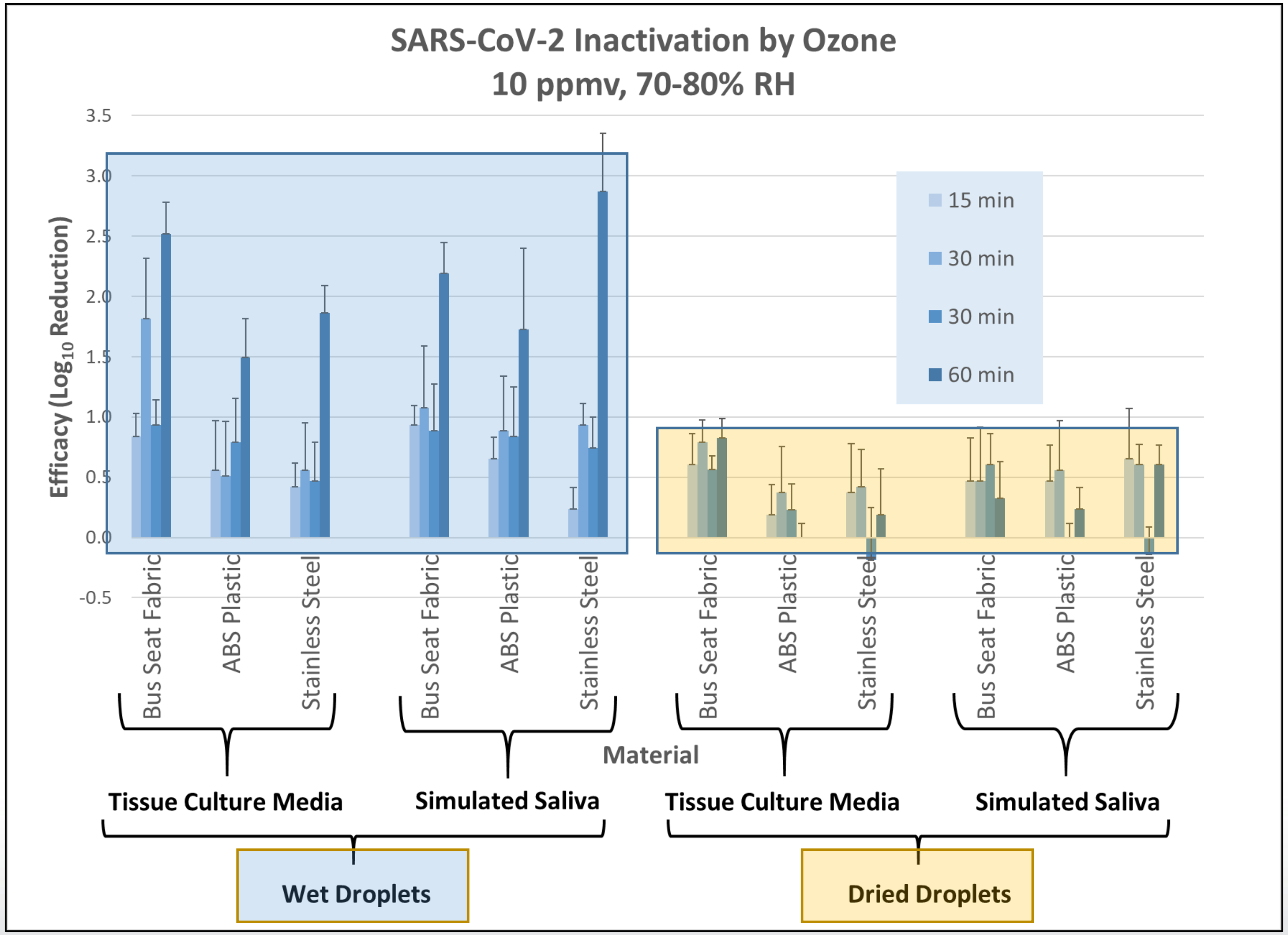


Dried droplets at start of ozone exposure; High RH (24 °C):

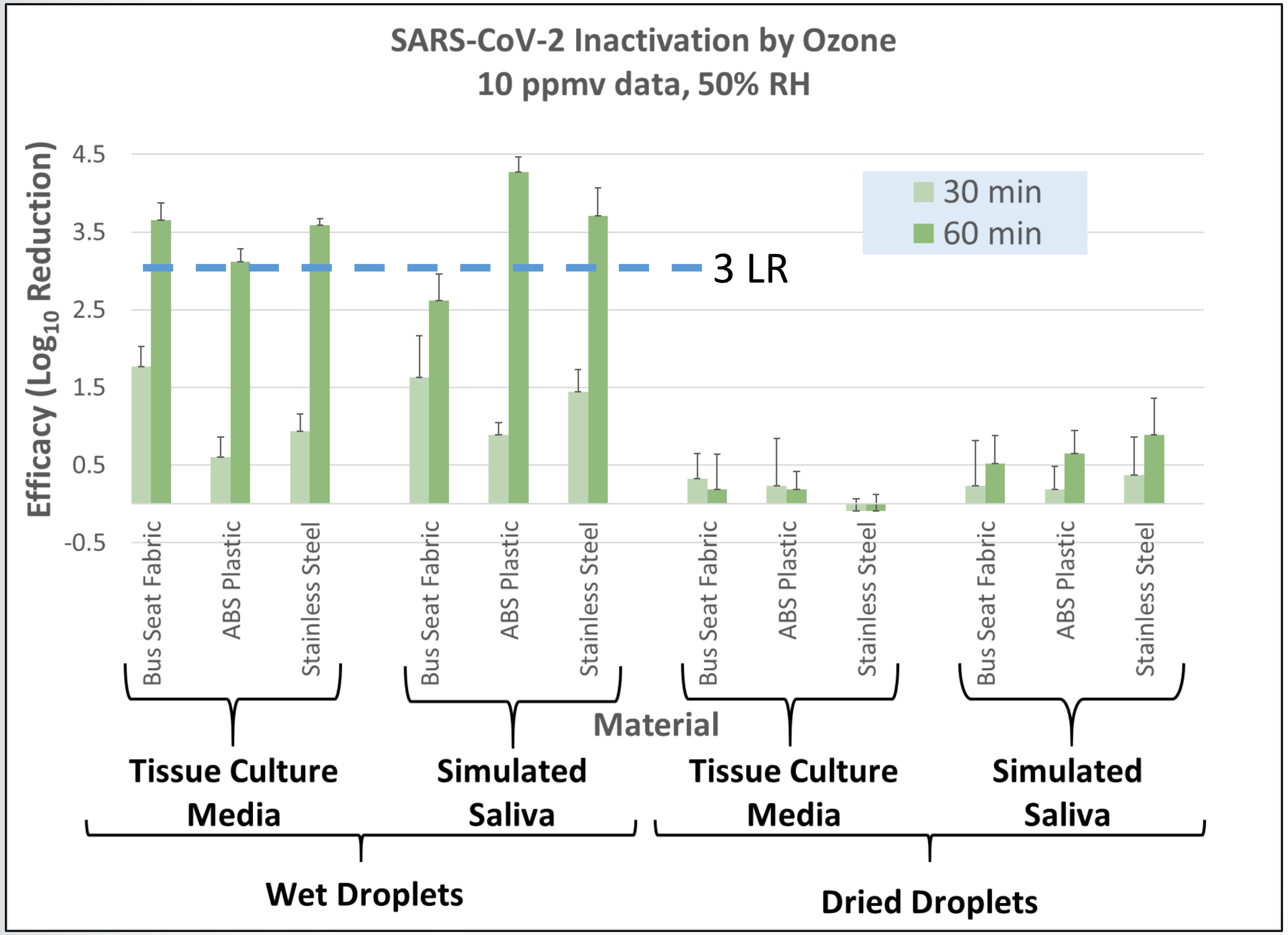
- Positive control recoveries were $>10^4$ PFU/sample for tissue culture media; $>10^3$ PFU/sample for simulated saliva
- Minimal (less than 1 log reduction; <90%)) inactivation of the SARS-CoV-2 virus
- No appreciable improvement in efficacy for longer exposure times
- No noticeable material dependence
- 30 min timepoint appears twice as it was the longest time for Test1 and shortest for Test2

Ozone Results at High RH

Side by side comparison of results for wet droplets vs dried droplets at start of ozone exposure



Ozone Results at Lower RH



Wet droplets and dried droplets at start of ozone exposure; (24 °C; 50% RH)

- Positive control recoveries were >10^5 PFU/sample (lower [10^4 range] for sim. saliva)
- Achieved a 3-log reduction level (99.9%) after 1 hour at 10 ppmv ozone for initial wet droplets for most material/media combinations
- Again, a significant difference in log reduction between wet and dried droplets with no noticeable material dependence
- Dataset at 20 ppmv ozone (not shown; same exposure times) show similar results in efficacy but the larger variability in recoveries from controls makes interpretation difficult

Ozone Disinfection Conclusions

- Inactivation of the SARS-CoV-2 virus on various surfaces via ozone fumigation (10 ppm; 1 hour) was observed [2-3 log reduction (99-99.9%)]

- SARS-CoV-2 is noticeably more difficult to inactivate when present in dried droplets on surfaces (most likely operational scenario)

- Lack of water (in dried droplets) diminishes inactivation

- Increase in RH (50% vs 70-80%) did not improve inactivation for dried droplets

- Wet droplet results are consistent with ozone inactivation data against SARS-CoV-2

