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# Evaluation of an Automated Truck Wash Modified with a Two-Stage Decontamination System for Sanitizing Transport Trucks At Large Farms or Animal Contaminant Facilities

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## ABSTRACT

An automated truck wash study was conducted at a large layer hen facility to determine the effectiveness of a modified decontamination system for sanitizing semi-trucks and other farm vehicles. The commercial automated power washing system was modified with a fixed gantry that applied a chlorine dioxide ($ClO_2$) disinfectant rinse as the truck exited the biosecurity facility. The truck decontamination study included the primary study plus one smaller *Bacillus atrophaeus* spore study, as well as air and water sampling. The goal of the field study was to determine the effectiveness of a two-stage automated decontamination system for sanitizing a large, semi-tractor trailer. The primary study objective was to evaluate two power washing techniques (power wash only with a surfactant or power wash with surfactant and a $ClO_2$ rinse). The second objective was to evaluate the decontamination methods on four coupon materials (glass, painted metal, plastic, rubber) to determine the effectiveness of the two-stage wash system on inoculated coupons. The third objective was to determine the effectiveness of the decontamination methods on coupon locations on the truck (front windshield, middle side of trailer, undercarriage). The fourth objective was to determine the effectiveness of the decontamination methods on coupon surface type (coupons coated with or without synthetic grime).

The primary study evaluated 48 decontamination treatments to assess their ability to inactivate the MS2 bacteriophage, which is the viral surrogate selected for the study. The results show that the two-stage decontamination treatments increased log10 reduction of the MS2 phage. Log10 reduction increased an average of 247% and 118% for the non-grimed and grimed coupons, respectively, when comparing the automated wash with and without $ClO_2$ rinse across all locations and material types. The average log10 reduction increased from 0.94 to 1.89 for the automated wash and the automated wash + $ClO_2$ rinse, respectively, for the grimed coupons, across all coupon locations and materials. The average log10 reduction increased from 1.23 to 2.17 for the automated wash without $ClO_2$ and the automated wash + $ClO_2$ rinse, respectively, for the non-grimed coupons, across all coupon locations and materials. These results show that combining the $ClO_2$ disinfectant rinse with the automated power wash increased viral efficacy by an average of one log (grimed coupons). Evaluation of the two-stage tuck decontamination system confirms that combining a power wash with a disinfectant rinse increases the ability of the system to sanitize transport trucks and increase farm biosecurity.

# 1. Introduction

Large agricultural production facilities are acutely aware that farm biosecurity is a crucial priority with the increasing frequency and severity of animal and even crop epidemics and global pandemics [1-3]. Once a high-risk animal pathogen or zoonotic disease is introduced and/or established, it can rapidly reach epidemic stages in agricultural regions due to the concentration and proximity of animal production facilities. The rapid spread of the porcine parvovirus among swine producers, or the highly pathogenic avian influenza (HPAI) among poultry producers, highlights the need to decontaminate all vehicles entering such animal production facilities. Although large commercial, automated decontamination systems are available for producers, there is a scarcity of knowledge about the effectiveness of such systems and which decontamination procedures are most cost-effective and efficient, based on the production facility scale and operating conditions. Evaluation of viable, cost-effective decontamination options for sanitizing trucks is a critical research need. Increased viral outbreaks are a strong impetus to implement effective and efficient perimeter biosecurity systems at farm entry points to help prevent disease transmission.

Semi-tractor trailers used to deliver feed or transport commodities require large, automated power washing systems that can rapidly sanitize trucks that arrive or leave with high frequency. Large, automated truck washing systems are commercially available, but they only feature power washing with a surfactant, a one-stage vehicle decontamination procedure. Also, large, automated power washing systems are typically designed to handle a limited range of truck sizes. Automated truck power washing systems generally have a fixed power wash gantry that cannot be adjusted to accommodate different truck sizes or shapes. Fixed gantry truck washing systems may vary in effectiveness due to decreased water pressure as the nozzle distance increases for smaller truck sizes and shapes [4].

Currently, no published decontamination studies have evaluated the effectiveness of a large, automated washing system for sanitizing all surfaces on a typical truck used by farms or large-scale animal producers. However, several field studies have tested the effectiveness of a portable power washing system involving a two-stage decontamination system [4-10]. The two-stage system included a power washing with a hand wand, followed by a hand wand disinfectant application. This field study evaluated an automated, commercial power washing system located at a large poultry production facility in Iowa.

In this study, a bacteriophage (MS2 phage) and a spore-forming bacterium (*Bacillus atrophaeus*) served as surrogate microorganisms to represent viral and bacterial pathogens that may enter poultry production facilities (Table **1**). The bacteriophage MS2 is a non-enveloped, positive-sense, single-stranded RNA, or (+) ssRNA virus that infects the *Escherichia coli* and other members of the *Enterobacteriaceae* [11-12]. Both MS2 and coronaviruses have the same structure ((+) ssRNA virus), making MS2 a rationale surrogate for most coronavirus efficacy studies. *B. atrophaeus* is a gram-positive, endospore-forming bacterium that is commonly found in the environment [13]. *B. atrophaeus* spores are good surrogates due to inexpensive culture and assaying costs and that they are extremely resistant to heat and radiation, along with a wide range of chemicals [14-17].

**Table 1:** Bacteriophage and spore-forming bacteria surrogate description.

| Microorganism | Description | Host or Cultivation Media |
|---|---|---|
| MS2 bacteriophage[1] (ATCC 15597-B1) | Non-enveloped, single-stranded RNA bacteriophage<br>Representative microorganisms for non-enveloped viruses | Bacterial host: *E. coli* ATCC 15597<br>37°C, LB broth |
| *Bacillus atrophaeus* (Mesa Labs)[2] | Gram-positive, spore-forming aerobic bacterium<br>Sterility test organism for ethylene oxide, dry heat sterilization (160°C), and formaldehyde fumigation | 30-35°C<br>Tryptic soy broth |

[1]**Reference:** Calfee *et al.* (2015) Evaluation of MS2 bacteriophage for use as viral decontamination biological indicators. U.S. EPA QAPP.
[2]**Reference:** https://biologicalindicators.mesalabs.com/apex/

Although this field study focused on vehicle decontamination, it builds upon a similar barn decontamination study conducted in 2016 that focused on sanitizing poultry barns infected by Highly Pathogenic Avian Influenza (HPAI), using the same bacteriophage and spore-forming bacteria surrogates. In the 2016 study, these surrogates were selected because they were more difficult to inactivate than an enveloped RNA virus such as HPAI. In particular, the bacteriophage surrogate (MS2) shares comparable resistance to disinfection as more hardy viruses, such as foot and mouth disease (FMD), porcine reproductive and respiratory syndrome (PRRS), and porcine epidemic diarrhea (PED).

