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FEDERAL TRANSIT ADMINISTRATION

COVID-19 Research Demonstration Program: Efficacy of UV Lights in Transit Applications

PREPARED BY
Rock Region Metropolitan Transit Authority

U.S. Department of Transportation
Federal Transit Administration

SEPTEMBER
2023

COVER PHOTO

Courtesy of Jon Wisniewski, Rock Region Metropolitan Transit Authority

DISCLAIMER

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PREPARED BY

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901 Maple St.
North Little Rock, AR 72114

SPONSORED BY

Federal Transit Administration
Office of Research, Demonstration and Innovation
U.S. Department of Transportation
1200 New Jersey Avenue, SE
Washington, DC 20590

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Metric Conversion Table

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
LENGTH				
in	inches	25.4	millimeters	mm
ft	feet	0.305	meters	m
yd	yards	0.914	meters	m
mi	miles	1.61	kilometers	km
VOLUME				
fl oz	fluid ounces	29.57	milliliters	mL
gal	gallons	3.785	liters	L
ft^3	cubic feet	0.028	cubic meters	m^3
yd^3	cubic yards	0.765	cubic meters	m^3
NOTE: volumes greater than 1000 L shall be shown in m^3				
MASS				
oz	ounces	28.35	grams	g
lb	pounds	0.454	kilograms	kg
T	short tons (2000 lb)	0.907	megagrams (or "metric ton")	Mg (or "t")
TEMPERATURE (exact degrees)				
^oF	Fahrenheit	5 (F-32)/9 or (F-32)/1.8	Celsius	^oC

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14. ABSTRACT

This report presents the results of the Rock Region METRO (METRO) Public Transportation COVID-19 Research Demonstration project performed with Environmental Services Company (ESC) and the University of Arkansas for Medical Sciences (UAMS). The purpose of this project was to demonstrate the efficacy of UV light in disinfecting applications for COVID-19. The tests were conducted with no passengers on board, after the vehicles were in service during normal operating hours.

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Abstract

This report presents the results of the Rock Region METRO (METRO) Public Transportation COVID-19 Research Demonstration project performed with Environmental Services Company (ESC) and the University of Arkansas for Medical Sciences (UAMS). The purpose of this project was to demonstrate the efficacy of UV light in disinfecting applications for COVID-19. The tests were conducted with no passengers on board, after the vehicles were in service during normal operating hours.

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Information and prevention guidelines from the Centers for Disease Control and Prevention (CDC) show that the COVID-19 virus (SARS CoV-2) is transmitted from infected individuals when they exhale droplets and particles that contain the SARS CoV-2 virus. Then, through close contact or aerosol form, the virus spreads in a population. To mitigate the transmission of COVID-19 and protect transit operators, who are a critical part of the public transportation infrastructure, transit agencies across the United States implemented temporary measures, some of which included restricting boarding to the rear doors, implementing frequent surface cleaning, issuing personal protective equipment (PPE), and installing droplet barriers around the operator workstation. Other measures included increased cleaning and disinfecting of buses using a variety of methods.

To demonstrate the efficacy of UV light in disinfecting applications for COVID-19, Rock Region METRO (METRO) in Arkansas performed this research project under contract with Environmental Services Company (ESC) and the University of Arkansas for Medical Sciences (UAMS) in support of the Federal Transit Administration (FTA) Office of Research, Demonstration & Innovation. The tests were conducted with no passengers on board, after the vehicles were in service during normal operating hours.

Objectives

This project has one main objective. To assess the effectiveness of UV light systems in disinfecting the COVID-19 virus, the team will:

a) Test the interior surfaces of transit buses for COVID-19 after the use of a chemical disinfectant

b) Test the interior surfaces of transit buses for COVID-19 after the use of UV disinfectant lights

A secondary objective is to compare the effectiveness of UV light systems to reduce bacteria (*Escherichia coli* and total coliform) on the same surfaces.

Section 1 Introduction

The COVID-19 public health emergency made a significant impact on transit operations. Ridership decreased dramatically due to reduced capacity from social distancing. The costs of disinfecting buses rose exponentially due to increased cleaning schedules. Public transportation carries a negative perception based on the common belief that public transportation is not sanitary. The COVID-19 pandemic increased the need to disinfect buses as quickly and cost-efficiently as possible.

Current disinfecting practices involve using a liquid chemical by either spraying or fogging buses. To reduce the use of chemicals and the films that they leave behind, a UV disinfecting light system will be used to disinfect buses. This project is intended to show that the use of UV light disinfecting systems is effective at eliminating COVID-19 on interior surfaces, thus creating a safe environment for passengers and operators while reducing the spread of COVID-19. Therefore, demonstration of a UV light disinfecting system will assess if UV lights are effective enough to reduce the amount of chemicals being used and reduce the manpower hours and resources required for disinfecting buses.