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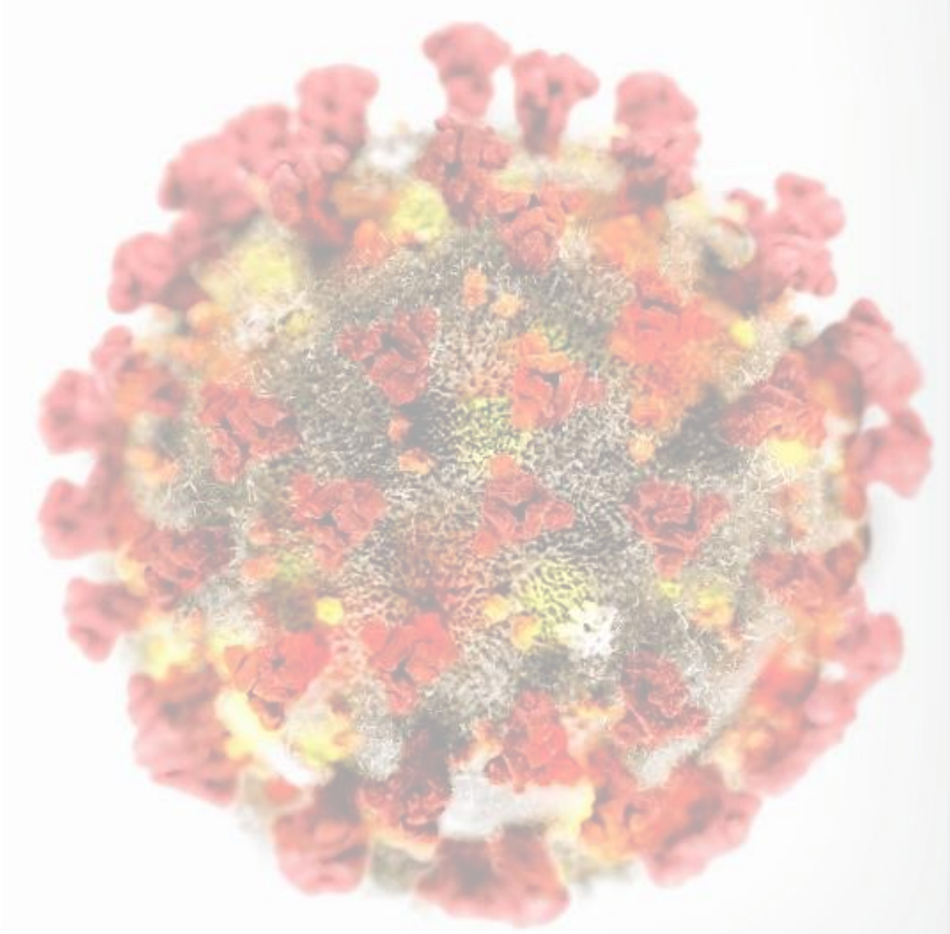
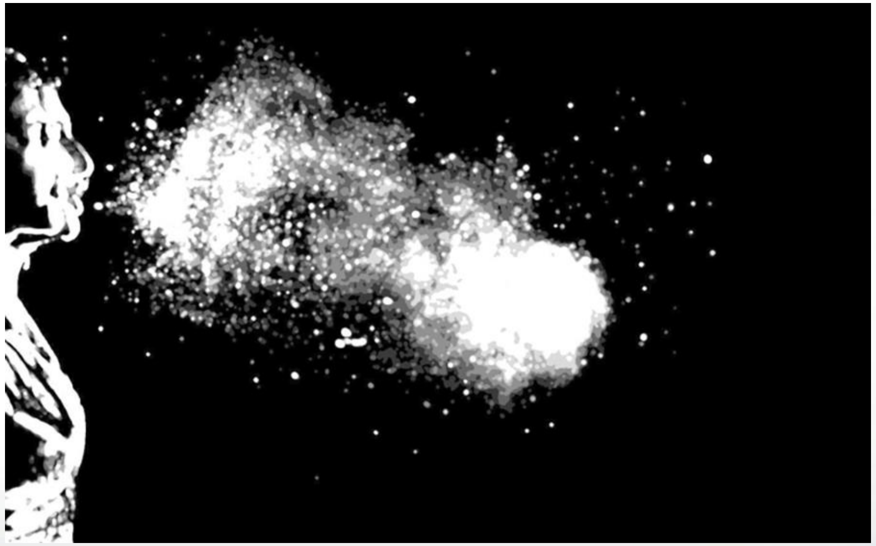


Aerosol Transmission and COVID-19

- Studies have documented the spread of COVID-19 in enclosed spaces (e.g., buses, hospital rooms, restaurants, offices)
- Growing focus on the transmission of COVID-19 via aerosolized SARS-CoV-2
- Increasing focus on **air treatment technologies**
  - Many technologies are devices, which are not registered by EPA.

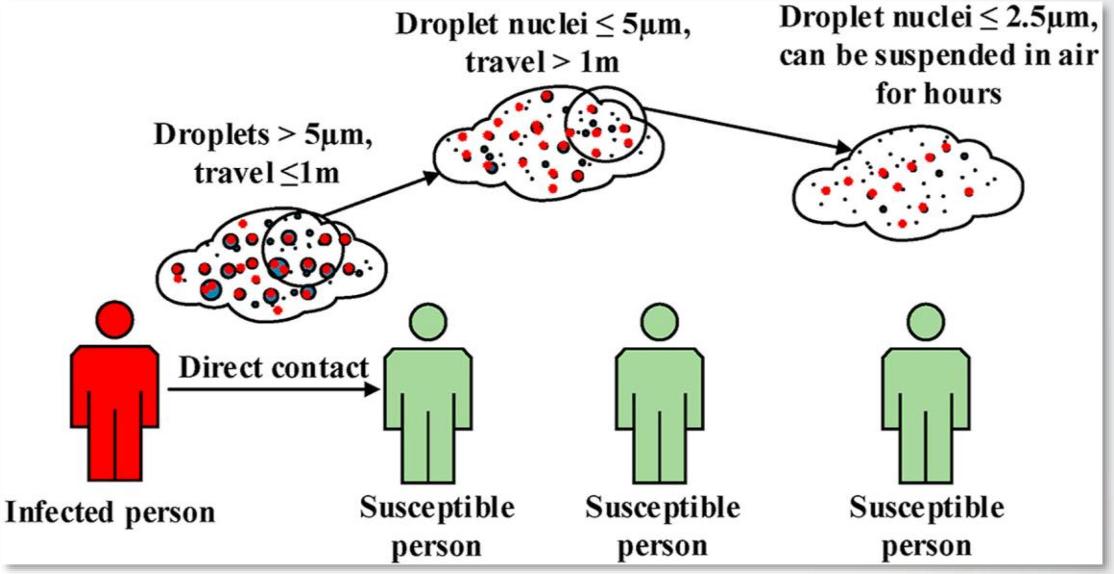
- **Challenges:**
  - Growing need and desire repopulate indoor spaces (schools, offices, restaurants, public transit, events and gatherings, etc.)
  - Social distancing not always feasible
  - Many air cleaning technologies on the market, lack of independent testing data



Aerosol Treatment: Project Objectives

- How effective are commercially-available aerosol treatment technologies at reducing concentrations of infectious SARS-CoV-2 in the air?

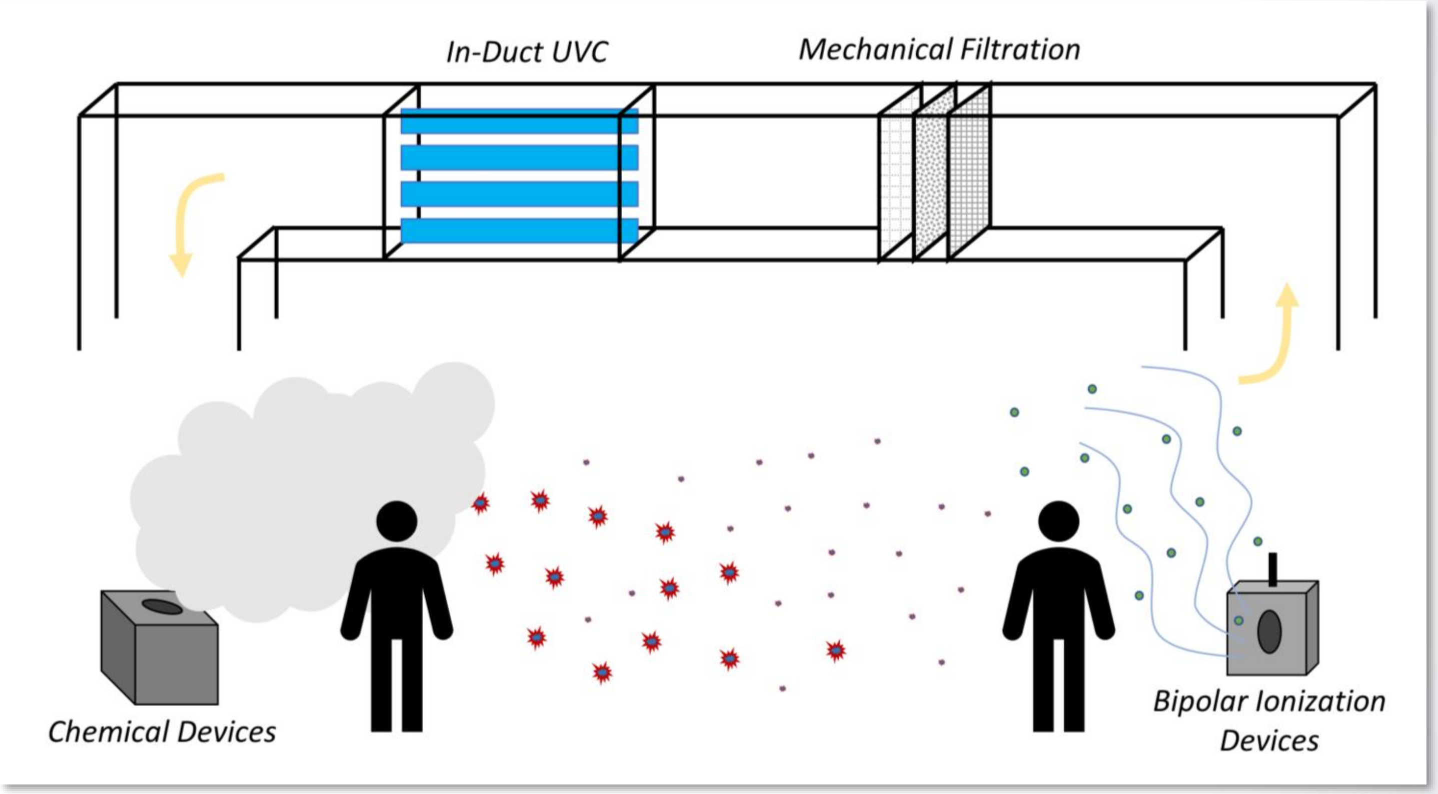
- **Research Goals:**
  - Assess efficacy of aerosol treatment products and devices at a real-world scale
  - Develop reliable and standardizable methods for testing air treatment technologies
  - Establish expertise to extrapolate understanding of treatment technologies to inform estimation of efficacy of novel, untested technologies