This field study evaluated an automated truck washing system modified to include a chlorine dioxide ($ClO_2$) disinfectant application. Chlorine dioxide formulated as a liquid disinfectant is $ClO_2$ gas dissolved in water along with stabilizer and other additives. It is a weaker oxidant than other chlorine disinfectants such as hypochlorous acid or ozone. However, it has a greater oxidation capacity at lower pH [18]. Chlorine dioxide is effective against bacteria, mycobacteria, viruses, fungi, and spore-forming bacteria [19].

The goal of the field study was to determine the effectiveness of a two-stage automated decontamination system for sanitizing a large, semi-tractor trailer. The primary study objective was to evaluate two power washing techniques (power wash only with a surfactant or power wash with surfactant and a $ClO_2$ rinse). The second objective was to evaluate the decontamination methods on four coupon materials (glass, painted metal, plastic, rubber) to determine the effectiveness of the two-stage wash system on inoculated coupons. The third objective was to determine the effectiveness of the decontamination methods on coupon locations on the truck (front windshield, middle side of trailer, undercarriage). The fourth objective was to determine the effectiveness of the decontamination methods on coupon surface type (coupons coated with or without synthetic grime).

## 2. Materials and Methods

The field study was a collaboration between two federal agencies and a private company specializing in decontamination services. The two federal agencies providing oversight and technical support were 1) U.S. Environmental Protection Agency (EPA), Chemical Biological Radiological and Nuclear (CBRN) Consequence Management Advisory Division (CMAD), and 2) the United States Department of Agriculture (USDA). The private company (SABRE bioWALL, Slingerlands, NY) supplied much of the equipment, supplies, study location, and personnel. The primary goal of the field study was to evaluate the ability of a commercial automated truck wash that was modified to include a disinfectant rinse application to decontaminate tractor-trailers entering a large poultry egg processing facility. The effectiveness of the truck wash was evaluated using the MS2 phage surrogate. A secondary study compared the decontamination techniques using a spore-forming bacteria using *B. atrophaeus*. Air and water samples were also collected during the power washing cycle for each of the treatments. Air samples were collected to determine whether the high-pressure power washing aerosolized the surrogates from the coupon surfaces. Water samples were collected to determine whether the high-pressure power washing dislodged the surrogates from the coupon surfaces and entered the wastewater stream.

The primary field trial was designed as a factorial study including four factors: 1) two power washing methods; 2) four surface materials; 3) three coupon locations on truck chassis; 4) two coupon surface types (grimed or non-grimed). The factorial study was conducted inside a decontamination building that contained an automated truck power washing system (Whiting System Inc, Alexander, AR) and a modified disinfectant rinse system developed by Sabre BioWALL (Image **1**). The automated truck washing system was operated under normal wash cycle conditions to evaluate the effectiveness of the commercial system operating under the facility's routine truck decontamination schedule.

Previous laboratory studies conducted by Sabre bioWALL were used to determine the most effective disinfectant rinse parameters. The parameters established by Sabre and used in this study were: 1) $ClO_2$ liquid concentration of 50 mg/l chassis rinse and 200 mg/l undercarriage rinse, and 2) a single pass disinfectant rinse. Sabre bioWALL established these parameters to balance multiple objectives such as decontamination effectiveness, cost savings, environmental issues, and health and safety concerns.

**Image 1:** Power washing a tractor-trailer with the Whitening power washing gantry system.

The study coupons consisted of four materials that represented four surfaces commonly found on large trucks. These materials were: 1) painted metal; 2) HDPE plastic; 3) glass; and 4) tire rubber. The coupons also had clean (un-grimed) and soiled (grimed) surfaces representing clean and trucks covered with road grime. The synthetic grime consisted of serum protein and organic dust. Serum protein is widely used in Biological Indicator (BI) preparations to mimic different levels of organic loading. The organic dust used was composed of poultry droppings. Experimental controls, including media blanks, procedural blanks (neutralization controls), positive control inoculums, and transit control, accounted for the potential of MS2 to be sequestered or inactivated by the inoculated materials. A description of the control and reference coupons is summarized in Table **2**.

**Table 2:** Description of control and reference coupons.

| Control and Reference Coupons | Parameter | Run | Treatment Methods | Material Types per Location | Locations per Run | Number of Coupons |
|---|---|---|---|---|---|---|
| Reference coupon (un-grimed) | MS2 phage | 8 | 2 | 4 | 3 | 48 |
| Reference coupon (grimed) | MS2 phage | 8 | 2 | 4 | 3 | 48 |
| Experimental disk (spore disk) | *B. atrophaeus* | 8 | 2 | 1 | 3 | 36 |
| Air samples (DFU) | MS2 phage | 8 | 2 | 1 | 2 | 2 |
| Air samples (PTFE) | MS2 phage | 8 | 2 | 1 | 2 | 4 |
| Water samples (during test) | MS2 phage & *B. atrophaeus* | 2 | 2 | - | - | |
| Water sample (background) | MS2 phage & *B. atrophaeus* | - | - | - | - | 1 |
| **Subtotal** | | | **145** | | | |

## 2.1. Preparation of MS2 bacteriophage, *E. coli* C-3000 stock, and B. *atrophaeus* spore strips

The viral surrogate, MS2 bacteriophage, was purchased from the American Type Culture Collection (ATCC) (product ATCC® 15597-B1™, Manassas, VA, USA)), which uses *Escherichia coli* (*E. coli*) strain C-3000 as host (ATCC® 15597™). When grown in the presence of *E. coli*, MS2 forms very hazy plaques with large halos in Luria-Bertani (LB) agar.

Microchem Laboratories (Round Rock, TX) prepared the MS2 phage by adding the phage to LB agar prior to the *E. coli* and LB agar overlay. The agar was prepared by adding 0.4 ml of LB broth to a vial with the phage pellet. Once mixed, the concentrate was added to a vial containing 5-6 ml of LB broth and then incubated at 35±2°C for 24 hr. After incubation, 2-3 sterile tubes containing 10 mL of LB broth were inoculated with 100 µL of the *E. coli* suspension and then placed into an incubator for 12-16 h at 37±2°C. Once incubation was completed, the 10 mL

tubes were aseptically combined into a sterile 50-ml centrifuge tube. The tube was centrifuged for 15 min at 5000 revolutions per minute (rpm), and the supernatant was decanted. The remaining cells in the tube were resuspended with 15 ml of 10 mM magnesium sulfate and stored at 2-8°C.

