EPA Office of Research and Development

HOMELAND SECURITY RESEARCH

COVID-19: UVC Devices and Methods for Surface Disinfection

HSRP Webinar Series

January 21, 2021

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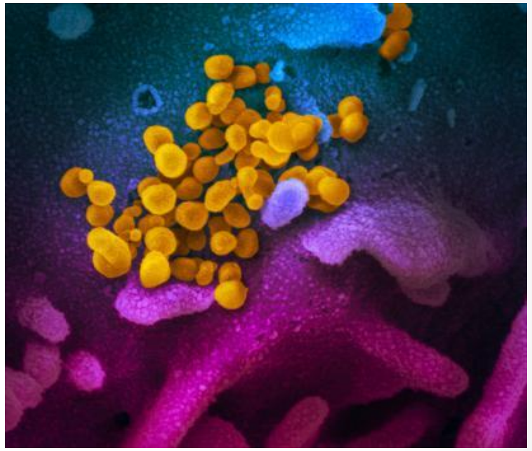


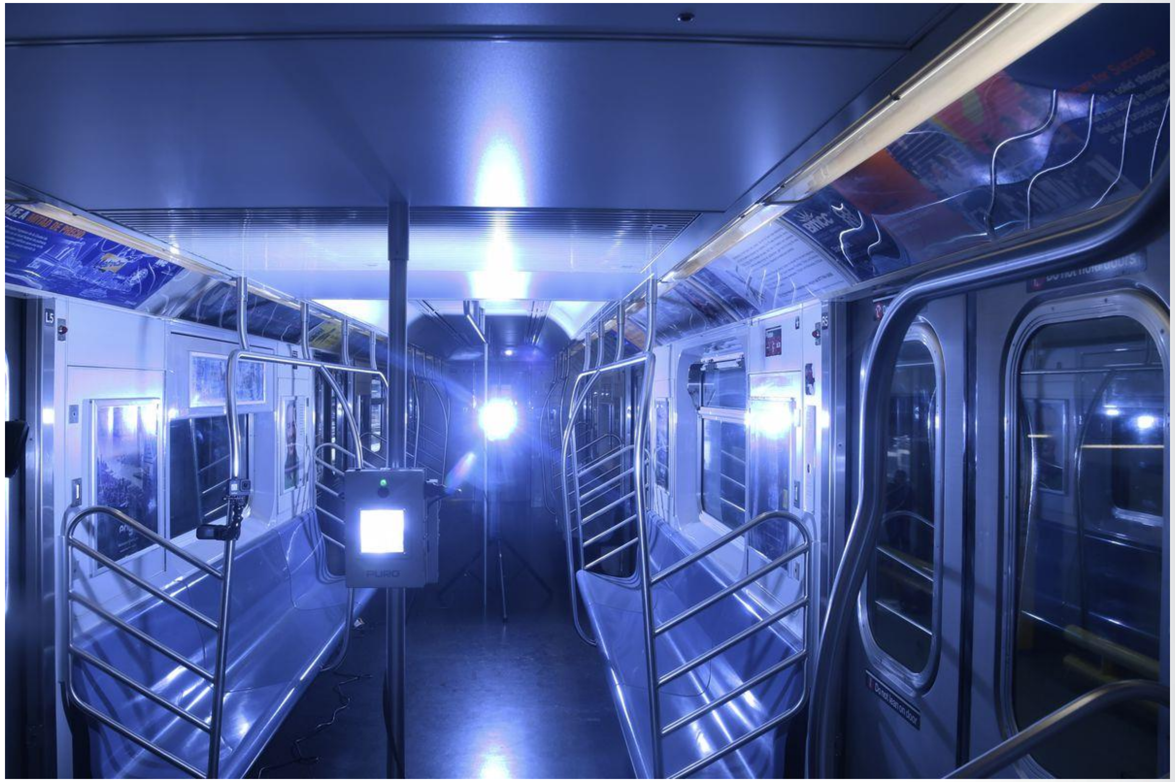
SEM image of SARS-CoV-2; credit NIAID-RML

Outline

- Background and Objectives
  - Definition of UV and UVC
  - Light sources and measurements
  - Viruses used in study
- Experiments
  - Field study with MS2 virus
  - MS2 and Phi6 results
  - SARS-CoV-2 results
- Conclusions
- Practical Applications/Challanges
- Next Steps

UVC and COVID-19

- Growing interest in UVC for surface disinfection as a result of pandemic
- Emerging UVC products are being widely marketed
- EPA does not register pesticide (UV) devices
- Increasing technical support requests for evaluating UVC technologies (e.g., from public transportation agencies)



New York Metropolitan Transport Authority invested $1 million on devices from Puro Lighting for their trains and buses

Project Objective / Goal

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

Initial selection:

1. **UV light – *topic of this webinar***
2. Ozone
3. Steam

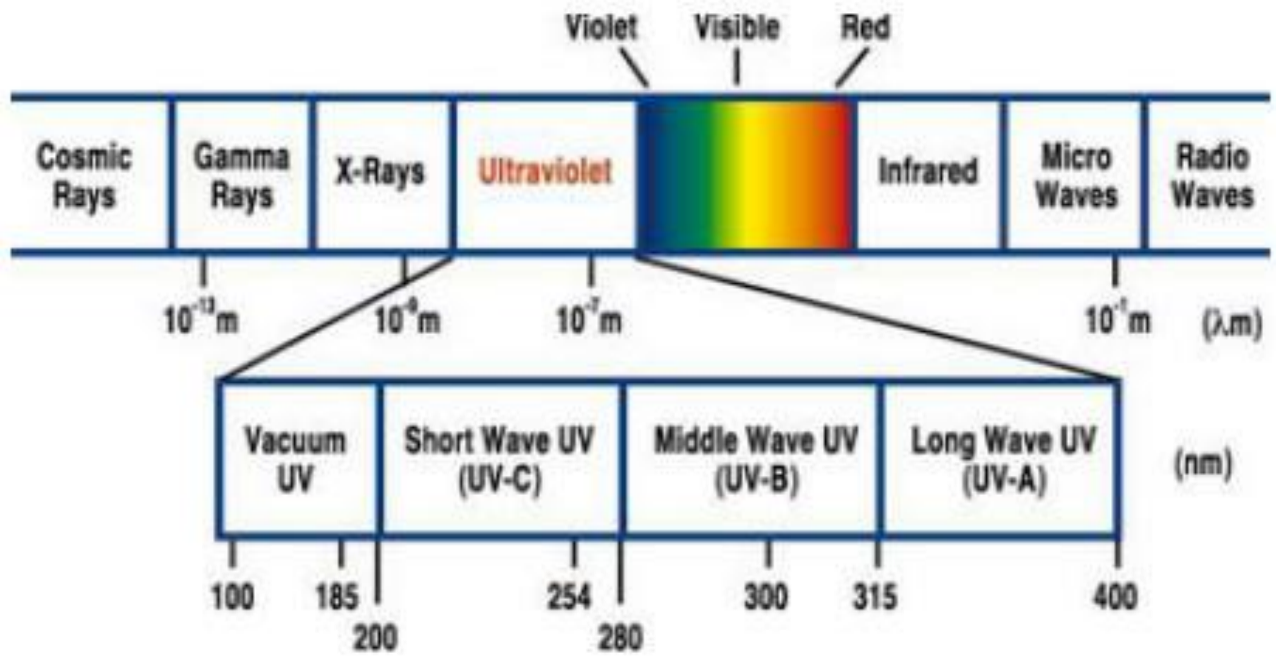
Disinfection Performance Goal:

- 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

UV Terminology

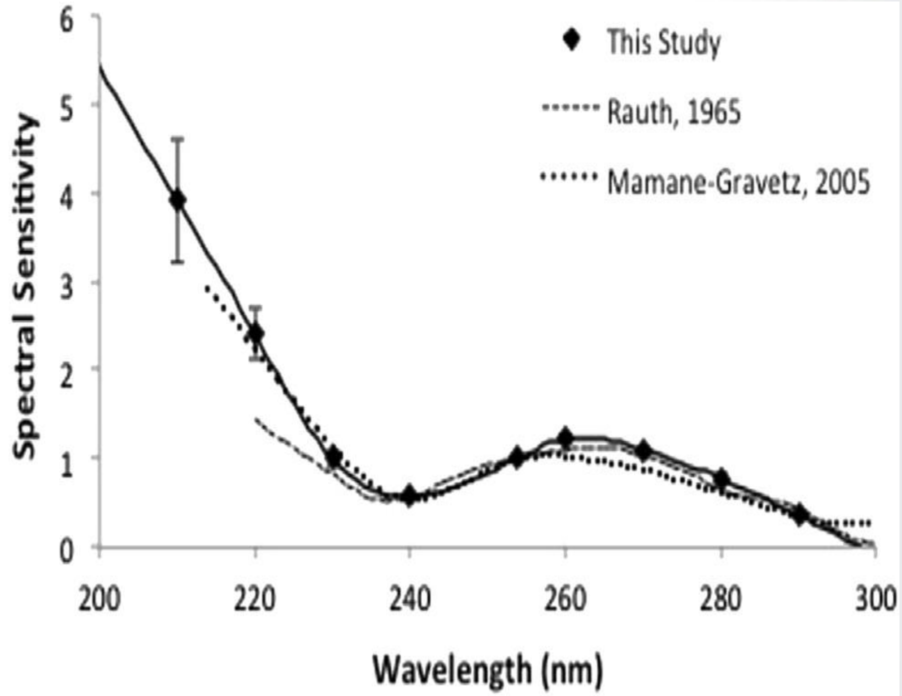
UV spectrum



https://www.cleanairoptima.com/information/193/UV-Clight

UV radiation is classified as a human carcinogen by US Department of Health and Human Services and the World Health Organization

UVC Action Spectrum: MS2

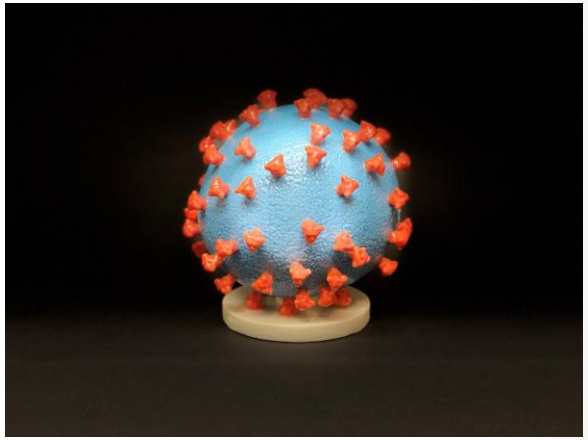


Relative spectral sensitivity of MS2 Coliphage to UV light; Beck et al. Water Research 70 (2015) 27-37

Action spectrum for SARS-CoV-2 has not been measured (Jan 2021)

UVC Disinfection Research

- At start of this study, very limited data existed on UVC disinfection against SARS-CoV-2
- Laboratory studies using various sources of UV are now being published
  - Includes some dose-response data
- Need more information for realistic field conditions (e.g., on different materials, inoculum type, realistic exposure conditions)
- Large variability in reported efficacies due to different test conditions



3D print of a SARS-CoV-2. Credit NIAID

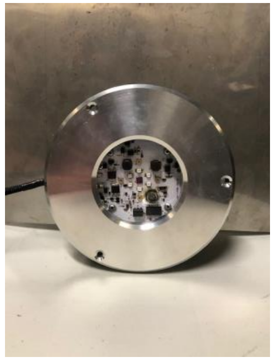
UVC Light Sources

Pulsed Xenon Light


Vendors:
- Puro Lighting / Violet Defense
- Xenex Robotic Units
- ...and many others

Expected Use:
- Addition to regular cleaning
- Short (<30 min) exposure
- Roll in / roll out approach

LED



Vendors:
- Transport Design Group (TDG) and Helios
- ...and many others

Expected Use:
- Addition to regular cleaning
- Build into metro car / office
- Long exposures (4-8 hrs)