The main objective of this project is to assess the effectiveness of UV light systems in disinfecting for the COVID-19 virus. The team will:

a) Test the interior surfaces of transit buses for COVID-19 after the use of a chemical disinfectant

b) Test the interior surfaces of transit buses for COVID-19 after the use of UV disinfectant lights

Section 2

Build and Test

The vehicles used for the test were Ford cutaway, 12-passenger vans. Two UV lights were placed inside the vans to allow the maximum coverage of the UV lights and increase the disinfecting potential. Figure 2-1 shows the test sampling areas.

Figure 2-1 *Sampling areas. Red boxes indicate the general sampling areas on the bus: (A) shows passenger door handles and seatbacks and (B) shows the wheelchair lift.*

Approach/Data Collection

Between November 16, 2021, and February 8, 2022, the third-party contractor Environmental Services Company, Inc. (ESC) completed 24 four sampling events at Rock Region METRO, located at 901 Maple Street, North Little Rock, Arkansas 72114.

In each event, ESC collected samples from four locations on each of two vans prior to sanitation with a disinfectant liquid fog and two vans prior to treatment with broad spectrum (UVA/B/C) UV lights. The same vans were then tested after sanitation. ESC also collected quality assurance samples.

Van interiors were treated with pulsed broad-spectrum UV light (UVA/B/C) using Sentry M1 mobile lamps (Puro, Lakewood, CO) for 30 minutes (dose range: 18-64 mJ/cm^2) or fogged with D-Germ spray (Wechem, Inc., Harahan, LA), an alkyl dimethyl benzyl ammonium chloride-based disinfectant.

COVID-19 Detection

Seven-hundred and sixty-eight samples were collected for COVID-19 detection, 384 before exposure to UV light or fog ("pre-treatment") and 384 after exposure ("post-treatment"). The samples were collected from the surfaces of handrails, seatbacks, or wheelchair lift handles on vans before and after UV light or chemical treatment using swabs wetted with MicroTest M4RT viral transport medium (ThermoFisher, Waltham, MA). The pre- and post-treatment samples from the handrails were collected from adjacent sites on the same rails and were therefore considered pairs. There was a total of 192 pre-treatment and 192 post-treatment handrail samples, half from the UV-treated vans and half from the chemical-treated vans. Pre-treatment samples from seatbacks were collected from a passenger-side seat while post-treatment samples were collected from a driver-side seat. For each sample type, there were a total of 96 pre-treatment and 96 post-treatment seatback samples, half from UV-treated vans and half from fog-treated vans. Finally, pre-treatment wheelchair lift samples were collected from the forward lift handle and post-treatment samples were collected from the rear lift handle. For each sample type, there were a total of 96 pre-treatment and 96 post-treatment wheelchair lift handle samples, half from UV-treated vans and half from fog-treated vans. SARS-CoV-2 was detected using a polymerase chain reaction (PCR) based nucleic acid detection method at American Esoteric Laboratories (Memphis, TN).

Bacterial Counts

Seven-hundred and sixty-eight samples were collected for measurement of *E. coli* and total coliforms. Sampling was performed as described for SARS-CoV-2 detection except that gauze pads wetted with phosphate-buffered saline were used instead of swabs. The populations of *E. coli* and total coliforms were

enumerated in each sample using the commercial assay kit Colilert-18 (IDEXX Laboratories, Westbrook, ME), which was adapted for bacterial enumeration on solid surfaces.

Statistical Analysis

To facilitate analysis, all test results for *E. coli* and total coliforms that were reported as <1 (below the limit of quantification) were converted to 0. Because the pre- and post-treatment handrail samples were collected from adjacent sites on the same handrails, they were considered paired samples. The data from the paired samples were visualized using matched pair plots and analyzed using the Wilcoxon Signed-rank Test. The pre- and post-treatment seatback samples were collected from different seats and the wheelchair lift handle samples were collected from different handles and therefore were not considered paired samples. These data were visualized using dot plots and analyzed using the Mann-Whitney Rank Sum Test.

Section 3

Evaluation Results

This project provided limited positive COVID-19 test results. Out of 768 samples, only 4 came back as positive. Of the positive samples, three tested negative post-treatment and one sample tested as indeterminate. Two of the positive results were from the vehicles that used the UV light treatment, and two positive samples were from the chemical treated vehicles. Overall, the positive sample results were likely due to random error or sampling noise. Based off the sample results, a direct conclusion cannot be drawn regarding the efficacy of UV light compared to chemical fogging. However, the dosage amounts of UV radiation measured on the vehicles exceeded the known amount that reduces active coronaviruses. Based off the amount of UV measured and the known levels needed to reduce coronaviruses, UV light in this application could provide the useful dosage to deactivate COVID-19.

One unexpected result is the finding that public transportation is more sanitary than it is perceived by the public. All the vehicles in this test carried passengers and were taken off duty to perform the testing. While this project does not provide sufficient data on COVID-19 testing, it should strengthen public confidence in the sanitary conditions of public transit.

Section 4 Conclusion

Rock Region METRO (METRO) performed this research project under contract with Environmental Services Company (ESC) and the University of Arkansas for Medical Sciences (UAMS) in support of the Federal Transit Administration (FTA) Office of Research, Demonstration & Innovation. The purpose of this project was to demonstrate the efficacy of UV light in disinfecting applications for COVID-19. The tests were conducted with no passengers on board, after the vehicles were in service during normal operating hours.