An active broth culture of *E. coli* C-3000 was prepared by inoculating a sterile tube containing 5 ml of LB broth with 100 µl of *E. coli* cell stock and incubating for 4-6 h at 35±2°C. After incubation, 100 µl of the broth culture was inoculated with 5 ml of LB top agar. The LB top agar and inoculum were mixed and immediately poured onto an LB agar plate. The freeze-dried pellet of MS2 was added to 0.5 ml of LB broth. The surface of the LB agar was then covered with 0.5 ml of the phage suspension. The phage suspension was incubated at 37±2ºC for 24 hr. Following incubation, the soft agar layer was added to a 50-mL centrifuge tube with 5 ml of SM buffer. The tube was centrifuged at 7,000 rpm for 15 min, and the supernatant was removed using a micropipette. The supernatant was cleaned by filtering through a 0.2 µm filter.

The BI spore discs were inoculated with *Bacillus atrophaeus* with an average of $10^6$ spores on a stainless-steel disc. One spore disk was collocated with the MS2 coupons during the runs with water only and with water and chlorine dioxide. Eighteen spore disks were used during each of the wash tests for a total of 36 spore disks.

## 2.2. Coupon Preparation, Inoculation, and Control Descriptions

The four reference materials were cut into coupons (13 x 13 mm), cleaned, and steam-sterilized at 121°C for 30 min (Tuttnauer Steam Sterilizer, Autoclave Model No. 3850M). Coupons were glued onto small magnets and stored in sealed containers prior to inoculation with MS2 phage suspension. The surface area of a coupon was approximately 169 $mm^2$, which is deemed acceptable for MS2 enumeration and inactivation estimates.

- Painted metal. Black iron (carbon steel) flat stock painted with a typical gloss paint used on vehicles (Port Welding Service, Albany, NY).
- HDPE plastic. High-density polyethylene plastic sheeting (Piedmont Plastics, Albany, NY).
- Glass. Non-porous; glass stock cut (Lowes, Albany, NY).
- Tire rubber. Porous; (Phillips Hardware, Delmar, NY).

The synthetic grime was applied by adding 75 µl of a sterile soil solution containing 133 mg/ml poultry waste dust to each autoclaved and labeled coupon and allowed to dry overnight. The organic poultry waste on the coupon surface averaged a loading density of 10 mg per coupon or 40 mg/$in^2$. Mesa Laboratories inoculated each of the four types of material coupons with 100 µ containing >1 × $10^8$ plaque-forming units (PFU) of the MS2 bacteriophage. Coupons were glued to steel washers attached to the truck with static magnets (Image **2**). Each coupon and spore disc were labeled with a unique numerical identifier to track each coupon from set up and preparation to data analysis.

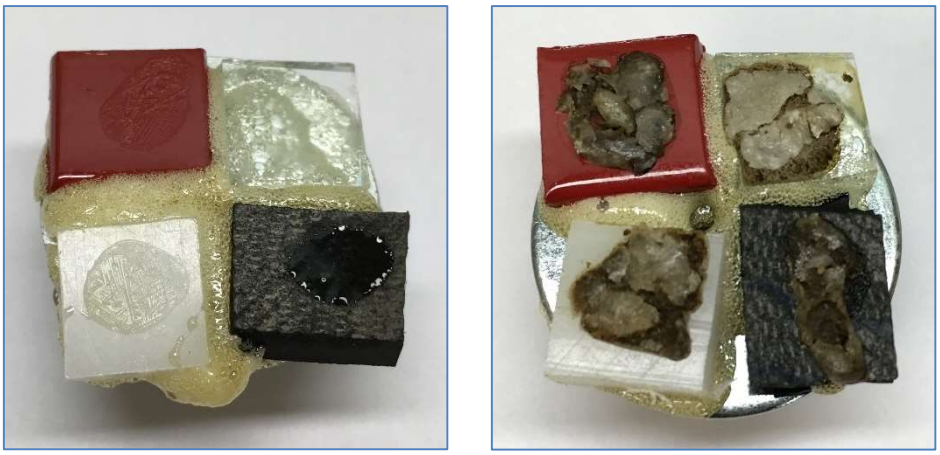

**Image 2:** Coupons with clean and grimed reference material coupons glued to washers.

The surrogates and material coupon methodologies employed in this research were agreed upon by EPA, USDA, and Sabre bioWALL collaborators and were evaluated in previous EPA scientific studies (U.S. EPA, 2014). The biological surrogates employed met several criteria: (1) they could be readily cultured; (2) they are representative of agricultural pathogens and possess a similar resistance to disinfectants or established pathogen equivalency; and (3) their bioassays have a proven track record.

Protocols for coupon preparation, packaging, surrogate inoculation, and microbiological analysis were jointly developed by Sabre and U.S. EPA. The spore discs and inoculated coupons prepared from four different materials with two surface conditions (grimed and non-grimed) were placed at three separate locations in the vehicle decontamination system (windshield, side middle, and undercarriage) (Image **3**). Following each treatment, the coupons and spore discs were removed, placed in sterile vials, labeled, and stored at 4º C (Image **4**). All MS2 coupons and spore discs were transported to the Mesa laboratory, assayed, and enumerated after the field study was completed. This study utilized 96 reference coupons (48 grimed, 48 un-grimed), 36 spore discs (36 clean), 5 water samples (4 during test, 1 background), and 8 air samples (2 Dry Filter Unit (DFU), 4 Teflon® (PTFE)), summarized in Table **2**. There were 42 different control samples which included positive controls, transit controls, and procedural blanks.

**Image 3:** Two coupons and steel washers were attached to the front windshield of the truck using an inside static magnet.

**Image 4:** Treated coupons are being prepared for labeling, storing, and transport to the Mesa laboratory for final assaying and enumeration of viable MS2 phages.

### 2.3. Automated Truck Power Wash and Disinfectant Rinse Description

The automated truck wash consisted of a power washing system (Whiting System Inc, Alexander, AR) and a modified disinfectant rinse system developed by Sabre bioWALL. The decontamination system was housed in a 38

x 24 x 10 m (126 ft x 23.75 ft x 32 ft) bay within the facility's biosecurity building (Figure **1**). All trucks entering the decontamination facility received a "de-mudder" wash (not used in this study), an under-carriage wash, and a four-pass truck wash. Following the power wash, a single disinfectant application with chlorine dioxide (DiKlor-W) was applied to the under-carriage and the truck chassis using a stationary gantry.