Niazi et al. (2020), *Environmental Pollution*

Aerosol Treatment Technologies

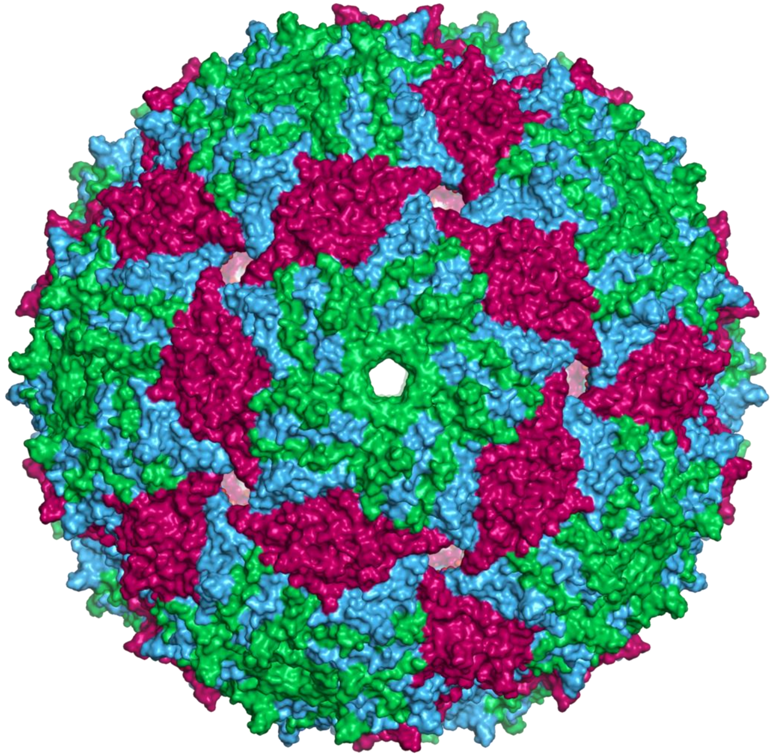
- <u>UVC devices</u>: e.g., upper-room germicidal UVC, in-duct UVC
- <u>Chemical products and devices</u>: in-room or in-duct; e.g., low-concentration ozone, low-concentration hydrogen peroxide, bipolar ionization
- <u>Physical removal</u>: e.g., MERV-13 and specialized filters, portable air cleaners
- Combinations of the above



Focus on air treatment technologies and methods that can be continuously operating in occupied spaces (in-room or in-duct)

Aerosol Treatment: Methods

- Evaluate efficacy of technologies against non-pathogenic virus (MS2)
  - Expected to be more resistant to chemical inactivation than SARS-CoV-2



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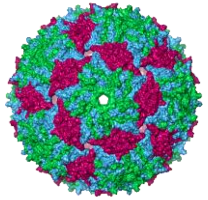
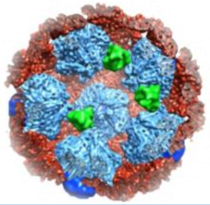
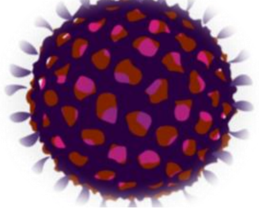
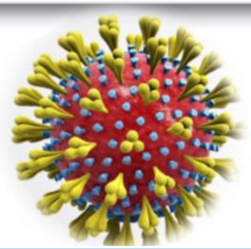
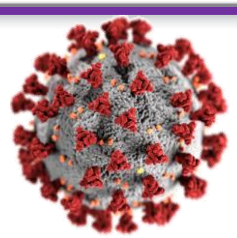
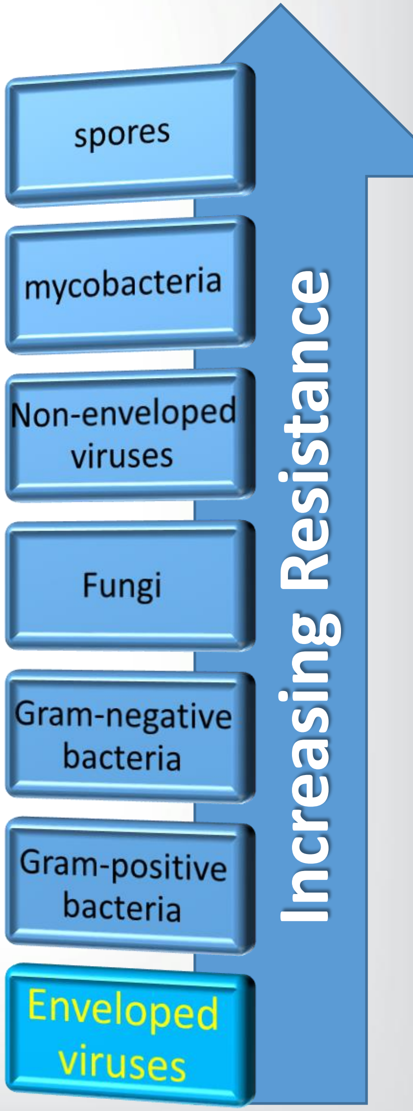
Aerosol Treatment: Methods

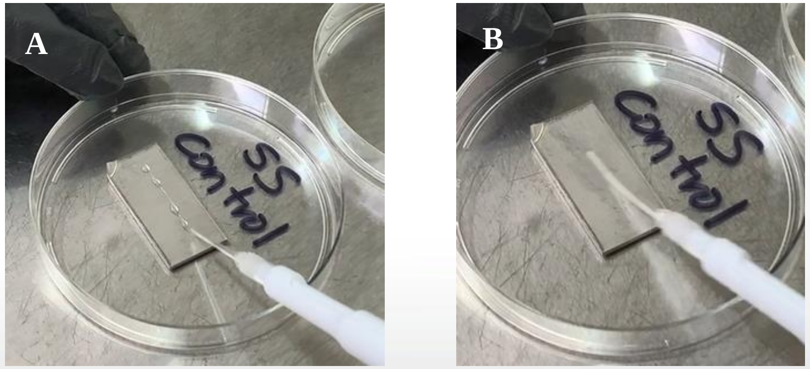






	MS2	Phi6	MHV	229E	SARS-CoV-2
Enveloped?	No	Yes	Yes	Yes	Yes
Host	Bacteria (*E. coli*)	Bacteria (*P. syringae*)	Mice	Humans	Humans
Genus	Levivirus	Cystovirus	Betacoronavirus	Alphacoronavirus	Betacoronavirus
BSL	1	1	2	2	3
Advantage	High resistance & persistence, fast and easy analysis	Moderate resistance & persistence, fast and easy analysis	Same genus as SARS-CoV-2, non-human pathogen	Same Family as SARS-CoV-2	Actual agent of COVID-19
Surrogate?	Comparison in progress			Regulatory surrogate	



Aerosol Treatment: Methods

- Evaluate efficacy of technologies against non-pathogenic virus (MS2)
  - Aerosolized using 6-jet Collison nebulizers
    - Count median diameter of aerosolized particles ~45 nm at the beginning of each test; increases in size over duration of test (100 nm at 120 min)
  - Air sampling:
    - SKC BioSamplers (enumerated via plaque assay)
    - CCDC-CBC TACBIO 2 (real-time)
  - Surface sampling:
    - Inoculated 2 cm x 4 cm stainless-steel coupons
    - Clean stainless-steel coupons for deposition
    - 5 pairs co-located on chamber floor
  - Onsite microbiology lab
  - Particle size & count measurements



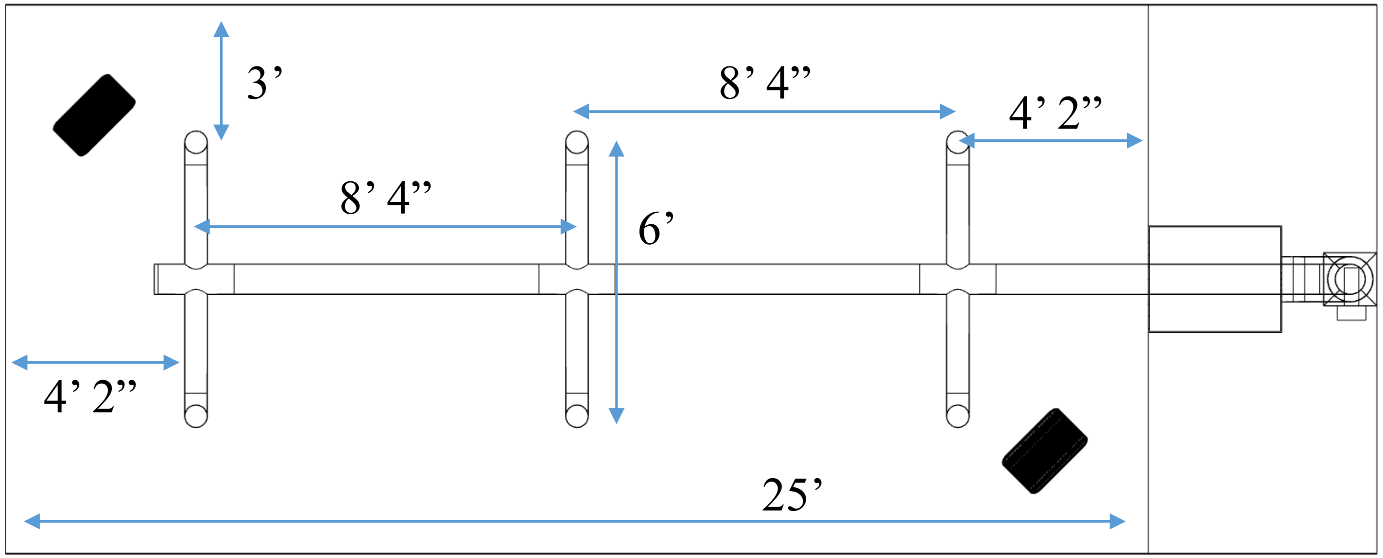
Aerosol Treatment: Methods

- Evaluate efficacy of technologies against non-pathogenic virus (MS2)