Overall, this study provides limited preliminary data supporting the effectiveness of UV light for the decontamination of vans used for public transit. The results for *E. coli* are inconclusive and the results for SARS-CoV-2 as well as total coliform bacteria on seatbacks and wheelchair lift handles are equivocal. However, the results for total coliforms from the paired handrail samples indicate that UV exposure may have successfully reduced bacterial load. In addition, the doses of UV radiation recorded from each sampled van far exceed the doses needed to inactivate most coronaviruses and bacterial species. These results taken together and interpreted within the context of prior literature documenting the germicidal effects of UV radiation indicate that UV light may be as effective as, or more effective than, chemical fogging. Nevertheless, additional studies are recommended.

Appendix A

Surface Sampling for Analysis of SARS-CoV-2 (COVID-19), *Escherichia coli* (*E.coli*), and Total Coliform Bacteria Inside Vans

Image courtesy of Rock Region Metropolitan Transit Authority

SEPTEMBER 2023

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Executive Summary

In 2021–2022, the Rock Region Metropolitan Transit Authority (Rock Region METRO) received funding in part to conduct a study designed to compare the effectiveness of two methods to disinfect vehicles used in public transit: fogging with a microbicidal chemical and ultraviolet (UV) light irradiation. Their primary endpoint of interest was the presence of detectable SARS-CoV-2 nucleic acid. Their secondary endpoints were two types of bacteria counts—*Escherichia coli* (*E. coli*) counts and total coliform bacteria. Rock Region METRO tasked Environmental Services Company, Inc., (ESC) of Little Rock, Arkansas, with conducting the sampling, data collection, and primary analysis, and contracted with the University of Arkansas for Medical Sciences (UAMS) for secondary analysis and evaluation of the results.

On each of 24 nonconsecutive days, ESC sampled four categories of frequently touched sites:

- Left-side passenger door handrail (left-side when boarding)
- Right-side passenger door handrail (right-side when boarding)
- Wheelchair lift handles
- Seatbacks

On each of the 24 days, Rock Region METRO and ESC treated two vehicles with chemical fogging using an alkyl dimethyl benzyl ammonium chloride-based disinfectant and two with pulsed broad-spectrum UV light. ESC sampled one area on each handrail, one wheelchair lift handle, and one seatback before treatment, and a different, nearby area from each handrail, a different wheelchair lift handle, and a different seatback after treatment. All sampled areas were tested for both the primary and secondary endpoints. A total of 768 samples were collected for SARS-CoV-2 detection and 768 for *E. coli* and coliform bacteria measurements. Half were collected before treatment and half after treatment. During the sampling process, ESC also measured the UV radiation fluence (dose) on at least one site for each UV-treated vehicle. They then provided the results to UAMS. UAMS considered most samples to be nonpaired and compared the aggregated means for the pre-treatment and post-treatment samples from each site. Pre- and post-treatment handrail samples were considered paired due to the proximity of the sampling sites and were analyzed as such using appropriate statistical methods.

Evaluation of the results at UAMS, in the context of the prior literature, revealed preliminary evidence to support the use of UV light to disinfect public transit vehicles. However, UAMS recommends additional studies based on the knowledge and experience gained in this project.

Findings

- The results of SARS-CoV-2 detection from chemical and UV treated vehicles were equivalent and equivocal.
- The results from *E. coli* measurement from chemical and UV treated vehicles were equivalent and equivocal.
- When considering all samples for total coliform measurement, the results from chemical and UV treated vehicles were equivalent and equivocal.
- When considering only the paired samples from handrails for which the pre-treatment sample had detectable total coliforms, UV light reduced bacteria in 87% of the pairs with median values of 3.1/25 cm^2 pre-treatment and 0/25 cm^2 (or <1) post-treatment, while chemical treatment reduced it in only 48% of pairs with no difference in median values.
- All measured UV radiation doses exceeded those reported to reduce SARS-CoV-2 and *E. coli* by ≥90%.

Section 1 | Introduction

Microbial contamination in public transit vehicles is a public health concern. Contaminated hand-touch sites may serve as fomites, spreading pathogenic microorganisms among passengers. For example, in a large-scale study on the subway in New York City, 12% and 31% of bacteria species found on surfaces in the subway system were either known human pathogens or potentially opportunistic pathogens, respectively, based on genome analysis [1]. Moreover, antibiotic-resistant bacteria were also recovered by culture methods from the surfaces on the subway [1]. Similarly, bacteria were detected in 95% of samples collected on hand-touch surfaces in the public transit system and in hospitals in London, with a median total aerobic count of 12 CFU/cm^2 [2]. While the U.S. Centers for Disease Control and Prevention considers respiratory droplets and airborne virus particles to be the major means of transmission of the novel sudden acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2), at least one study demonstrated that it could remain viable on plastic and stainless steel surfaces for at least 72 hours [3]. Therefore, reduction of the microbial contamination level on hand-touch surfaces could decrease the probability of transmitting pathogens, including SARS-CoV-2, among public transit users.