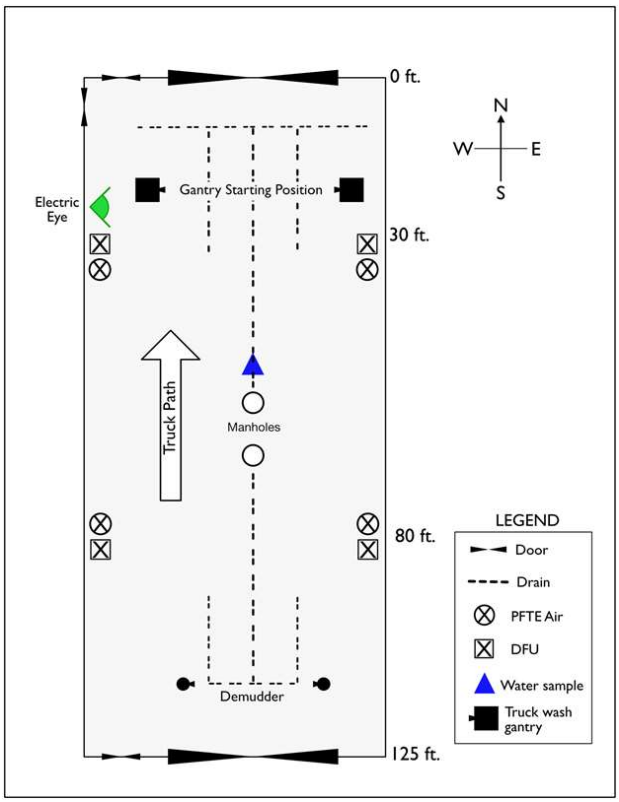


**Figure 1:** Schematic of automated power wash system for large trucks.

The Whiting truck wash could be manually operated or run with the automated Whiting software program that allowed truck drivers to decontaminate their trucks without leaving their cab. The truck wash-only treatment included the under-carriage wash and the truck wash. The truck wash and disinfectant rinse treatment included the under-carriage wash, the truck wash, and the disinfectant rinse (Figure **2**).


**Figure 2:** Power wash and disinfectant rinse run time.

The stationary under-carriage wash included two side manifolds and a recessed manifold in the bay floor, with a nozzle capacity of 8.8 l/s (140 GPM) using twenty-one nozzles. Eight nozzles were located on each of the two side manifolds, and five nozzles were in the floor manifold. The de-mudder washer was turned off during this study due to excessive nozzle pressure that could have dislodged the test coupons and spore disks.

After the under-carriage wash, the truck was signaled to pull forward for the chassis wash that utilized the overhead gantry system. The three-sided gantry (4 x 5 m) traveled on 27 m (90ft) tracks, and the gantry completed four passes over each truck. The gantry was designed to sequentially dispense cleaning solution, low-pressure rinse water, and a high-pressure wash and rinse during the four truck passes. The cleaning fluid pump rate was 1.26 l/s (20 GPM) using thirty-three ¼-inch nozzles evenly dispersed around the gantry. The third pass was a high-pressure wash, using ten tri-headed spinning motors, five nozzles on each side of the gantry, and nine high-pressure nozzles on top of the gantry. The pump rate was 8.83 l/s (140 GPM) with a pressure of approximately 2,068 Kpa (300 PSI) at the nozzle. The fourth pass was a high-pressure rinse. This rinse included stationary nozzles with a pump rate of 8.83 l/s (140 GPM) and a pressure of approximately 2,068 Kpa (300 PSI) at the nozzle.

The disinfectant rinse included two chlorine dioxide concentrations. The DiKlor-W concentrations for the chassis and under-carriage rinse were 50 mg/l and 200 mg/l, respectively. After completing the power wash, the driver was directed to slowly proceed through the DiKlor-W rinse to achieve a disinfectant contact time of approximately 30 seconds. The DiKlor-W pump rate for the undercarriage/tire wash supply was (40 GPH) to achieve the correct dosage (200 mg/l). The pump rate to achieve the desired concentration in the stationary halo (50 mg/l) was 10 GPH. This supplied the wash that utilized twenty-one nozzles in the stationary halo and twenty-one nozzles recessed within the concrete floor, ensuring complete coverage for each truck. The DiKlor rinse air-dried as the truck exited the decontamination station.

Air and water samples were collected to evaluate the potential for the power washing to remove or aerosolize the MS2 phage from the coupons. Air samples were collected with two types of air sampling instruments during each of the truck runs. The first air sample instrument was a dry filter unit (DFU) sample that collected samples from four separate bay locations simultaneously (Image **5**). The air samples were collected next to the power washing gantry, where the MS2 phages were most likely to be aerosolized. The four air filters were composited into one sample to increase the probability of MS2 detection. The second set of air samples were collected using two SKC pumps (SKC Inc., Eighty Four, PA) which were collocated with two DFUs, collected an air sample through a polytetrafluoroethylene (PTFE) filter. The filter pumps were left on during each test day and were not turned off between tests. The average airflow through the DFUs was 1,110 and 1,161 l/min for the power wash with water only and the power wash with the DiKlor-W rinse tests, respectively. The airflow rate through the PTFE filters was 5 l/min.

**Image 5:** Preparing the SKC air sampling instrument inside the automated power washing bay.

Water samples were also collected to determine whether the rinse/wastewater was contaminated with the MS2 phage. At the end of all the decontamination treatments, wastewater samples were collected from the water drains on the bay floor. Two samples were collected from the wash floor drain, two samples from the disinfectant rinse drain, and one water sample represented the background control. In addition, one media water blank, two equipment water samples, and one field blank were also collected. The average surface area for a 23 m (75 ft) truck is approximately 334 $m^2$, and the total surface area for 96 inoculated coupons is 0.0154 $m^2$. Therefore,

collecting air and water samples after all the decontamination treatments were completed, based on the total surface area of 96 coupons, represented only a small fraction of the total truck surface area that was decontaminated.

In addition to the water and air monitoring for surrogate contaminants, two non-replicated pilot tests were conducted to monitor $ClO_2$ vapor concentrations inside the truck cab and localized $ClO_2$ concentrations next to the disinfectant rinse station. Both truck operator exposure tests were conducted to determine the appropriate level of Personal Protective Equipment (PPE) needed for the truck operators. A portable C16 $ClO_2$ data logger/sensor (PortaSens II Model C16, Analytical Technology Inc., Collegeville, PA) for both tests. The portable sensor was placed next to the air vents in the truck cab to monitor and collect air quality data. The $ClO_2$ sensor had an accuracy range of 1 to 5 mg/L, a sensitivity of 0.01 mg/l, and collected data at 10 s intervals. Measurements were taken inside the cab of the truck using the C16 analyzer for five out of the nine runs in which chlorine dioxide was applied. An additional run was conducted with the air conditioning operating in the recirculation mode. The sensor was turned on before the truck entered the facility and was left running approximately three to five minutes after exiting the disinfectant rinse station. The facility operator test monitored air quality at 1.5 m height and within five m (16 ft) of the bay door next to the disinfectant rinse station for approximately 3 min after the rinse cycle was completed.