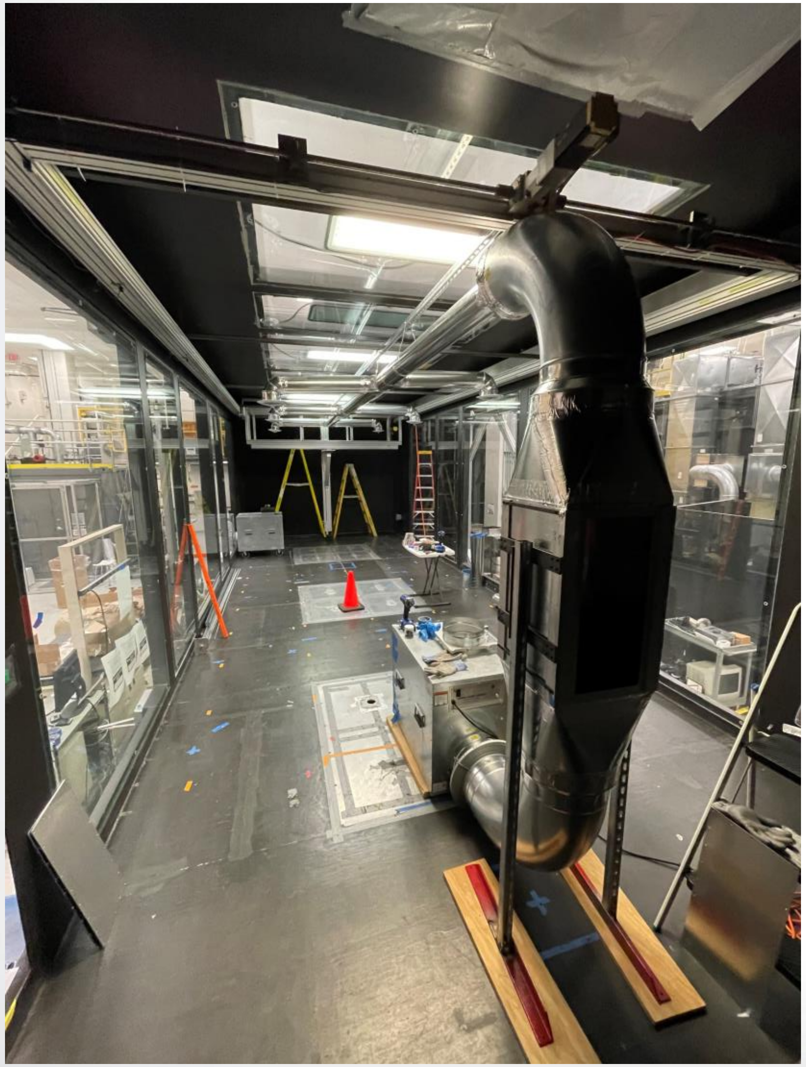
- Utilize specialized Aerosol Test Facility in Research Triangle Park, NC
  - Large air treatment test chamber 10 x 12 x 25 ft (3000 ft^3)
  - Controlled temperature / humidity
    - 22 ± 2 °C
    - 30-35 % RH
  - Mixing fans

Aerosol Treatment: Methods

- Evaluate efficacy of technologies against non-pathogenic virus (MS2)
- Utilize specialized Aerosol Test Facility in Research Triangle Park, NC
- Mock HVAC system designed and installed for evaluating technologies
  - Negative air machine simulates mock cold air return with adjustable flow
    - 350 CFM, ~7 air changes per hour
  - Six branches (6”) from main line (8”), each outlet fitted with diffuser
  - Galvanized steel duct materials



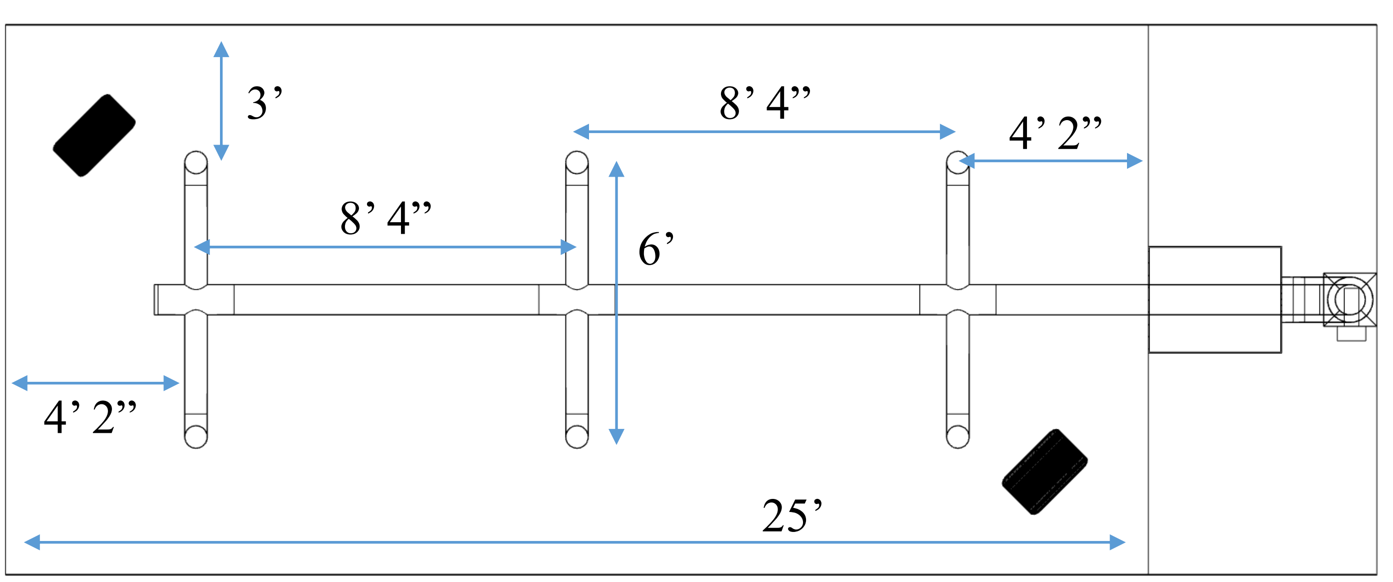
Test Chamber & HVAC System



Aerosol Treatment: Methods

- Evaluate efficacy of technologies against non-pathogenic virus (MS2)
- Utilize specialized Aerosol Test Facility in Research Triangle Park, NC
- Mock HVAC system designed and installed for evaluating technologies

Testing objective: Obtain high enough recoveries in control conditions throughout duration of testing to demonstrate a 3-$\log_{10}$ reduction



Aerosol Treatment: Technologies

Technology Type	Intended Use
Bipolar Ionization	Installed in-duct; intended for residential, commercial, industrial, education, health care settings
Grignard Pure (active ingredient: Triethylene Glycol)	Dispersed either in-room or in-duct; intended for use in indoor spaces for essential economic activities as determined by the state; e.g., health care, transportation, food processing
Knorr 3-Stage System: Electrostatic Filter, UVC, and Bipolar Ionization modules	3-stage air filtration and purification system designed to be installed (either new or as retrofit) in the HVAC system of rail transit vehicles; plan to evaluate components operating together & in isolation
Photocatalytic	General indoor use; transit vehicles
Filtration	For use in residential, commercial, transit settings

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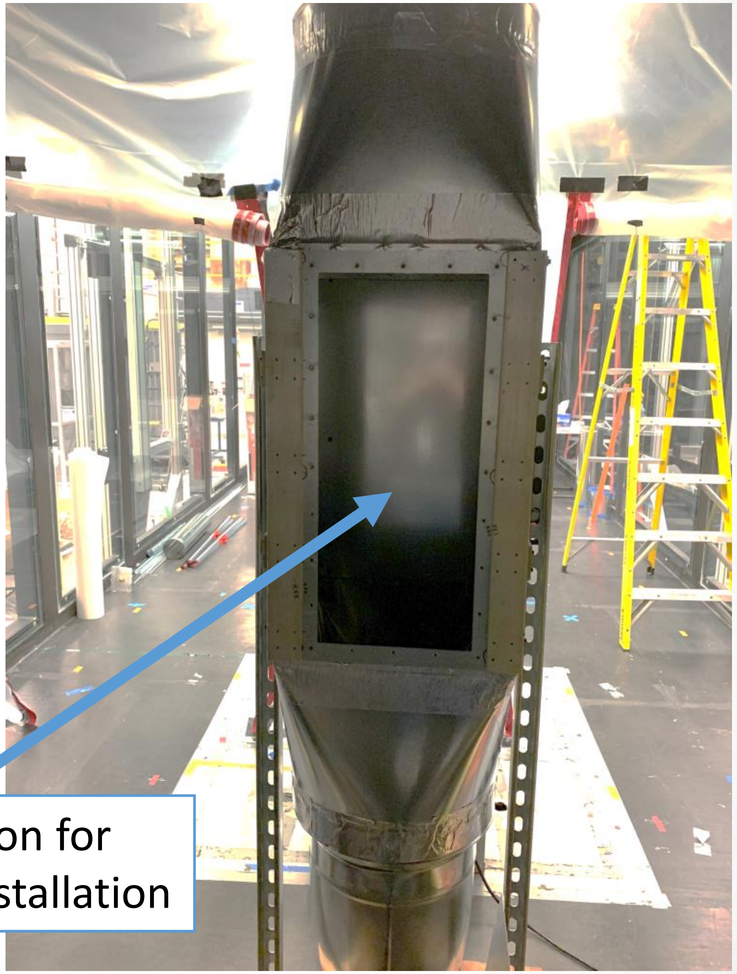
✓ Knorr 3-Stage System: Electrostatic Filter, UVC, and Bipolar Ionization modules	3-stage air filtration and purification system designed to be installed (either new or as retrofit) in the HVAC system of rail transit vehicles; plan to evaluate components operating together & in isolation
* Photocatalytic	General indoor use; transit vehicles
* Filtration	For use in residential, commercial, transit settings