Disinfectants are agents that can be used to decontaminate surfaces. Two of the most used types of disinfectants are microbicidal chemicals and ultraviolet (UV) radiation. UV radiation has major advantages over chemicals, including lower toxicity and more convenient handling and storage methods. It has been used successfully in drinking water and wastewater treatments, food processing, HVAC airduct decontamination, and surface disinfection in hospitals. It kills microorganisms primarily by direct DNA damage, forming pyrimidine dimers in DNA via photochemical reaction [4].

However, although UV radiation is known to inactivate bacteria and viruses in well-controlled laboratory settings, it may be less effective in real-world environments. Direct exposure at a sufficient fluence (dose) and duration is required for pathogen inactivation by UV radiation and that may not be easily achieved in a complex environment with numerous obstructions, varying surface materials and contours, and varying distances between those surfaces and the UV light source. In addition, not all UV radiation is created equal. Various UV lamps can emit radiation at different wavelengths, including UVA (320 to 400 nm), UVB (280 to 320 nm), and UVC (220 to 280 nm), but UVC light is the most effective for microbial inactivation [5]. Finally, the sensitivity of microorganisms to UV light varies greatly. For example, a dose of 5-10 mJ/cm^2 UV radiation is required to inactivate over 99.9% of most *E. coli* strains [6]. It is estimated that the median dose required to reduce 90% of SARS-CoV-2 is approximately 10.6 mJ/cm^2 [7], but SARS-CoV-2 viruses were partially (>90%) inactivated at 10 mJ/cm^2 and completely (99.999%) inactivated only at 40 mJ/cm^2 at 254 nm [8]. Thus, it is critical to assess the efficacy of UV irradiation for disinfection of real-world

public spaces, especially with reference to currently established disinfection methods.

Project Goal/Hypothesis

This project was designed to evaluate a portable broad-spectrum (UVA/B/C) UV light source (Puro Sentry M1) for disinfection of hand-touch surfaces in a public transit system compared with a currently used chemical disinfectant method. The primary endpoint was SARS-CoV-2 nucleic acid on commonly touched surfaces on public vans operated by the Rock Region Metropolitan Transit Authority (Rock Region METRO) in Pulaski County, Arkansas, USA. *E. coli* and total coliform counts were secondary endpoints.

Section 2 | Approach/Data Collection

Between November 16, 2021, and February 8, 2022, third-party contractor Environmental Services Company, Inc. (ESC) completed 24 sampling events at Rock Region METRO located at 901 Maple Street, North Little Rock, Arkansas 72114.

In each event, ESC collected samples from four locations on each of two vans prior to sanitation with a disinfectant liquid fog and two vans prior to treatment with broad spectrum (UVA/B/C) UV lights. The same vans were then tested after sanitation. ESC also collected quality assurance samples.

Van Treatment. Van interiors were treated with pulsed broad-spectrum UV light (UVA/B/C) using Sentry M1 mobile lamps (Puro, Lakewood, CO) for 30 minutes (dose range: 18-64 mJ/cm^2) or fogged with D-Germ spray (Wechem, Inc., Harahan, LA), an alkyl dimethyl benzyl ammonium chloride-based disinfectant.

Figure 2-1 *Sampling areas. Red boxes indicate the general sampling areas on the bus: (A) shows passenger door handles and seatbacks and (B) shows the wheelchair lift.*

SARS-CoV-2 Detection. Seven-hundred and sixty-eight samples were collected for SARS-CoV-2 detection, 384 before exposure to UV light or fog ("pre-treatment") and 384 after exposure ("post-treatment"). The samples were collected from the surfaces of handrails, seatbacks, or wheelchair lift handles (Figure 2-1) on vans before and after UV light or chemical treatment using swabs wetted with MicroTest M4RT viral transport medium (ThermoFisher, Waltham, MA). The pre- and post-treatment samples from the handrails were collected from adjacent sites on the same rails and were therefore considered pairs. There was a total of 192 pre-treatment and 192 post-treatment handrail samples, half from the UV-treated vans and half from the chemical-treated vans. Pre-treatment samples from seatbacks were collected from a passenger-side seat while post-treatment samples were collected from a driver-side seat. For each sample type, there were a total of 96 pre-treatment and 96 post-treatment seatback samples, half from UV-treated vans and half from fog-treated vans. Finally, pre-treatment wheelchair lift samples were collected from the forward lift handle and post-treatment samples were collected from the rear lift handle. For each sample type, there were a total of 96 pre-treatment and 96 post-treatment wheelchair lift handle samples, half from UV-treated vans and half from fog-treated vans. SARS-CoV-2 was detected using a polymerase chain reaction (PCR) based nucleic acid detection method at American Esoteric Laboratories (Memphis, TN).