### 2.4. Analytical Methods

The study design was developed with JMP software (SAS Institute Inc., Cary, NC, USA), using the Design of Experiment (DOE) program to reduce the number of samples. The DOE design utilized hidden replication by limiting interaction terms to two-way interactions, which resulted in a design with 19 statistical replications. There were 48 power washing and rinse treatments (2 x 3 x 4 x 2 = 48). The actual coupon number averaged two replicates per treatment.

The study factors were: 1) coupon surface with or without synthetic grime (2 levels); 2) coupon location – front windshield, middle side of the trailer, undercarriage (3 levels); 3) coupon material – glass, painted metal, plastic, rubber (4 levels); and 4) automated wash status – power wash only, or power wash with $ClO_2$ rinse (2 levels). The factorial analyses were limited to only two-way interaction terms. The Generalized Linear Model (GLM) program was used to analyze the MS2 surrogate data utilizing a binomial distribution program. The GLM model was also used to analyze the *B. atrophaeus* viable spores. The significance level ($\alpha$) was set at 0.05, which is a 5% risk of concluding that an association exists.

The response variable was log10 reduction of the MS2 bacteriophage. Log10 reduction was calculated as:

A was the number of viable MS2 phages recovered from the control treatment surfaces, and B was the number of viable phages recovered from the treated surfaces:

$$Log10\ Reduction\ of\ viable\ MS2\ phages = Log(^{A}/_{B})$$

## 3. Results

Sabre bioWALL conducted a preliminary MS2 phage survival study to determine whether there would be adequate phage counts for statistical reliability when conducting the multi-day field study. Their preliminary research revealed that MS2 survival rates were low, especially when MS2 phages were inoculated on clean coupon surfaces. Therefore, estimating the survival rate was critical for this field study, where the coupons would be transported and stored several days before they could be assayed at the Mesa Laboratory. The MS2 recovery study was conducted over 12, 24, and 48 h on four coupon materials that were either clean surfaces or coated with synthetic grime. The coupon materials were glass, painted metal, plastic, and rubber. The study revealed that MS2 survival/recovery rates declined over a 48 h period (Fig. **3**). The phage recovery rate for coupons with clean surfaces declined by 1 to 1.5 $log_{10}$ as the elapsed time increased from 12 to 24 h. The synthetic grime-coated coupons had a higher MS2 survival rate than the non-grimed coupons across coupon material types and elapsed time periods. The synthetic grime provided an organic substrate that stabilized the MS2 virus and increased its

recovery rate. The phage survival rates for MS2 showed that the quicker the coupons could be treated and assayed, the phage survival rates increased, and the overall results would be more reliable and accurate.

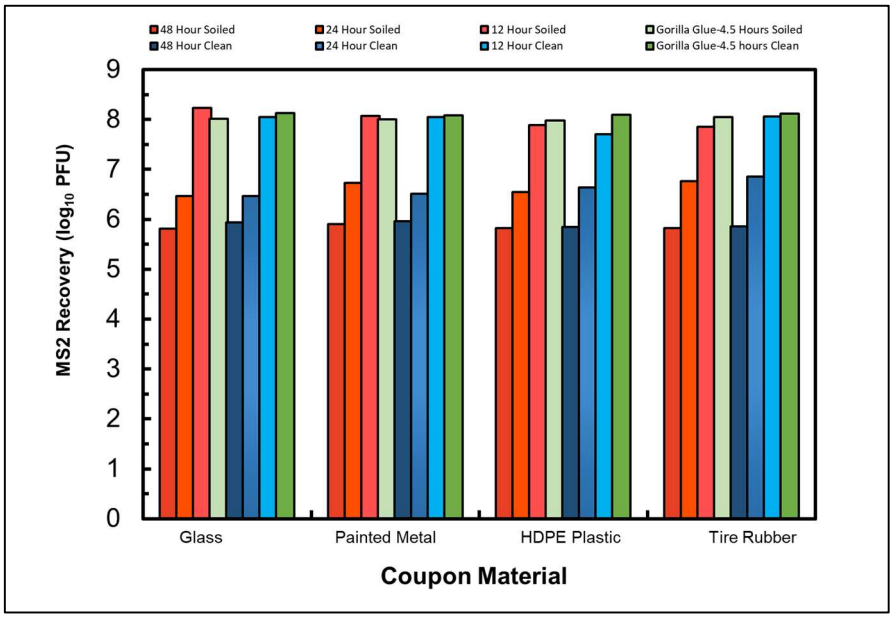


**Figure 3:** Average MS2 survival/recovery rate for four coupon materials, based on 12, 24, and 48-hour elapsed time and grimed (red bars) and non-grimed (blue bars) coupons.

The MS2 phage raw data were converted into binomial data using the transit control data. The final GLM model included all four study factors and four two-way interaction terms (Table **3**). The interaction terms require that all 48 decontamination treatments be listed in the MS2 phage, $\log_{10}$ reduction tables (Tables **4**, **5**, **6**, and **7**). The GLM model revealed that all four study factors contributed to the effectiveness of the decontamination methods and interacted with other study factors to either increase or decrease the overall phage inactivation rate.

**Table 3:** Generalized Linear Model (GLM) results for study factors and their interactions.

| Source | ChiSquare | Prob>ChiSq |
|---|---|---|
| Synthetic Grime | 5.37 | 0.0204 |
| Location | 9.96 | 0.0069 |
| Coupon material | 50.25 | <.0001 |
| Water Wash or Water + $ClO_2$ rinse | 71.90 | <.0001 |
| Synthetic Grime* Location | 29.04 | <.0001 |
| Synthetic Grime* Coupon material | 58.18 | <.0001 |
| Location* Coupon material | 49.75 | <.0001 |
| Location* Water Wash or Water + $ClO_2$ | 38.55 | <.0001 |

The $\log_{10}$ reduction tables show that the two-stage decontamination treatment had higher MS2 reduction rates compared to the single-stage, automated power wash treatment. The four tables are arranged to list the predicted $\log_{10}$ reduction for either a single (Tables **4** and **5**) or two-stage (Tables **6** and **7**) decontamination treatment and by the synthetic grime status of coupons. The log10 reduction tables also show that the coupon materials and coupon location influenced MS2 phage efficacy.