Traditional Hg Lamp

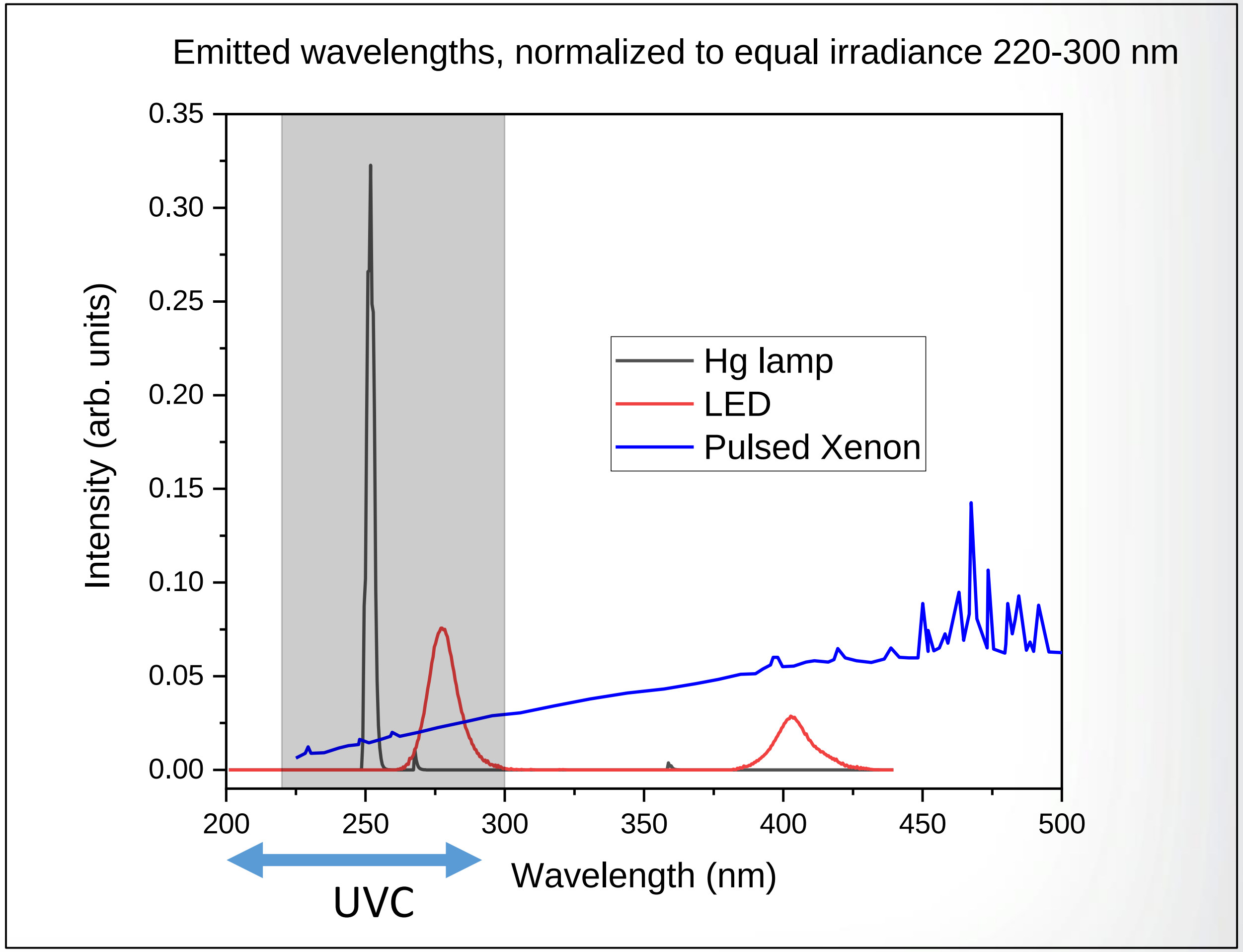
Vendors:
- Standard UVC in Bio Safety cabinet

Expected Use:
- Addition to regular cleaning
- Build into infrastructure
- Long exposures (hours)
- Concern of containing Hg

Emitted Wavelengths

Characteristics:

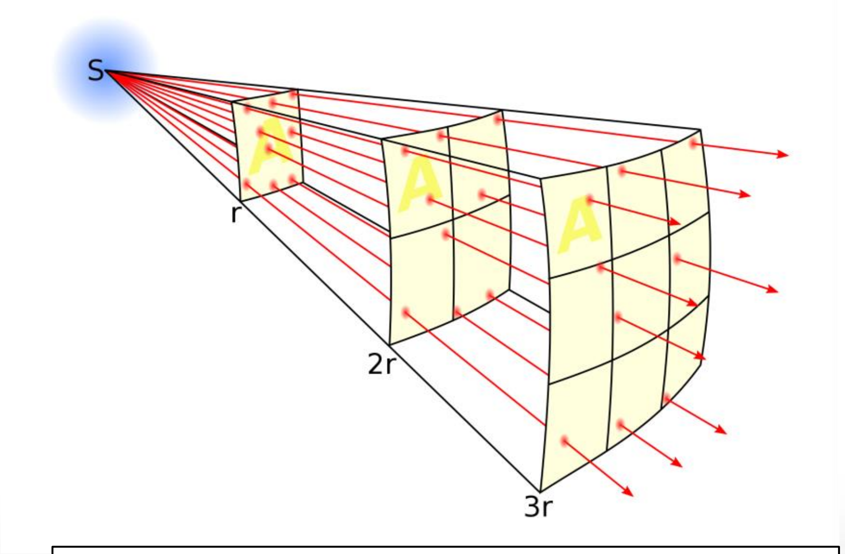
- Xenon light
  - Broad wavelength spectrum,
  - Pulsed (msec); 1 pulse/6 sec
- LED
  - 270 nm and 400 nm wavelength
  - Continuous (110 mW)
- Hg
  - 254 nm wavelength
  - Continuous



Puro Light Spectrum, Courtesy of Brian Buckley, Environmental and Occupational Health Sciences Institute, Rutgers University (September 2020)

Light Measurements

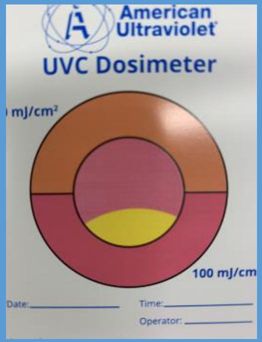
- UVC dose measurements are required to establish dose-response curves for the inactivation of SARS-CoV-2 or other viruses:
  - Dose [mJ/cm^2] = Intensity [mW/cm^2] x Exposure Time [s]
- *Inverse Square Law:* Intensity ~ $1/r^2$ with r the distance from the light to the contaminated surface



https://en.wikipedia.org/wiki/Inverse-square_law#/media/File:Inverse_square_law.svg

Dose measurements:

- Dosimeter paper



- UVC Light Measurement

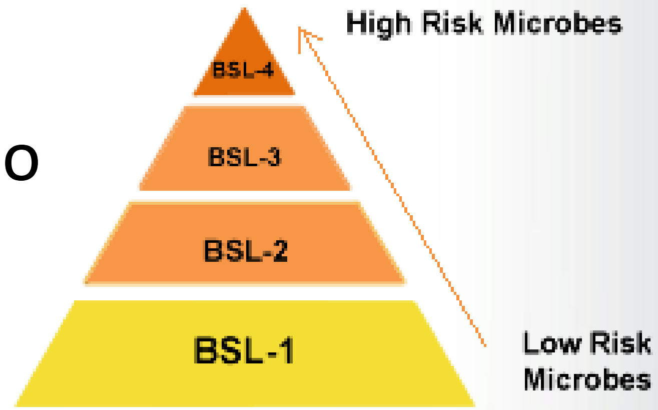
International Light Technologies ITL2500 meter with SED270C or SED270 sensor

SED270C: 220-280 nm range

Targeted Viruses

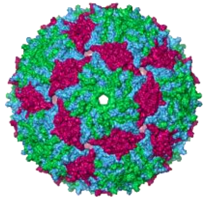
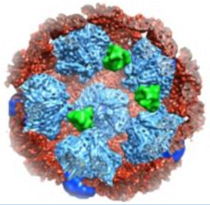
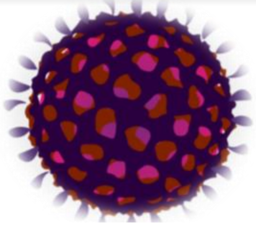
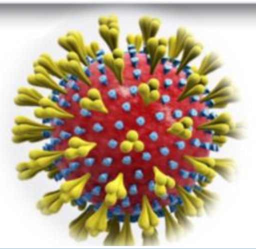
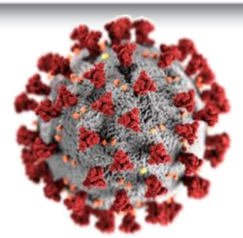
- EPA studies have focused on:
  - MS2, bacteriophage and potential surrogate for SARS-CoV-2
  - Phi6, bacteriophage and potential surrogate for SARS-CoV-2
  - SARS-CoV-2

- Since SARS-CoV-2 research must be conducted in a Bio Safety Level (BSL) 3 (or higher) laboratory, research with this virus is expensive and limited to dose response data

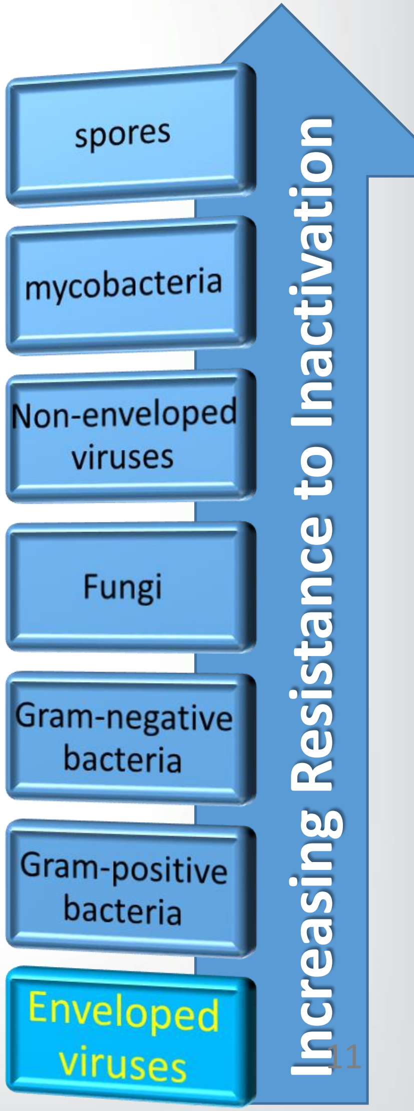


- Objectives of the surrogate virus research is to assess additional impacts of other materials and inoculum conditions on disinfection efficacy

Virus Testing: Comparison*

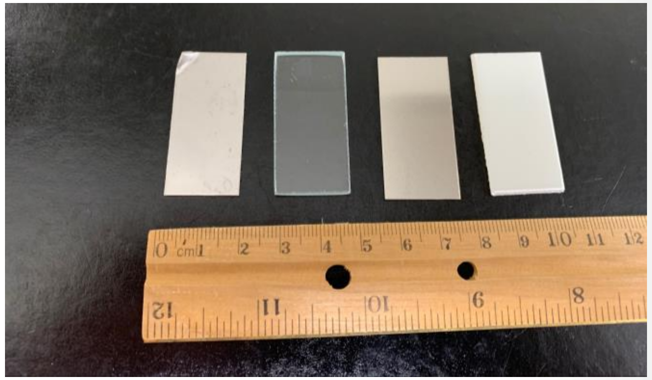
	MS2	Phi6	MHV	229E	SARS-CoV-2
Enveloped?	No	Yes	Yes	Yes	Yes
Host	Bacteria (*E. coli*)	Bacteria (*P. syringae*)	Mice	Humans	Humans
Genus	Levivirus	Cystovirus	Betacoronavirus	Alphacoronoavirus	Betacoronavirus
BSL	1	1	2	2	3
Advantage	High resistance and persistence, fast and easy analysis	Moderate resistance and 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



*** Credit: Dr. Worth Calfee, US EPA**

Test Matrix

	MS2 / Phi6	SARS-CoV-2
UV Light Sources	Pulsed xenon, LED, Hg	Pulsed xenon, LED
Materials	304 Stainless Steel Glass 301 Stainless Steel ABS Plastic	301 Stainless Steel ABS Plastic Bus Seat Fabric (pile; 85% wool, 15% nylon)
Inoculum Application	10 µL Droplet & Spread 10 µL Droplet	10 x 10 µL Droplets
Inoculum Matrix	Phosphate-Buffered Saline (PBS) with 5% Fetal Bovine Serum (FBS)	Tissue Culture (TC) Media + 5% FBS & Simulated Saliva
Inoculum Presence	Wet / Dry Droplets Wet / Dry Spread	Wet / Dry Droplets
Dose	Multiple (n > 10)	3 Doses / Light Source

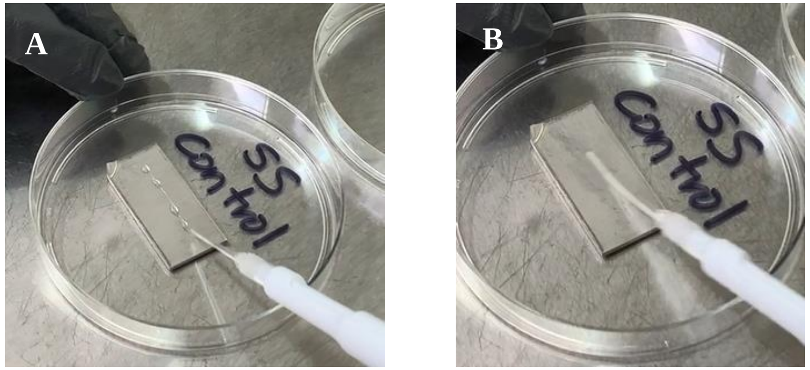


304 SS, glass, 301 SS, ABS plastic; 2 cm x 4 cm

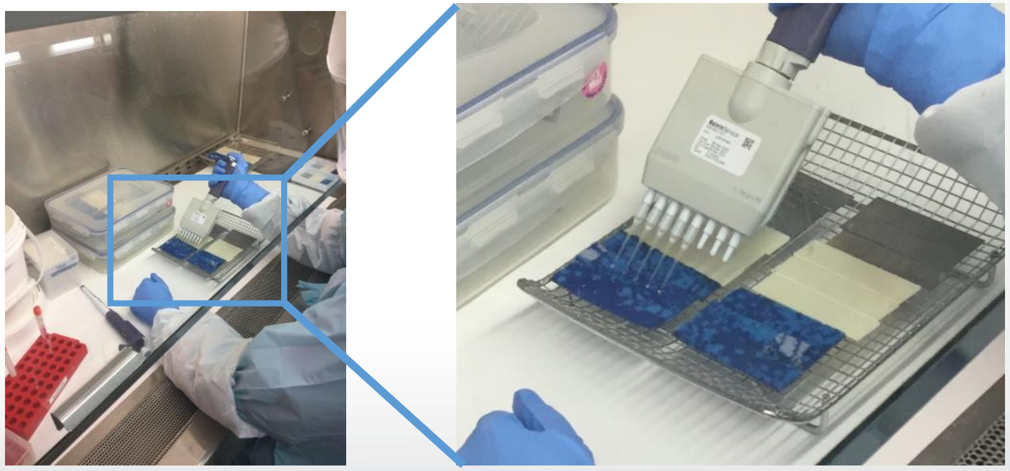
Spread vs droplet inoculum (MS2)