Bacterial Counts. Seven-hundred and sixty-eight samples were collected for measurement of *E. coli* and total coliforms. Sampling was performed as described for SARS-CoV-2 detection except that gauze pads wetted with phosphate-buffered saline were used instead of swabs. The populations of *E. coli* and total coliforms were enumerated in each sample using the commercial assay kit Colilert-18 (IDEXX Laboratories, Westbrook, ME), which was adapted for bacterial enumeration on solid surfaces.

Statistical Analysis. To facilitate analysis, all test results for *E. coli* and total coliforms that were reported as <1 (below the limit of quantification) were converted to 0. Because the pre- and post-treatment handrail samples were collected from adjacent sites on the same handrails, they were considered paired samples. The data from the paired samples were visualized using matched pair plots and analyzed using the Wilcoxon Signed-rank Test. The pre- and post-treatment seatback samples were collected from different seats and the wheelchair lift handle samples were collected from different handles and therefore were not considered paired samples. These data were visualized using dot plots and analyzed using the Mann-Whitney Rank Sum Test.

Section 3 | Evaluation Results

SARS-CoV-2. SARS-CoV-2 was detected in only 8 samples out of 768 (1%), 4 pre-treatment and 4 post-treatment. Among the four positive pre-treatment samples, three had negative corresponding post-treatment samples and one post-treatment sample was reported as indeterminate. Two of the positive pre-treatment samples with a corresponding negative were collected from UV-treated vans, while the remaining positive pre-treatment sample with a corresponding negative as well as the pre-treatment sample with the indeterminate corresponding sample were collected from chemical-treated vans. While the trend from positive to negative after sanitation is consistent with an effect of UV light, it is important to note that the four positive post-treatment samples had negative corresponding pre-treatment samples, indicating randomness. Taken together, the positive results are likely due to random error or sampling noise. Overall, the data from SARS-CoV-2 testing are equivocal and direct conclusions could not be drawn regarding the efficacy of UV light compared to chemical fogging to reduce the SARS-CoV-2 load.

Importantly, however, the mean dose of UV radiation recorded on the vans during the sanitation procedure was 33.5±8 mJ/cm^2 (mean±SD). This value meets or exceeds most doses that have been reported to reduce active coronaviruses by ≥90% in prior studies [7,8], including SARS-CoV-2 [8]. These data indicate that the UV treatment regimen tested here may well be effective to reduce active SARS-CoV-2 despite the limited data from PCR testing. A major caveat, however, is that the distance of the UV radiation detectors from the UV light sources for each measurement is not known nor how those distances compare to the distances between the lights and the sampling sites.

E. coli. *E. coli*, a specific bacterial species in the coliform group, was detected in only four samples and the count never exceeded 1 per 25 cm^2 sampling area, which could be due to random error. Thus, the results for *E. coli* are inconclusive.