** Testing ongoing in August 2021*

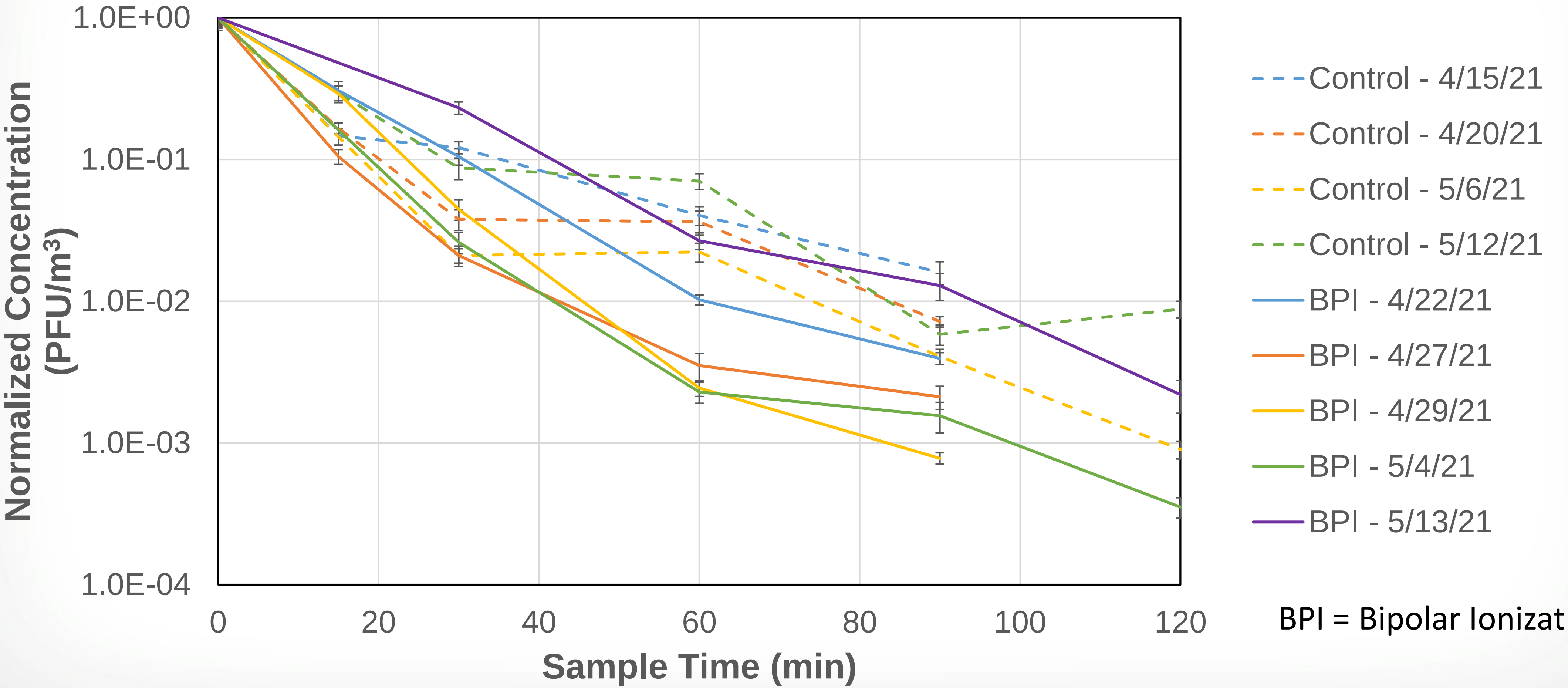
Bipolar Ionization Device

- Bipolar ionization generates charged ions that react with airborne contaminants, including viruses
- Cold plasma bipolar ionization device selected for evaluation
  - Sized to treat 2000-4000 ft^2 of living space
- Installed in-duct
- 30 to 90-minute ion buildup times in chamber prior to testing, resulting in ion counts of 1000-6000 $ions/cm^3$



duct location for technology installation

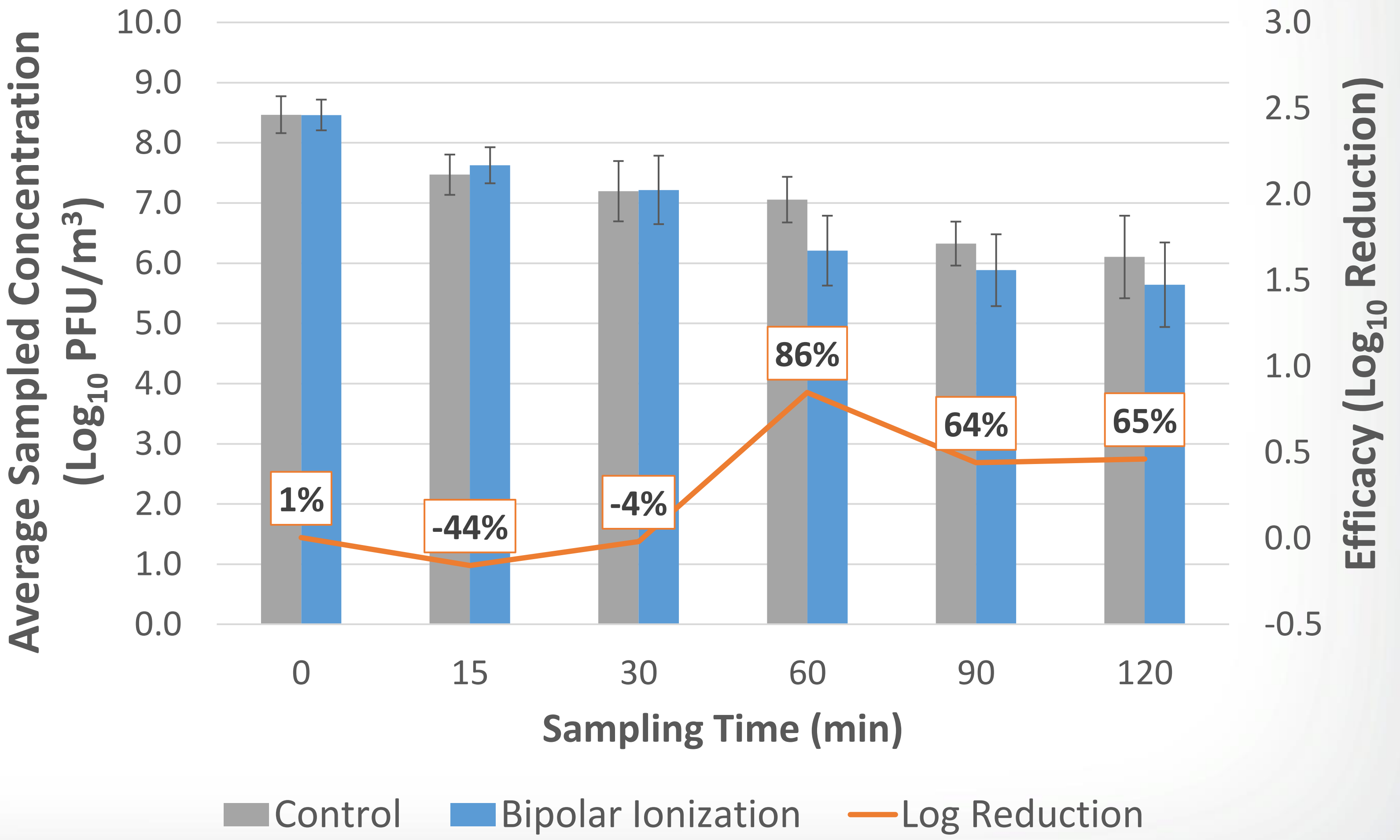
Bipolar Ionization Device



BPI = Bipolar Ionization

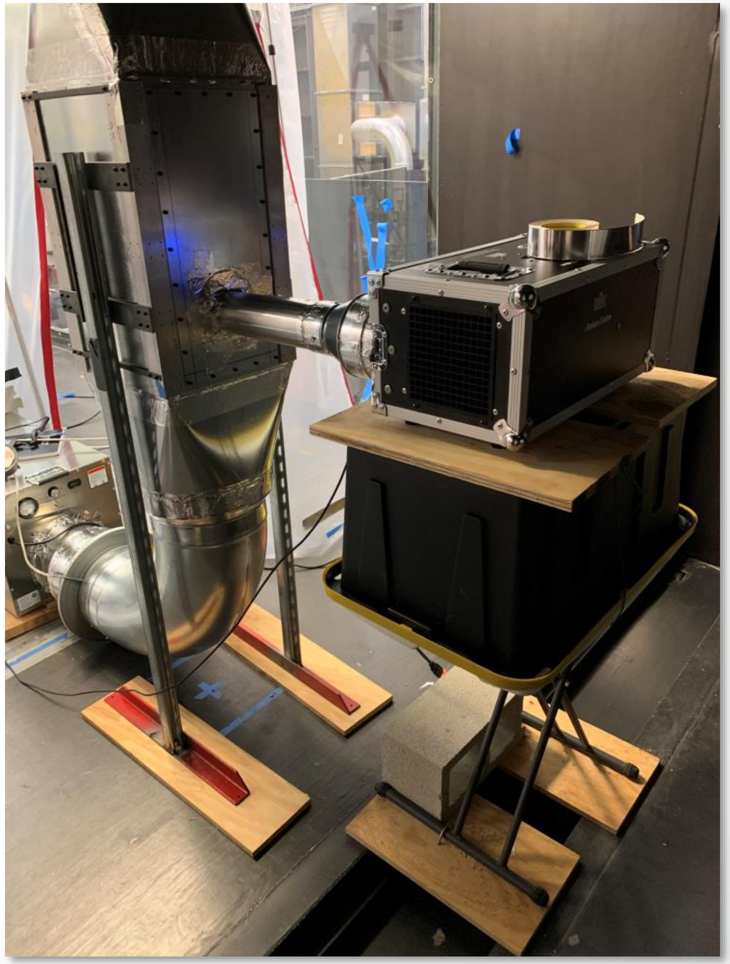
Bipolar Ionization Device

- Average log_{10} reductions in aerosolized MS2 from initial tests range from -0.16 to 0.85 throughout test duration
- No additional virus recovered from surfaces
- No surface inactivation observed