Material Inoculation

- <u>MS2 and Phi6</u>: Materials were inoculated with viruses contained in PBS + 5% FBS by application of droplet(s) followed by spreading (applies to most of MS2 and Phi6 virus research)

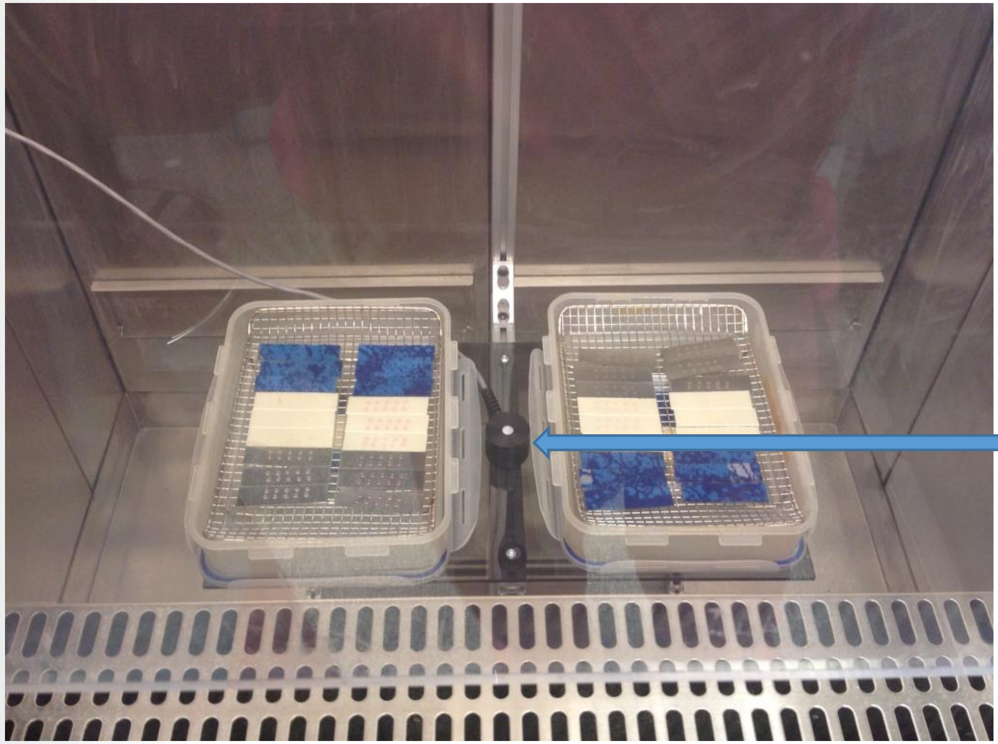


- <u>SARS-CoV-2</u>: Materials were inoculated with virus in either TC media or simulated saliva liquids by application of 10 x 10 µL droplets



Experimental Setup

SARS-CoV-2 Setup (Light source not shown)

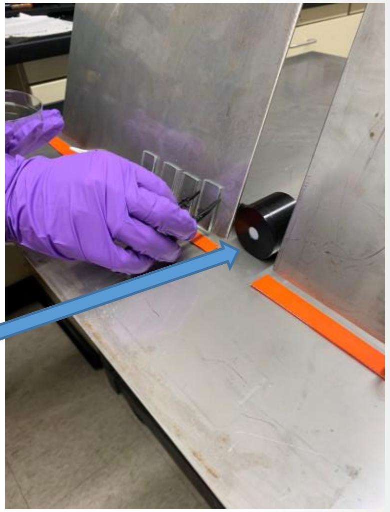


(Left) Top-Bottom: Seat fabric – ABS plastic – stainless steel

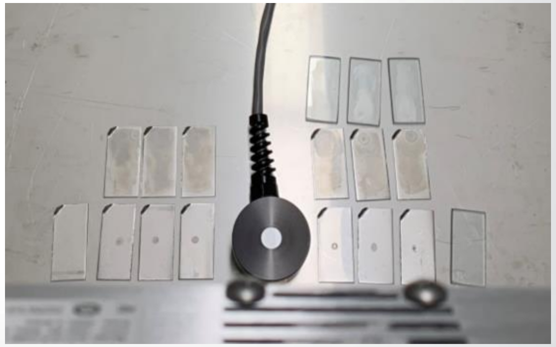
Power/Energy Detector

MS2 / Phi6 Research

Tripod with xenon light



Vertical support stand

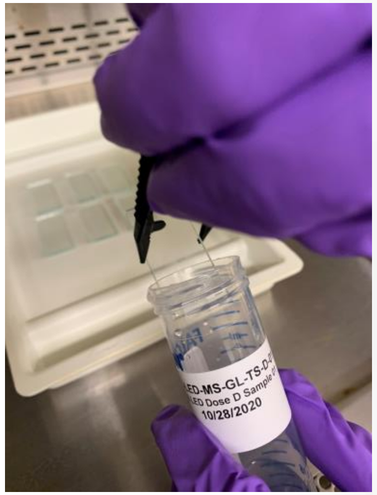


Horizontal layout

Virus Recovery

- Aseptic retrieval of material coupon followed by placement into sterile conical tubes with extraction buffer
- Vortexing (2 min)
- Dilution series prepared
  - MS2: Plaque assay, *E. coli* C-300 (ATCC 15597)
  - Phi6: Plaque assay, *P. syringae* LM2489
- Overnight incubation (35 / 21 °C for MS2 / Phi6)
- Plaque forming unit (PFU) enumeration

- For SARS-CoV-2, samples were split with one part stored for RV-PCR analysis (*in progress*)
- SARS-CoV-2 Eluents were tested for viable virus by cell culture (Median Tissue Culture Infectious Dose, TCID50 assay)



Efficacy:

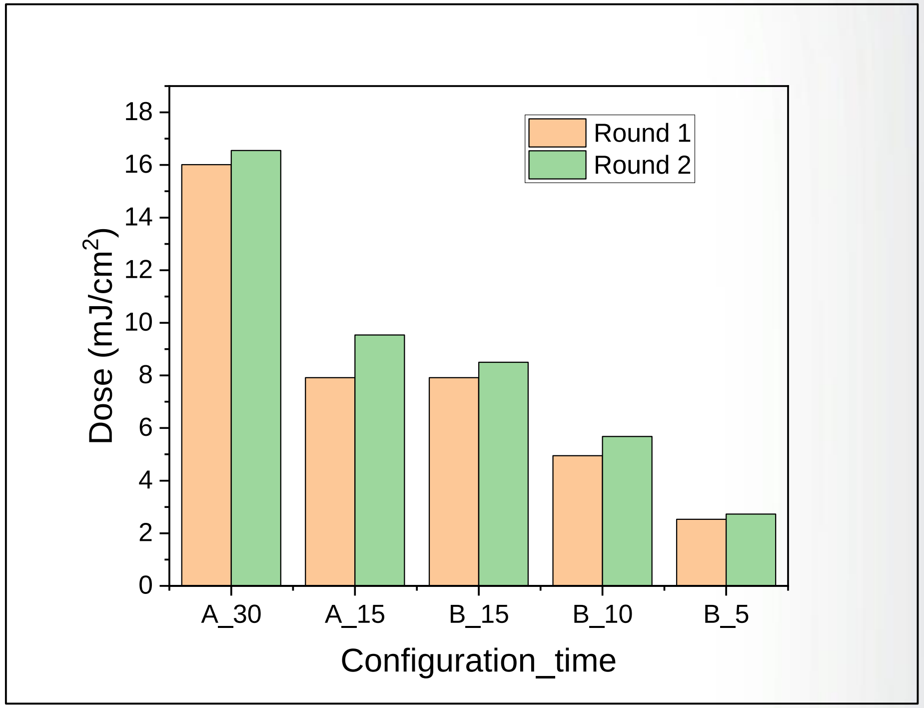
Log Reduction (LR) = Mean $\log_{10}$ recovery (Positive Controls) - Mean $\log_{10}$ recovery (Test Coupons)

Realistic Test Conditions

Use of UVC light to augment disinfection practices in a metro car

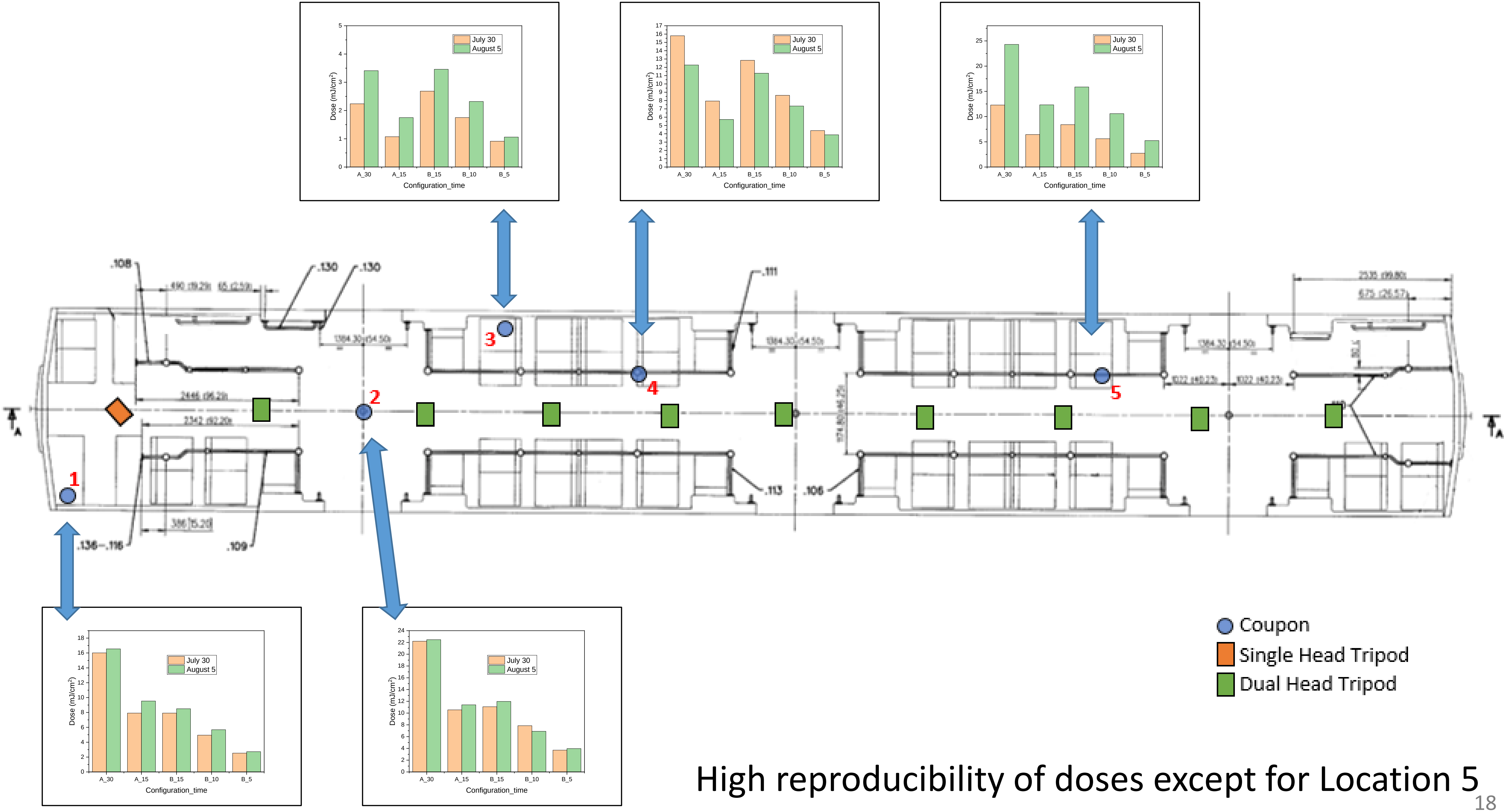
LA Metro Field Study

- Los Angeles County Metropolitan Transport Authority (LA Metro) conducted a field study to evaluate practicality of pulsed xenon UVC units (ease of use, setup time, durability, electrical load, functionality, etc.) for two lamp configurations and achievable UV dose in a metro car
- EPA supplied MS2-inoculated material coupons to incorporate in this field test
- LA Metro measured UV dose* for each coupon location / exposure time



A_30: Config A (11 lights/car), 30 min
A_15: Config A (11 lights/car), 15 min
B_15: Config B (19 lights/car), 15 min
B_10: Config B (19 lights/car), 10 min
B_5: Config B (19 lights/car), 5 min