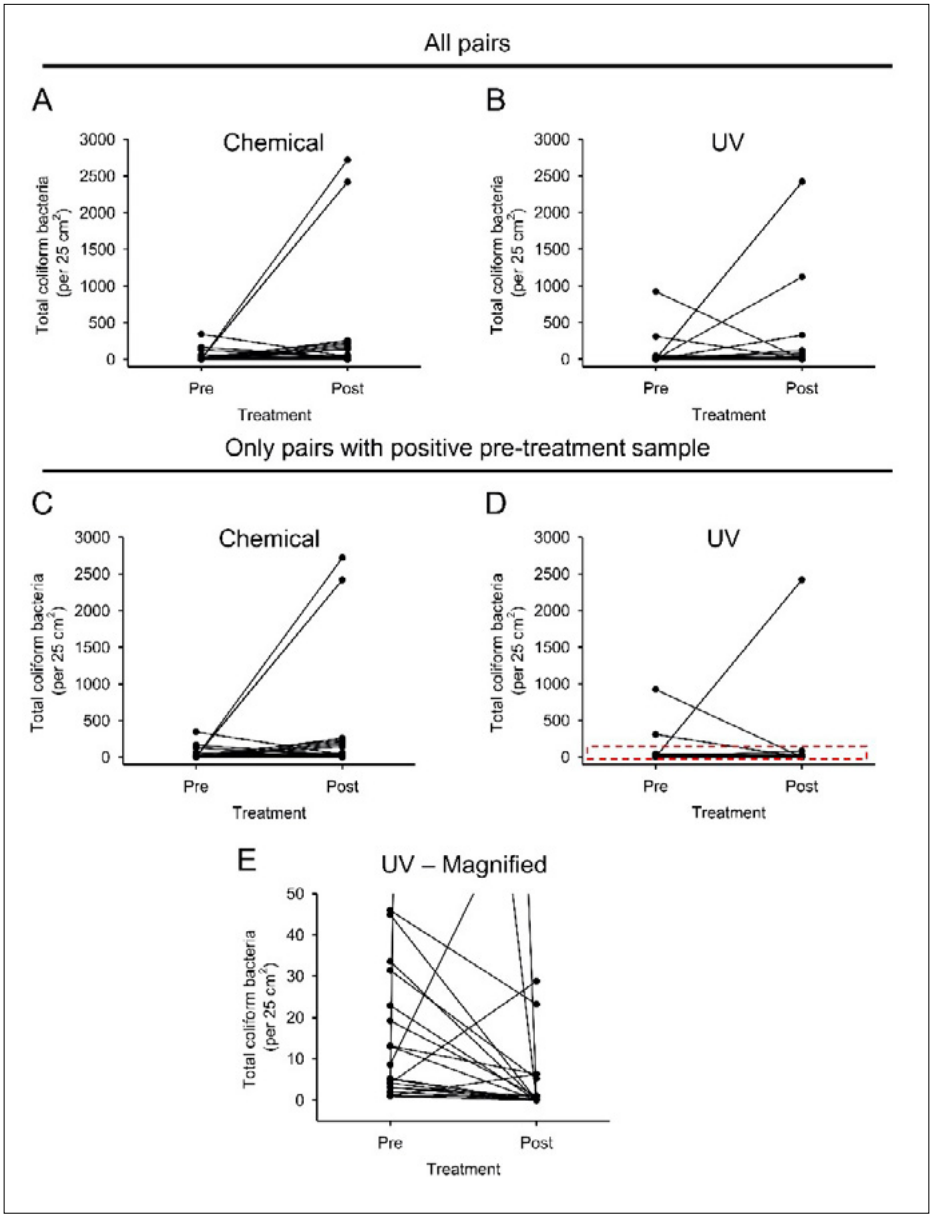

Figure 3-1 *Total coliform bacteria from paired handrail samples. (A) Total coliform counts before (Pre) and after (Post) chemical treatment for all paired samples. (B) Total coliform counts before and after UV treatment. (C) Total coliform counts before and after chemical treatment for only sample pairs with positive pre-treatment values. (D) Total coliform counts before and after UV treatment for only sample pairs with positive pre-treatment values. (E) Magnification of the area in panel D indicated by the red dashed box.*

Total Coliform Bacteria. For total coliforms, a measure of all bacterial species in the coliform group, a total of 179 individual samples had a detectable level so analysis was possible (Figure 3-1). Paired analysis of the handrail samples from

the chemical-treated vans revealed that 10.4% (10 of 96 pairs) had lower total coliform counts in the post-treatment samples compared to the pre-treatment samples, and the mean count was significantly greater post-treatment ($p = 0.003$) (Figure 3-1A). When considering all paired handrail samples from the UV-treated vans, 13.5% (13 of 96) had lower total coliform counts in the post-treatment samples and there was no significant difference between the mean counts for the pre-treatment and post-treatment samples ($p = 0.746$) (Figure 3-1B).

It is difficult to interpret data from the paired handrail samples in which total coliforms were below the limit of quantification (<1 per 25 cm^2) in the pre-treatment sample. Those pairs do not provide useful information because the value cannot further decrease post-treatment. Thus, it was considered appropriate to separately analyze the results after exclusion of those pairs. When considering only paired samples in which the pre-treatment sample had detectable total coliform bacteria (15 pairs from the UV-treated vans and 21 from the chemical-treated vans), chemical treatment reduced the counts in 48% of the pairs (10 out of 21) with median values of 3.0 per 25 cm^2 pre-treatment and 3.1 per 25 cm^2 post-treatment (Figure 3-1C). UV treatment, on the other hand, reduced total coliform count in 87% of the pairs (13 out of 15) with median values of 3.1 per 25 cm^2 pre-treatment and 0 per 25 cm^2 (or <1) post-treatment ($p = 0.013$) (Figure 3-1D,E). This is consistent with previous studies that demonstrated the effectiveness of UV treatment for microbial reduction on a railcar [9] and in hospitals [10,11,12] and indicates that UV light may be more effective than chemicals, or at least as effective, for reduction of total coliforms. However, this is a preliminary conclusion, and it is advised to confirm this result with additional studies.

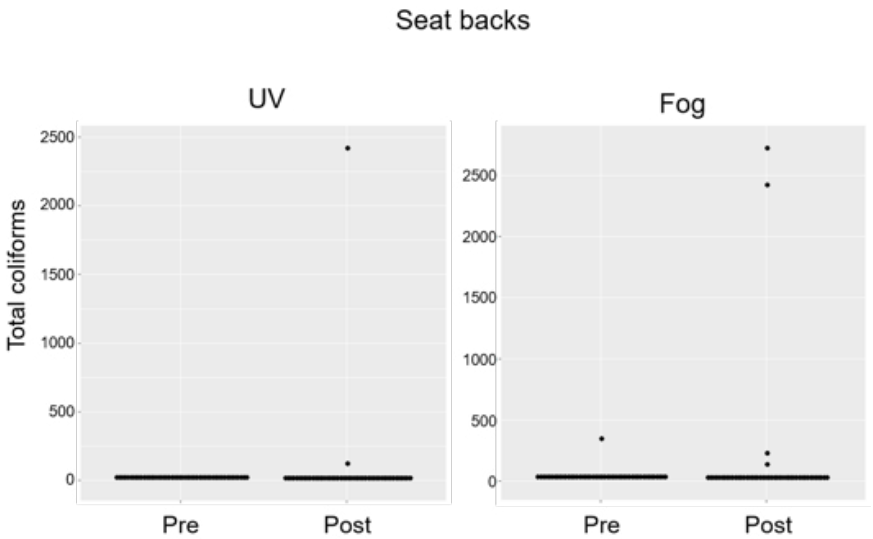

Figure 3-2 *Total coliform counts from seatbacks. Total coliform bacteria counts per 25 cm^2 from seatbacks before (Pre) and after (Post) treatment with UV light or chemical fog (Fog). Each dot represents one data point. Most points aggregated at or near 0, creating the appearance of a line.*