**Table 4:** Predicted $\log_{10}$ reduction of MS2 phage and 95% CI, based on sample location, grime status, and sample material, for the automated wash without the DiKlor disinfectant rinse and without synthetic grime.

| Location | Material | Predicted Log Reduction-Median | Lower 95% CI for mean test | Upper 95% CI for mean test |
|---|---|---|---|---|
| Windshield | Glass | 0.48 | 0.31 | 0.68 |
| Windshield | Painted Metal | 1.88 | 0.01 | 5.25 |
| Windshield | Plastic | 1.09 | 0.81 | 1.38 |
| Windshield | Rubber | 0.03 | 0.01 | 0.11 |
| Side Middle | Glass | 5.71 | 0.00 | 168.38 |
| Side Middle | Painted Metal | 0.14 | 0.02 | 0.65 |
| Side Middle | Plastic | 1.07 | 0.80 | 1.35 |
| Side Middle | Rubber | 0.29 | 0.15 | 0.50 |
| Undercarriage | Glass | 1.10 | 0.60 | 1.67 |
| Undercarriage | Painted Metal | 0.25 | 0.04 | 0.84 |
| Undercarriage | Plastic | 1.81 | 1.20 | 2.45 |
| Undercarriage | Rubber | 0.93 | 0.53 | 1.41 |

**Table 5:** Predicted $\log_{10}$ reduction of MS2 phage and 95% CI, based on sample location, grime status, and sample material, for the automated wash without the DiKlor disinfectant rinse and with synthetic grime.

| Location | Material | Predicted Log Reduction-Median | Lower 95% CI for mean test | Upper 95% CI for mean test |
|---|---|---|---|---|
| Windshield | Glass | 0.47 | 0.31 | 0.67 |
| Windshield | Painted Metal | 2.06 | 0.96 | 3.20 |
| Windshield | Plastic | 1.15 | 0.64 | 1.72 |
| Windshield | Rubber | 0.63 | 0.40 | 0.92 |
| Side Middle | Glass | 0.82 | 0.54 | 1.13 |
| Side Middle | Painted Metal | 0.76 | 0.54 | 1.00 |
| Side Middle | Plastic | 0.88 | 0.54 | 1.28 |
| Side Middle | Rubber | 1.03 | 0.61 | 1.50 |
| Undercarriage | Glass | 1.29 | 0.82 | 1.79 |
| Undercarriage | Painted Metal | 0.57 | 0.41 | 0.76 |
| Undercarriage | Plastic | 1.03 | 0.51 | 1.63 |
| Undercarriage | Rubber | 0.54 | 0.34 | 0.79 |

The MS2 $\log_{10}$ reduction for the single-stage, automated power wash without synthetic grime on the coupons is reported by coupon location and material type (Table **4**). The glass coupons had the highest $\log_{10}$ reduction (5.71) for coupons placed on the side of the truck. The lowest $\log_{10}$ reduction (0.03) occurred for rubber coupons placed on the truck windshield. The upper 95% confidence interval (CI) for the painted metal coupon on the windshield and glass coupon located on the side of the trailer were substantially higher in comparison to all the other predicted values. The coupons in both treatments had a non-porous surface, with no synthetic grime added, which may explain the range of variation in $\log_{10}$ reduction.

The MS2 $\log_{10}$ reduction for the single-stage, automated power wash with synthetic grime on the coupons is reported by coupon location and material type (Table **5**). Painted metal coupons had the highest $\log_{10}$ reduction (2.06) for coupons placed on the windshield. Glass coupons had the lowest $\log_{10}$ reduction (0.47) for coupons placed on the windshield. In the power wash-no grime analysis (Table **5**), the non-porous painted metal coupon on the windshield had the highest upper 95% CI level. The 95% CI levels for the single-stage, automated power wash with grime were much more uniform, which may have resulted from adding synthetic grime to the coupons, which increased phage survival rates.

There are few differences in $\log_{10}$ reduction between Table **4** and Table **5**. These results show that the addition of synthetic grime to the coupons had little effect on the overall efficacy of the automated power washing system. The hydraulic pressure from power washing was high enough to dislodge the viral surrogate on the non-grimed coupons and the surrogate/grime complex on the grimed coupons.

The MS2 $\log_{10}$ reduction for the two-stage (power wash + disinfectant rinse) with synthetic grime on the coupons is reported by coupon location and material type (Table **6**). The two-stage treatment with the highest $\log_{10}$ reduction (4.31) was found for painted metal coupons placed on the windshield. The lowest $\log_{10}$ reduction (0.51) was found for plastic coupons placed on the undercarriage. For all four coupon materials, the sample location with the lowest average $\log_{10}$ reduction was the undercarriage. Overall, the glass coupons had the highest $\log_{10}$ reduction regardless of location.

**Table 6:** Predicted $\log_{10}$ reduction of MS2 phage and 95% CI, based on sample location, grime status, and sample material, for the automated wash with the DiKlor disinfectant rinse and synthetic grime.

| Location | Material | Predicted Log Reduction-Median | Lower 95% CI for mean test | Upper 95% CI for mean test |
|---|---|---|---|---|
| Windshield | Glass | 1.71 | 1.10 | 2.35 |
| Windshield | Painted Metal | 4.31 | 2.92 | 5.71 |
| Windshield | Plastic | 2.37 | 1.37 | 3.38 |
| Windshield | Rubber | 2.89 | 1.95 | 3.83 |
| Side Middle | Glass | 1.59 | 1.00 | 2.20 |
| Side Middle | Painted Metal | 2.36 | 1.65 | 3.08 |
| Side Middle | Plastic | 1.51 | 0.86 | 2.19 |
| Side Middle | Rubber | 2.78 | 1.87 | 3.69 |
| Undercarriage | Glass | 0.85 | 0.56 | 1.18 |
| Undercarriage | Painted Metal | 0.87 | 0.62 | 1.15 |
| Undercarriage | Plastic | 0.51 | 0.31 | 0.76 |
| Undercarriage | Rubber | 0.93 | 0.56 | 1.34 |

The MS2 $\log_{10}$ reduction for the two-stage (power wash + disinfectant) treatment without synthetic grime on the coupons is reported by coupon location and material type (Table **7**). The highest $\log_{10}$ reduction (7.78) was seen for glass coupons placed on the side of the truck. The lowest $\log_{10}$ reduction (0.17) was for painted metal coupons on the undercarriage. The sample location with the lowest average $\log_{10}$ reduction was the undercarriage, and the coupon type with the lowest $\log_{10}$ reduction was rubber.