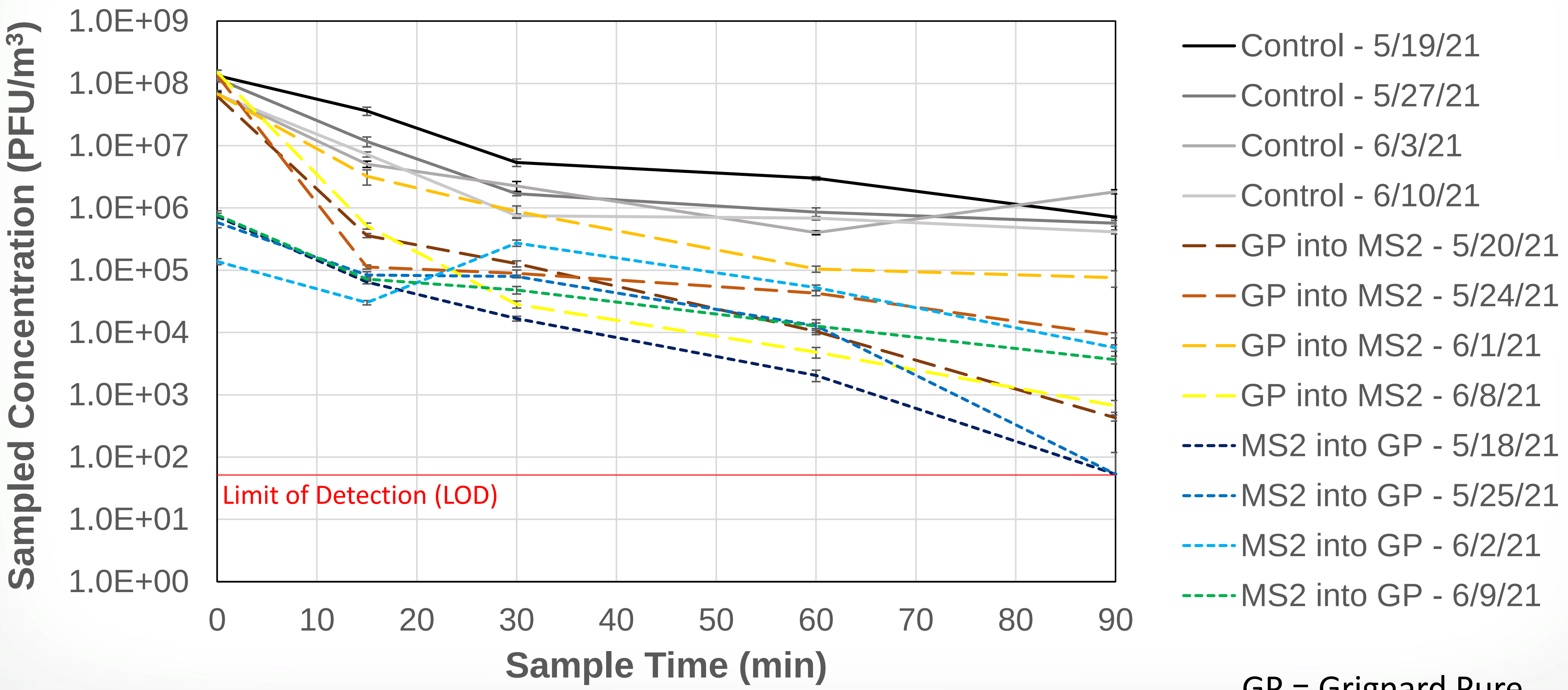


Grignard Pure

- Antimicrobial air treatment product
  - Section 18 Emergency Exemptions for indoor use in GA, MD, NV, PA, TN, TX
- Triethylene glycol (TEG) active ingredient
  - Commonly used in theatrical fog machines
  - Historic publications on air disinfection date to 1940’s
  - 1.2 – 1.5 mg/m^3 concentration of TEG during testing (NIOSH Method 5523)
- Two different test sequences evaluated:
  - Introduce product into aerosolized MS2
  - Introduce MS2 into product in chamber already at target concentration