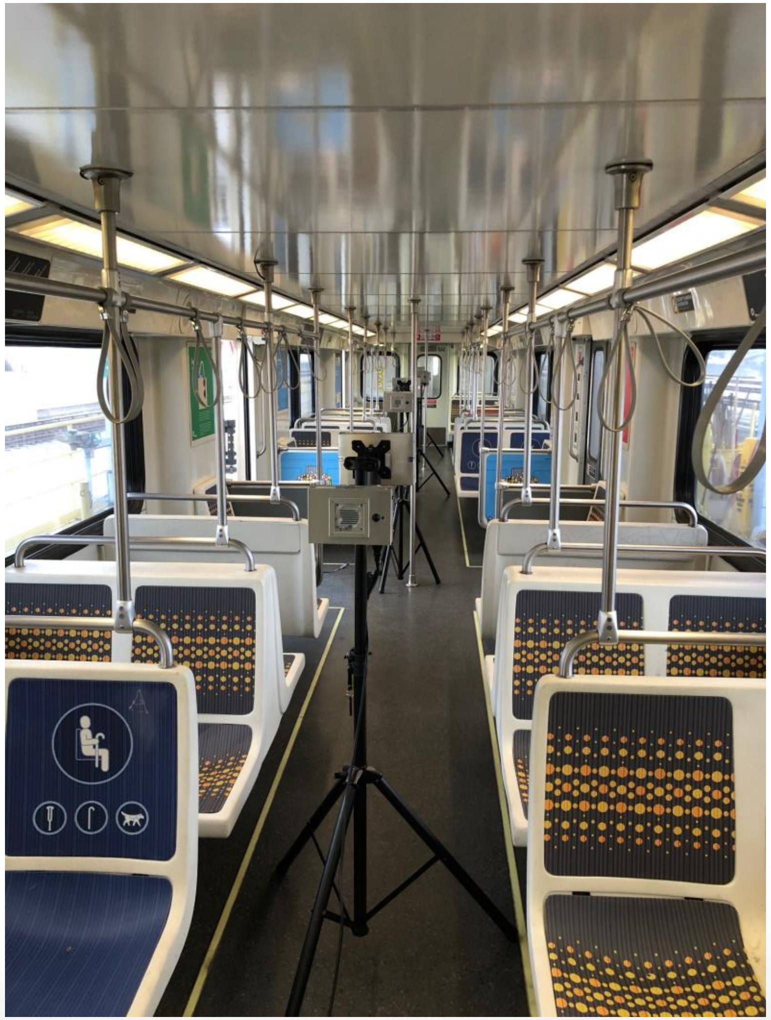
* Dose as measured with ILT SED270 light sensor in metro car (which includes UVC + UVB + UVA)



High reproducibility of doses except for Location 5

LA Metro Field Study-Results

- Measured range of doses* in LA Metro tests: 1-22 mJ/cm^2
- Lowest doses at locations outside of direct line of sight or at large distances
- Highest dose for location at ~60” directly in front of light, 30 min exposure time
- High reproducibility in doses between two tests run on different days
- *No significant reduction in MS2 on coupons exposed to UVC in LA Metro test*
    - Additional lab tests needed to understand this lack of virus inactivation

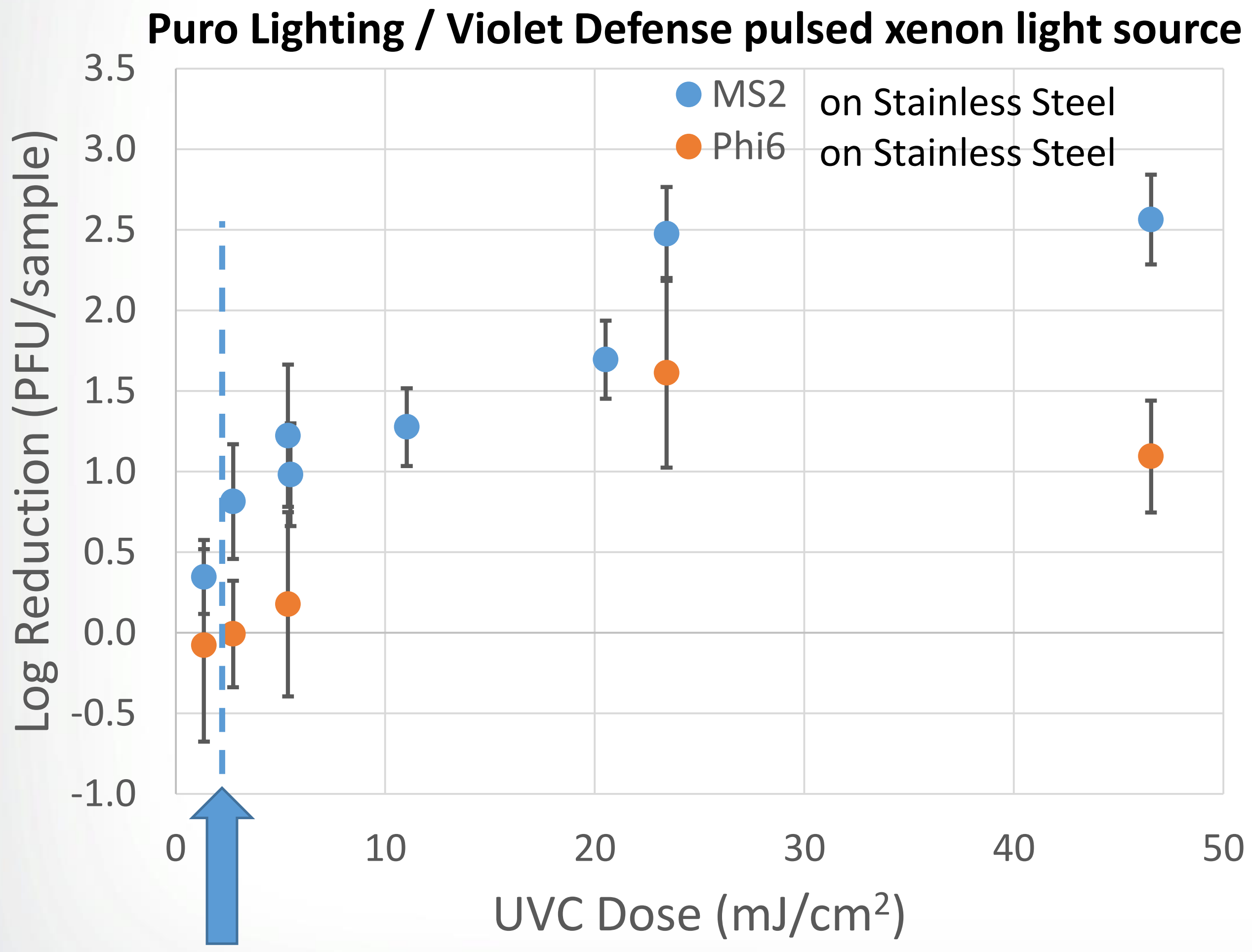


* Dose as measured with LA light sensor (ILT SED270) in metro car (which includes UVC + UVB + UVA)

Laboratory Research

MS2 and Phi6 Disinfection via UVC light

Initial Dose-Response Results

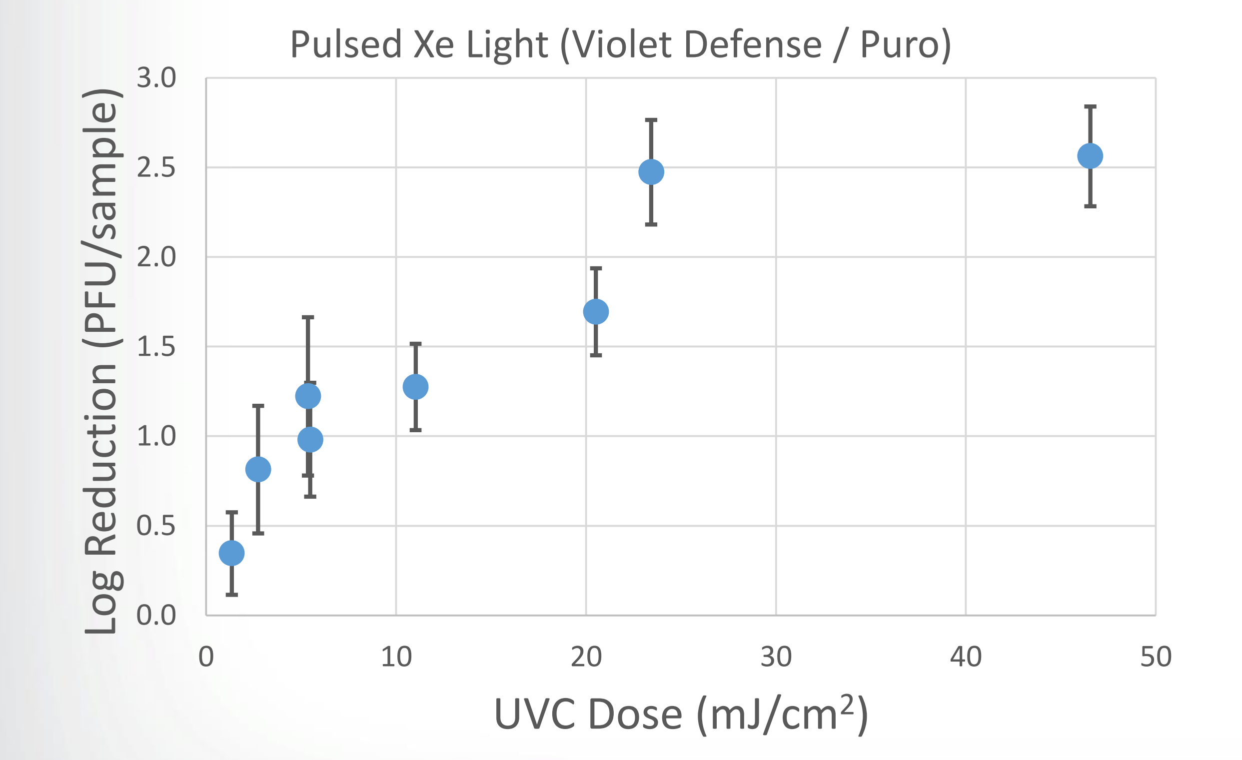


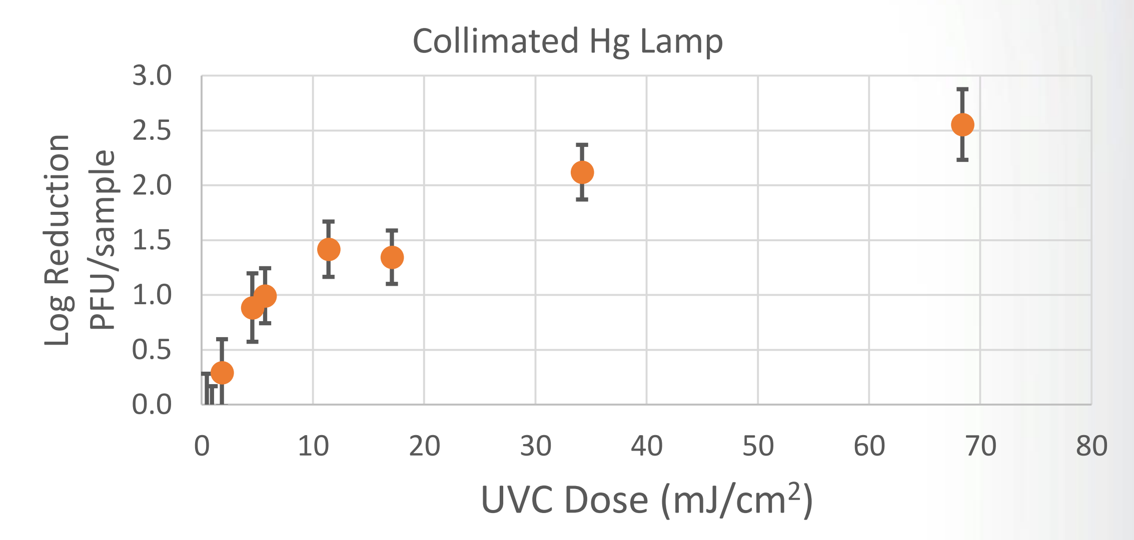
LA Metro dose (at ~60”, 30 min)

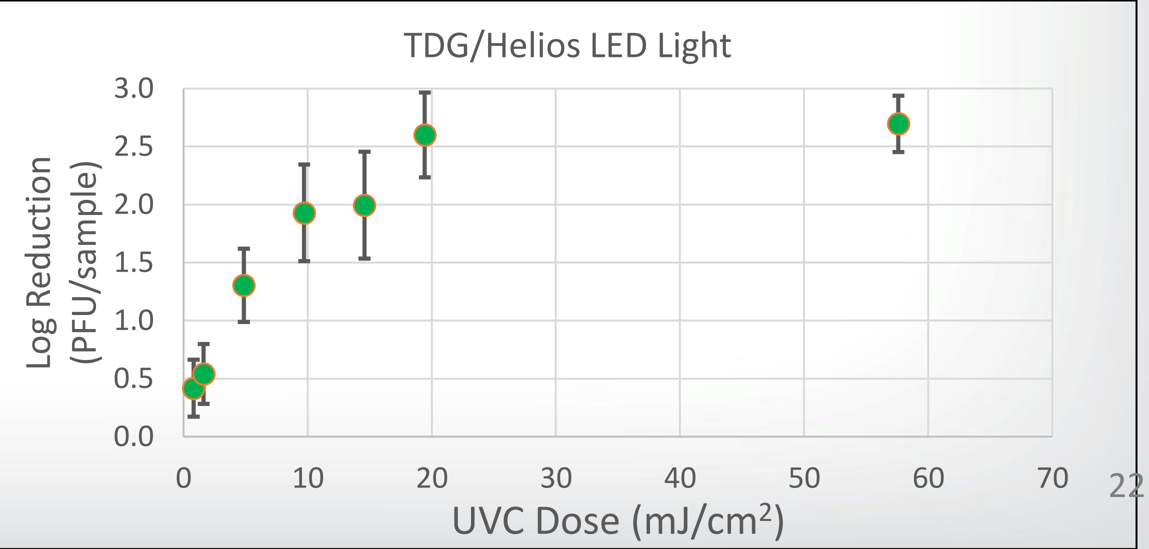
- Positive control recoveries were >10^5 PFU/sample
- UVC light inactivates MS2 and Phi6 virus
- Did not achieve 3-log reduction (“disinfection”)
- Observed non-linear relationship between dose and log reduction for stainless steel
- Highest dose from LA Metro study:
    - ILT SED270C detector: 3.5 mJ/cm^2
    - ILT SED270 detector: 22 mJ/cm^2
- Agreement (MS2 results) between lab study and LA Metro field test