The average viable spore recovery for the *B. atrophaeus* spore discs was 5.48 $\log_{10}$ for the power wash-only treatments. Power washing alone only resulted in a modest 0.52 $\log_{10}$ reduction compared to the transit control reference value. The average viable spore recovery for the *B. atrophaeus* spore discs was 7.27 $\log_{10}$, for the power wash + DiKlor rinse treatments, which was a 1.79 $\log_{10}$ decrease in spore efficacy when compared to the power

wash only treatments. These results are challenging in that the two-stage decontamination treatments should have increased the spore inactivation rates. One possible explanation is that the spore discs were contaminated with other *Bacillus* species, which may have increased the final CFU counts. The overall results indicate that power washing did not dislodge many of the spores from the coupons and that the rinse treatments did not improve spore inactivation.

**Table 7:** Predicted $\log_{10}$ reduction of MS2 phage and 95% CI, based on sample location, grime status, and sample material, for the automated wash with the DiKlor disinfectant rinse and without synthetic grime.

| Location | Material | Predicted Log Reduction-Median | Lower 95% CI for mean test | Upper 95% CI for mean test |
|---|---|---|---|---|
| Windshield | Glass | 2.95 | 1.43 | 4.49 |
| Windshield | Painted Metal | 3.54 | 0.25 | 7.18 |
| Windshield | Plastic | 3.01 | 1.98 | 4.05 |
| Windshield | Rubber | 0.39 | 0.20 | 0.65 |
| Side Middle | Glass | 7.78 | 0.00 | 170.46 |
| Side Middle | Painted Metal | 0.75 | 0.14 | 1.76 |
| Side Middle | Plastic | 2.41 | 1.53 | 3.29 |
| Side Middle | Rubber | 0.83 | 0.47 | 1.27 |
| Undercarriage | Glass | 1.83 | 0.65 | 3.11 |
| Undercarriage | Painted Metal | 0.17 | 0.01 | 0.90 |
| Undercarriage | Plastic | 1.88 | 1.23 | 2.56 |
| Undercarriage | Rubber | 0.52 | 0.29 | 0.82 |

The air monitoring test showed that the composited DFU filters collected an average of $4.12 \times 10^6$ PFU for the MS2 phage, with an average volume of $2.14 \times 10^5$ l for the power washing alone treatments. Air monitoring for the power washing + the DiKlor rinse treatments averaged $4.40 \times 10^6$ PFU, with an average volume of $3.02 \times 10^5$ l with the composited DFU filters. Air monitoring with the SKC filters resulted in a measurement of non-detect on the first filter and $5.03 \times 10^6$ PFUs on the second filter, with an average volume of $9.65 \times 10^2$ l. for the power wash alone treatments. The power wash + DiKlor rinse treatment with the SKC filters resulted in a measurement of non-detect on both filters, with an average volume of $1.3 \times 10^3$ l. The air monitoring results from the DFU and SKC filter methods differed because of the lower air volume rates for the SKC pumps. The DFU filters for both the power washing alone and the power washing + DiKlor treatments showed that MS2 was aerosolized from the coupons during the power washing treatments. The wastewater monitoring tests resulted in no detectable MS2 phage counts from any of the water samples.

The truck cab air monitoring test did not detect any $ClO_2$ levels above the sensitivity limits of the PortaSens C-16 sensor (0.01 mg/l). The air monitoring near the DiKlor rinse gantry recorded $ClO_2$ concentrations up to 3 mg/l within 5 m of the bay exit door.

# 4. Discussion

The Highly Pathogenic Avian Influenza outbreak in 2015-16 among poultry farms in the midwestern U.S. prompted poultry producers to increase biosecurity at each farm or production facility. Also, the swine porcine (PRRSV) outbreak in 2013-14 in the U.S. stimulated swine producers to follow suit and increase their biosecurity methods to prevent farm to farm transmission of viral diseases. One of the farm biosecurity methods involved installing automated truck washes at each production facility to mitigate the transmission of any animal diseases by any vehicles entering the facilities. To the author's knowledge, this is the first study that investigated the

decontamination effectiveness of a two-stage automated washing system on a large truck chassis. Such studies are vitally important for preventing vehicle transmission of animal diseases and reducing the risk of another widespread viral outbreak in the U.S. animal feed industries.

Three decontamination studies evaluated the effects of disinfectants for viral efficacy. The first study conducted by Alphin *et al*. [9] evaluated the effects of several disinfectants on the Newcastle Disease Virus (NDV). The authors found only one disinfectant that significantly reduced the NDV titer, which was glutaraldehyde (1%) applied as an aerosolized fog to coupons placed inside and on the outer surface of the equipment. A study by Dee *et al*. [10] evaluated the effects of power washing, power washing followed by fumigation treatments, and power washing followed by heat treatment on PRRSV efficacy, using swine trailer equipment. The authors state that two out of four treatments had significant PRRSV viral efficacy. The first treatment was power washing followed by glutaraldehyde: quaternary ammonium treatment had 0 out of 19 trailer sample swabs return with positive PCR readings for the PRRSV virus. The second treatment with power washing followed by overnight drying had 0 out of 20 trailer sample swabs return with positive PCR readings for the PRRSV virus.

A third study by Guan *et al*. [8] evaluated the effects of power washing followed by disinfectant treatments on *Geobacillus stearothermophilus* and Infectious Bursal Disease Virus (IBDV) efficacy on-field equipment during cold weather conditions. The authors evaluated a combination of four-stage decontamination methods involving dry cleaning, power washing, disinfectant treatment, and final rinse. They evaluated two disinfectants, bleach (5,200 mg/l) and Virkon-S (2% or 20,000 mg/l) for the four-step method. They also found that the $\log_{10}$ reduction of the IBDV virus ranged from 1.4 to 3.4 when tested for three locations, four power washing variables, three disinfectants, and three equipment locations. The highest $\log_{10}$ reduction of IBDV viral surrogate was 3.3, which involved a four-step decontamination process.

In contrast, the highest MS2 $\log_{10}$ reduction for this study was 7.8 for the automated power wash, followed by a $ClO_2$ rinse (DiKlor at 200 mg/l) for non-grimed, glass coupons placed on the side of the truck trailer. The average $\log_{10}$ reduction was 2.7 and 1.9 for the four-step decontamination study Guan *et al*. [8] and this study (based on grimed coupons with power washing followed by a DiKlor rinse), respectively. These studies used different viral surrogates, different power washing, different disinfectants, and different decontamination methods (manual versus automated). However, the final $\log_{10}$ reduction results for the viral surrogates were comparable between the two studies, which authenticates both study designs and implementation.