As stated, the mean dose of UV radiation recorded on the vans during the sanitation procedure was 33.5±8 mJ/cm^2 (mean±SD). Importantly, this value far exceeds doses required to reduce most bacteria, including *E. coli* and other coliforms, by ≥99.9% in various media (water, solid surfaces, etc.) [13]. This is consistent with the results from the paired handrail samples and further indicates that the UV irradiation procedure used in this study is likely effective. But, again, the distances between the UV detector and light source compared to the distances between the lights and the sampled surfaces is not known.

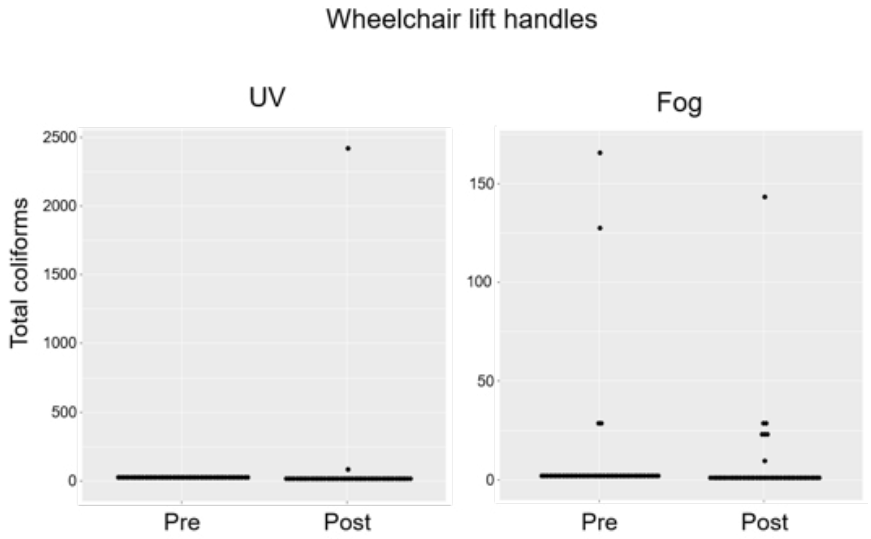

Figure 3-3 *Total coliform counts from wheelchair lift handles. Total coliform bacteria counts per 25 cm^2 from wheelchair lift handles before (Pre) and after (Post) treatment with UV light or chemical fog (Fog). Each dot represents one data point. Most points aggregated at or near 0, creating the appearance of a line.*

The results from the seatback samples and wheelchair lift handles were less clear. These samples were collected from different seats and different wheelchair lift handles, so paired analysis was not appropriate for them. Instead, the data from these sites were analyzed by comparing the overall means from the pre-treatment and post-treatment samples. The data are displayed as dot plots in Figures 3-2 and 3-3. Most samples had undetectable levels of total coliform bacteria per 25 cm^2. UV treatment and fog treatment were equivalent in the sense that there were no significant differences between the pre-treatment and post-treatment means for either the seatbacks or wheelchair lift handles regardless of treatment method. All p values were in the range of 0.223 to 0.879.

Chain of Custody. Within the initial reports from Rock Region METRO and ESC, UAMS received chain of custody forms that included the names and signatures of the individuals who collected samples for both SARS-CoV-2 and bacteria

testing, as well as dates and times of sampling and relevant details about the sampling method. UAMS reviewed each document in detail. Almost all the documents were appropriately filled out and complete. One chain of custody form from November 23, 2021, included a date for field blank collection but not a time. The documents from several dates included revisions, but all revisions appeared to be within reason and appropriately documented. No additional chain of custody information was provided to UAMS documenting possession of the samples after collection and before or during analysis.

Section 4 | Conclusions

Overall, this study provides limited preliminary data supporting the effectiveness of UV light for the decontamination of vans used for public transit. While the results for *E. coli* are inconclusive and the results for SARS-CoV-2 as well as total coliform bacteria on seatbacks and wheelchair lift handles are equivocal, the results for total coliforms from the paired handrail samples, and when considered in the context of the greater literature documenting the germicidal effects of UV radiation, indicate that UV exposure may have successfully reduced bacterial load. In addition, the doses of UV radiation recorded from each sampled van far exceed the doses needed to inactivate most coronaviruses and bacterial species. Nevertheless, additional studies are advisable. For instance, it may be beneficial to perform the study again using total aerobic count as an indicator for the evaluation of sanitation effectiveness, as this is a more sensitive endpoint. Alternatively, surfaces in the vehicles could be inoculated with nonpathogenic strains of bacteria before testing. Commercial contact plates supplemented with neutralizers (e.g., tryptic soy agar with lecithin and Tween-80), which inactivate potential residues of disinfectants and therefore reduce the risk of false negative results, could also be used as an efficient and convenient method for environmental surface microbial monitoring [14]. Finally, viral cultures may be better for detection of viable SARS-CoV-2 than nucleic acid-based methods. Little is known about the effect of UV radiation–induced lesions in the SARS-CoV-2 genome on PCR nucleic acid detection. PCR detection methods amplify and quantify small portions of the viral genome and it is possible that the UV-induced lesions do not occur in those portions. Furthermore, even if the lesions do occur in the sites that are targeted for amplification, it is not known with certainty that they would prevent the amplification. Thus, false positives could occur.