Other UVC Light Sources

Light source dependence (same stainless-steel material, same spread inoculum with MS2 bacteriophage)



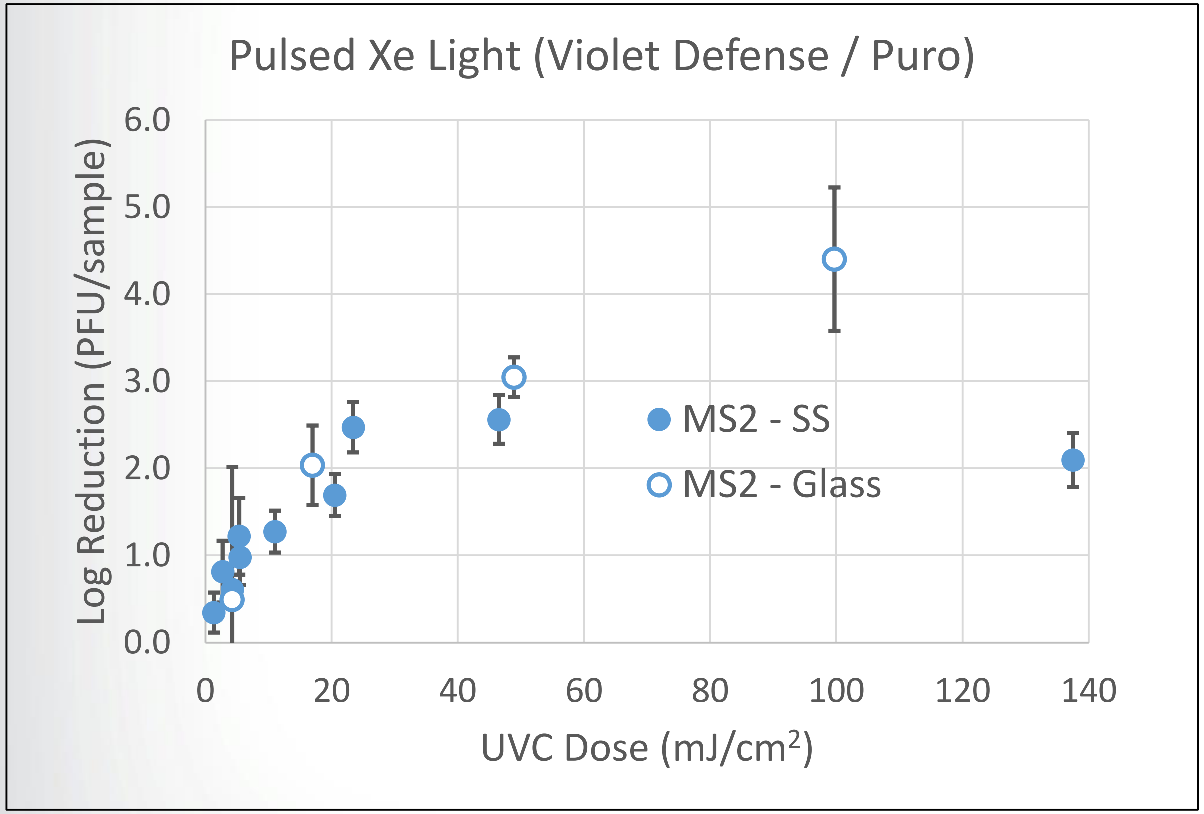


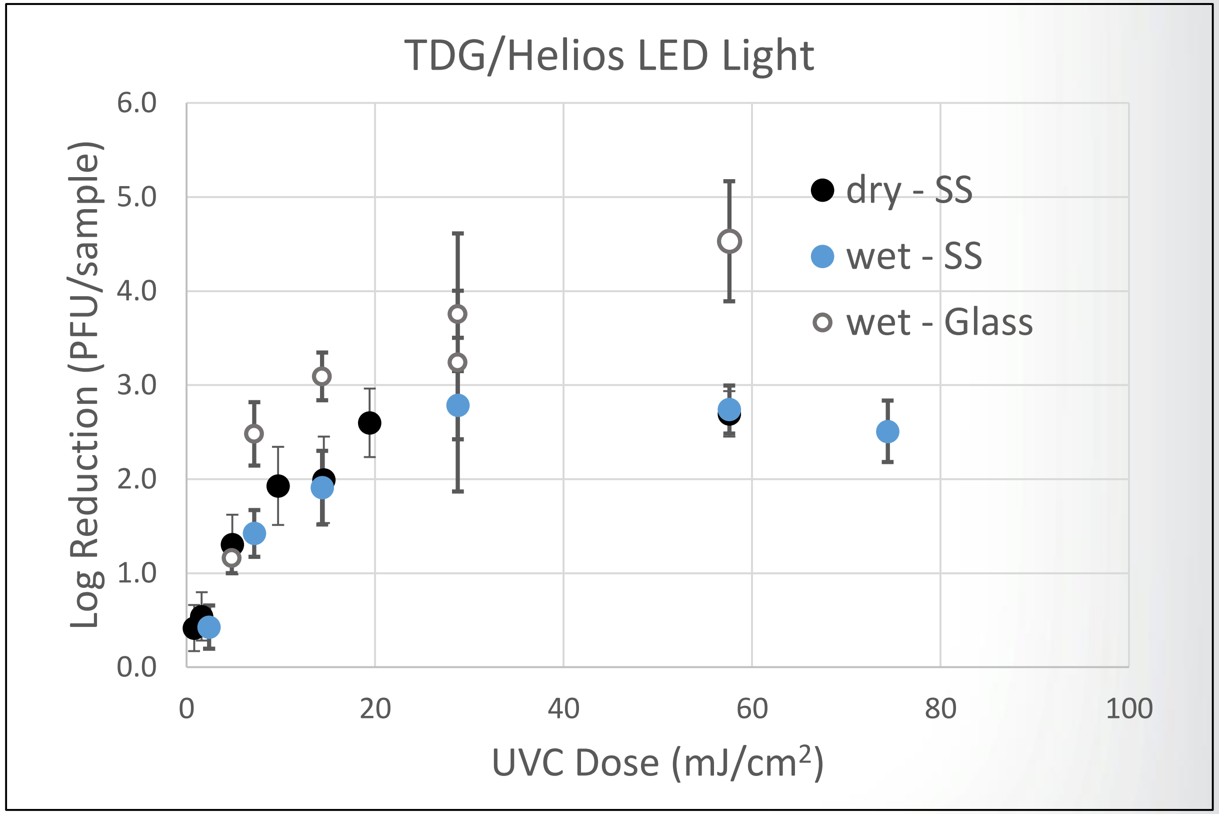


Nonlinear behavior appears for all three light sources

Other Materials

Material Dependency (same spread inoculum with MS2 bacteriophage)



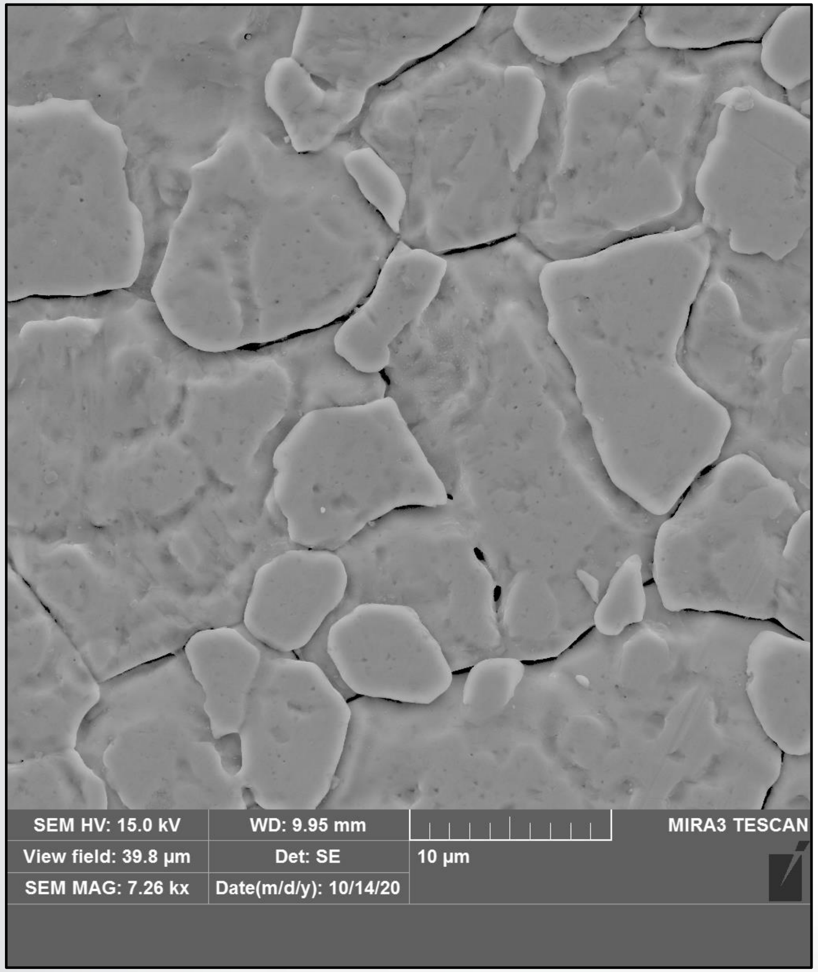


SS: 304 Stainless Steel

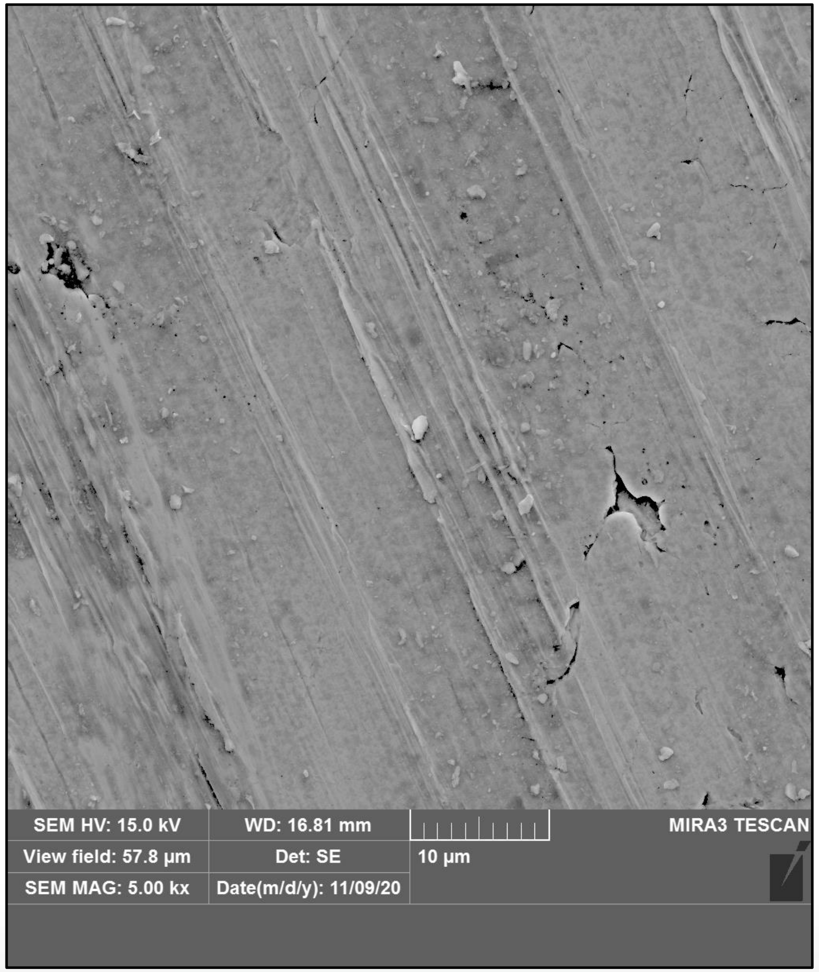
Nonlinear relation also appears for glass.
LRs for glass are noticeably higher than for stainless steel (both light sources)