In general, the two-step process of the automated power wash followed by the $ClO_2$ rinse increased $\log_{10}$ reduction. $\text{Log}_{10}$ reduction increased an average of 247 and 118% for the non-grimed and grimed coupons, respectively, when comparing the automated wash alone to the automated wash + $ClO_2$ rinse across all locations and material types. The average $\log_{10}$ reduction increased from 0.94 to 1.89 for the automated wash and the automated wash + $ClO_2$ rinse, respectively, for the grimed coupons, across all coupon locations and materials. The average $\log_{10}$ reduction increased from 1.23 to 2.17 for the automated wash and the automated wash + $ClO_2$ rinse, respectively, for the non-grimed coupons, across all coupon locations and materials. These results show that adding the $ClO_2$ disinfectant rinse to the automated power wash increased viral efficacy by an average of one log (grimed coupons), which should justify the economics of this decontamination treatment.

Non-grimed coupons generally had a higher $\log_{10}$ reduction (2.2) when compared to grimed coupons (1.9) across all treatments. Any reduction in MS2 survival/recovery rates due to non-porous and non-grimed surfaces increased the variation in phage efficacy levels among the treatments. The addition of grime to the coupons increased the MS2 survival rate due to the organic material acting as a substrate for the virus. The grimed coupons also partially encapsulated or absorbed the virus during the inoculation process, limiting dislodging and/or inactivation of the virus during power washing. Under real-world vehicle decontamination conditions, it is expected that the vehicles would have road grime covering a large percentage of the vehicle.

Four materials were evaluated in this study to determine the ability of power washing and disinfectants to sanitize porous (plastic, rubber) and non-porous materials (glass, painted metal). Glass was the coupon material with the highest $\log_{10}$ reduction (7.78). Rubber was the coupon material with the lowest $\log_{10}$ reduction (0.03). The average $\log_{10}$ reduction was 1.19 and 2.21 for the porous and non-porous coupon materials, respectively, for the

non-grimed coupons, across all locations and washing/decontamination treatments. The average $\log_{10}$ reduction was 1.35 and 1.47 for the porous and non-porous coupon materials for the grimed coupons, respectively, across all locations and washing/disinfectant treatments. The porous materials reduced viral efficacy by approximately one log for the non-grimed coupons. Tire surfaces would probably be heavily contaminated due to soil dust and organic debris pressed into the tire treads. Unfortunately, the porous surface of rubber tires also reduces the effectiveness of most decontamination methods. There is a real-world trade-off between increasing the undercarriage power washing pressure and total water volume to improve decontamination of the truck tires and the increased cost of sanitizing the large volume of recycled wastewater.

The three locations for the coupons evaluated the ability of power washing and disinfectants to decontaminate exposed and non-exposed surfaces on the truck chassis. The locations were the front windshield, side panel, and undercarriage. The coupon location with the highest $\log_{10}$ reduction was the side panel (7.78). The coupon location with the lowest $\log_{10}$ reduction was the front windshield (0.03). The average $\log_{10}$ reduction was 1.67, 2.37, and 1.06 for the front, side panel, and undercarriage, respectively, for the non-grimed coupons across all coupon materials and washing/decontamination treatments. The average $\log_{10}$ reduction was 1.95, 1.47, and 0.82 for the front, side panel, and undercarriage, respectively, for the grimed coupons across all coupon materials and washing/decontamination treatments. Coupons placed on the truck undercarriage reduced viral efficacy by approximately one log for the grimed coupons. These results show that decontamination of vehicle undercarriage remains a daunting challenge even with high pressure, automated power wash systems using wash manifolds directed at the entire undercarriage.

This study was designed as a factorial study, where the four study factors could be tested for their interactions with each other. Data analysis shows that all the study factors interact, i.e., all the study factors influence each other's decontamination effectiveness. The results show that a two-stage decontamination method (power wash + disinfectant rinse) improves the overall decontamination effectiveness. The results also show that vehicle tires and undercarriage are the most challenging surfaces to sanitize on vehicles, and future research could focus on these critical locations. Other areas of interest for future studies could include the total length of power washing time, improved undercarriage power washing manifolds, increasing disinfectant concentrations, increasing the disinfectant contact time, or double automated washes for animal emergencies or high-risk infectivity events.

Automated power wash systems for tractor-trailer trucks generally have a water use rate of about 3,785 l/m (1,000 GPM) [20]. The estimated total water volume needed to power wash one 15 to 18 m (50 – 60 ft) truck is about 7,570 to 15,141 l (2,000 to 4,000 gal), depending on whether de-mudding manifolds are used. If this wastewater is recycled and filtered, the contaminated water would still need to be sanitized before reusing it for decontamination of other vehicles. This large wastewater volume for each truck needs to be considered when designing a water recycling system for automated washing systems.

The truck cab air quality was monitored during the power wash + DiKlor disinfectant runs (5 of the 9 runs) to determine if any $ClO_2$ vapors would enter the cab. The results show that no $ClO_2$ vapors were detected during any of the runs. In addition, air monitoring during the extra run with the air conditioning operating in the recirculation mode also showed no $ClO_2$ vapors detected inside the cab. The OSHA Permissible Exposure Limit (PEL) $ClO_2$ is 0.1 mg/l, and the Short-Term Exposure Limit (STEL) is 0.3 mg/l, based on a 15 min exposure time [21]. This preliminary test shows that the truck driver was not exposed to any health risks from the $ClO_2$ rinse, as the air quality inside the cab during the power washing + DiKlor rinse treatments met the PEL and STEL limits.

The operator exposure test for working near the disinfectant rinse station was conducted to determine the appropriate level of personal protective equipment (PPE) for the operators. The $ClO_2$ concentration reached as high as 3 mg/l over the monitoring time. However, the measurements were collected 5 m from the wash area, and the $ClO_2$ rapidly dissipated in the open air. Ultra-violet radiation in sunlight degrades low $ClO_2$ concentrations to non-detect levels in less than a minute.

In the past two decades, the surge in zoonotic diseases has forced large agricultural facilities to consider investing in biosecurity systems that include vehicle decontamination facilities. Evaluation of decontamination and biosecurity systems and methods will help producers find cost-effective methods and reduce their risks for the

farm-to-farm spread of high-risk pathogens [1-3]. Designing field studies to evaluate biosecurity or decontamination systems or methods should be well thought out and planned. A well-designed decontamination study may include multiple surrogates, secondary studies for environmental monitoring, and 'real-world scenarios" to fully assess the effectiveness of the decontamination system. This study attempted to fully evaluate the automated truck wash by including secondary environmental studies. The authors are hopeful that this field study will provide much-needed decontamination information so that producers can evaluate the effectiveness of large, automated power washing systems.

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