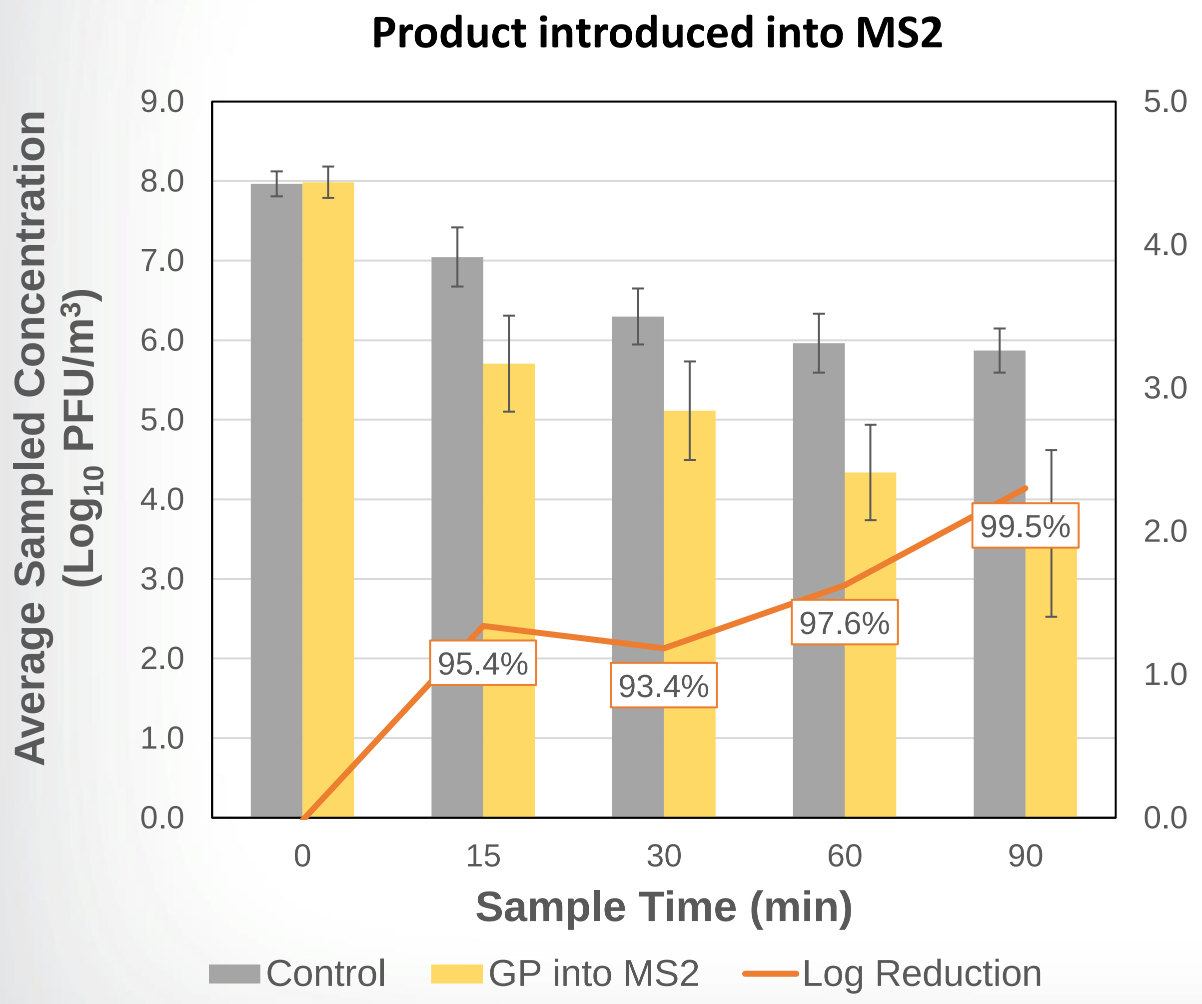


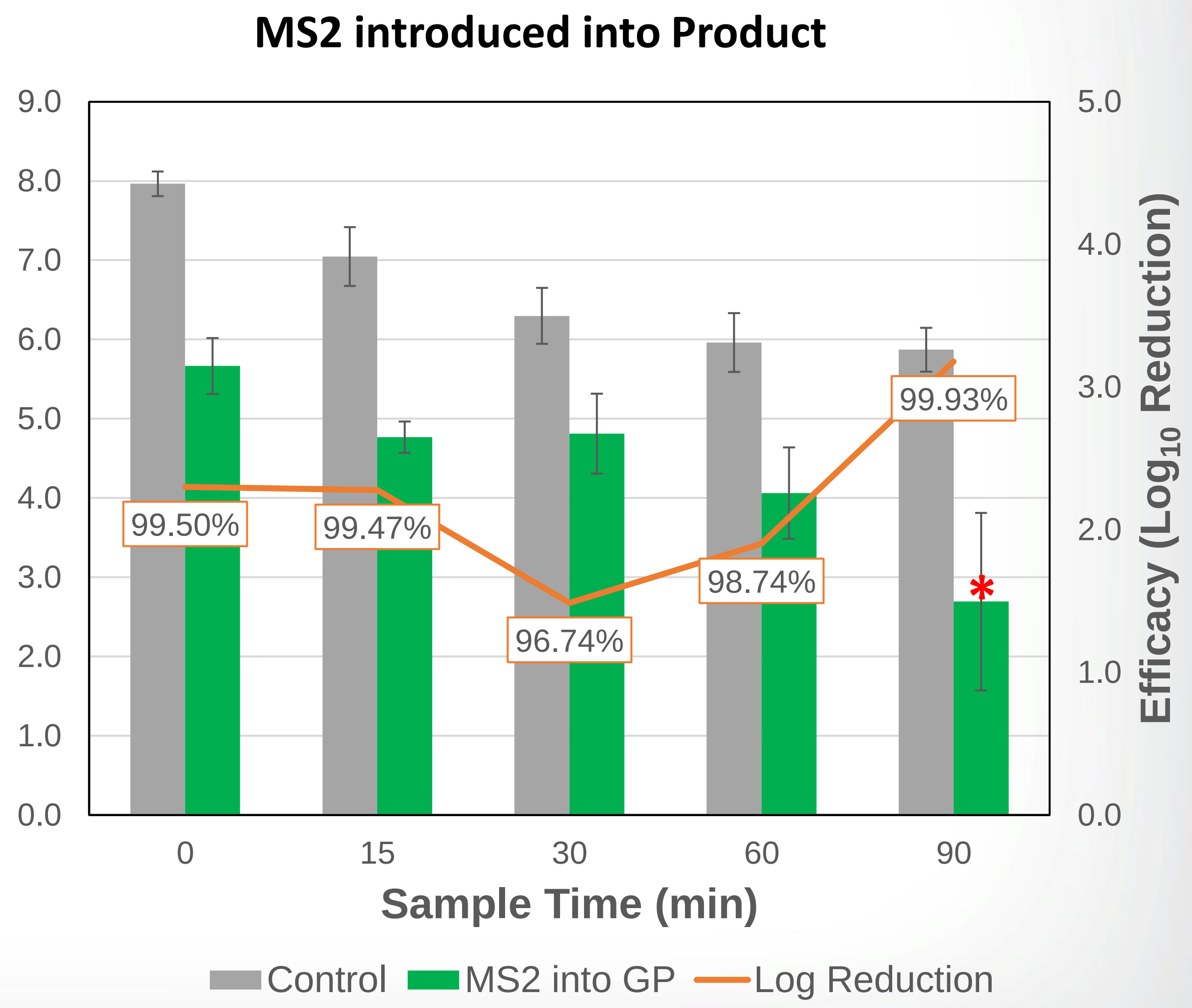
Grignard Pure



GP = Grignard Pure

Grignard Pure

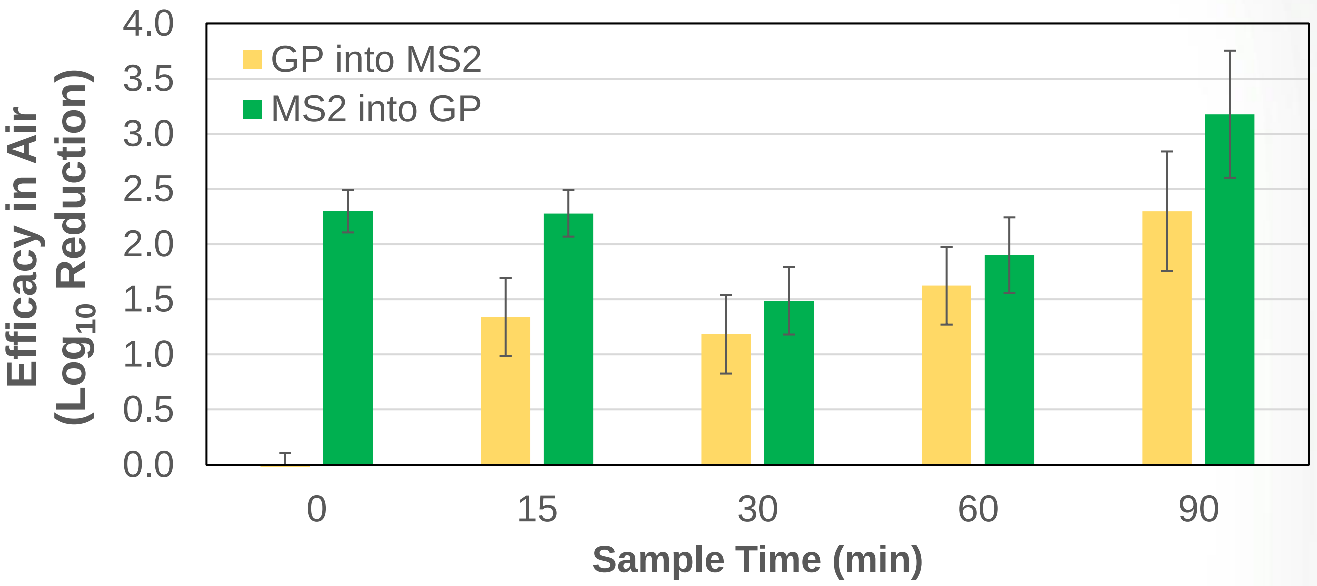


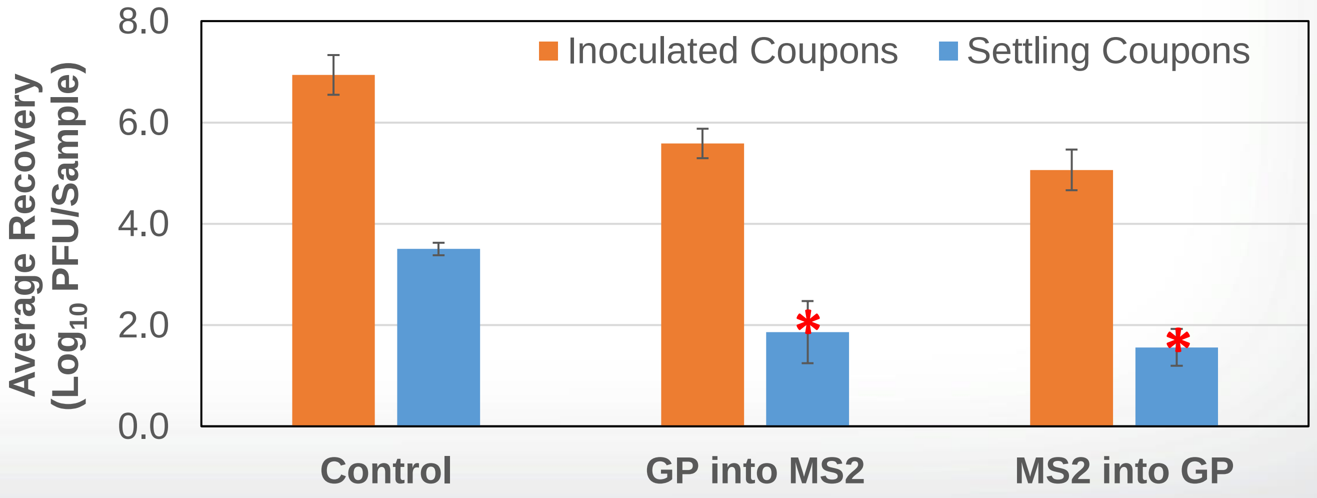


** 2 of 4 replicates at LOD*

Grignard Pure

- Higher calculated efficacy when MS2 aerosolized with product present in test chamber
- Surface inactivation observed on inoculated coupons
  - GP into MS2: average $\log_{10}$ reduction 1.6 ± 0.4 PFU/coupon
  - MS2 into GP: average $\log_{10}$ reduction 1.9 ± 0.2 PFU/coupon
- Reduced MS2 recoveries on deposition coupons



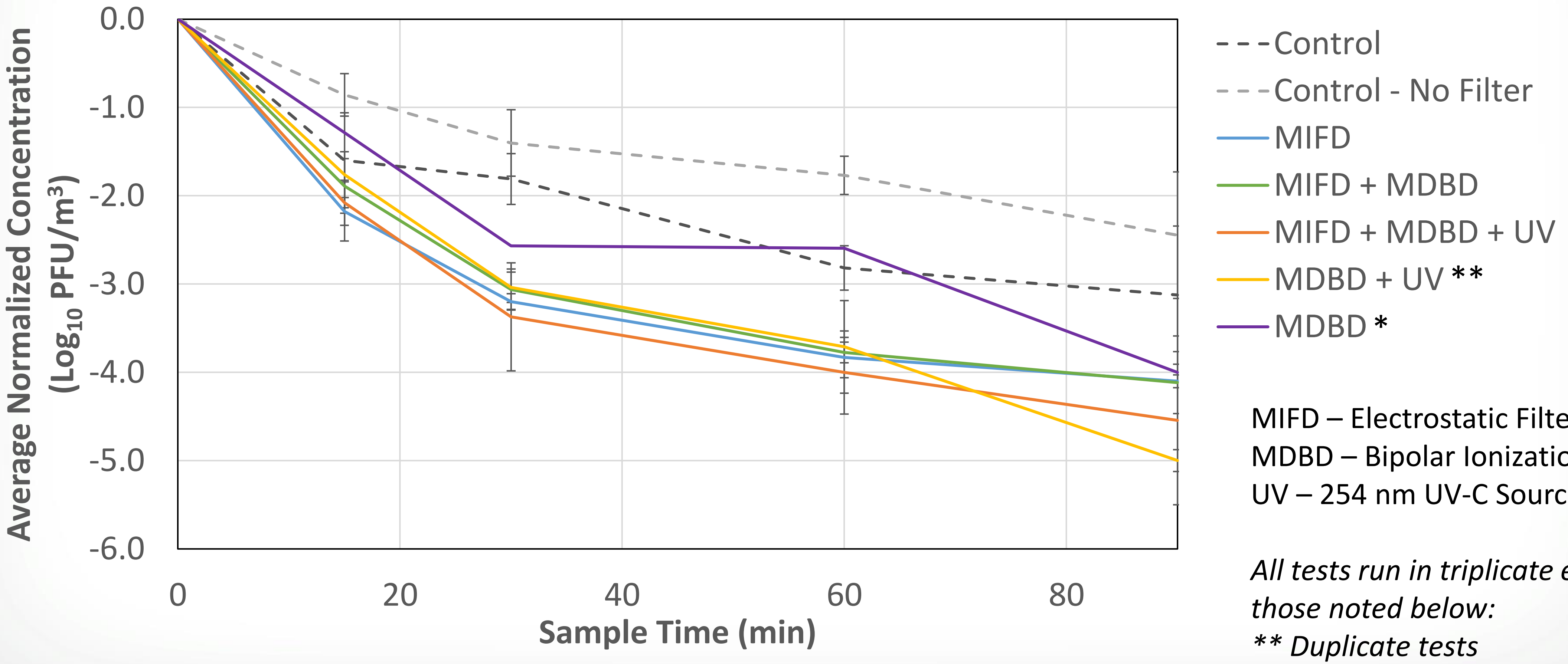


** Some replicates at LOD (1.0 $\log_{10}$ PFU/Sample)*

3-Stage Air Filtration & Purification

- System developed by Knorr Brake Company for transit vehicles
  - Electrostatic filter – Merak Intense Field Dielectric (MIFD) filter
  - Bipolar ionization – Merak Dielectric Barrier Discharge (MDBD) device
  - UV-C radiation – low-pressure mercury vapor lamp (wavelength = 254 nm)
- Test unit in center of chamber
  - Knorr blower: ~25 air changes per hour
  - Recirculating HVAC still operating
- Controls with and without MIFD arrest filter