A closeup… literally

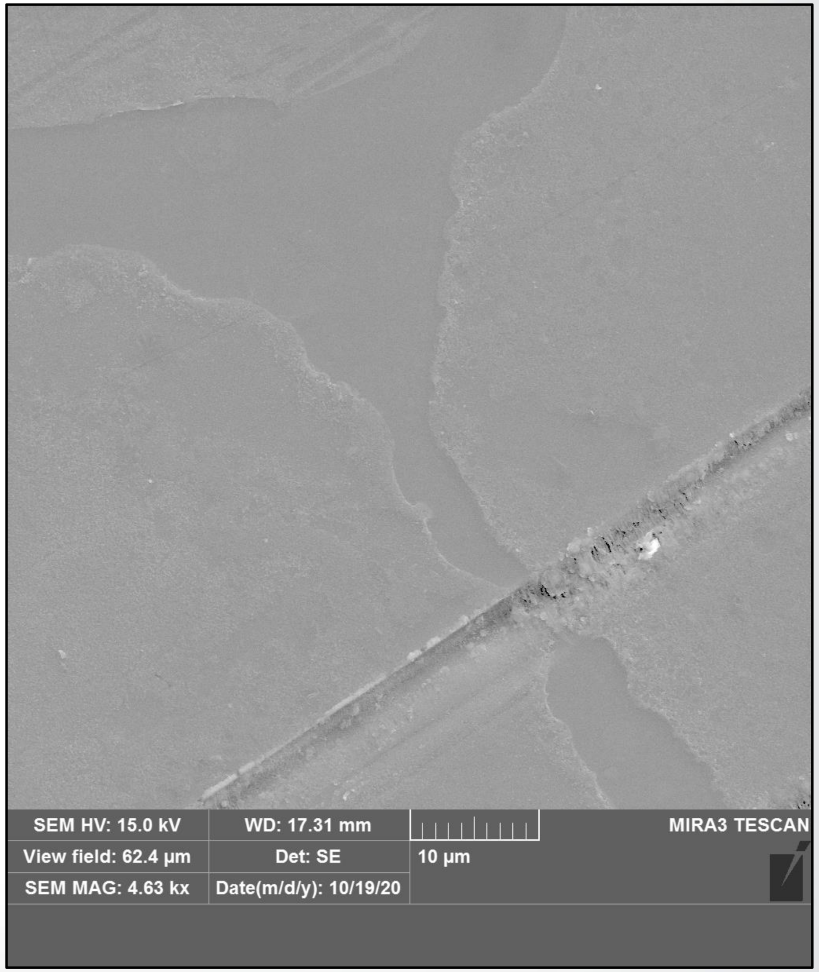
Milled 304 Stainless Steel (MS2/Phi6 study)



301 Stainless Steel (SARS-CoV-2)

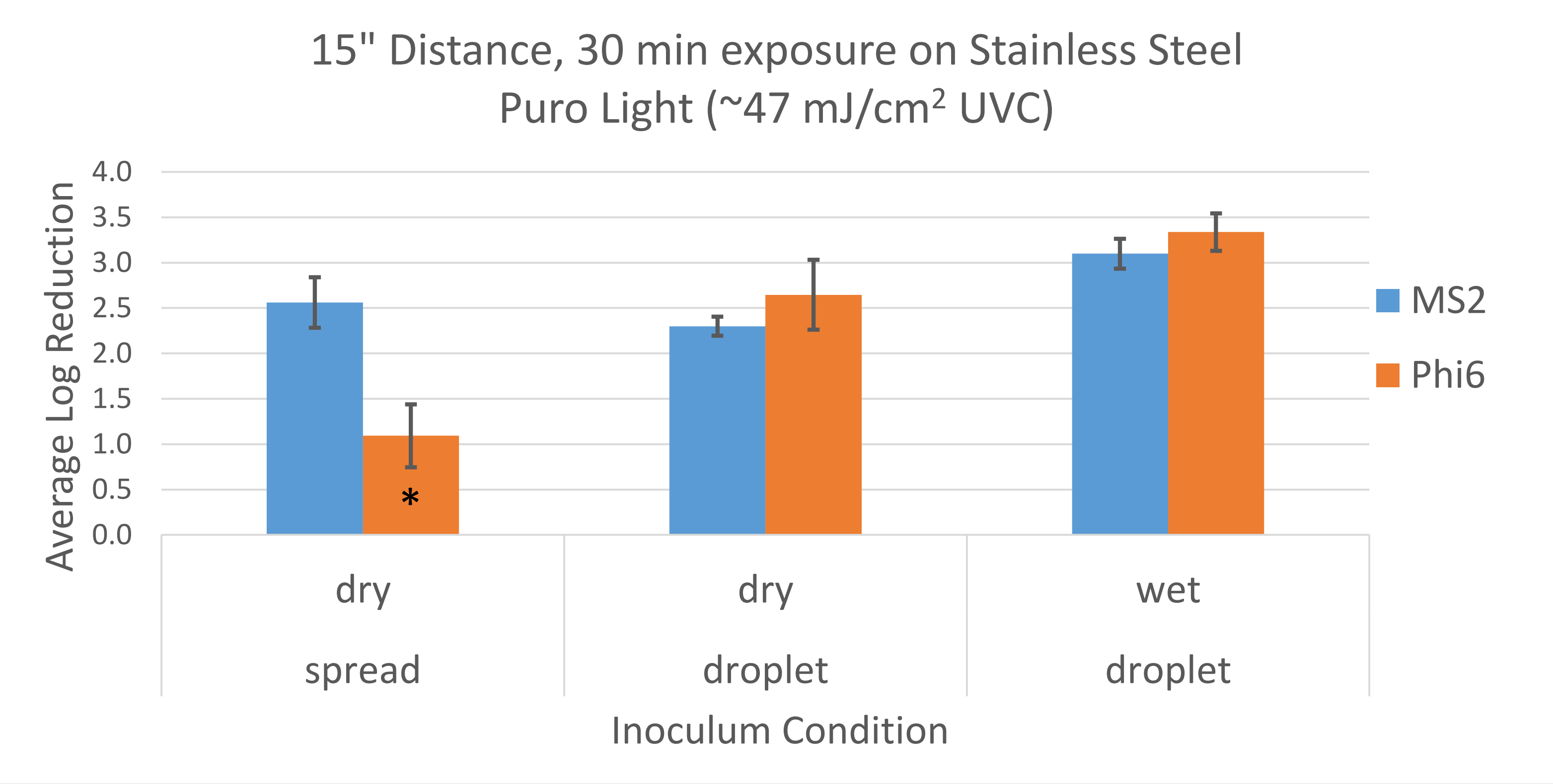


Glass Coupon (MS2/Phi6 study)



Inoculum Start Conditions

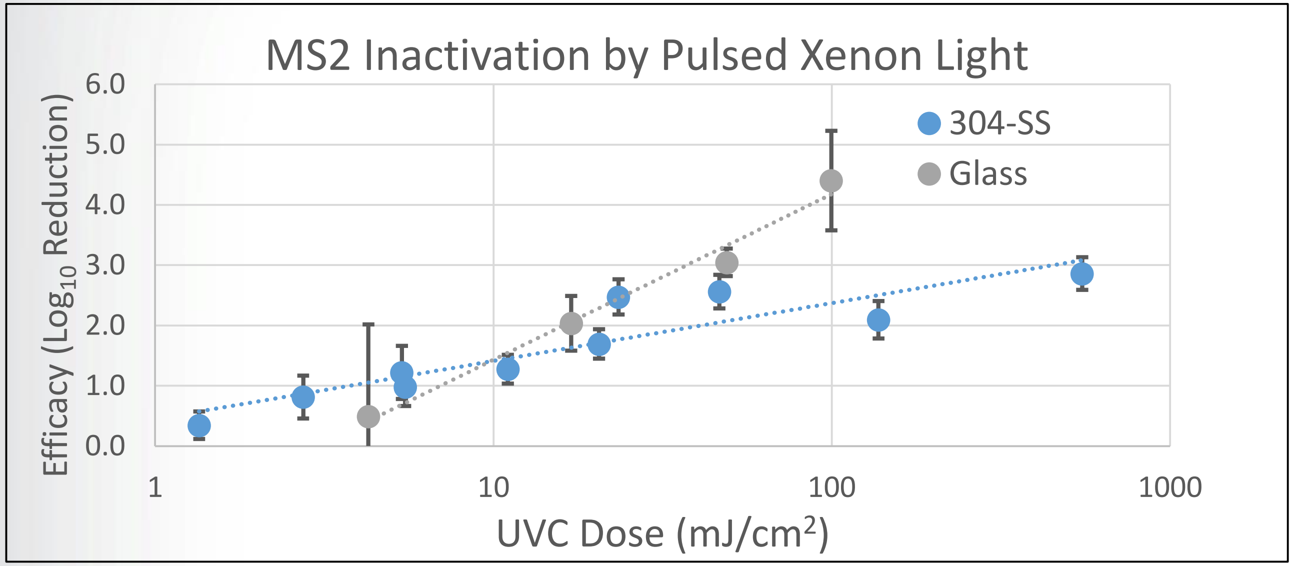
Relevancy of dry droplet, wet droplet, or dried spread inoculum

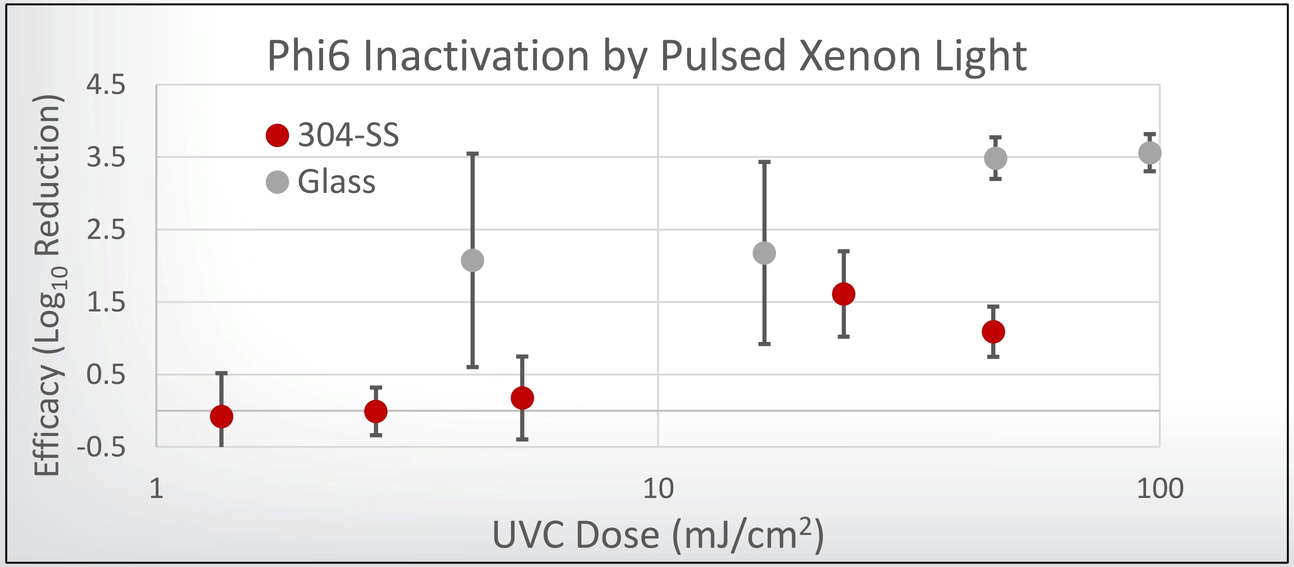


* To be verified in future tests

LRs are higher for wet droplet over dried droplet at start of UV light exposure; Minimum impact on log reduction of the inoculum spreading

Summary MS2 and Phi6 Research





- Nonlinear dose-response behavior appears for all three light sources and (two) materials
  - Relying on reported "90%" efficacy data to get to (linear extrapolation) 99.9% "disinfection" values is inaccurate for these test conditions
- Log reductions are higher for wet droplet over dried droplet; minimum impact from the inoculum spreading
- Log reductions on glass were noticeably higher than on stainless steel (for both UVC light sources)

Laboratory Research

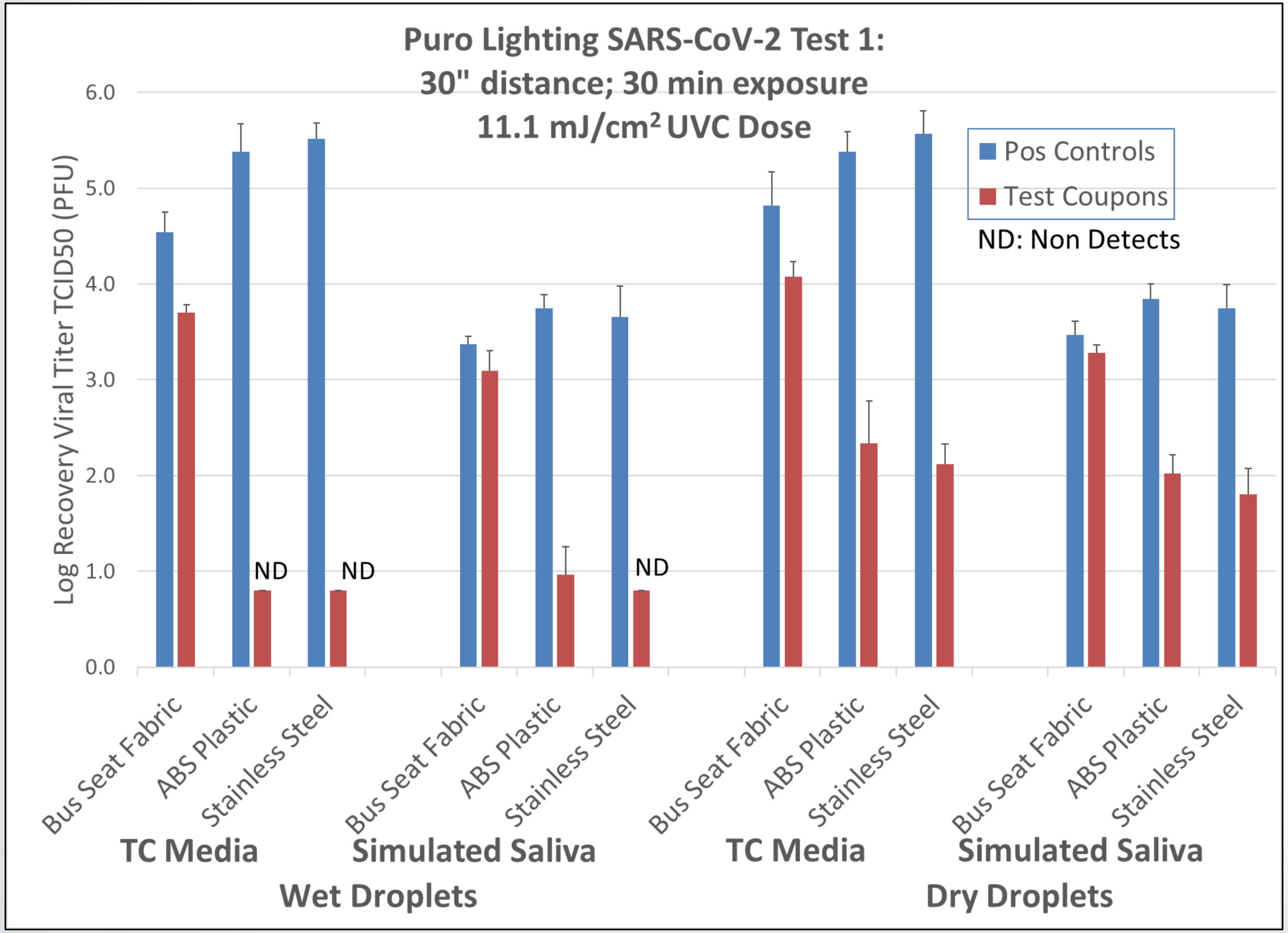
SARS-CoV-2 Disinfection via UVC light

Method Development

Prior to the UVC inactivation testing, we demonstrated that:

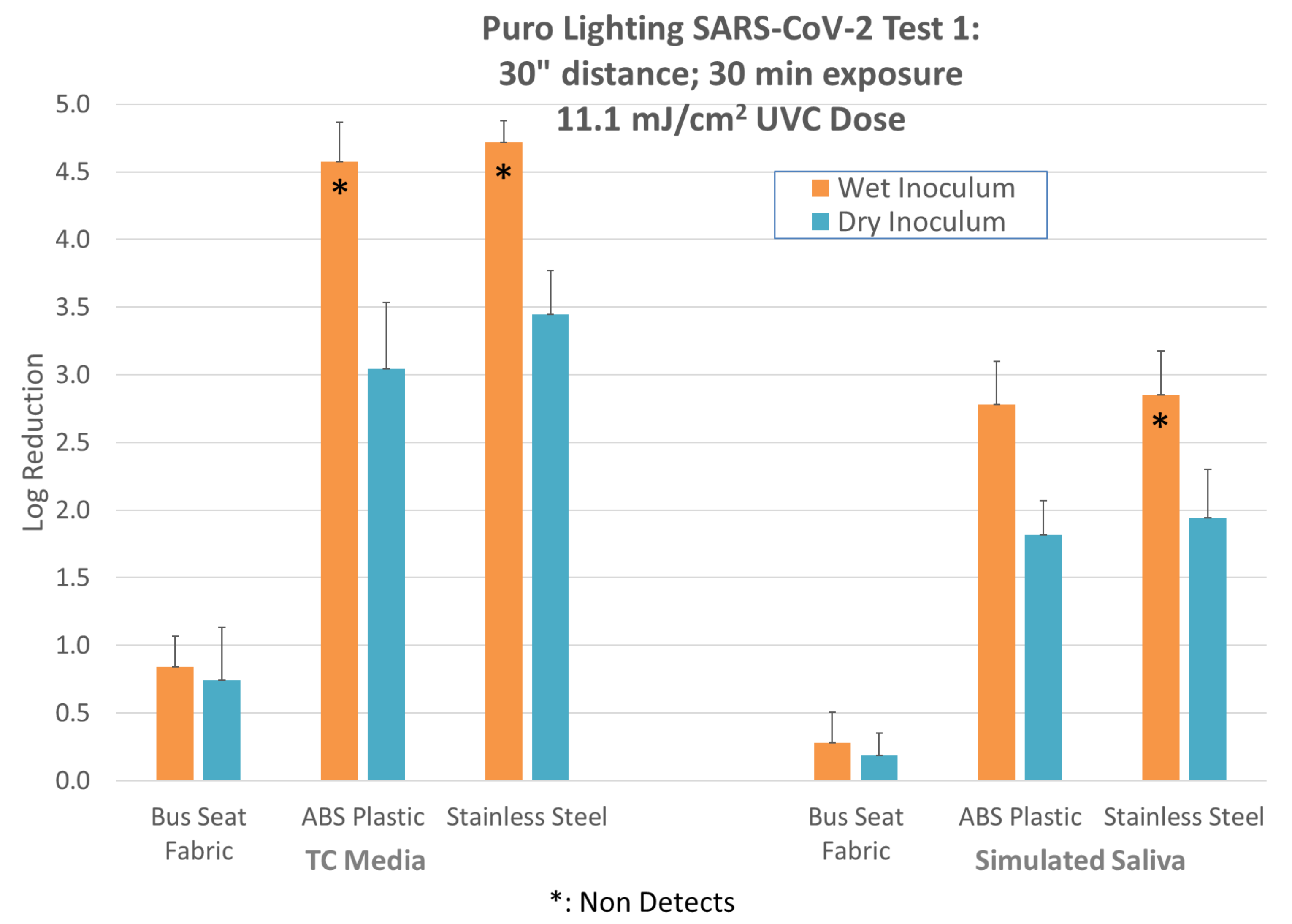
- Sufficient SARS-CoV-2 high recoveries (mid 10^4 PFU/coupon) can be obtained from all materials (after drying + 1 hr)
  - Lower recoveries for SARS-CoV-2 in simulated saliva (mid 10^3 PFU/coupon)
- No cytotoxicity of materials observed
- Based on TCID50 procedures, a detection limit of 6.3 virons/coupon was established

Pulsed Xenon Light Recoveries



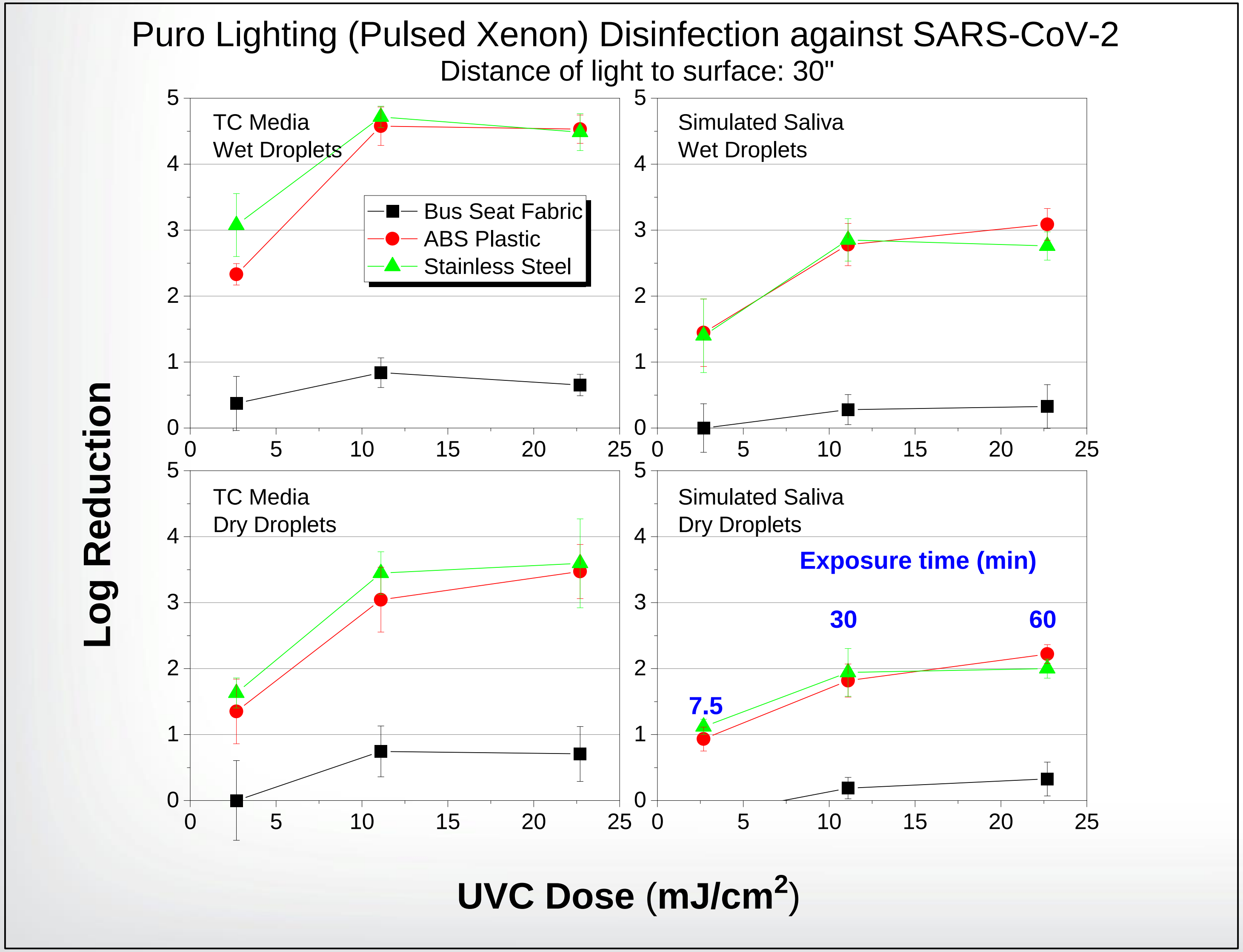
- Positive Controls (non-exposed):
  - High recoveries in TC media
  - Lower recoveries in simulated saliva
  - Minimal material dependence
- Test Coupons (exposed):
  - Non-detects (less than 6.3 virons) in three occasions
  - Significant high recoveries for bus seat fabric
  - Higher recoveries in simulated saliva than in TC media

Pulsed Xenon Light Efficacy



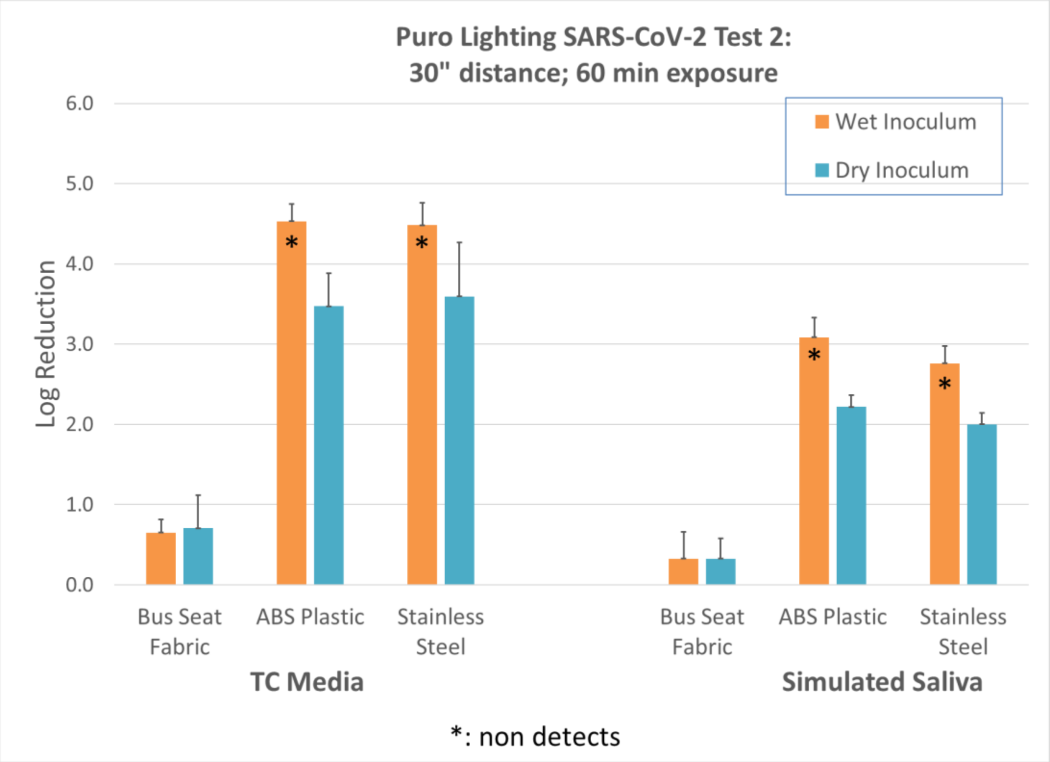
- Minimal reduction in viable virus for bus seat material
  - Non-smooth, porous material
- Higher (> 3 LR) efficacy on ABS plastic and stainless steel when SARS-CoV-2 is applied in TC media
- Lower (< 3 LR) efficacy on ABS plastic and stainless steel when SARS-CoV-2 is applied in simulated saliva
  - Some of the UVC gets absorbed in saliva (mucus and salts)
- Virus in dried inoculum is more difficult to inactivate