Acronyms and Abbreviations

CFU	Colony-forming unit
cm^2	Square centimeters
E. coli	Escherichia coli
ESC	Environmental Services Company, Inc.
mJ	Milljoules
nm	Nanometers
PCR	Polymerase chain reaction
Pre	Pre-treatment
Post	Post-treatment
Rock Region METRO	Rock Region Metropolitan Transit Authority
SARS-CoV-2	Severe acute respiratory syndrome – coronavirus 2
UAMS	University of Arkansas for Medical Services
UV	Ultraviolet

References

1. Afshinnekoo, E., Meydan, C., Chowdhury, S., Jaroudi, D., Boyer, C., et al. (2015). "Geospatial Resolution of Human and Bacterial Diversity with City-Scale Metagenomics." *Cell Systems* 1(1): 72–87.
2. Otter, J. A., and French, G. L. (2009). "Bacterial contamination on touch surfaces in the public transport system and in public areas of a hospital in London." *Letters in Applied Microbiology* 49(6): 803–805.
3. van Doremalen, N., Bushmaker, T., Morris, D. H., Holbrook, M. G., Gamble, A., et al. (2020). "Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1." *The New England Journal of Medicine* 382: 1564–67.
4. Kebbi, Y., Muhammad, A. I., Sant'Ana, A. S., do Prado-Silva, L., Liu, D., and Ding, T. (2020). "Recent advances on the application of UV-LED technology for microbial inactivation: Progress and mechanism." *Comprehensive Reviews in Food Science and Food Safety* 19(6): 3501–27.
5. FDA. (2021). UV Lights and Lamps: Ultraviolet-C Radiation, Disinfection, and Coronavirus.
6. Chang, J. C., Ossoff, S. F., Lobe, D. C., Dorfman, M. H., Dumais, C. M., Qualls, R. G., and Johnson, J. D. (1985). "UV inactivation of pathogenic and indicator microorganisms." *Applied and Environmental Microbiology* 49(6): 1361–65.
7. Heßling, M., Hönes, K., Vatter, P., and Lingenfelder, C. (2020). "Ultraviolet irradiation doses for coronavirus inactivation – review and analysis of coronavirus photoinactivation studies." *GMS Hygiene and Infection Control* 15: Doc08.
8. Patterson, E. I., Prince, T., Anderson, E. R., Casas-Sanchez, A., Smith, S. L., et al. (2020). "Methods of Inactivation of SARS-CoV-2 for Downstream Biological Assays." *Journal of Infectious Diseases* 222(9): 1462–67.
9. American Public Transportation Association. (2020). "Cleaning and Disinfecting Transit Vehicles and Facilities During a Contagious Virus Pandemic." White paper. https://www.apta.com/research-technical-resources/standards/security/apta-ss-iss-wp-001-20/
10. Casini, B., Tuvo, B., Cristina, M. L., Spagnolo, A. M., Totaro, M., Baggiani, A., and Privitera, G. P. (2019). "Evaluation of an ultraviolet C (UVC) light-emitting device for disinfection of high touch surfaces in hospital critical areas." *International Journal of Environmental Research and Public Health* 16(19): 3572.
11. Hosein, I., Madeloso, R., Nagaratnam, W., Villamaria, F., Stock, E., and Jinadatha, C. (2016). "Evaluation of a pulsed xenon ultraviolet light device for isolation room disinfection in a United Kingdom hospital." *American Journal of Infection Control* 44(9): e157–e161.
12. Villacís, J. E., Lopez, M., Passey, D., Santillán, M. H., Verdezoto, G., et al. (2019). "Efficacy of pulsed-xenon ultraviolet light for disinfection of high-touch surfaces in an Ecuadorian hospital." *BMC Infectious Diseases* 19(1): 575.
13. Masjoudi, M., Mohseni, M., and Bolton, J. R.(2021). "Sensitivity of bacteria, protozoa, viruses, and other microorganisms to ultraviolet radiation." *Journal of Research of the National Institute of Standards and Technology* 126, Article No. 126021.
14. Pinto, F., Him, S., Girdlestone, S., and Maillard, J. Y. (2009). "Evaluation of the effectiveness of commercially available contact plates for monitoring microbial environments." *Letters in Applied Microbiology* 48(3): 379–82.

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