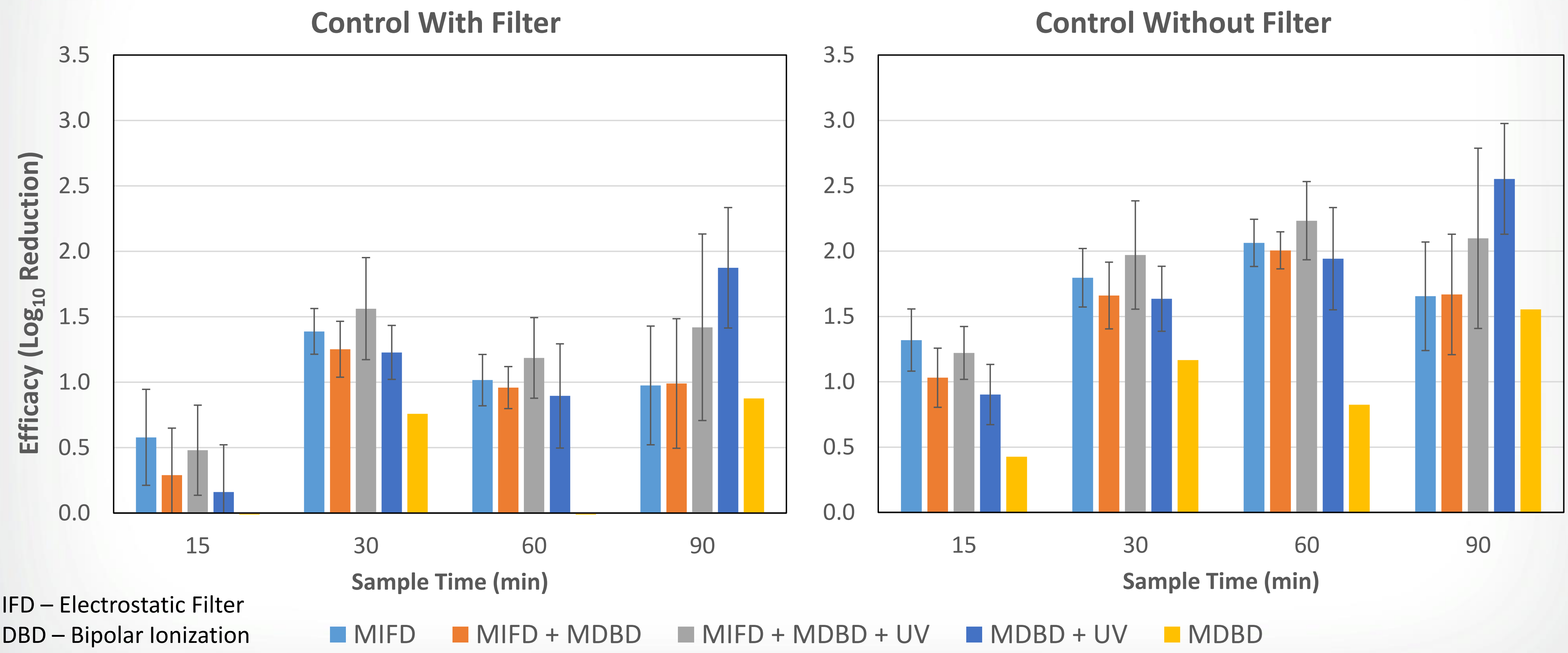
3-Stage Air Filtration & Purification



MIFD – Electrostatic Filter
MDBD – Bipolar Ionization
UV – 254 nm UV-C Source

All tests run in triplicate except those noted below:
*** Duplicate tests*
** Single test*

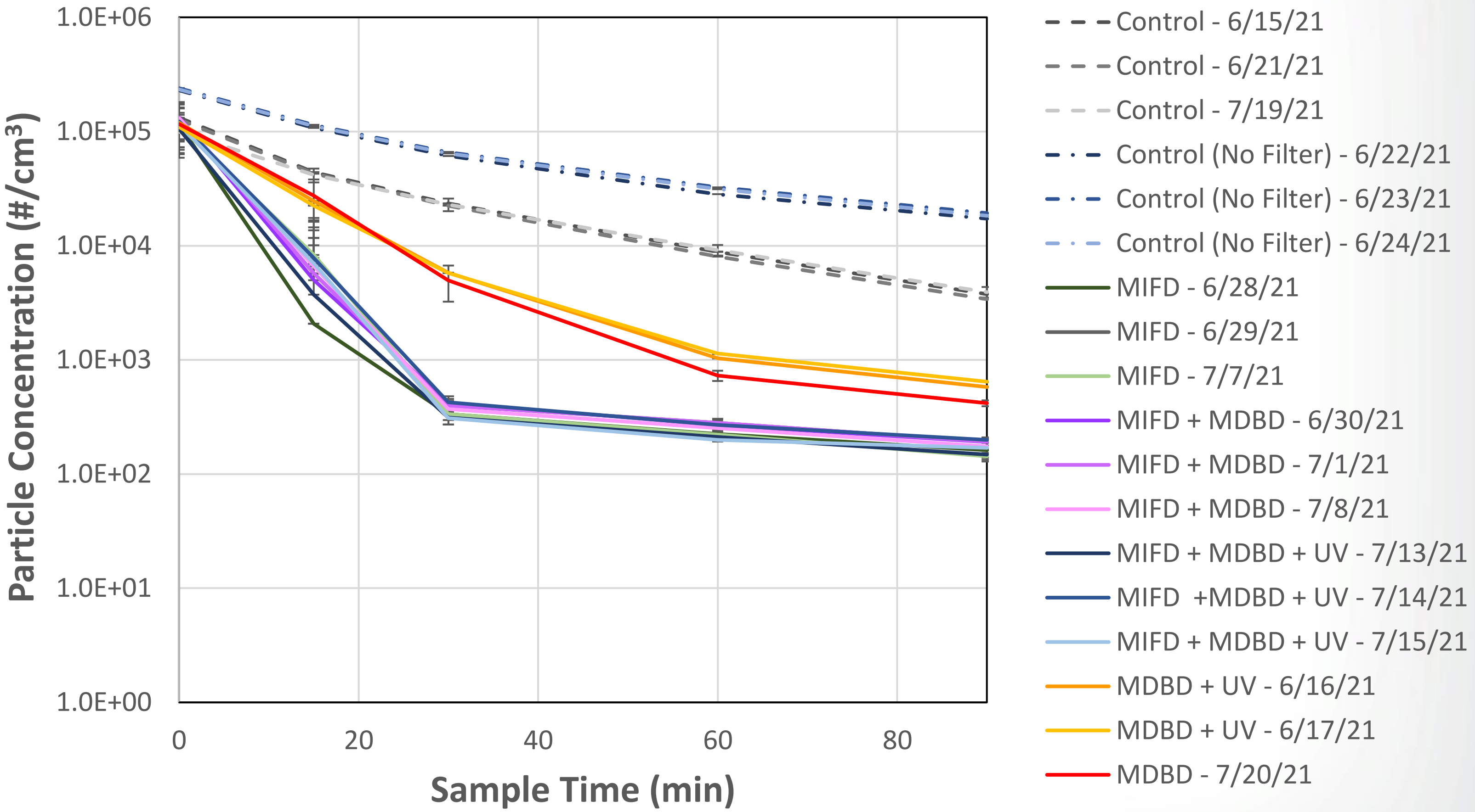
3-Stage Air Filtration & Purification



MIFD – Electrostatic Filter
MDBD – Bipolar Ionization
UV – 254 nm UV-C Source

3-Stage Air Filtration & Purification

- Greater particle capture with MIFD operating
- MIFD arrest filters capture ~0.5 $\log_{10}$ particles alone (without applied charge)
- MDBD and UVC components appear to contribute to MS2 inactivation
  - Reduced particle capture, but efficacy against MS2 is similar when MIFD is operating



Aerosol Treatment Conclusions

- Efficacy of different types of air treatment technologies can be evaluated using similar testing methodologies
  - Demonstrated efficacies range from < 1-log reduction to > 3-log reduction, depending on technology type and test sequence

- Testing at large scale needed to extrapolate results to real world
  - Still need to consider many other factors, such as temperature/humidity, HVAC system design, air change rates, etc.

- Design of control conditions impacts calculated efficacy

- *Next steps: evaluate photocatalytic devices and filters (August 2021)*

Acknowledgements

- John Archer, Lance Brooks, Worth Calfee, Shawn Ryan, and members of EPA Project Teams
- EPA Program Offices
  - Office of Chemical Safety and Pollution Prevention
  - Office of Land and Emergency Management
- EPA Regional Offices
- EPA ORD Safety, Health, and Environmental Management
- NYC MTA and LA Metro
- Battelle Task Order 68HERC20F0241 (ozone studies):
  Will Richter, Michelle Sunderman, Megan Howard
- Jacobs Technology Group Task Order 68HERC21F0063 (aerosol treatment studies):
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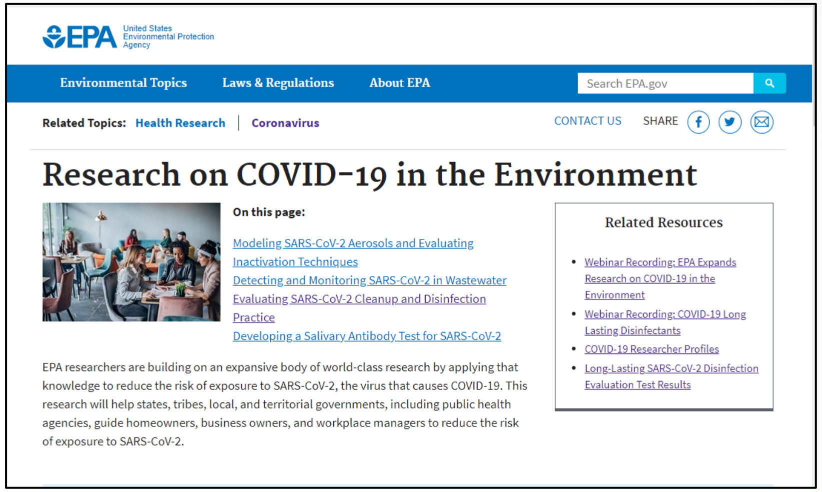

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