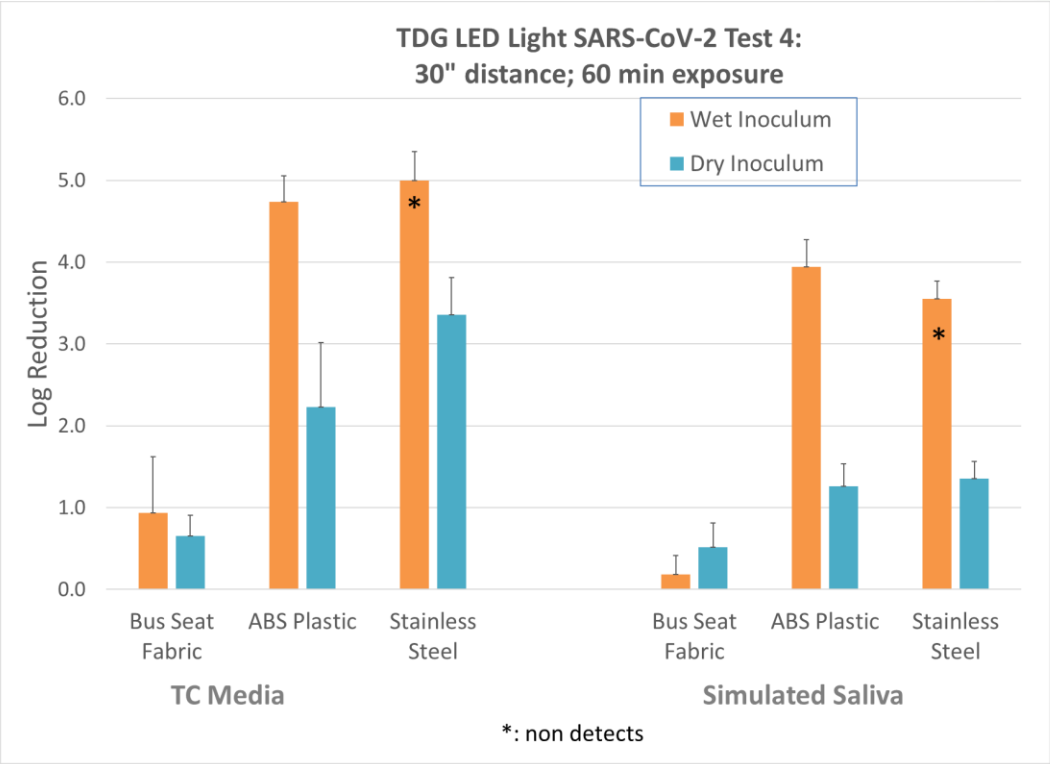
Pulsed Xenon Light Efficacy



- High log reductions for smooth materials (ABS plastic and stainless steel)
- Low log reductions for rough, porous surface (bus seat fabric)
  - Virus shielded from UVC light within material fibers
- SARS-CoV-2 in a dried saliva is most difficult to inactivate.
  - Absorption of UVC in saliva may explain this difference
- LA Metro's highest UVC dose recorded for a surface at 60" distance from a light and a 30 min exposure time was 3.5 mJ/cm^2

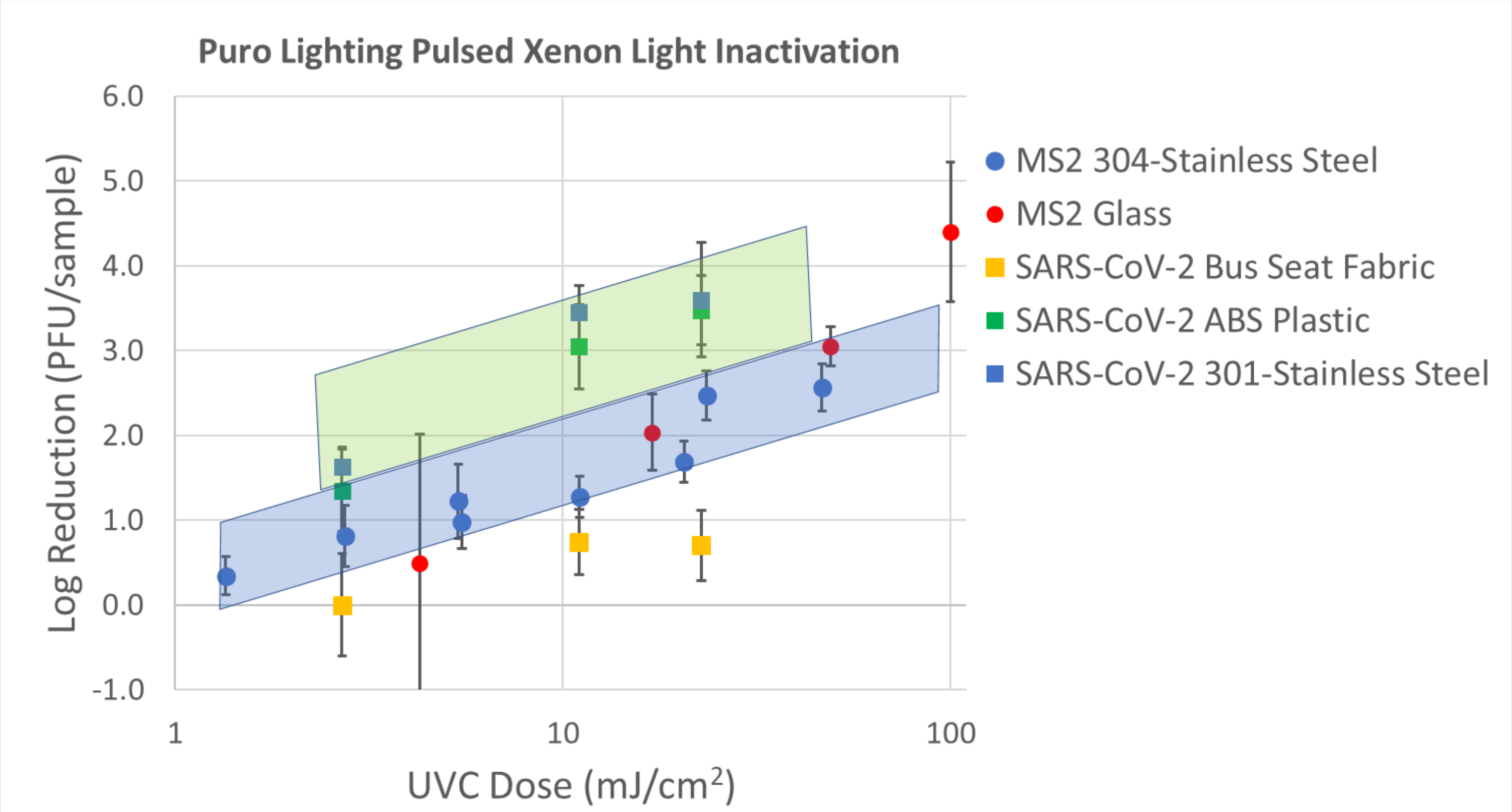
UVC Efficacy (First) Comparison





- Efficacy for bus seat fabric is low (independent of UV light source)
- Both UV light sources show similar efficacy in inactivating SARS-CoV-2
  - Log reduction of dried inoculum is generally higher with pulsed xenon than LED
- Less than 3-log reduction for most challenging (dried saliva) condition
  - *Higher dose measurements with LED light are in progress*

SARS-CoV-2 vs MS2



- MS2 appears to be a good surrogate for SARS-CoV-2 for UVC disinfection studies, with higher efficacy observed for SARS-CoV-2 vs. MS2 under nearly same conditions

- <u>Difference in test conditions:</u>
  - Stainless steel surfaces are not identical between studies
  - Inoculum composition
  - Droplet inoculum (SARS-CoV-2) vs spread inoculum (MS2)

- Comparison against Phi6 in progress (less stable virus)

Conclusions

- UVC light emitted by pulsed xenon light or LED inactivates SARS-CoV-2 and bacteriophages MS2 and Phi6
- Efficacy/log reductions are dependent on
  - Material/substrate,
  - Inoculum matrix, and
  - Wet or dry droplets vs. spreading of inoculum
- Microscopic surface features of materials may lead to shielding and subsequent lower efficacy of UVC inactivation
- MS2 appears to be a good surrogate for SARS-CoV-2 for UVC testing, as it is slightly more difficult to inactivate
- The large number of variables that have an impact on efficacy makes side by side comparisons with other disinfection studies difficult
  - A standardized method for UVC disinfection would allow for better evaluation and comparison of UVC light emitting devices

Practical Applications/Challenges

- The estimated UVC dose needed to achieve a 3-Log reduction of SARS-CoV-2 in the conditions presented here ranges from ~ 6 mJ/cm^2 to >22 mJ/cm^2, depending on multiple variables
- Such doses can only be obtained with the tested UVC light sources at relatively short (30” or shorter) distances for operationally feasible exposure times (<60 min)
  - Longer exposure times (> 1 hr) could improve efficacy
  - Additional lights at different locations may reduce shading

- UVC light is only effective where there is a direct line of sight between the light source and the contaminated surface
- Porous materials will shield some of the virus from light, leading to only partial inactivation

Next Steps

- Continue to investigate UVC to inform and improve on its application
  - Address impact of other variables and
  - Comparison to published log-reduction values
- Continuation of BSL-3 research with SARS-CoV-2
  - UVC
  - Ozone and Steam research will follow
- Additional UVC research:
  - Characterization of UVC sources and UVC reflectivity with different materials
  - Material compatibility tests

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    EPA Region 10

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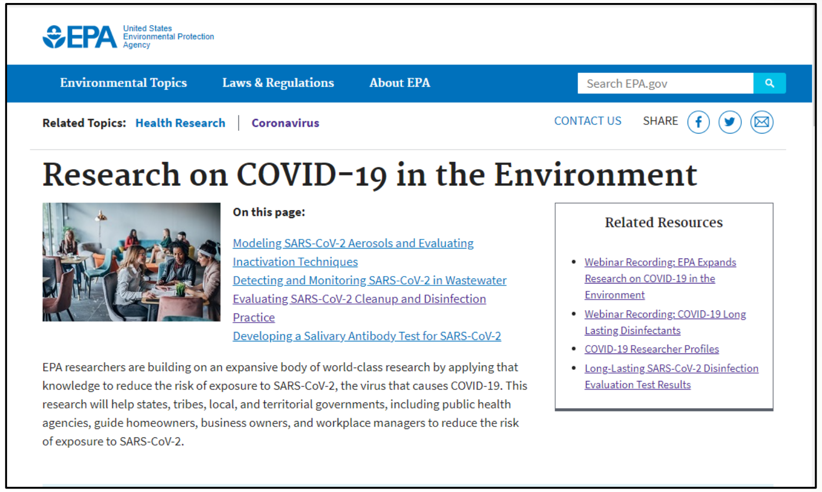
- Los Angeles Metro
- Members of EPA project team

Points of Contact



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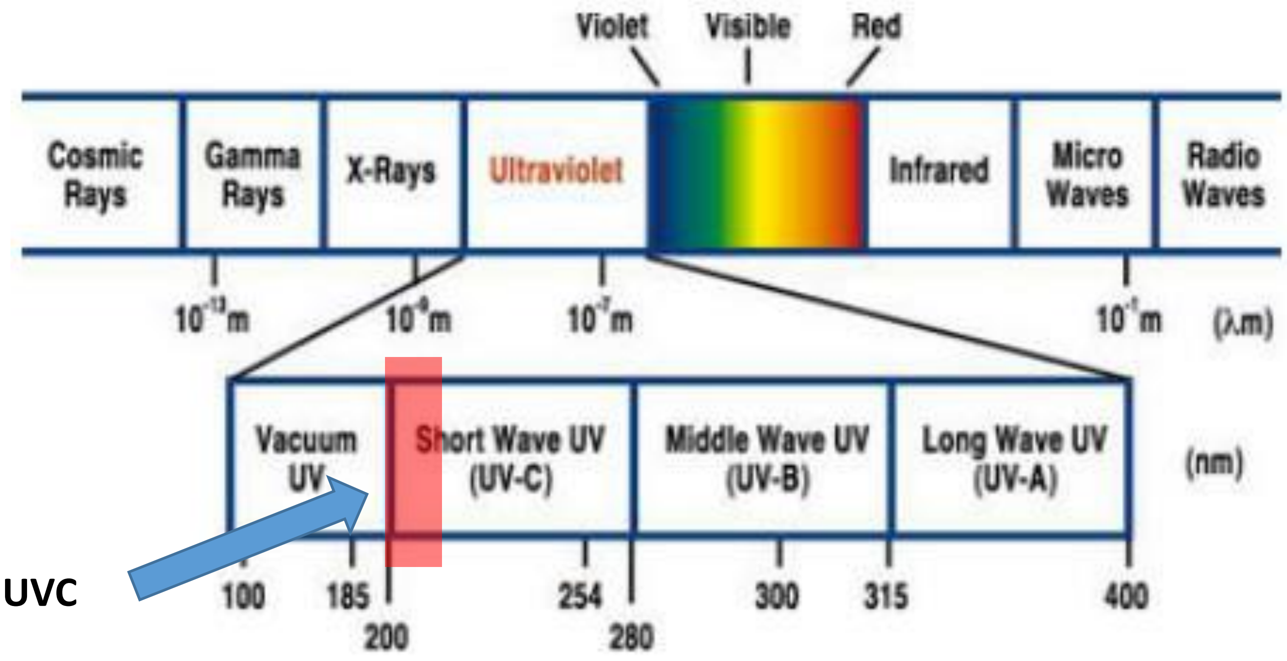
Data presented herein did not undergo a formal quality assurance review as outlined in “U.S. EPA Office of Research and Development’s Quality Management Plan for Scientific Research”. If these data are included in future EPA reports or other publications, they will be subjected to this review.

Backup Slides

Far UVC



https://www.cleanairoptima.com/information/193/UV-Clight

While UVC is damaging to skin and eyes, far UVC has been proposed for use in occupied spaces as its wavelength (200-220 nm) does not appear to cause damage to skin or eyes. However, such studies have been limited to relative short-term exposure studies.